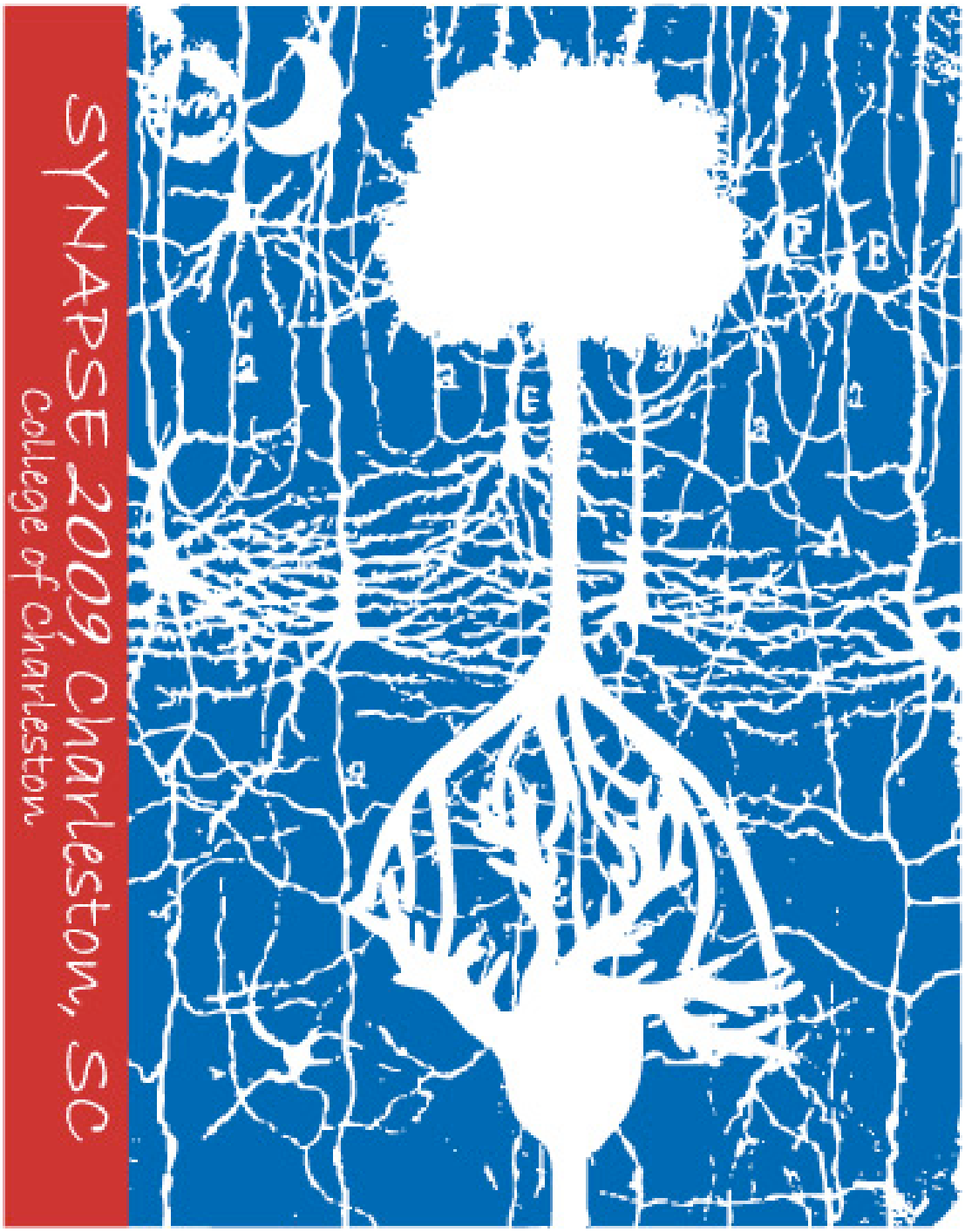

Symposium for Young Neuroscientists and Professors of the Southeast 2009

This symposium was funded by a research symposium grant from the South Carolina EPSCoR/IDeA Program (www.scepscoridea.org).

SC EPSCoR/IDeA

Support was also provided by the College of Charleston's Undergraduate Research and Creative Activities Office (www.cofc.edu/UR) and the Howard Hughes Medical Institute.

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HHMI

We would also like to say a special thanks to Vanessa McNamara in the Office of the Dean of the School of Science and Math for all of her help with scheduling the conference and to Barbra Bannan for providing the administrative support required to run the conference smoothly.

Symposium Schedule

Human Disease Research in the 21st Century

Saturday, March 28, 2009

8:00-9:00a.m. Breakfast, Registration and Student Poster Set-up

9:00-10:00a.m Welcome: Drs. Chris Korey and Beth Meyer-Bernstein
(*Physicians Auditorium)*

Keynote: **Huntington's Disease - Genetics and Neuroscience**
Marcy E. MacDonald, Ph.D.
Center for Human Genetic Research, Harvard Medical School

10:00-10:45a.m. Student Platform Session I (*Physicians Auditorium*)

- **Novel GABAA Receptor Mutations Exhibit Subunit-Selective Effects on Receptor Properties**
  Shana Dykema, University of South Carolina
- **Long-Term Potentiation in a Sprouted Hippocampal Pathway**
  Sarah Rhodes, Davidson College
- **The Role of Dopamine D2 and D3 Receptor in Adolescent Nicotine Sensitization and Conditioned Hyperactivity in Rats D2-receptor Primed as Neonates: Sex Differences**
  Julia Lehmann, East Tennessee State University

10:45-11:00a.m. Coffee Break

11:00-12:00p.m. Poster Session I (Even Posters in *Alumni Hall*)

12:15-1:45p.m. Lunch/Student Workshops (*Rita Liddy Hollings Science Center*)

- Session I: 12:15-1:00 p.m.; Session II: 1:00-1:45 p.m.

2:00-3:00p.m. Poster Session II (Odd Posters in *Alumni Hall*)

3:00-3:30p.m. Student Platform Session II (*Physicians Auditorium*)

- **Altered Peripheral and Spinal Innervation Patterns in a Rodent Model of Fetal Alcohol Exposure: Implications for Somatosensation and Nociception**
  Jeet Guram, University of South Carolina
- **Self-Administration of Heroin and Incubation of Drug-Seeking Behavior in Adolescent and Adult Male Rats**
  James Doherty, Georgia State University

3:30-4:30 p.m. Closing Keynote: **And the Crowd Goes Wild: The Eruption of Personal Genomics**
Misha Angrist, Ph.D.
Duke Institute of Genome Science and Policy

4:30 p.m. Closing Remarks

Lunch Workshops

Each workshop discussion will last 45 minutes and will be presented twice (12:15-1:00p.m. and 1:00-1:45p.m.).

Workshop: Scientific Writing
Location: Science Center Room 108
Leader: Jennifer G. Schnellmann, Ph.D., E.L.S., Medical University of South Carolina

Workshop: AMCAS Enhancement Tips
Location: Science Center Room 218
Leader: Karen Eippert, Director of Pre-professional Health Advising, College of Charleston

Workshop: Applying to Graduate School
Location: Science Center Room 106
Leader: James Buggy, Ph.D. Dean of the Graduate School, University of South Carolina

Workshop: Alternative Careers in Neuroscience
Location: Science Center Room 239
Leaders: Christine Lauay, Ph.D. (Medical Writer), Yashmin Karten, Ph.D. (Technology Transfer)

Workshop: Personal Genomes: Have we arrived?
Location: Science Center Room 317
Leader: Dana Waring, Personal Genetics Education Project-Harvard Medical School

The following workshops will only be presented only once:

Workshop: Teaching Undergraduate Neuroscience in the Digital Age
Location: Science Center Room 227
Leader: Chris Korey, College of Charleston

Workshop: Brain Awareness Week
Location: Science Center Room 277
Leader: Open Discussion

SYNAPSE Participants

Marcy E. MacDonald, Ph.D.: Marcy MacDonald is Professor of Neurology at Harvard Medical School. She earned a B.Sc. in Honors Biology from the University of Ottawa before receiving a PhD in Medical Biophysics from the University of Toronto. In 1983, Marcy, Jim Gusella, and colleagues mapped the Huntington's gene to the 4th chromosome. As part of the Huntington's Disease Collaborative Research Group, Marcy's lab continued to play a major role in the final identification of an expanded CAG repeat as the disease culprit in 1993. Since then, her lab at Massachusetts General Hospital's Center for Human Genetic Research has continued to be at the forefront of Huntington's Disease research. Marcy has received several awards in recognition of her major contributions to the understanding of Huntington's Disease, including the National Health Council Award for Medical Research and the Milton Wexler Award for Huntington's Disease Research.

Misha Angrist, Ph.D.: Misha Angrist is Assistant Professor of the Practice at the Duke University Institute for Genome Sciences & Policy. He earned a Ph.D. degree in Genetics from Case Western Reserve University, an MFA in Writing and Literature from the Bennington Writing Seminars, and was formerly a board-eligible genetic counselor. He has covered the biotechnology industry as market-research analyst and worked as an independent life sciences consultant, writer and editor. His current research interests include genetic privacy, gene patents and the ethical, legal and social issues surrounding affordable, large-scale DNA sequencing. In April 2007 he became the fourth subject in Harvard geneticist George Church's Personal Genome Project. He is writing a book about that experience as well as the larger emergence of personal genomics, to be published by HarperCollins in 2010.

Workshops:

Karen Eippert: Karen Eippert came to the College of Charleston in March, 2006. Prior to accepting her position as Director of Pre-professional Health Advising, she worked for 13 years at the Medical University of South Carolina in admissions, recruitment, and student development. Ms. Eippert earned a B.S. in Psychology from the College of Charleston and completed a master's degree in Career Development at John F. Kennedy University in the San Francisco Bay area. Her professional career has been devoted to helping students prepare for careers in health care.

James Buggy, Ph.D.: James Buggy has been with the Graduate School at the University of South Carolina since 2006, first as Associate Dean for Academic Affairs and currently as Interim Dean. He had previously served as Assistant Dean for Graduate Studies and Academic Director for the Biomedical Science and Nurse Anesthesia graduate programs for the USC School of Medicine and Department of Pharmacology, Physiology, and Neuroscience. He has been recognized with the USC School of Medicine Faculty Research Award in 1983 and School of Medicine Teaching Advancement Awards in 1997, 1998, 2000, 2003, and 2005. Previously, he was an instructor in Physiology and fellow at the Cardiovascular Center of the University of Iowa, earned the PhD in Psychobiology from the University of Pittsburgh, and was introduced to the science laboratory as a

work-study undergraduate at the University of Pennsylvania.

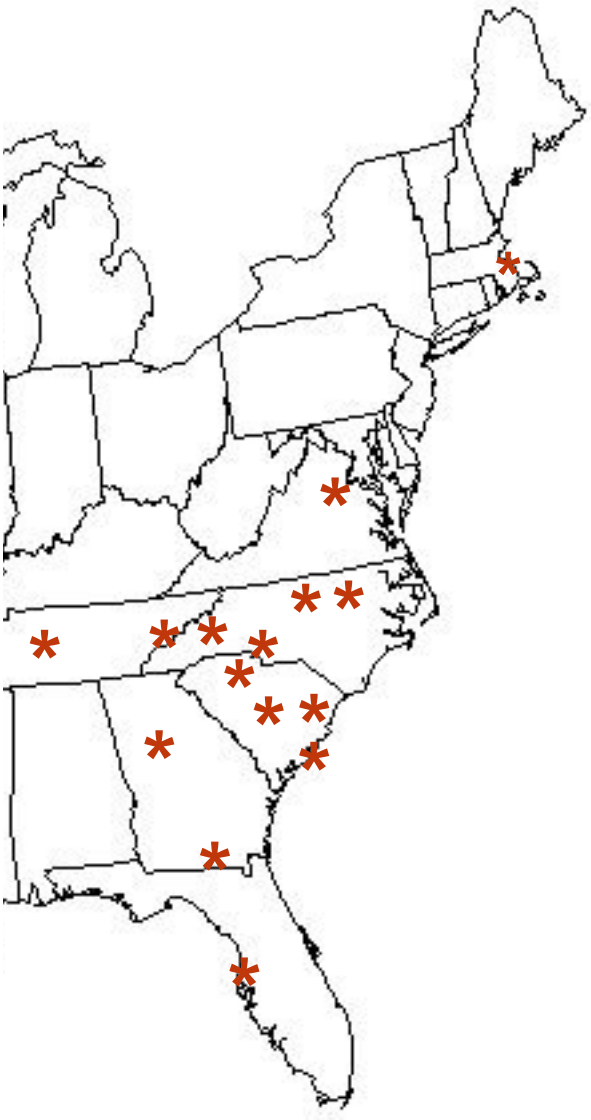
Jennifer G. Schnellmann, Ph.D., E.L.S.: Jennifer G. Schnellmann is the Director of the MUSC Office of Scientific Editing and Publications at MUSC. She received a PhD in pharmacology and toxicology from the University of Arkansas for Medical Science in Little Rock, AR and completed a postdoctoral fellowship in neurotoxicology with the FDA at the National Center for Toxicological Research. She joined MUSC in 2001 as a board-certified editor in the life sciences where she serves as science/medical writer/editor.

Christine Lauay, Ph.D.: Dr. Christine Lauay received her Ph.D. in Biopsychology from the Department of Psychology of Cornell University in 2003. She did postdoctoral research at Northwestern University from 2003 to 2004, after which she began her career as a medical writer. Dr. Lauay is currently employed by Delta Pharma, Deerfield, Illinois. She works with pharmaceutical companies to write a variety of regulatory documents required during clinical drug development, including new drug applications, clinical study protocols, clinical study reports, and annual reports.

Yashmin Karten, Ph.D.: Yashmin Karten is Licensing Manager at the MUSC Foundation for Research Development. Her role is to develop and implement commercialization strategies for MUSC inventions and technologies. She was a postdoctoral researcher at the National Institute of Mental Health and at the University of Arizona and has several years of research experience in the field of neuroscience. She received her Ph.D. degree in Medical Biology from the University of Amsterdam in 2000.

Dana Waring: Dana Waring is a co-founder of the Personal Genetics Education Project at Harvard Medical School. Her current interests are the intersection of personal genomics and privacy, healthcare, and public policy. In addition to regularly presenting to diverse audiences about personal genomics and society, Dana develops curricular materials for use in high school, college, and community settings. Dana has a special interest in the ways that information about one's genome might impact reproductive decisions and family dynamics. She earned her BFA from Syracuse University and her Master's degree in Women's Studies at the Harvard University Extension School.

Schools Represented at SYNAPSE 2009



Appalachian State University (NC)
Belmont University (TN)
College of Charleston (SC)
Davidson College (NC)
Duke University (NC)
East Tennessee State University (TN)
Elon University (NC)
Francis Marion University (SC)
Furman University (SC)
George Mason University (VA)
Georgia State University (GA)
Harvard Medical School (MA)
Medical University of South Carolina (SC)
North Carolina A&T State University (NC)
University of South Carolina (SC)
University of Tampa (FL)
Valdosta State University (GA)
Wake Forest University (NC)

SYNAPSE 2009 Abstracts

Student Platform Presentations:

Novel GABAA Receptor Mutations Exhibit Subunit-Selective Effects on Receptor Properties

Dykema SK & Fisher JL
Department of Pharmacology, Physiology and Neuroscience, University of South Carolina School of Medicine and the South Carolina Honors College

The GABAA receptor is a ligand-gated ion channel found throughout the brain, and is responsible for mediating the effects of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the CNS. A novel mutation in the GABAA receptor has recently been associated with febrile seizures (FS), which account for the majority of childhood seizure disorders, affecting 2-5% of children under five years of age. This mutation resulted in the substitution of a glycine for a highly conserved arginine residue in the external N-terminus (R139G) of the γ2 subunit. To investigate whether the R139G shift caused subunit-specific effects on receptor function, we created this mutation in the α1, β3, and γ2 subunits of the GABAA receptor. In the α1 subunit, the mutation caused reduced GABA sensitivity, sped deactivation, and slowed desensitization. In the β3 subunit, this mutation caused a slight increase in sensitivity to GABA and slowed deactivation, but did not alter desensitization. Finally, in the γ2 subunit, the R139G mutation reduced GABA sensitivity, sped deactivation, and completely eliminated desensitization in response to GABA concentrations up to 10 mM. These findings reinforce the hypothesis that this mutation in the γ2 subunit could be linked to inherited forms of epilepsy, and that changes at this site exhibit subunit-selective effects on receptor activity.

Long-Term Potentiation in a Sprouted Hippocampal Pathway

Rhodes S, Robinson M, Lang K, & Ramirez JJ
Center for Interdisciplinary Studies, Davidson College

In the rat, unilateral damage to the entorhinal cortex destroys a primary input to the dentate gyrus of the hippocampus. The result is a temporary deficit in spatial working memory. Beginning 8 to 12 days after an entorhinal lesion, the crossed temporodentate pathway (CTD) undergoes axonal sprouting, expanding its connections with the dentate gyrus and reversing the mnemonic impairment. Performing a progressive lesion over two surgeries separated by 6 days accelerates sprouting and causes less memory impairment as early as 4 days after the brain injury. To elucidate the electrophysiological mechanisms that underlie spatial working memory, this study examines the emergence of the capacity for long-term potentiation (LTP) in the sprouted CTD after one-stage and progressive entorhinal lesions, at a time point just after behavioral recovery is known to occur following a one-stage lesion. Fifteen days after lesion completion, responses of the CTD to electrical stimulation were recorded before and after a high-frequency tetany protocol designed to induce LTP. Neither the maximum amplitude nor the maximum rising slope of the responses underwent a statistically significant increase after tetany, indicating that the sprouted CTD is not capable of supporting LTP 15 days after one-stage or progressive lesions. Because LTP does not emerge as rats recover memory function, LTP does not appear necessary for spatial working memory. Both the amplitude and slope for progressive cases were statistically greater than for control cases, while one-stage cases did not differ from controls. Progressive lesions, therefore, enhance synaptic efficacy for up to 15 days post-lesion.

The role of dopamine D2 and D3 receptor in adolescent nicotine sensitization and conditioned hyperactivity in rats D2-receptor primed as neonates: Sex differences

Lehmann J, Sheppard AB, Cope ZA & Brown RW
Department of Psychology, East Tennessee State University

Smoking cigarettes often begins in adolescence, and smoking behavior is approximately 3-5 times more likely in psychosis compared to the general population. Both male and female Sprague-Dawley rats were neonatally treated with either saline or the dopamine D2 /D3 receptor agonist quinpirole from postnatal days (P) 1-21. This treatment produces long-term D2 receptor priming, consistent with psychosis. Beginning on P33, all animals were first injected with the D2 receptor antagonist eticlopride (0.1 mg/kg) the D3 antagonist nafadotride (1 mg/kg) or saline followed 10 min later by either nicotine (0.5 mg/kg free base) or saline. Approximately 10 min after the nicotine or saline injection, all rats were placed into an arena and locomotor behavior measured. On P50, animals were given saline before being placed into the same locomotor arena and conditioned activity measured. Results showed that eticlopride was more effective at blocking nicotine sensitization in D2-primed females compared to males, and eticlopride produced locomotor depression in control females. On the drug free test, eticlopride blocked nicotine conditioned hyperactivity, and produced conditioned hypoactivity in control females. The D3 antagonist nafadotride blocked nicotine sensitization more effectively in males compared to females, but on the drug free test nafadotride equally blocked nicotine-conditioned hyperactivity in males and females. In conclusion, the D2 receptor in adolescent females appears to be more sensitive the locomotor activating effects of nicotine as well as locomotor depression produced by D2 antagonism compared to males, and the D3 receptor appears to play a more important role in nicotine sensitization in males. Supported by the East TN State Univ Honors College.

Altered Peripheral and Spinal Innervation Patterns in a Rodent Model of Fetal Alcohol Exposure: Implications for Somatosensation and Nociception

Guram JS, Sanders DL, McKelvy AD, & Sweitzer SM
South Carolina Honors College

Fetal alcohol syndrome disorder (FASD) occurs in 1-10 out of every 1,000 births. Our laboratory has shown that fetal ethanol exposure leads to decreased sensitivity to non-noxious mechanical stimuli and increased sensitivity to noxious thermal stimuli. Our hypothesis is that fetal ethanol exposure results in aberrant termination patterns of large Aβ- and small C-fibers in the skin and spinal cord, increasing capsaicin-induced nociception. In our FASD model, ethanol was administered to rats from postnatal day 1-9. On postnatal day 21, capsaicin-induced thermal hyperalgesia was assessed and spinal cords and hindpaws were collected for immunohistochemical localization of Aβ- and C- primary afferents and neuronal activation. In the skin, fetal ethanol exposure decreased the overall number of peripheral nerve endings as measured with the pan neuronal marker PGP9.5, but increased the number of nerve endings immunoreactive for CGRP (a marker for C terminals). In the spinal cord, a decrease in NF200 immunoreactivity (a marker for Aβ- terminals) and an increase in CGRP immunoreactivity corresponded to an increase in both capsaicin-induced thermal hyperalgesia and neuronal activation (as measured by c-fos expression) in ethanol-exposed animals compared to control animals. In conclusion, we detected in both the spinal cord and the periphery of ethanol-exposed animals an increased prevalence of pain-associated nerve endings and decreased prevalence of touch-associated nerve endings, and this finding corresponded to an increase in capsaicin-induced nociception and neuronal activation. These studies suggest that fetal alcohol exposure results in dramatic changes in pain-touch peripheral and spinal innervation patterns and function.

Self-Administration of Heroin and Incubation of Drug-Seeking Behavior in Adolescent and Adult Male Rats

Doherty JM & Frantz KJ
Neuroscience Institute & Department of Biology, Georgia State University

Despite steady levels of heroin abuse among human adolescents and the fact that early-onset drug use increases the chances of later drug addiction, few laboratory experiments address adolescent vulnerability to heroin using animal models. In lab animals, drug intake and relapse are modeled with self-administration and reinstatement of drug-seeking after periods of abstinence, respectively. We tested heroin self-administration in adolescent and adult rats, followed by extinction and cue-induced reinstatement after 1 or 12 days of withdrawal. Daily food intake, body weight and fecal boli were quantified to measure the somatic influence of heroin. After recovery from catheterization, adolescent (35 days old) and adult (86 days old) male rats acquired lever-pressing maintained by heroin on a FR1 schedule of reinforcement in 3 hr daily sessions (0.05 mg/kg/infusion for the first 6 sessions, followed by 0.025 mg/kg/infusion for 7 sessions). Adolescents took slightly more heroin, exhibited higher non-reinforced responding, and displayed less somatic signs of heroin use compared to adults. Following 1 or 12 days of withdrawal, rats that self-administered heroin as adolescents displayed less context-induced heroin-seeking behavior under extinction conditions, compared with adults. Cue-induced reinstatement was similar across age groups. Reinstatement of drug-seeking incubated over time, as higher rates of responding occurred after 12 vs. 1 day of withdrawal from heroin. These data suggest younger rats are less sensitive to both acute and long-term effects of heroin exposure, compared to adults. Thus, adolescence may reveal important neuroprotective factors that could be utilized for relapse prevention in humans.

Poster Presentations:

1. Conditioned Place Preference to EtOH is Enhanced by Stress in b-Endorphin Deficient Mice

Locklear MN, Crawford J, Pujara M, Duan A, Broderick L & Grisel JE
Department of Neuroscience, Furman University

The purpose of this study was to investigate the hypothesis that the stress response acting through b-endorphin circuitry modulates EtOH reinforcement. To test this hypothesis, a conditioned place preference (CPP) study was designed. This type of study is used in animals (in this case, mice) to test the reinforcing effects of a drug (in this case, EtOH) through observation of the animal's preference for a context associated with drug effects. The CPP apparatus consists of three chambers. The two outer chambers differ in scent, floor texture, and wall pattern while the central chamber is intended to be stimulus neutral. The experiment takes place over four days. The mice are habituated to the apparatus on Day 1, where they move freely between all chambers. Days 2 and 3 are conditioning days where the mice receive either 2.5 g/kg EtOH or equivolume saline injections, and are relegated to either side of the chamber. Injection order and conditioning side are counterbalanced. Day 4 is the test day and the mice are placed in the center chamber and allowed access to all chambers while activity is recorded. Prior to being placed into the chamber on Days 2-4, mice were either stressed by 15 min in a restraining tube or were individually housed for equal time. Also, a portion of the mice were given 5 mg/kg Naloxone, an opioid antagonist, 15 minutes prior to the injection of ethanol. Mice with greater amounts of b-endorphin showed greater preference for the apparatus side where ethanol was given. Also, stress increases the response to ethanol only in b-endorphin deficient mice. Finally, opiate antagonism completely blocked the ethanol place preference,

further indicating that ethanol reward in this paradigm is mediated by endogenous opioids. Our findings indicate that the reinforcing properties of EtOH are influenced by b-endorphin in a stress dependent manner, and point the way toward understanding the relationship between this peptide and alcoholism.

2. Role of the Delta Opioid Receptor in the Regulation of Chronic Neuropathic Pain

Mohammad H, Spears WE, Velazquez KT & Sweitzer SM
Department of Pharmacology, Physiology, and Neuroscience, University of South Carolina

Millions of people suffer from chronic neuropathic pain as a result of a broad range of injuries as well as diseases such as type II diabetes. These conditions remain difficult to treat and high dose opioid analgesics are limited by side effects such as constipation, mental degradation, development of tolerance, and fear of addiction. Opioid analgesics work by binding to and activating opioid receptors such as the delta opioid receptors (DOR) in peripheral tissues, spinal cord, and the brain. Recent behavioral studies in our laboratory have shown that using Herpes Simplex Virus (HSV) to increase expression of DOR in nociceptors increases pain, but has no effect on opioid analgesia. In contrast, infection with HSV vectors that decrease expression of DOR in nociceptors results in decreased pain, and increased opioid analgesia. My project expands on these recent behavioral studies by quantifying HSV mediated increases and decreases in expression of DOR in nociceptors that innervate the spinal cord. On day seven following a nerve transection, mice were given several complementary DNA sequences: delta opioid receptor in the sense direction (VDOR), delta opioid receptor in the anti-sense direction (VADOR), delta opioid receptor in the anti-sense direction and mu opioid receptor in the sense direction (VADOR + VMOR), and a control virus (VCONT). Using immunohistochemical procedures, we observed increased DOR immunoreactivity following day nine and day sixteen post-viral infection in the VDOR group and decreased DOR immunoreactivity in the VADOR group. These results paralleled the previous behavioral studies showing that infection with VDOR results in an increase in pain by day nine post-infection and that infection with VADOR results in a decrease in pain by day nine post-infection.

3. Small Group Based Elementary School Activities for Brain Awareness Week

Sanders D, Ali R, Barrett S, Guram G, Haymond T, McKelvy A, Velazquez K & Sweitzer SM
Dept. of Pharmacology, Physiology and Neuroscience, University of South Carolina School of Medicine

For Brain Awareness Week 2008, four days of activities were developed for K-5th grades that promoted an elementary understanding of neuroscientists and neuroscience in their daily life. The event took place at The Center for Knowledge a public magnet elementary school in Columbia, South Carolina. On the first day grades K-5 were gathered for an introductory presentation on “What is a Scientist?” which introduced media stereotypes of scientists, different areas of science, and the scientific method. The grades were split for four days of grade specific activities. Each day an introductory presentation was given, followed by small group activities lead by undergraduate and graduate students, followed by a wrap-up by a group demonstration, reading a children’s book related to the topic of the day or by presenting the collected data to explain trends and discoveries. Kindergarteners explored the comparative anatomy of skulls from carnivores, herbivores, and omnivores followed by an egg drop experiment to demonstrate the importance of helmet safety. First graders explored the critical role of the brain in the 5 senses. Second graders explored comparative brain anatomy across different species. Third through 5th graders learned about neurons, neurotransmission and neural circuits. The activities were assessed by teacher evaluations. This event successfully augmented the elementary science curriculum to include a basic understanding of neuroscience and to inspire an interest in science using fun hands-on activities.

4. Transient Inflammation in the Skin and Prolonged Over-expression of Enkephalin and Mu-opioid Receptor in Primary Afferent Neurons using Herpes Simplex Viral Vectors

Ali R, Mohammad H, Wilson SP, Raja S & Sweitzer SM
Department of Pharmacology, Physiology, and Neuroscience, University of South Carolina

Chronic pain affects millions of Americans decreasing their quality of life. Current drugs and treatments are inadequate in dealing with chronic pain. Our laboratory has previously shown that over-expression of the endogenous opioid ligands and receptors using Herpes Simplex Virus-1 (HSV-1) can decrease nociception and enhance opioid analgesia in a mouse L5 spinal nerve transaction model of persistent pain. Our hypothesis is that HSV-1 vectors can be used to over-express the mu-opioid receptor and enkephalin primary afferent neurons with minimal inflammation. Five groups were used in this study: mu-opioid receptor vector infected, preproenkephalin vector infected, a combination of mu-opioid receptor and enkephalin vectors, control (E. Coli lac Z gene) vector infected, and a sham group. To examine over-expression in the skin and lumbar spinal cord, immunohistochemistry for mu opioid receptor and enkephalin expression was performed on days 1, 9, and 16 days post-infection. H&E staining was used to assess skin inflammation at day 1 post-infection. At one day post-infection a significant increase in enkephalin in the skin was observed in both mu-opioid receptor and enkephalin vector infected mice when compared with the control groups. A viral vector dependent transient skin inflammation was observed on day 1 post-infection. The combination vector group demonstrated the greatest amount of inflammation, followed by the enkephalin vector, with minimal inflammation in the mu opioid receptor vector. Changes in enkephalin and mu opioid receptor expression in the spinal cord are not observed until day 9 post-infection. In summary, these HSV-1 constructs drive over-expression of the mu-opioid receptor and enkephalin in the peripheral and central terminals of primary afferent neurons. Also, the combination group consisting of both the mu-opioid receptor and enkephalin produces significant inflammation in the skin. This approach to treating neuropathic pain may one day be used as a way to help human chronic pain patients.

5. Protein Kinase C Epsilon Inhibition Decreases Enodthelin-1 Induced Nociception and Neuronal Activation

Mark IM & Sweitzer SM
Department of Pharmacology, Physiology, & Neuroscience, University of South Carolina

Chronic pain is defined as pain that persists longer than the temporal course of natural healing associated with a particular type of injury or disease state. Chronic pain lessens the quality of life and leads to lost wages and medical expenses. By finding ways to decrease pain, quality of life can be improved. Endothelin-1 (ET-1) is a chemical mediator released by the body at site of injury. Protein kinase C is a potential therapeutic target for the treatment of chronic pain. We hypothesize that administration of a protein kinase C epsilon (PKCe) inhibitor will reduce endotholin-1 induced pain-associated behaviors and neuronal activation. ET-1 was administered intraplantar into the left hind paw of each animal immediately followed by subcutaneous administration of the PKCe inhibitor. The nociceptive response (hind paw flinching and holding the paw up) of each animal was observed and videotaped for seventy-five minutes. At two hours post-ET-1, animals underwent perfusion fixation. Spinal cords were sectioned for immunohistochemical analysis for c-fos, a marker of neuronal activation. The experimental findings showed that 25 ug/kg of PKCe inhibitor decreased nociceptive responses over the course of seventy-five minutes. There was a peak in behavioral response at about twenty-five minutes, decreasing over the course of seventy-five minutes post-ET-1. Immunohistochemical results showed the least amount of neuronal activation was present in the spinal

cords from animals treated with 25 ug/kg of PKCe inhibitor. In conclusion, the PKCe inhibitor dose-dependently reduced both paw flinching and neuronal activation. These results are a good indicator of the ability of the PKCe inhibitor to reduce pain-associated behaviors and neuronal activation and should further be studied as a potential therapy for decreasing chronic pain in humans.

6. Nalbuphine Attenuates Behavioral Sensitization to the Locomotor and Positive-Reinforcing Effects of Cocaine

Cole KT, Walker KA & Smith MA
Department of Psychology and Program of Neuroscience, Davidson College

Sensitization refers to an increase in sensitivity to the effects of a drug and is believed to play a role in the etiology of substance use disorders. Identifying drugs that can prevent or attenuate the development of sensitization is a major aim of preclinical research. The aim of the present study was to determine whether the mixed mu/kappa agonist nalbuphine attenuates the development of sensitization to the locomotor and positive- reinforcing effects of cocaine. Different groups of male Long-Evans rats were habituated to a locomotor activity chamber and treated with either saline (1.0 ml/kg, ip), cocaine (10 mg/kg, ip), or cocaine + nalbuphine (10 mg/kg, ip) every day for 10 days. After 10 days, all rats were implanted with venous catheters and trained to self-administer cocaine on a fixed-ratio (FR) schedule of reinforcement. Rats treated with cocaine exhibited a progressive increase in locomotor activity over the 10-day treatment period, but this effect was not observed in rats treated with cocaine + nalbuphine. In the drug self-administration procedure, cocaine produced an inverted U-shaped dose-effect curve in all groups of rats. Rats treated with cocaine exhibited higher levels of responding for a threshold dose of cocaine (0.03 mg/kg/infusion) than rats treated with saline. Rats treated with cocaine + nalbuphine did not differ from rats treated with saline and exhibited lower levels of responding for a threshold dose of cocaine than rats treated with cocaine. These data suggest that nalbuphine attenuates the development of sensitization to the locomotor and positive-reinforcing effects of cocaine.

7. Temporal Effects of PKCε and γ in Capsaicin-Induced Mechanical Allodyniaε

Revels BN, Liu J & Sweitzer SM
Department of physiology, pharmacology, and neuroscience: USC school of medicine

The PKC family of isozymes function as key signal transducers in cells allowing them to regulate many cellular functions and making them an attractive therapeutic target for a number of human diseases. Within the PKC isozymes, PKCε has been identified in the primary afferent C fibers responsible for transmitting nociceptive signals from the peripheral site of injury to the superficial dorsal horn of the spinal cord, whereas PCKγ has been identified in the superficial lamina of the spinal cord. This study was undertaken to determine if small peptide inhibitors that specifically block the translocation of PCKε (PPC1) or γ (PPC2), respectively, could attenuate mechanical allodynia associated with capsaicin, the pungent ingredient in hot peppers. PPC1 dose-dependently attenuated mechanical allodynia when administered 10 minutes after capsaicin, but not when administered 60 minutes post-capsaicin. PPC2 dose-independently attenuated mechanical allodynia when administered 60 minutes post-capsaicin. These results suggest that nociceptive signals may be regulated in at least two levels of the neuroaxis: primary afferents in the periphery and within the dorsal horn of the spinal cord. Immunohistochemical results show a dose dependent decrease in c-fos positive neurons, a marker of neuronal activation, that parallels the attenuation of mechanical allodynia by PKCε inhibition. This may illustrate that peripheral sensitization via PKCε may be driving the central sensitization associated with capsaicin via PKCγ.These findings emphasize the importance of PKC isozyme

specific mechanisms in nociception and may prove to be a useful track for the discovery of successful treatment options for acute and chronic pain.

8. Investigation of Leadmium Green as a useful fluorescence sensor of cadmium in living neurons

Cook MD, Kirkland WM, Alford AM, Dineley KE & Malaiyandi LM
Department of Biology, Francis Marion University

Cadmium is a common industrial pollutant, and its accumulation is toxic to energy intensive tissues such as brain, heart, liver and kidney. The study of cadmium cytotoxicity is difficult in part due to the absence of a probe that can detect cadmium in living cells. Recently, Invitrogen™ developed the metal-sensitive fluorophore Leadmium Green, which was used to monitor nanomolar levels of lead and micromolar levels of cadmium in live Jurkat cells using flow cytometry. Using the cell-impermeant form, we evaluated the selectivity of Leadmium Green in MOPS-buffered solution with spectrofluorometry. Consistent with manufacturer's claims, Leadmium Green responded robustly to lead or cadmium in a concentration-dependent manner, and furthermore proved insensitive to calcium. However, it also responded to zinc at low micromolar concentrations. Using fluorescence microscopy, we then evaluated the response of Leadmium Green inside living cells. We observed large fluorescence increase in HT-22 cells treated with zinc and the ionophore pyrithione. Moreover, the metal chelator TPEN, which has high affinity for zinc, reversed these changes. Given the abundance and ubiquity of zinc in living systems, these results question the utility of Leadmium Green as a live-cell cadmium and lead sensor.

9. High-Risk Tanning Behaviors, Ultraviolet Light Dependency, and Responses to the Addiction Potential Scale in University Undergraduates: A Preliminary Investigation

Olszewski EA & Gendle MH
Department of Psychology, Elon University

Recreational tanning increases a person's exposure to ultraviolet light (UVL) and can lead to the development of skin cancer. Previous studies have examined the psychologically reinforcing effects of UVL exposure using metrics based on substance abuse disorders. The Addiction Potential Scale (APS, a supplementary scale of the Minnesota Multiphasic Personality Inventory-2) is a measure highly correlated with states of dependence which does not ask questions directly pertaining to substance use behaviors. The present research intended to replicate earlier findings documenting rates of UVL dependency in various samples utilizing previously used measures (the modified (m)CAGE (Cut Down, Annoyed, Guilty, Eye-opener) Questionnaire and modified (m)DSM-IV-TR criteria) in conjunction with the APS. A questionnaire assessing tanning behavior was administered to a random sample of 156 first-year university students. Wilcoxon rank sum tests were used for statistical comparisons, which focused exclusively on the female tanners. Female responders reported a high rate of tanning (78%) and of those tanners, 18% met the mCAGE criteria for UVL dependence and 21.25% met the mDSM criteria for UVL dependence. The females tanners who met the mDSM criteria for UVL dependence had significantly higher scores on the APS ($P=.005$) than those who did not. Female tanners who met both the mCAGE and mDSM criteria for UVL dependence also had elevated APS scores compared to female tanners that did not, however, this comparison was not classically significant ($P=.11$). These results indicate that the APS may be useful in identifying UVL dependence within populations of frequent tanners.

10. Nociceptive Threshold is Altered during Epileptogenesis in the Pilocarpine Model of Temporal Lobe Epilepsy in Rats

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Individuals with temporal lobe epilepsy have elevated pain threshold. Antiepileptic drugs are analgesic, making it difficult to determine whether the reduced sensitivity to pain in these individuals is due to their epilepsy or to its treatment. We have used the pilocarpine model of TLE in rats to determine whether epilepsy itself can alter nociceptive thresholds. Adult male Sprague-Dawley rats were treated with pilocarpine to induce status epilepticus (SE). SE was terminated after 2 hours with diazepam. Sham animals were treated with saline and diazepam. Beginning 24 hours post-SE, nociceptive thresholds to mechanical (Von Frey filaments), thermal (heat and cold) and painful (blunt needle) stimuli were measured. Nociceptive thresholds were measured every third day over a period of 24 days. This time window corresponds to the period of epileptogenesis, (when the animals are developing TLE) and epilepsy (when spontaneous seizures are present). Beginning on day 22, SE-experienced animals were video monitored for 72 hours to assess for behavioral seizures. On day 25 animals were transcardially perfused and their brains removed for histological analysis. SE-experienced rats exhibited a significant elevation in the nociceptive threshold to mechanical stimuli by 7 days post-SE that returned to control by 15 days post-SE. As opposed to the transient change in response to mechanical stimuli, nociceptive threshold to both cold and painful stimuli were persistently increased throughout the 24 day period. In contrast, paw withdrawal latency in response to thermal stimuli did not differ at any time point from the sham group. Histological analysis of SE-experienced rats revealed substantial cell loss in somatosensory cortex, suggesting that seizure-induced neurodegeneration in this brain area could contribute to the observed alteration in nociceptive thresholds. These findings suggest that epilepsy itself can produce changes in pain perception and that these changes in pain thresholds may become apparent before the onset of frank seizures.

11. Age-dependent MK801-induced caspase-3: co-localization with GAD67-rich puncta

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Injection of postnatal day 7 (P7) rat pups with the N-methyl-D-aspartate receptor (NMDAR) blocker MK801 promotes rapid and robust induction of the pro-apoptotic marker activated caspase-3 (AC3). Further, NMDAR antagonism at P7 leads to loss of the GABAergic marker GAD67 at P56, suggesting that at P7 NMDAR blockade-induced AC3 should occur in GAD67 positive cells. To test this prediction, we injected P7 rat pups with vehicle or MK801 and after 8 hours (peak of AC3 induction) we examined brain sections for AC3, GAD67, or both markers. As expected, MK801 profoundly induced AC3 in all brain regions examined (compared to vehicle) but co-expression of GAD67 in the same cells was not observed. However, in brain regions where punctate (synaptic) GAD67 was abundant (for example the barrel fields of the somatosensory cortex), AC3 was robust. These data suggest that whereas somatic expression of AC3 and GAD67 may be non-overlapping, areas that exhibit punctate GAD67 (and are high in synaptic turnover) may be more vulnerable to MK801 exposure.

12. Oral activity does not induce context-dependent memory when flavor is held constant

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Recently there has been increased interest in the effects of oral activity on cognition. For instance, it has been shown that chewing gum increases immediate and delayed recall of information. One reported influence of gum-chewing on memory is a context-dependent effect in which memory is improved by a match between the context in which information is learned and that in which it is recalled, versus a mismatch between the learning and recall contexts. However, the apparent context-dependent effects of chewing gum may actually be due to the flavor match between the two conditions, rather than due to the chewing match. Although Miles & Johnson (2008) partially addressed this problem, their design still allowed for the possibility of an interaction between chewing and flavor such that chewing only causes context-dependent memory effects in the presence of flavor and not in the absence of flavor. The present study clarifies some of the ambiguity surrounding the effects of oral activity on memory by examining whether context-dependent memory effects can be induced when flavor is held constant. Participants studied lists of words while chewing cinnamon gum or sucking on cinnamon candy (see Zoladz & Raudenbush, 2005), completed a math distracter task, then chewed cinnamon gum or sucked on cinnamon candy while recalling previously studied words. No context-dependent effect of oral activity in the presence of cinnamon flavor was found. These results extend previous findings by casting further doubt on the ability of oral activity to induce context-dependent memory effects.

13. Neonatal quinpirole induces amphetamine conditoned place preference in adolescent rats

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Conditioned place preference (CPP) has been prevalently used to analyze the associative effects of addictive drugs. This behavioral paradigm has shown that psychostimulant drugs including amphetamine, nicotine, and cocaine increased associative preference for a particular context. Past work from this laboratory has shown that neonatal quinpirole (dopamine D2/D3 agonist) treatment produces increases in dopamine D2/D3 receptor sensitivity that persists throughout the animal's lifetime. Increases in D2 sensitivity are relevant to a number of psychotic conditions, including schizophrenia. Psychotic individuals have shown a high prevalence of comorbidity with psychostimulant abuse. In the present study, we analyzed conditioned place preference using amphetamine in adolescent male rats D2-primed as neonates with the dopamine D2/D3 agonist quinpirole. Male rats were treated with quinpirole (1mg/kg) or saline from postnatal days (P)1-11. Animals were weaned at P21, raised to P30, and habituated to a locomotor arena. Beginning on P33, rats were treated with amphetamine (1mg/kg) or saline, and 10 min later were placed in a two-chamber shuttle box painted either black or white on either side of chamber. Amphetamine was always paired with the white context. Results showed that although non D2-primed rats did not demonstrate amphetamine-induced CPP, D2-primed rats demosntrated a strong preference with the chamber paired with amphetamine. This study appears to show that D2-priming induced conditioned place preference in adolescent male rats, and ehanced the reinforcing properties of amphetamine. These results are congruent with past work in this laboratory showing that psychstimulant sensitization is enhanced in D2-primed rats.

14. Schizophrenic Pigeons? Cognitive Rigidity Induced by Lesions in the Pigeon Nucleus Accumbens

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The nucleus accumbens septi (Acc) of mammals plays a critical role in reinforcement, addiction, and goal-directed behavior. The Acc also has extensive connections with prefrontal cortex (PFC); faulty dopaminergic regulation in these areas has been implicated in schizophrenia (e.g. cognitive rigidity in the Wisconsin Card Sorting Task). Comparatively little is known whether other amniotes (i.e., reptiles and birds) have an analogous, and possibly homologous, brain structure to Acc. We performed immunochemical labeling of calcium-binding proteins (CaBPs), and tract-tracing experiments in the medial portion of avian medial striatum (mMSt). The results were consistent with traits of Acc (e.g., connections between mMSt and both Wulst and medial mesopallium, which may have functional correspondence to PFC). Guided by these results, we evaluated functional similarities between mMSt and Acc. Lesions of Acc impair reversal learning (indicative of cognitive rigidity) with few effects on sensory discrimination. Electrolytic lesions of mMSt had no effect on a visual discrimination in a two-key operant chamber. However, lesioned subjects performed significantly worse than controls on reversal learning tasks using these stimuli. Errors in lesioned subjects indicated cognitive rigidity (i.e., fixation on the previously rewarded stimulus). These results provide new insights regarding the evolutionary origin of Acc in amniotes, as well as potential comparative models using birds in future schizophrenia research.

15. A dose-response analysis of nicotine sensitization in adolescent rats D2-primed as neonates.

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Past work from this laboratory has shown that neonatal quinpirole treatment produces long-term increases in dopamine D2 receptor sensitivity that persists into adulthood, referred to as D2 priming. Recent work has shown that adult rats D2-primed as neonates demonstrate an enhanced sensitization to the psychostimulants amphetamine and nicotine. In the present study, both male and female Sprague-dawley adolecent rats were administered quinpirole HCl (1.0 mg/kg) or saline from postnatal days (P)1-21 and raised to adolescence at P30. After three days of habituation to a locomotor arena, animals were administered saline or a 0.3, 0.5, or 0.7 mg/kg free base dose of nicotine tartarate. Approximately 10 min after each injection, animals were placed into a locomotor arena and behavior was analyzed using an automated behavioral scanning system every other day from P33-49. Analyses are still underway, but results appear to show that D2-priming as produced by neonatal quinpirole treatment eliminated nicotine-induced hypoactivity early in testing, and enhanced sensitization to the 0.5 and 0.7 mg/kg dose in males, but not in females. It appears that D2-primed males demonstrate an overall higher level of activation to the 0.7 mg/kg dose of nicotine in adolescence. Further behavioral analyses will include behavioral stereotypic behaviors including immobility time and immobility episodes, as well as circling behavior. These results indicate that D2 priming enhances the behavioral activating effects of nicotine, however these effects are more prominent in adolescent males as compared to adolescent females.

16. Electrogenic calcium pump derives cell depolarization in a computational model of dopamine neuron

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A minimal model of dopamine neurons was implemented using a single-compartment conductance-based paradigm. The essential currents involved in fast spiking oscillatory phenomena are calcium and apamin-sensitive SK potassium current. Numerical simulations revealed a sudden transition to very slow plateau potentials if SK current is blocked. The repolarization of dopamine cell is ensurend by a slowly activating potassium current. Although all experiments indicated that calcium plays an essential role, previous mechanisms assumed a nonelectrogenic calcium pump. Our results show that a (partially) electrogenic calcium pump could drive cell's depolarization.

17. Changes in Kainate Receptor Subunit Expression Following Status Epilepticus

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Temporal lobe epilepsy (TLE) can develop after a brain injury. TLE is distinguished by spontaneous recurrent seizures which often originate in the hippocampus. Frequently implicated in the development of epilepsy, kainate receptors (KARs) are a subtype of excitatory amino acid glutamate receptor in the brain. They are composed of combinations of five different subunits which include KA2 and GluR6. KAR function is determined by the subunit composition of the receptor. When present, KA2 subunits increase the conductance of the receptor and enhance its affinity for glutamate. Changes in the subunit composition of KARs after a brain injury could contribute to the development of epilepsy. We examined changes in expression of KA2 and GluR6 in the pilocarpine model of epilepsy. Animals were treated with pilocarpine (saline as control) and allowed to remain in status epilepticus (SE) for two hours to induce a brain injury; they were sacrificed two weeks later. We immunostained sections from control and pilocarpine-treated SE-experienced (PTSE) animals separately for KA2 and GluR6 and analyzed them qualitatively. Control animals showed a similar pattern of GluR6 and KA2 expression in the hippocampus, except in the dentate molecular layer (DML) where GluR6 was more abundant than KA2. In PTSE animals KA2 expression decreased in CA1 and the dentate hilus, and increased in the DML. In these same PTSE animals GluR6 expression decreased in CA1 and CA3 and increased in the piriform cortex. We suggest that these changes in KA2 and GluR6 expression produce alterations in the subunit composition of KARs in PTSE animals. Given the dependence of KAR function on subunit composition, we suggest that KAR function is altered in epilepsy. Agents targeted to these altered KARs may represent novel therapeutic drugs for TLE. Supported by the SC Research Foundation and the Magellan Scholars Program from the Office of Undergraduate Research at USC

18. Selectivity of SB-205384 on the αα subunits of the GABA(A) Receptor

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γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter of the central nervous system. The GABA(A) receptor is a ligand-gated ion channel that is permeable to chloride ions and consists of a pentamer of subunits (α1-6, β1-3, γ1-3, δ, ε, π, and θ). SB-205384 is an anxiolytic drug that has been historically associated with being α3 selective. However, only the α1-3 subunits have been examined. The goal of this work was to evaluate the possible effects of SB-205384 on the α4-6 subunits and the α1/α6 and α6/α1 chimera subunits. Using recombinant receptors and the patch clamp method, the potentiation by SB-285384 on the GABA(A) receptor was measured. The responses indicated that the α1,2, 4 subunits had different sensitivities to SB-205384, but all commonly displayed minimal sensitivity. The α5 subunit displayed moderate sensitivity, whereas the α3 and α6 subunits displayed high sensitivity. α3 and α6 also displayed a slowed decay rate. The α1/α6 chimera subunit displayed a low sensitivity similar to the α1 subunit, indicating that SB-205384 bonds to the α1 portion of the chimera. The α6/α1 chimera displayed a high sensitivity similar to the α6 subunit, indicating that SB-205384 bonds to the α6 portion of the chimera. These results indicate that SB-205384 is not as selective as previously thought. α6 subunits are predominantly located in the adult brain and reside in the cerebellum, which controls motor coordination. Since SB-205384 was found to be at α6 containing receptors, the potential adverse effects of the drug (loss of motor control) could be diminished through the development of a more α3 selective drug.

19. Scientific Publishing and IMPULSE: an Integral Part of a Complete Neuroscience Undergraduate Education.

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While recognition of the importance and practice of involving undergraduates in primary research has been advancing steadily in recent years, there is still a huge chasm between that experience and the real world of publishing results. Students rarely have the follow-up experience of writing up, submitting, and then publishing their findings. An experience that included opportunities both to write and review manuscripts would provide for a more complete scientific experience. The journal IMPULSE (http://impulse.schc.sc.edu; hosted at the University of South Carolina) was created in 2003 to fill this void, and has been presented at the Society for Neuroscience for the last six years to publicize this opportunity (Soc. Neur. Abs.: 29:25.3; 30:28.6; 31:20.19; 32:26.13; 33:26.13; 34:224.5). Students doing primary research or literature reviews while in college/university now have a place to submit their own work and have it reviewed by peers, other students at the same educational level and from over 15 universities worldwide. Reviewer Training Sites include the host site, Middlebury College, and Appalachian State University, where students are either formally trained in a Scientific Publishing class (see JUNE Spring 2006 Vol. 4, Issue 2 http://www.funjournal.org/results.asp?juneid=159), or informally but rigorously guided by a neuroscience faculty member. This collaboration between students and Faculty Advisors promotes understanding of the review process, and training modules are currently being developed to aid remote reviewers who do not have the advantage of constant communication with a Faculty Advisor. IMPULSE welcomes neuroscience researchers and educators from other programs to start a reviewer training site at their institution.

20. Thalamic Morphometrics: Sex Differences in Asymmetry

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We examined the normal average human thalamus using T-1 weighted MRI scans. The sample included 17 women and 29 men, five of which were right handed. The volumes of each thalamus were delineated by hand using a computer program after the MRIs were normalized in stereotaxic space. Contrary to the results of a preliminary study, the right and left thalami were measured separately and no significant asymmetries were found. On average, men were found to have larger thalami than women.

21. Role of the Homer 2 protein in the mammalian circadian system

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Within living organisms, patterns of molecular and physiological activity revolve around a 24-hour time period known as a circadian rhythm. Mammalian circadian rhythms are generated by the suprachiasmatic nucleus (SCN) within the brain and synchronized to a 24-hour cycle by light. Based on their known association with components crucial to light resetting, a group of proteins called the Homer proteins in the SCN are likely involved in the ability of light to reset this biological clock. Using running wheel activity as a behavioral output of the circadian clock, we have evaluated the ability of Homer 2 deficient mice to regulate their rest: activity schedules in response to changes in their light: dark cycles. Homer 2 deficient mice were able to adjust their circadian rhythms in response to light under most conditions, but these mice often had an altered response to light, possibly due to an increased sensitivity to light. When the light:dark cycle was shifted to turn on eight hours early, the wildtype mice became active earlier each day until they were resynchronized to the new schedule. However, mice with little or no Homer 2 protein adjusted to the new light schedule by progressively delaying their onset of activity each day. Based on our preliminary data, we believe that the disruption of the Homer 2 protein alters the ability of light to reset the circadian clock in mice, thereby suggesting that the Homer 2 protein might play a role in the appropriate adjustment of circadian activity to photic input.

22. The Rewarding Properties of Sugar in an Animal Model Fetal Alcohol Spectrum Disorders

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There is a high co-morbidity of Fetal Alcohol Spectrum Disorders and drug abuse. There is evidence that suggests that prenatal ethanol exposure alters the neural mechanisms involving reward and possibly increase the tendency to respond to rewarding substances. Sucrose water is a motivating reward for rats, and activates the same nucleus accumbens- ventral tegmental area pathway as many drugs of abuse. An animal model of FASD was used to investigate the hypothesis that ethanol exposure during development increases the rewarding properties of sugar. There were three treatment groups of Long Evans rats: one group received ethanol throughout the period equivalent to all three trimester equivalents, one group received the administration procedures but no ethanol, and one group was not treated in any way. At adulthood all rats were taught to bar press for sugar water, and eventually breakpoints were determined for each animal. The rats were then tested for sugar preference. Finally, the rats were given the opportunity to bar press for sugar for thirty minutes and then perfused. Tissue was processed for c-fos immunoreactivity

in the nucleus accumbens. Ethanol-exposed animals showed an increased preference for sugar, and an increased breakpoint for sugar when compared with controls suggesting enhanced sensitivity to reward. The tissue collection and processing for c-fos immunoreactivity is currently underway. These results suggest that the reward pathways in FASD are altered in such a manner as to make the individuals vulnerable to drug abuse. Supported by a Magellan Fellowship from USC to CEO and NIAAA 11566 to SJK

23. Safety, Tolerability and Efficacy of High Doses of Left Prefrontal Daily rTMS in an Academic Clinical Setting

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There are currently a wide variety of treatments for depression from mild forms of behavioral and cognitive therapy, to more extreme forms of treatments such as multiple drugs and Electro Convulsive Therapy (ECT). Repeated transcranial magnetic stimulation (rTMS), which has recently received FDA approval for the treatment of depression, offers an alternative to extreme treatments as ECT. This study represents data collected over a year and a half from 19 patients. In general, treatment was given for a week followed by a battery of questionnaires. Patients ranged in age from 17 to 57, and all showed some level of treatment resistance. The majority of these patients were on medication and continued medication throughout treatment, thus rTMS was used as an adjunct treatment. We measured depression, quality of life, suicidal ideation, and social and physical functioning. Not only did all of these dimensions show improvement, many showed improvement in just 1 to 2 weeks. Furthermore, suicidal ideation diminished in 67% of the patients after just one treatment, which could have implications for highly suicidal patients. Not only does this study show the effectiveness of rTMS in an academic clinical setting, it illustrates the safety of the treatment. The average dose was 6000 magnetic pulses per session (30,000 pulses a week), and one subject received 983,000 pulses and is still continuing treatment, all with no adverse effects.

24. Evaluation of Working Memory in Zebrafish, Danio rerio, Using Three Different Testing Paradigms

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Danio rerio are small tropical fish, typically found in fresh water streams and rice paddies of East India, Bangladesh, and Burma. Commonly known as zebrafish, Danio rerio are a model organism historically utilized for studies of vertebrate development, but recent studies have used zebrafish for the study of behavioral plasticity and normal learning patterns. The current study utilizes zebrafish to explore working memory and factors that may affect it. Working memory refers to the ability to retain information in the prefrontal cortex and progressively modify it for the purpose of responding to different situations. Three different tasks were used to assess working memory: a rapid conditioning paradigm for spatial preference, a T-maze and a hole maze. The rapid conditioning test requires a tank divided into three sections by moveable dividers. The zebrafish is placed in the middle chamber. The dividers are removed, and the zebrafish is allowed to swim to either side of the tank. The side chosen first becomes the “wrong” side– established by punishment. The zebrafish is then placed back in the middle and this procedure is repeated ten times. Learning is assessed by how many times the zebrafish swim to the correct side of the tank. In addition, fish were treated with caffeine, nicotine or testosterone prior to testing in order to determine whether these drugs influence working memory. The T-maze and hole maze were used to obtain converging

lines of evidence for the demonstrated effects. In summary, zebrafish are a useful model organism for evaluating working memory.

25. The Catalytic Effect: Facilitation of LTP in the Sprouted Crossed Temporodentate Pathway

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The entorhinal cortex (EC) serves as a major source of input to the dentate gyrus (DG) of the hippocampus. Following unilateral one-stage lesion of the entorhinal cortex, rats lose their ability to perform a working memory task. Between 8 to 12 days post-lesion, the crossed temporodentate pathway (CTD), which originates from the intact contralateral EC, proliferates and forms new connections with the denervated DG. Our lab found that conducting the lesion within two stages separated by 6 days accelerates axonal sprouting and results in less memory deficits. The sprouted CTD develops the ability to facilitate long-term potentiation (LTP), a likely mechanism important for learning and memory. The purpose of this experiment was to determine if extending the inter-lesion interval (ILI) from 6 to 12 days would enhance synaptic efficacy of the CTD. Maximal sprouting occurs 4 to 13 days between the first and second stages, therefore we hypothesize that an extended ILI will elicit a catalytic response from the CTD after electrical stimulation. Following 15 days post-lesion, the responses of the CTD were recorded before and after a high-frequency tetany protocol (8 trains of 8 pulses at 400Hz) designed to induce LTP. The criterion used to assess the emergence of LTP was a 15% increase in maximum slope or amplitude of evoked potentials from the CTD. We observed that a 12 day ILI did not result in increased LTP emergence or enhancement of maximum evoked potentials.

26. Social Environment Alters Central Distribution of Estrogen Receptor Alpha in Juvenile Prairie Voles

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It is well established that social environment, particularly isolation, has a significant impact on social behaviors and neuroendocrine responses. Estrogen receptor alpha (ERα) expression in limbic structures and associated nuclei is related to the display of social behaviors. We hypothesized that the stress of isolation would cause changes in the pattern of ERα expression in the brain. Using a highly social (typically monogamous and biparental) rodent species, the prairie vole (Microtus ochrogaster), we housed juvenile voles with a sibling, stranger or in isolation for either 4 days or 21 days. Housing manipulations began following weaning from parents and group housed siblings. Rodents may be particularly sensitive to manipulations of their social environment during this juvenile period. In particular, female prairie voles are induced ovulators, reliant upon exposure to an unrelated male (male urine) to become reproductively active. ERα immunoreactivity was quantified in the medial preoptic area (MPOA), bed nucleus of the stria terminalis (BST), ventromedial nucleus of the hypothalamus (VMH) and medial amygdala (MeA). Significantly fewer ERα immunoreactive (ERα-ir) cells were labeled in the MPOA and BST of females isolated for 21 days compared with stranger housed females. Similar, but non-significant patterns were shown in the VMH and MeA. No differences were found in voles isolated for four days. These results suggest that female prairie voles may be more sensitive to manipulations of their social environment during the juvenile period. Future studies will examine the interaction between ER alpha and neurogenesis.

27. In Situ Description of Neuroanatomy and Caudal Sensory Structures in the Late Aquatic Phase of the Tiger Salamander (Ambystoma tigrinum)

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Salamanders are a source of great curiosity because they undergo such drastic morphological change during ontogeny. Studies of amphibian neuroplasticity during development reveal that structures required for life on land must develop while the organism is still aquatic, and just as there are morphological changes to the body, there are changes which occur in the brain as well. The tiger salamander (Ambystoma tigrinum) goes through numerous developmental stages throughout its life. This study examines the brain of the tiger salamander in situ at a late aquatic stage of development when all appendages were ready for the transition onto land, but gills were still present. The in situ preparation allows for analyses of caudal sensory structures in relation to the brain. Horizontal sections from the brain were stained (hemotoxylin and eosin) and compared with existing data on the mature terrestrial adults for developmental insights and for detailed analysis of existing brain structures. The late aquatic stage salamander has a well formed telencephalon and diencephalon, as well as many other neuroanatomic features. For sensory systems, a defined retina, vestibular, auditory and olfactory structures are identified. There was a distinct difference in the visual system of this individual because the lens was not yet fully developed. Although there was a minute difference in the visual system between late aquatic and adult animals, the data collected implies that during the late aquatic stage of development, the organism is equipped with sensory and brain structures needed for life on land.

28. Early Stress and Alcoholism: A Neurobiological Analysis of the Serotonin System

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The prevalence of alcohol disorders has a considerable impact on mortality rates, economic costs, and health care expenditures worldwide. Early life stress has been linked to later alcohol abuse and dependence. The serotonin system's role in the stress response and drug abuse reinforcement makes it of considerable interest to understanding this well documented relationship. Serotonergic changes in the limbic system were targeted due to its involvement in both the stress response and the reward pathway. Mother and nursery-reared male rhesus macaques were trained to self administer ethanol or a control solution in a two-by-two experimental design that implemented a schedule-induced polydipsia technique. Self-administration occurred during 22 hour sessions for 12 months. At necropsy, brains were blocked and flash-frozen. Brain regions of interest were then prepared and in vitro autoradiography was used to measure expression of extracellular serotonin concentration by targeting serotonin transporter (SERT) and 5-HT1A receptor densities. Preliminary results show a greater expression of SERT in amygdala and dorsal raphe regions of mother-reared drinkers compared to nursery-reared and mother-reared non-drinkers ($p=0.005$, $p=0.0001$). In addition, mother-reared drinkers showed significantly greater 5-HT1A receptor density in the dorsal raphe than nursery-reared drinkers ($p=0.04$). These region specific alterations in the serotonin system suggest that nursery-reared monkeys who have undergone early life stress lack the development of an adaptive response to alcohol exposure. Our involvement in this ongoing study was comprehensive, focusing on behavioral training, drinking protocol, data collection, and analysis.

29. Exercise and Neuroplasticity: Effects of Exercise on Neuroplasticity in two Hippocampal Afferents

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The ability of the mammalian brain to change and adapt is termed neuroplasticity and is the neurobiological basis of learning and memory. Exercise has been shown to affect neuroplasticity in a variety of experiments. This study examines neuroplasticity of the hippocampus electrophysiologically and anatomically in a group of exercising rats as compared to a group of sedentary rats. After 10 weeks of living with or without access to a running wheel, each rat received a unilateral electrolytic lesion to the right entorhinal cortex. This damage destroys the perforant pathway, which provides a primary source of input to the hippocampus. The loss of input triggers compensatory growth in other pathways - notably the crossed temporodentate and septodentate pathways. Six days after the lesion, electrophysiological data, based on a paired-pulse protocol, were collected from each rat to assess the capabilities of the newly sprouted crossed temporodentate pathway. The brain tissue from the rats in the study was stained to discern sprouting by the cholinergic septodentate input to the hippocampus. Analysis of both electrophysiological and anatomical data failed to reveal significant differences between groups; possible explanations for this unexpected result are discussed.

30. Neurobiology of Cocaine Relapse

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Persistence of relapse from drugs of abuse is the biggest problem in drug dependence treatment. This study focused on understanding the neural mechanisms involved in relapse from drugs. The dorsolateral caudate-putamen (dlCPu) is a brain area that receives dopaminergic terminals and has been associated with habit formation. We proposed that the persistence of relapse to drug addiction is based on the fact that cocaine-seeking becomes a habit after extensive use and long periods of abstinence and that changes in dopamine neurotransmission underlie that effect. A self-administration paradigm was used with rats to model relapse in humans. The rats were trained to self-administer cocaine by lever-pressing for a cocaine infusion. The subjects were then divided into two groups: one receiving 5 days of access to cocaine (short access) and the other receiving 15 days of access to cocaine (long access). After these periods of time, the subjects were no longer given access to cocaine in order to mimic abstinence. At one, fourteen, and sixty days after the start of abstinence, tests were done to assess the level of drug-seeking behaviors (indicating relapse) triggered by cues or stressors, and dopamine was measured. Preliminary studies from our lab have proven that the involvement of the dlCPu on cocaine-seeking behaviors becomes more important after extensive use or longer periods of abstinence. While the experiment is not complete, we predict that this behavior is based on higher release of dopamine in the dlCPu after extensive cocaine use or longer periods of abstinence.

31. Gliomagenesis

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Gliobastoma multiforme (GBM) is one of the most common and most malignant of all glial tumors, as it is fatal within 36 weeks. Little is known about how and why the brain tumor reoccurs. In our research, we are trying to identify the cellular origin of GBM using stem cell markers from the subventricular zone (SVZ). We

hypothesized that we can control the reoccurrence of GBM by suppressing the activity of the SVZ. Two patients with reoccurring GBM were given injections of DepoCyt, a chemical which has been shown to prevent the migration and division of certain brain cells. MRIs indicated that the tumors disappeared after these injections, suggesting that DepoCyt may be an effective treatment for GBM. The understanding of GBM and the stabilization of the patient's disease calls for further research in clinical trials to help prevent reoccurring GBM.

32. The Effects of Chronic Ethanol Intake on Brain Glucose Utilization in CB1 Knockout Mice

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The mesolimbic dopamine system is most implicated in the mediation of reward and pleasure and is thought to be moderated by the CB1 receptor in the case of alcohol administration. Results from previous studies show significantly decreased binding rates of [3H]2-deoxyglucose in the brains of CB1 KO mice after acute alcohol consumption. The current study investigates the effects of chronic alcohol consumption using the same paradigm. We hypothesized that, as in the previous study, there would be a decreased rate of [3H]2-DG binding in the brains of the CB1 knockout mice. CB1 knockout and Swiss-Webster (n=18) mice were assigned to control (non-alcoholic beer), low (0.5 g/kg) and high (1.5 g/kg) ethanol dosage groups. The mice received their respective doses one hour daily for four weeks. On test day, the mice were injected with [3H]2-DG prior to receiving their ethanol dose. After drinking for one hour, the animals were sacrificed and blood samples were taken for glucose and ethanol analysis. Brains were removed and flash frozen for imaging.

33. Estrous State as Determined by the Visual Method in Mice

Matson LM, Usala JM, Locklear M, Grisel JE & Allen SA
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Experimenters generally use the lavage method to account for variability as a result of the estrous cycle. The lavage method tends to be quite tedious and can potentially interrupt estrous cycling. Evidence was found that a visual method is as reliable, if not more so, than the swabbing method. A clearly defined visual method would provide a simpler, accelerated approach to identifying estrous states in mice, while alleviating stress on the animals. Further, it would allow experimenters to account for the variability of female subjects, which would allow incorporation of females into more studies.

34. The Effects of Pentobarbital as an Agonist on the K289M Mutation of the Alpha-1 and Alpha-6 subunits of the GABAA Receptor

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The neurotransmitter GABA acts through three classes of GABA receptors: GABAA, GABAB, or GABAC. The GABAA receptors are ligand-gated chloride channels which are the targets for many clinically used sedative drugs, including pentobarbital. Pentobarbital acts through at least two different binding on the GABAA receptor. At low concentrations (μM), they act as positive allosteric modulators. At higher concentrations, they can directly activate the receptor as agonists. This agonist action depends upon the

subunit composition of the receptor, and pentobarbital is a more effective agonist than GABA only at receptors containing an $\alpha 6$ subunit. The conformation change that translates GABA binding into channel opening is known to involve lysine289, located in an extracellular domain of the receptor. Mutations of this residue disrupt activation of the channel by GABA. Pentobarbital binds to the receptor at a different site than GABA, but may use a common signal transduction mechanism. To address this question, we used patch clamp recordings from transfected HEK-293T transfected cells to compare the effectiveness of pentobarbital and GABA at GABAA receptors carrying a mutation at lysine289 in either the $\alpha 1$ or $\alpha 6$ subunits.

35. An In Situ Descriptive Study of the Brain of the Spotted Salamander *(Ambystoma maculatum)* with Specific Reference to Caudal Sensory Structures

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The amphibian brain shares a similar structural organization of a nervous system common to all vertebrates except on a smaller, more simplified scale. While much is known about the neurology of the tiger salamander *(Ambystoma tigrinum)*, surprisingly little is known about the spotted salamander *(Ambystoma maculatum)*. In our study, we examined the brains of the spotted salamander *in situ* in the late aquatic stage of embryogenesis just as the animal was transitioning to a terrestrial existence. Studying the specimen *in situ* allowed us to describe the brain in relation to caudal sensory structures. Horizontal and saggital sections were mounted then stained (hemotoxylin and eosin). The sections were then imaged using a Nikon 80i light microscope and photographed with a SPOT CCD camera. The central nervous system features were well-developed and component organs of the telencephalon, diencephalon, and mesencephalon are identified. We were also able to describe a number of sensory structures and the way in which they articulate with the brain. Olfactory, visual and vestibular/auditory structures were described including their component parts. We conclude that there does not appear to be any significant difference between the sensory and nervous system of this earlier phase of ontogeny compared to the adult plan. This indicates that by the time the salamander leaves the water it has all of the adult nervous system features for a terrestrial existence.

36. Structural disparities between the late aquatic stages of the Tiger (*Ambystoma tigrinum*) and Spotted Salamander (*Ambystoma maculatum*) Retina

Harden AL & Milliken GW
Department of Biology and the Department of Psychology, College of Charleston

Retinal structure of a Tiger Salamander (*Ambystoma tigrinum*) and a Spotted Salamander (*Ambystoma maculatum*) in the late aquatic stage of embryogenesis were examined *in situ*. Hemotoxylin and Eosin stained retinas were imaged at 20x magnification to reveal that both species have the same general design and number of retinal layers. In each retinal layer of the Spotted Salamander, there were more layers of cells than in the Tiger Salamander. The Ganglion cell layer of the Spotted Salamander had approximately two layers of cells whereas in the Tiger Salamander; it contained only one layer of cells. The outer plexiform layer is more distinguished and the receptors are significantly more defined in the Spotted Salamander. The receptor layers of both the Spotted Salamander and the Tiger Salamander consist primarily of rods. The pigment and choroid layers of the Tiger Salamander's retina are very loosely compressed, while the Spotted Salamander's pigment and choroid layers are densely compressed. The outer and inner nuclear layers in the Spotted Salamander's retina have twice the layers of cells as those layers in the Tiger Salamander's retina. Comparing the slides of both salamander species' retinas at 10X magnification, the

lens of the Spotted Salamander is closer to full development than that of the Tiger Salamander. In addition, the overall size of the Tiger Salamander's eye is much larger than that of the Spotted Salamander. These results are discussed in terms of the ocular anatomy of the Fire Salamander (*Salamandra salamandra*).

37. Deficits in Verbal, Spatial, and Object Working Memory after Thalamic Stroke

Absher J & Schneidewind JA
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Strokes of the thalamus can cause numerous varying cognitive impairments. One rarely studied effect of thalamic stroke is deficits in working memory. Some recent models suggest that the thalamus functions as a gate to disinhibit the prefrontal cortex (PFC) in response to salient stimuli. Activation of the PFC allows information to be updated and kept in working memory. The specific function of the thalamus and the thalamic nuclei involved in this circuit are poorly understood. To further elucidate the involvement of the thalamus in working memory, we performed working memory tasks with several patients who had isolated thalamic strokes. The paradigm we used was an n-back task, using verbal, spatial, or object stimuli. In this paradigm, participants are asked to determine whether stimuli presented are identical to those presented n (1, 2,or 3 depending on the difficulty of the trial) back in a sequence of stimuli. As previously found, verbal deficits were shown more than spatial and object deficits. Continuing research will involve performing these tasks in an fMRI and analyzing brain activity in impaired vs. normal patients.

38. Age Effects in Memory Acquisition of CHL1 Knockout and Wild-type Mice in a Radial Arm Maze Task

Meyer AE & Buhusi CV
Department of Psychology, College of Charleston

Normal brain aging is associated with deficits in learning and memory. The hippocampus is frequently implicated in aging-related learning deficits. This study investigates the role of the cell adhesion molecule CHL1 (close homologue to L1) in the age-related changes found in hippocampal pyramidal neurons and associated learning deficits in a radial arm maze task testing for both working memory and reference memory capabilities. Subjects consisted of young and old CHL1 knock-out (-/-) mice and their littermate controls. Stereological techniques were implemented to assess the number of CA1 and CA3 pyramidal neurons in the four groups which were then correlated with behavioral performance. We hypothesize that aging processes will result in biophysical alterations in the pyramidal neurons in the older mice, the lack of the CHL1 molecule will accentuate these processes, and that the decline in pyramidal neurons will correlate with behavioral performance.

39. The effects of chronic ethanol intake on brain glucose utilization in mice lacking the CB1 receptor

Rice OV, Schonhar D, Rubel K, Piyis Y, Michaelides M, & Thanos PK
Neuroscience Program, Furman University

The mesolimbic dopamine system is most implicated in the mediation of reward and pleasure and is thought to be moderated by the CB1 receptor in the case of alcohol administration. Results from previous studies show significantly decreased binding rates of [^{3}H]2-deoxyglucose in the brains of CB1 KO mice after acute alcohol consumption. The current study investigates the effects of chronic alcohol consumption using the same paradigm. We hypothesized that, as in the previous study, there would be a decreased rate of [^{3}H]2-

DG binding in the brains of the CB1 knockout mice. CB1 knockout and Swiss-Webster (n=18) mice were assigned to control (non-alcoholic beer), low (0.5 g/kg) and high (1.5 g/kg) ethanol dosage groups. The mice received their respective doses one hour daily for four weeks. On test day, the mice were injected with [3H]2-DG prior to receiving their ethanol dose. After drinking for one hour, the animals were sacrificed and blood samples were taken for glucose and ethanol analysis. Brains were removed and flash frozen for imaging.

40. Differing Sensitivities in Wild-Type C57BL/6J and β-Endorphin Deficient C57BL/6J Mice to Drugs Affecting the GABAA Receptor Are Due to Fundamental Differences in GABAA Receptor Makeup

Crawford JT, Spence T, Locklear M, Pujara MS, & Grisel JE
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In order to examine the opioid-mediated behavioral responses to alcohol a battery of behavioral tests were performed using wild type C57BL/6J mice (WT) and β-endorphin deficient (POMCX*4 β-endorphin knockout) C57BL/6J mice (bEKO). Among the behaviors examined was the sedation of each strain to a variety of drugs including ethanol, pentobarbital, THIP, and zolpidem. In each of these drug studies, the dependent measure of sedation was loss of righting reflex. In examining the data from the drug sedation studies, we found that the two strains exhibited differing sensitivities to each drug. Since all of these drugs are known to act upon GABAA receptors in order to produce their effects, and since modifying an organism's genome can have effects on the rest of the genome, we decided to investigate whether or not the two strains differed in the makeup of their GABAA receptors. Several brain areas known to contain high concentrations of GABAA receptors were dissected from naïve WT and bEKO mice. These samples were then analyzed using real-time reverse-transcription quantitative PCR to measure which receptor subunits were being expressed in each strain. In investigating the heterogeneity of receptor subunits between the strains we found a significant difference in the amount of α2 and δ subunits by strain. We believe this fundamental difference in GABAA receptor makeup to be partially responsible in determining the effect β-endorphin has on ethanol sensitivity, as well as sensitivity to other drugs.

41. Correlation between the role of RGS4 and the activation of ERK during relapse in rats abstinent from chronic cocaine use

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It has been hypothesized that enduring neuroadaptations in the brain circuitry including dorsal striatum (dSTR) underlie the high vulnerability to cocaine relapse even after long periods of abstinence. However, precise character of these changes is poorly understood. The chronic use of cocaine has already been shown to decrease levels of the protein termed regulator of G-protein signaling 4 (RGS4) in the dSTR. RGS4 is known to limit signaling of several G-protein-coupled receptors, therefore controlling downstream second messenger cascades, activation of protein kinases and gene expression. One of the kinases inhibited by RGS4 is the extracellular signal-regulated kinase (ERK) pathway. Our experiment sought to determine the correlation of RGS4 regulation in the dSTR to the activation of ERK via phosphorylation (pERK) after chronic cocaine self-administration and relapse to cocaine-seeking using male Sprague-Dawley rats as animal models. Understanding the role of RGS4 in cocaine-seeking may lead to novel pharmacotherapies of relapse.

Further Reading

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- Personal Genetics Education Project Teaching Resources (Dana Waring)
- Medical School Application Tips (Karen Eippert)
- Applying for Grants and Fellowships (Chris Korey, Ph.D.)
- Careers in Technology Transfer (Yashmin Karten, Ph.D.)
- Careers in Medical Writing (Christine Lauay, Ph.D.)
- Neurons in Action 2 Resources (Ann Stuart, Ph.D.)

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Huntington's disease: seeing the pathogenic process through a genetic lens

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Thirteen years ago, the culmination of genetic rather than biochemical strategies resulted in the identification of the root cause of Huntington's disease: an expanded CAG trinucleotide repeat that leads to an elongated polyglutamine tract in the huntingtin protein. Since then, biochemical and cell biological attempts to elucidate pathogenesis have largely focused on N-terminal polyglutamine-containing huntingtin fragments. However, continued application of genetic strategies has suggested that the disease process is, in fact, triggered by the presence of expanded polyglutamine in intact huntingtin. An increased emphasis on the earliest presymptomatic stages of the disease, facilitated by incorporating genetic lessons from human patients into the search for biochemical targets, could provide a route to a rational treatment to prevent or slow the onset of this devastating neurodegenerative disorder.

An exceptional opportunity to prevent neurodegeneration

Huntington's disease (HD) has been a flagship for the study of inherited neurological disease, from initial chromosomal localization of the gene without any prior understanding of its nature, through identification of the molecular defect without knowledge of the gene or its function, to characterization of pathogenesis using gene-based models. Unlike other common neurological disorders, HD has a single cause in all patients, which enables investigators to focus on a single fundamental disease mechanism. Genetics has made it possible to approach that mechanism from its initial stages, without having to work backwards from end-stage pathology. This feature of HD contrasts with the heterogeneous etiologies of well-known disorders such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis that force therapeutic efforts to be trained upon shared biochemical events that occur late in each disease process. It is notable, however, that, even in these heterogeneous disorders, much of the current knowledge is owed to the identification of genetic defects in the small proportion of cases with evident genetic etiology.

The single starting point for HD pathogenesis offers the special opportunity for discovering a rational therapy that blocks the initial triggering event or its immediate biochemical consequences. However, despite this unique advantage, much HD research has not capitalized on it, being focused instead on cell biological and biochemical events that occur relatively late in the disease process, particularly the formation of insoluble aggregates of truncated protein. Recently, biochemical and other phenotypes have been described that are caused by the expression of endogenous full-length huntingtin and occur long before the appearance of huntingtin fragments or insoluble aggregates. These findings, combined with the capacity to apply genetic criteria to dissect the disease mechanism, suggest that it is timely to increase the focus of experimentation on the earliest stages of pathogenesis – it is these stages that offer the promise understanding the presymptomatic disease state and could lead to targeted therapies that prevent disease onset.

Here, we consider the current status of the understanding of HD pathogenic process prior to late-state-stage neurodegeneration, as guided by genetic studies in HD patients and accurate genetic models.

Clinical and neuropathological features of HD

HD patients are typically recognized by their peculiar writhing movements, but they also suffer behavioral and intellectual deficits [1]. Disease manifestations can begin at any time in life, although the vast majority of cases display onset in middle-age. HD symptoms are associated with a distinctive underlying neuropathology that starts with the death of GABAergic medium-sized spiny projection neurons in the caudate nucleus (a tail-shaped region bulging into a lateral ventricle of the brain) and then progresses to neurons in other brain regions. Motor symptoms begin subtly as minor spontaneous movements that progress to continuous, involuntary jerky movements, giving way to eventual rigidity. At the time of first motor onset, it is estimated that 20–30% of the caudate nucleus neurons have already been lost. Psychiatric symptoms, including chronic depression, irritability, impulsiveness and aggression, are variable and sometimes precede motor onset by years. Intellectual decline occurs with disease progression, which further contributes to the loss of capacity to function in daily life. Death in HD typically occurs ~15 years after motor onset due to complications of the disorder, such as aspiration pneumonia.

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Inheritance of the HD genetic defect

HD has traditionally been defined, like other diseases, in terms of its clinical and neuropathological description. However, these are only evident in mid-life and actually represent relatively late aspects of a process that is encoded in the DNA from conception. All cases of HD are caused by inheritance of an expanded CAG trinucleotide repeat. This repeat normally comprises 6–34 CAG units, but expansion beyond this range causes onset of disease symptoms within a normal life span [1]. Interestingly, when a CAG repeat is in the pathogenic range (>34 CAG units), the number of CAG units inherited is meiotically unstable: in most parent-to-child transmissions, the child's CAG repeat is one or a few units longer or shorter than the parent's (i.e. a mutation rate that approaches 1). Inheritance of the *HD* gene from fathers might occasionally show much larger size jumps due to particular instability of the *HD* CAG repeat in spermatogenesis in some males.

The length of the *HD* CAG repeat is the primary determinant of the age at which clinical symptoms will appear. An increase in CAG length correlates with a decrease in age of onset of neurological symptoms, with the longest CAG repeats causing juvenile-onset HD (Figure 1). This is an extremely strong functional relationship, with the CAG-repeat length alone accounting for 70% of the variance observed for age at onset [2]. The remaining variance, which accounts for a range of onset ages of ±19 years around the mean for most CAG-repeat lengths, is also heritable (proportion of phenotypic variance attributable to genetic variance, $h^2 = 0.56$), indicating the actions of modifier genes [2,3]. Interestingly, in the same dataset, there is no correlation between CAG length and duration of disease from neurological onset to death (Figure 1). Similarly, in a recent extensive study of clinical phenotypes in a large HD cohort, it was found that there is only a small effect of CAG length on progression of overall neurological signs, motor impairment and cognition, but no significant effect on progression of chorea or activities of daily life [4]. These and other studies of progression (see Ref. [4] and references therein) indicate that, although the process that initiates HD pathogenesis is highly dependent on CAG-repeat length, the mechanisms that lead from overt symptoms to eventual death are not. Thus, the search for treatments aimed at blocking disease progression might require fundamentally different approaches from the search for therapeutics to prevent disease onset.

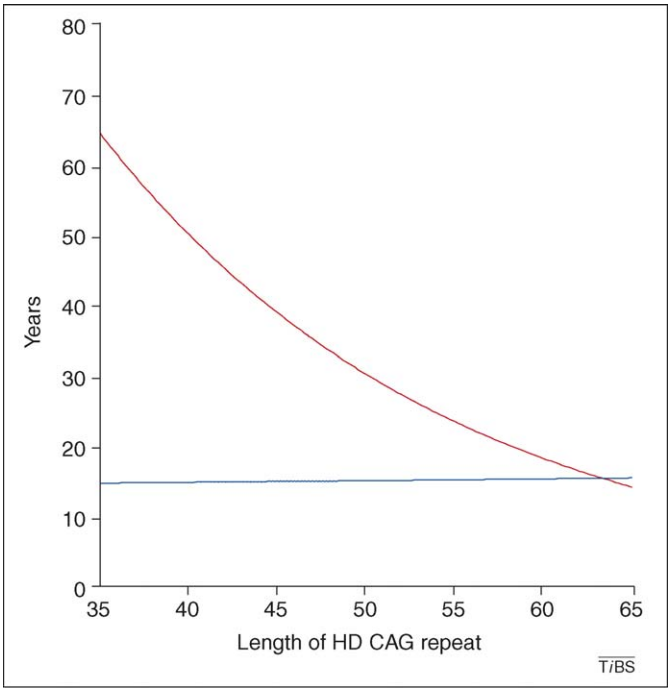

Figure 1. Correlation of HD CAG-repeat length with age at onset. Best-fit curves for age at neurological onset (red) and duration of disease from onset to death (blue), plotted against CAG-repeat length for the expanded mutant allele from Huntington disease (HD) patients. Age at onset is strongly correlated with the CAG-repeat length ($r^2 = 0.54$; $p < 0.001$), whereas duration of disease shows no correlation with the CAG-repeat length, suggesting that factors independent of the original trigger of pathogenesis predominate after onset of HD to determine rate of progression. Based on the data from Ref. [75].

Two special circumstances in which this striking relationship between CAG-repeat length and age at onset has been investigated are also revealing concerning both the inheritance and functional characteristics of the mutation. First, this relationship, together with the occasional larger increases in repeat size seen in paternal transmissions of the disease gene, explains the previously enigmatic observation that most juvenile-onset HD patients have inherited their *HD* gene from a father. Second, although most HD patients possess a single copy of the genetic defect, rare cases have been reported whereby an individual has inherited two expanded copies, one from each affected parent. Investigations of these rare 'HD homozygotes' show that neither the presence of two copies of an HD expanded CAG repeat nor the absence of a normal CAG repeat alters significantly the age at neurological onset predicted by the longer of the two expanded disease alleles [5–8]. Thus, at least with respect to age at neurological onset, HD exhibits phenotypic dominance.

The HD genetic defect in context

The CAG repeat is located in the coding sequence near the 5′ end of the *HD* gene. It encodes a variable length polyglutamine tract beginning 18 amino acids from the N terminus of the large (>3100 amino acid) huntingtin protein. Huntingtin is expressed widely from conception in both neuronal and non-neuronal tissues, and, within the brain, is not limited to the neurons that are vulnerable in HD. Indeed, this pattern in which neuronal susceptibility is not explained by preferential protein expression parallels the observations in a series of other inherited neurodegenerative disorders in which expanded CAG repeats encode lengthened glutamine tracts in different proteins (Figure 2). However, in each of these disorders, a different neuronal population is the primary target. For example, in spinocerebellar ataxia 1, the glutamine tract is within the 815 amino acid ataxin 1 protein. Despite being expressed in both medium spiny neurons in the caudate and Purkinje cells of the cerebellum, this mutant protein leads to primary loss only of the latter neurons. Similarly, huntingtin is expressed in both neuronal populations but the mutant protein leads to primary loss of medium spiny neurons. The striking similarities and notable differences in these neurodegenerative disorders suggest a mechanism by which the effect of the altered

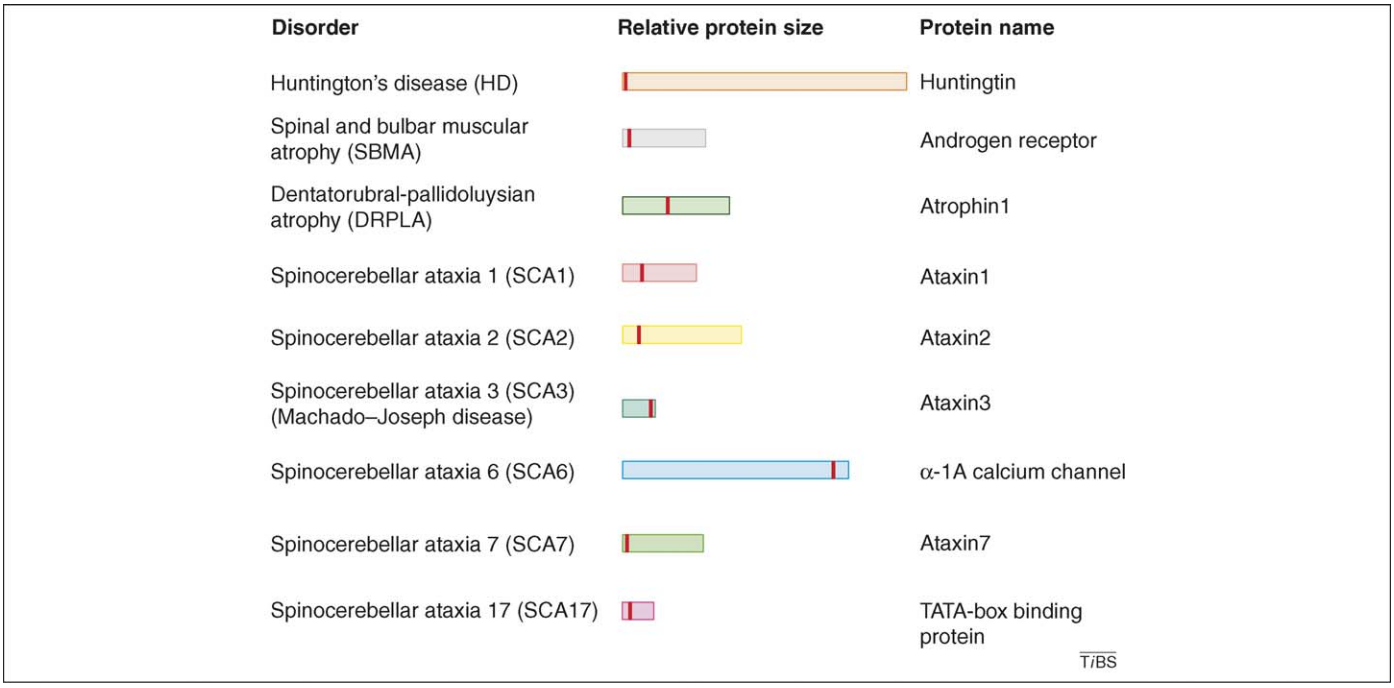

Figure 2. Protein context of the polyglutamine expansion determines which neuronal cell populations are the most vulnerable. Shown is the location of the polymorphic polyglutamine tract in nine different protein contexts (drawn to scale) that, when expanded, causes the specific loss of neurons from different brain regions and lead to distinct inherited neurodegenerative disorders.

polyglutamine combines with its specific protein context to trigger the characteristic pathology in each different disorder.

How does the HD genetic defect kill neurons?

The toxic effects of the *HD* polyglutamine tract have most often been studied within a small N-terminal fragment (hereinafter termed 'h') of the huntingtin protein [9,10]. This approach has been spurred by the observation in post-mortem HD brain of the h in inclusions within remaining neurons [11]. In a wide variety of model systems, including both neuronal and non-neuronal cells in culture, yeast, nematode, fruit fly and rodents, expression of h leads to formation of intracellular inclusions in the cytoplasm and/or nucleus. The correlation of inclusions with cellular toxicity has been extremely variable, with some studies reporting toxic effects and others reporting protective effects [12,13]. This extensive literature has been reviewed often. However, a recent careful quantitation of the time course of inclusion formation and cell death in primary striatal neurons expressing h shows that such inclusion formation is not the source of polyglutamine-mediated toxicity in HD [14]. This has been supported by two other reports in which lentivirus-driven or HD-promoter-driven h produced intraneuronal inclusions without correlation with neuronal loss [15,16].

In contrast to h fragments with polyglutamine lengths in the normal range, those with polyglutamines in the pathogenic range rapidly form inclusions in model systems and aggregated insoluble amyloid *in vitro*. This supports the suggestion that the expanded polyglutamine confers an altered physical property on the protein. An elegant series of studies has shown that this conformational property is dependent on both the polyglutamine tract and its surrounding sequence context [17–19]. Their studies suggest that amyloid formation is nucleated at the level of the single molecule by a thermodynamically unfavorable misfolding that leads to formation of an aggregate structure with alternating elements of extended chain and turn. Some of the predictions from these studies have been reproduced in cultured cells that express variants of the polypeptide encoded by exon 1 of the 67-exon *HD* gene [20]. These types of experiments, combined with the HD genotype–phenotype relationship described, have led to the view that HD is a conformational disorder in which the pathogenic pathway is triggered in some manner by protein misfolding and its consequences.

Whereas an altered physical property is readily measured in the context of the h fragment, its effects on the full-length huntingtin protein are not as well understood. For example, Cong *et al.* [21] have shown that distinct differences on native gels between soluble h with glutamine tracts in the mutant and normal size ranges are abolished in larger fragments that included more of the downstream huntingtin sequence. These results suggest that the huntingtin sequence dampens the acute effects of the conformational difference between mutant and normal-sized polyglutamine tracts [21]. As expression of full-length mutant huntingtin occurs throughout life and precedes the detection of h in human patients, the possibility that pathogenesis is triggered via a novel property conferred on the huntingtin protein has been tested in a variety of mouse models.

What is the effect of polyglutamine on huntingtin?

A route used by several laboratories to test the consequences of an elongated polyglutamine tract in intact huntingtin has been to create *Hdh* (the mouse *HD* gene

ortholog) CAG knock-in lines of mice as true genetic models of HD. These lines express mutant huntingtin with 50–150 glutamines from the endogenous mouse *Hdh* promoter in a manner comparable to the expression of mutant huntingtin in HD patients [22]. These mouse models exhibit a variety of abnormalities that are detected at every level, from the biochemical, molecular and cellular level to phenotypes that can be measured in the whole animal. As expected based upon HD in humans, changes are first apparent in the striatum (i.e. the caudate nucleus, putamen and globus pallidus). Molecular phenotypes include: (i) increased levels of the ribosome signaling protein Rrs1; (ii) an altered conformation or accumulation of huntingtin in the nucleus of medium-sized spiny neurons; (iii) striatal *Hdh* CAG-repeat instability; (iv) altered enkephalin mRNA level; (v) altered Ca^{2+} sensitivity of striatal mitochondria; and (vi) altered intracellular signaling affecting at least protein kinase A, protein kinase B (Akt), glycogen synthase kinase 3β (GSKβ), and serum- and glucocorticoid-inducible kinase (SGK) pathways [22–25]. Abnormal behaviors such as nocturnal hyperactivity manifest at a young age, well before signs of overt neuronal cell pathology or neurodegeneration [22]. At much older ages, h, insoluble amyloid, and intranuclear and cytoplasmic inclusions become evident. As is the case for neurological onset in humans, the onset of these phenotypes is hastened by longer polyglutamine-repeat lengths and is more sensitive to polyglutamine length than to huntingtin dosage. In animals with the longest glutamine tracts, age also brings striatal atrophy, loss of medium-sized spiny striatal neurons, gait deficits and mildly decreased survival. Notably, homozygotes for the knock-in alleles have a slightly earlier onset of these phenotypes than heterozygotes. At first glance, this seems to differ from the phenotypic dominance observed in humans for age at neurological onset. However, this small difference of a matter of weeks might also manifest in human HD patients but might be undetectable, being overwhelmed by the normal ±19-year variation around the mean for neurological onset.

An alternative approach has involved the analysis of YAC72 and YAC128 transgenic mice, created with modified *HD* 4p16.3 genomic DNA YAC transgenes [26–32]. These mice ubiquitously express copies of human huntingtin from human *HD* promoter elements. The YAC128 mice have recently been shown to exhibit a similar striatal-specific huntingtin nuclear localization phenotype to the knock-in models. As in *Hdh* CAG knock-in mice, this coincides with onset of early behavioral abnormalities and is also followed much later by intranuclear inclusions and striatal-cell degeneration. More dramatic effects of mutant huntingtin have recently been achieved in a tetracycline-off conditional PrP-tTA-6/iFL148Q transgenic line, in which a prion promoter drives conditionally regulated expression of human huntingtin with 148 glutamine residues from a full *HD* cDNA transgene [33]. Transgene expression from the prion promoter is highest in regions other than the striatum. These mice exhibit mutant h and inclusions in regions beyond the striatum, and early death [33]. It will now be important to determine whether inappropriate regulation of mutant huntingtin leads to early presymptomatic disease phenotypes, and in which cell types.

Early presymptomatic effects of properly regulated mutant huntingtin have also been studied in cell culture HD models. Primary striatal cell cultures and clonal immortalized striatal cells derived from *Hdh* CAG knock-in mice and from YAC transgenic mice exhibit a variety of phenotypes comparable to the early striatal-specific phenotypes seen in the mice, but do not manifest h fragments or inclusions [34]. Thus, the accurate expression of intact mutant huntingtin in YAC transgenic and *Hdh* CAG knock-in mice has revealed early phenotypes long before those associated with symptomatic HD, thereby, providing models for the presymptomatic and early symptomatic disease period. The early phenotypes first manifest in striatal neurons, confirming the special vulnerability of this cell population. In addition, they occur in the absence of overt h, indicating that, although aggregate formation might have a role downstream, it does not trigger the disease process, which, instead, begins with an effect of the intact mutant huntingtin.

The HD pathogenic process in humans

The existence of a protracted pathogenic process in humans, as seen in mice, is increasingly apparent from brain imaging and neuropsychological and cognitive studies of presymptomatic individual, made possible by HD CAG testing. These have revealed both morphometric and functional changes in the brain that occur prior to the onset of marked neurological symptoms [35–45]. It is not known how early in life these differences begin, but they have often been presumed to result from a direct effect of mutant huntingtin on a neuron-specific target. Interestingly, it is now also being recognized that presymptomatic individuals display abnormalities due to mutant huntingtin in non-neuronal tissues such as skeletal muscle, fibroblasts, platelets, blood-nucleated cells and cultured lymphoblasts [46–53]. These findings present an alternative model for the HD disease process, suggesting that the effect of mutant huntingtin might alteration of the fundamental state of any cell in a CAG-length-dependent manner. Although most cells have the capacity to survive the CAG-length-dependent state, striatal medium-sized spiny neurons eventually exhibit dysfunction and succumb owing to some peculiarity that makes them unable to cope (Figure 3). In this model, striatal specificity lies not in the nature of the direct target of mutant huntingtin, but in the special physiology of striatal neurons that makes them vulnerable. Deciphering the nature of this striatal specificity could provide clues to treatment that cannot be identified using other cell types.

Impact of polyglutamine on huntingtin normal function

Huntingtin deficiency is lethal both in mouse embryogenesis and in adult cells and, although increasing huntingtin levels might help cells to survive certain stresses, the actual function(s) of the protein is not known [54]. The existence of HD homozygote patients with no normal *HD* gene and whose age at neurological onset is comparable to equivalent HD heterozygotes has made it clear that the expanded CAG does not greatly impair the essential developmental function(s) of huntingtin. Neither does it cause onset by a mechanism that can be rescued by one

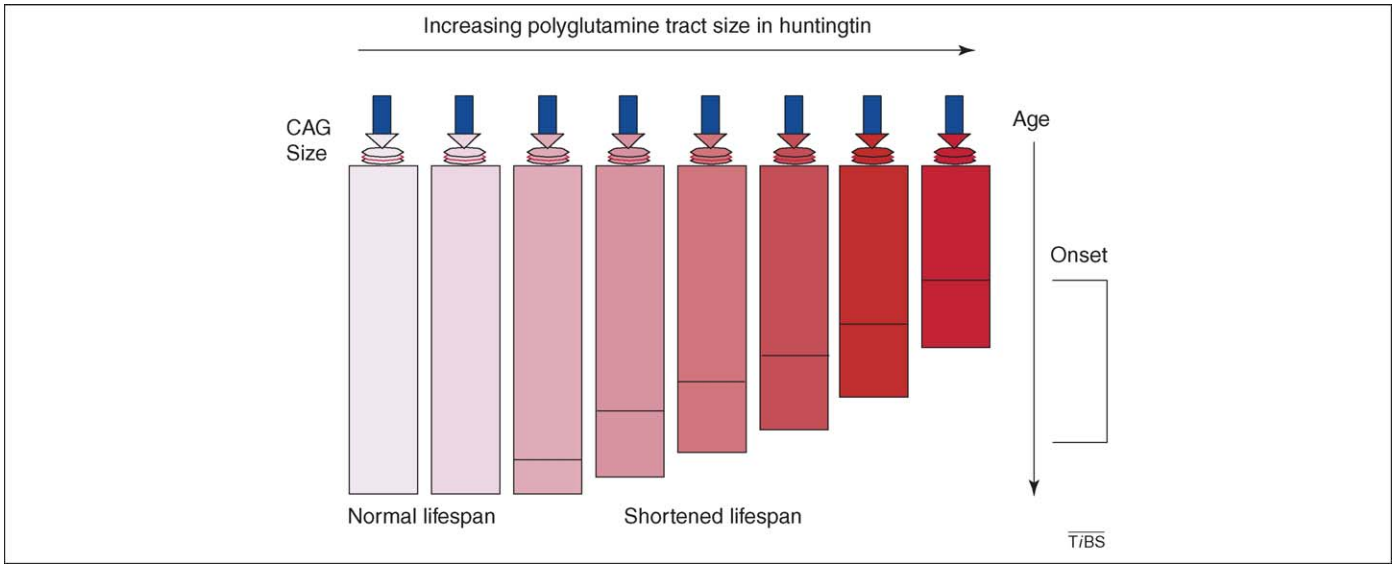

Figure 3. A quantitative trigger might determine a constitutional physiologic state. The schematic diagram depicts a model for a hypothetical quantitative effect of the *HD* CAG repeat that might manifest in both non-pathogenic and pathogenic size ranges in many cell types throughout life, as suggested by the quantitative effect of *HD* CAG size on the cellular ATP:ADP ratio [53]. The blue rectangle above each box represents huntingtin and the arrow at its base represents its polyglutamine tract, the increasing color intensity of which indicates increasing polyglutamine length. Huntingtin interacts with an unknown cellular target (denoted by the octagon) with intensity or frequency of interaction also increasing with polyglutamine length (denoted by increasing color intensity). This huntingtin–target interaction determines a physiologic state (denoted by the large rectangle) that lasts throughout life and is dependent on the glutamine-tract length. The physiological state becomes increasingly intense (denoted by red color intensity) with increased glutamine size within the normal size range (<35 CAGs), but is not sufficient to disable striatal neurons, does not lead to onset of HD and supports a normal lifespan. CAG lengths of 39 and higher produce a progressively more intense physiological state with which striatal neurons cannot cope, thereby leading to onset of overt clinical symptoms (black vertical bars) at progressively earlier ages. Premature death ensues after a course of ~15 years, but is not influenced by repeat size. Intermediate glutamine tracts (35–38 CAGs) might cause onset late in life, <15 years before the average normal lifespan.

allelic equivalent of the normal protein. One report suggests that HD homozygotes display more rapid progression after onset, indicating that wild-type huntingtin might possess a protective activity [6]. However, as neither the progression nor the duration of illness after neurological onset is strongly correlated with CAG length, this protective activity might act on processes secondary to the initial trigger and to the neuronal dysfunction that causes disease onset.

Still, the fact that the disease process triggered in HD is different from that triggered in other polyglutamine disorders indicates that some aspect of the structure, binding partners, subcellular localization or activity of huntingtin is crucial to this specificity. Huntingtin is composed largely of consecutive HEAT repeats, which ate ~38 amino acid degenerate motifs that are named for their presence in huntingtin, elongation factor 3, regulatory A subunit of protein phosphatase 2A and TOR1 (target of rapamycin 1) [55,56]. Each HEAT repeat consists of two α-helical domains separated by a short linker. X-ray crystallography of other HEAT repeat proteins, such as β-importin and the regulatory A subunit of protein phosphatase 2A has revealed a stacking of consecutive HEAT repeats that forms a flexible solenoid-like structure. A recent preliminary biochemical characterization of recombinant full-length human huntingtin is consistent with this model, with huntingtin being a much larger protein that might form an elongated superhelix [57]. Based on its HEAT-repeat structure, huntingtin has been suggested to function as a scaffold, organizing members of dynamic complexes for transport and/or activity [56]. Huntingtin interacts with membranes and a wide range of other proteins that represent many different cellular functions, and is subject to a variety of post-translational modifications [54,58,59]. It shuttles between cytoplasmic and nuclear compartments and subsets of the protein are differentially detectable by immuno-staining, which indicate different conformations or epitope availability in different locations. Huntingtin has been implicated in facilitating a variety of cellular processes, including transcriptional regulation, mRNA processing, vesicular transport, and organellar location and morphology.

Interestingly, the polyglutamine segment is dispensable because its removal from mouse huntingtin leads to animals that are fully viable [60]. However, they develop subtle motor and behavioral differences from wild-type mice, suggesting that the glutamine tract might modulate a normal function of huntingtin. Cultured fibroblasts from these mice display elevated ATP levels. Notably, mouse striatal cells from *Hdh* knock-in mice with an elongated glutamine tract show reduced ATP levels, which could be an effect of polyglutamine length. Examination of human lymphoblasts representing both the pathogenic and non-pathogenic size ranges has revealed a clear correlation between polyglutamine length in endogenous huntingtin and the cellular ATP:ADP ratio [53]. These findings on the effect of the polyglutamine tract combine to implicate huntingtin either directly or indirectly in regulation of energy metabolism, which might also be consistent with the recent description of an effect of huntingtin dosage on body weight in mice [26].

Modifying the pathogenic process

The understanding of the structure and function of huntingtin is not yet sufficient for small-molecule drugs

to be targeted with certainty to the huntingtin-specific trigger of pathogenesis. Continued delineation of the earliest events in pathogenesis and further examination of the structure and function of huntingtin will be required. However, both can be informed by the identification of pharmacological and genetic modifiers of the disease process either in model systems or in human patients. Many drug screens have been performed in cellular models, typically with different results in each, confirming that the polyglutamine has different consequences in different contexts and different cell types [61]. This suggests that the many cellular assays do not, as a group, capture the same fundamental mechanism. As a conformational property is thought to trigger the pathogenic process, several drug screens have been aimed at altering this property *in vitro*. Testing of these compounds in mouse models represents the current best hope for a drug that blocks the beginnings of pathogenesis. It is hopeful that some compounds identified in this manner rescue a phenotype caused by intact mutant huntingtin in cultured striatal cells from *Hdh* knock-in mice [62]. In the absence of a small molecule treatment, blocking the pathogenic trigger might require manipulation of endogenous mutant huntingtin expression via siRNA, intrabodies or other techniques [63–66].

Another approach that might provide clues to presymptomatic treatment is the search for those genes that reveal the heritable variance of age at onset that is not due to the HD CAG repeat. Several potential genetic modifiers have emerged from candidate association studies in HD patients, of which only the gene encoding the GluR6 subunit of the kainate-type glutamate receptor (*GRIK2*) and the gene encoding ubiquitin C-terminal esterase L1 (*UCHL1*) have yet been replicated in more than one population [67–71]. These modifiers, which might impact by altering glutamate-mediated signaling and huntingtin clearance, respectively, explain a relatively small portion of the variance in age at onset. However, when confirmed, even modifiers with a small but significant impact can provide potential drug targets because they have been validated as having a measurable effect on HD pathogenesis in human patients. Candidates have also been tested in the mouse, where DNA repair has emerged as a potential drug target [72,73]. Another hopeful direction is to identify genetic modifiers in humans by unbiased genetic linkage and association studies. An initial linkage scan by an international consortium of HD investigators has implicated several chromosomal regions as potentially harboring common modifiers of HD [74]. Although this strategy might take longer than candidate searches, it has the potential to yield the unexpected and cast entirely new light on the pathogenic process.

Concluding remarks

The homogeneous genetic nature of HD offers a tremendous opportunity to identify and block the very earliest stages of the disease process, before widespread neuronal dysfunction occurs. Although examination of the final stages of dysfunction might provide functional drug targets to treat particular phenotypes, genetics has provided the means for describing the characteristics of the initial trigger mechanism and its consequences in model systems. Rational treatments based on the biochemical nature of the trigger and/or early steps in the disease process could prevent all subsequent phenotypes from developing. However, identification of valid biochemical targets at which to aim these rational therapies will require paying close attention to the information provided by the use of genetic strategies in human clinical studies, in experimental models and in drug development.

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Hunting Down Huntington's

12.01.1993

From the shores of Maracaibo to the halls of Washington, Nancy Wexler has spent 25 years stalking her mother's killer.

by Andrew Revkin

Joseph Hartman and his wife, Marilyn, smile stiffly as they sit in the Ben Franklin room of the University of Pennsylvania's student union. He is gaunt and pale, with a long face and cropped, graying hair. She has the delicate frame of a songbird and is prim and neat in glasses and a green and white floral-print dress. They might be posing for a picture--a modern version of American Gothic--except that Joseph Hartman's body is in constant motion.

Two dozen physicians and biologists politely but intently inspect Hartman as his case is presented by his neurologist. Hartman (not his real name) first sought medical help 13 years ago, complaining of irritability, lack of sleep, the jitters. He soon became unable to work and began to lose his short-term memory.

Only after many years did the chorea begin. The word comes from the Greek for dance, and that is exactly what Hartman is doing now. His hands dart and float in the air as if manipulated by an inebriated puppeteer. His head has a looseness that recalls those toy puppies whose heads once bobbed in the rear windows of American sedans. He watches his own limbs as if from a distance, occasionally reasserting control long enough to force a hand to grasp at the fabric of his pant leg or scratch the nape of his neck. Inevitably, his body rebels and resumes its dance. It is a dance of death.

This is Huntington's disease: a devastating inherited condition that often waits until midlife to strike. A rare genetic flaw, present in one of every 10,000 people, selectively destroys two small regions of the brain--the putamen and the caudate nucleus--that help control movement. Eventually the muscles cannot be controlled at all; many Huntington's patients die because they choke on food they can no longer swallow.

The presentation of Hartman's case is the opening act in a two- day workshop aimed at generating research on how the gene kills brain cells. Hartman and his wife respond to questions. Do you like sweets? asks the woman seated next to Hartman's wife. The woman could not present a more dramatic contrast to Joseph Hartman. Her blue eyes are clear and probing; she holds her shoulders square and steady. Indeed, Nancy Wexler, a psychologist and president of the Hereditary Disease Foundation, which organized the session, is as elegant and sharp as the tailoring of her cream suit.

After a pause, Hartman replies, I like fruit. He speaks in a shallow voice that seems only marginally under his control. I've practically given up candy. My grandchildren say my loss of candy is not a good thing. The joke elicits polite chuckles from the scientists.

Wexler comments to the gathering that Huntington's victims often crave sweets and rich foods, perhaps to provide the extra energy it takes to keep the body in perpetual motion. Sometimes they'll get up in the middle of the night and fix themselves enormous dinners, she notes.

Wexler's familiarity with Huntington's is not merely academic. Twenty-five years ago, when she was 23, Wexler learned that her mother, Leonore, had Huntington's disease. That means there is a fifty-fifty chance that she has inherited the genetic mutation and will eventually die just as her mother finally did--just as the man in front of her is slowly dying now.

The specter of the disease has dogged Wexler every day since 1968--Any time I trip on a curb or drop something, I think, 'Is this it?' --but she, in turn, has hounded the disease. For more than two decades she has devoted all her energy to finding the destructive genetic misprint and a way to cure it.

In March of this year, a consortium of 58 researchers--including Wexler--announced that they had accomplished the first goal. The gene hunters, as the group was informally called, had discovered Huntington's lethal mutation: an extended, stuttery repetition of a single DNA instruction. Now many labs are racing to create cell cultures and gene- altered mice that may lead to treatments or, eventually, a way to repair the defect through gene therapy.

Wexler has hardly paused to celebrate. The Philadelphia workshop is the first of a new wave of post gene workshops, one in a series of dozens of similar sessions she has organized over the years. When the presentation of Joseph Hartman's

case draws to a close, Mrs. Hartman grabs some muffins and a glass of orange juice for her husband. Wexler glances at the food and pats Joseph Hartman's stomach. Keep eating! she says, and hugs him tightly, as if he were a long-lost relative whom she might not see again. In a way, he is.

It is almost impossible to talk about Huntington's disease without talking about Nancy Wexler. In the fight against the disease, she has functioned not only as scientist but as catalyst, cheerleader, even den mother. Her quest has taken her from lavish fund-raising dinners to congressional hearings, from genetics laboratories across North America and Europe to squalid shantytowns on the shores of a Venezuelan lake. Her personal struggles--with her mother's illness, with the decision about whether to be tested for the gene--have only added to her credibility. And through it all, she has remained an eloquent advocate for victims of the disease. In September she received the prestigious Albert Lasker Public Service Award in recognition of her work on their behalf.

Yet when asked, Wexler credits her father with sparking her crusade. There's something very fundamental about how a family or any human being faces a critical problem, she says. The choices in my family have very much been determined by my father's reactions to the crisis--how he dealt with it.

Just a few minutes apart, on a hot August day in 1968, Nancy and her sister, Alice, arrived in Los Angeles, answering their father's invitation to celebrate his sixtieth birthday. Nancy thought something was up; theirs was not a family that was big on birthday parties. Milton Wexler was a tall, urbane man who had succeeded in three careers--as a lawyer, a commander in the Navy, and a psychoanalyst. He has since added another, collaborating with friend Blake Edwards on several successful screenplays. Alice, then 26, was well on her way to a doctorate in history at Indiana University; today she is writing a book about Huntington's disease. Nancy had recently graduated from Radcliffe and was about to begin graduate studies in psychology at the University of Michigan.

Their father did not tell them anything until he had driven them back to his apartment. There he broke the news that their mother, from whom he had been divorced for several years, was doomed. (In retrospect, he says, many of the behavior patterns that led to the split--depression, irritability, and difficulty relating to others--read like a textbook on the early symptoms of the disease.)

Nancy says she doesn't really remember much else about that day, except that she knew her mother was dying and that she and her sister decided never to have children. Milton tried to tell them what was known about the disease, but there wasn't much to tell. Huntington's afflicts 30,000 people in the United States and places another 150,000 at risk. The gene that causes the disease is dominant, meaning that if a child inherits just one copy of it, he or she will get the disease. As Milton spoke, Nancy's mind rushed back to the early deaths of Leonore's three brothers. The brother closest to her, Seymour, was a fantastic clarinetist, she says. When he died, Mother was very upset and went back east. I asked what was going on, and I was told that all of her brothers had died of a hereditary disease, but that we were not at risk.

Once the shock had passed, Nancy, Alice, and Milton rallied around Leonore. But they didn't stop with that. Milton got in touch with the widow of the disease's most famous victim, folk singer Woody Guthrie. Marjorie Guthrie was organizing victims' families into the first organization to call for research, the Committee to Combat Huntington's Chorea, and Milton decided to form a California chapter of the group. Guthrie's organization, however, focused on building state chapters and lobbying; Wexler wanted to fund scientific research directly. My father felt very strongly--because it was such a devastating disease--that even though care could certainly be improved, the crucial thing was to find a treatment that took away the symptoms and cured the disease, says Nancy. That no matter how much you padded a bed with lamb's wool so someone wouldn't bash their legs and get black and blue on the railings, it was much better to get them out of bed. And that's been the guiding philosophy.

Eventually the differences forced a split--in 1974 Milton Wexler created the Hereditary Disease Foundation. Even before then, he began pursuing his scientific agenda by setting up a series of freewheeling discussions on Huntington's; he lured the best and brightest young minds to these workshops by offering free travel and $1,000 honoraria.

He was frustrated by the first meetings, at which young scientists gave old-fashioned presentations bogged down by slides and charts. So he refashioned them. From then on, sessions were held in the round, with no set agenda. What we ask for are people's imagination, energy, and affection for a weekend, Nancy Wexler explains. If, however, anybody feels inspired to actually tackle some of these problems, we're quite interested in knowing that.

Allan Tobin, a young neuroscientist from Harvard who knew Wexler from her undergraduate days, says the early workshops were like a game of the blind man and the elephant, where no speculation was too wild. In addition, he fondly recalls how Milton Wexler would sometimes use his contacts in the entertainment community--many of whom also

happened to be his patients--to arrange a Hollywood-style party as the centerpiece of meetings held in Los Angeles. There the researchers hobnobbed with the likes of Jennifer Jones, Candice Bergen, and Cary Grant.

Eventually Tobin moved to UCLA and became scientific director of the Hereditary Disease Foundation. He also became the moderator for the Huntington's workshops, refining a style that one of his colleagues calls a cross between Socrates and Geraldo Rivera. Over the years he and Nancy Wexler have developed the habit of sitting side by side while they watch the assembled scientists play intellectual volleyball. That's so I can reach out and kick him if he intrudes too much, she jokes. And vice versa.

Wexler found her true mission, however, not at one of her own workshops but at an outside meeting. It was 1972, and she was attending a symposium marking the 100th anniversary of Long Island physician George Huntington's landmark paper describing the disease that would come to bear his name. At the meeting Ramón Avila Girón, a Venezuelan psychiatrist, talked about a large population of Huntington's cases clustered along the shores of Lake Maracaibo, a 130-mile-long brackish lake best known for the vast oil reserves beneath its muddy bottom. Then he turned down the lights and showed a grainy black-and-white film, replete with a tinny sound track of patriotic music.

One after the other, people walking down the streets or sitting in cafés danced to the tune of the Huntington's gene. Inbreeding, isolation, and a tendency toward large families--one woman had 18 children; one man, 34--had produced an extraordinary, possibly unique, concentration of the mutant gene. It was a total shock, Wexler says. Here were all these Huntington's cases, practically in every household, not shut away in nursing homes like they are here, not being stared at, but accepted as part of a community.

Though Wexler felt drawn to these people, it would be several more years before she'd have the chance to meet any of them in person. In 1974, after completing her doctoral thesis on the psychology of people at risk for Huntington's disease, she moved to New York City to teach. Less than two years later she was named executive director of a new congressional Huntington's Disease Commission to set priorities for federal funding to fight the disease, and she moved to Washington, D.C. At this point Milton Wexler realized that it was time for him to step back a bit from the project he had started: the science was getting more complicated, and Nancy and Allan Tobin had the workshops under control. From there on, says Milton, the story is Nancy's.

One of the first things she did was as- semble a Venezuela Working Group so that she could follow her instincts and see that the lakefront population was studied. In 1977 her commission gave its recommendations to Congress, and federal funding began to flow to the gene hunt.

Her triumphs were tempered by her mother's death, on Mother's Day of 1978. Wexler sadly recalls visiting her mother in nursing homes as the disease took its toll. She was extremely frail. All of us were always afraid when she took a step that she would go careening forward onto the concrete. It wasn't a soft environment; everything was unfriendly--concrete floors, hard walls, the chairs weren't soft, the bed wasn't soft.

Spurred by the loss of her mother, Wexler made preliminary trips to Venezuela in July 1979 and April 1980. In March 1981 she made the first of what would become annual expeditions to collect blood and chart this sprawling Huntington's family tree. The lineage is now the largest ever documented, numbering more than 13,000 individuals. Day after day, assailed by insects and heat, she and about ten other researchers explored the barrios around Maracaibo and traveled to outlying villages. They worked in local dispensaries or government-built clinics. It was a sauna, Wexler says, describing one of these facilities. We had to scream over people's heads. The room was packed because this was such a novelty. The Venezuelans were not the only ones to find the experience both odd and compelling. Here was this setting that couldn't have been more different from anything I'd seen in my life, and yet here was this totally familiar disease, Wexler adds. I was exhilarated and frightened. I felt connected and alienated.

She and her colleagues wanted to take blood samples to use in studies of how Huntington's does its damage to the body, but because few of the people had ever had blood drawn, they were scared. It was hard to describe why we wanted the blood of healthy as well as sick people, Wexler says. She explained over and over again that the large family tree around Maracaibo could provide special clues to a disease that was hurting people around the world. I told them my mother had this, she says, and that I was at risk. I told them that very far back we were family. They felt that bond. She returned to the United States with blood samples, a crude pedigree, and high hopes.

Those hopes were bolstered by an ongoing revolution in molecular biology. For several years researchers had been refining new tools called restriction enzymes; these enzymes, they believed, which could be used to snip DNA into manageable pieces, would someday let them pick out specific disease-causing genes from the huge, bewildering tangle

of DNA that makes up a person's entire genetic endowment. Each restriction enzyme recognizes its own specific sequence of four to eight nucleotide bases--the building blocks of genes. Wherever it spots that sequence, it makes a cut in the DNA. Over a long stretch of DNA, a restriction enzyme will excise snippets of varying sizes, depending on the number of nucleotides sitting between its cutting sites. Once researchers have the bits of DNA, they can copy them and determine the order of their bases--that is, they can sequence the gene or genes contained in that DNA.

Although more than 99 percent of human DNA is exactly alike from one individual to the next, at certain spots along the chromosomes short stretches of DNA display distinctive variations, called polymorphisms, and these differences are passed within a family from one generation to the next. The variations may mean that a cutting site that exists on one person's chromosome is missing from the same spot on another person's chromosome, or that extra nucleotide bases are added between two cutting sites on one person's chromosome but not on another's. In either case, when restriction enzymes go to work on the same chromosome from two different people, the resultant fragments may vary in length or weight, and so they can be used to distinguish one person's DNA from another's.

It was quite possible, the reasoning went, that some of these easy-to-spot polymorphisms sat very close to a disease-causing gene on a chromosome. If so, then the polymorphism and the gene would be likely to stay together, even when the chromosomes get shuffled around, as they do when eggs and sperm are produced. In other words, if you could find a particular polymorphism that consistently traveled with a particular disease, you could use that polymorphism as a marker to tell you that the disease gene was located somewhere nearby. And you'd know that, in any given family, anyone who had the polymorphism would be at risk for the disease.

When Wexler first encountered the idea of using polymorphisms as markers, it was just that--an idea. It was October 1979, and she was hosting yet another workshop. She listened intently as key theorists in the field explained their vision for the future of gene hunting. All the people there were true believers, she says. Once you accepted the premise that you really could find markers, then it was just a matter of time to find the gene. It might take you a million years, but it wasn't a complete wild-goose chase where if you miss then you end up with nothing. The whole idea looked beautiful.

One of that workshop's leaders was David Housman from MIT, a friend of Tobin's. After the meeting, he returned to Cambridge and persuaded one of his graduate students, James Gusella, to focus on finding Huntington's markers. Gusella, who soon graduated and moved to Massachusetts General Hospital, began identifying and collecting DNA probes--bits of DNA that are made up of the nucleotide bases complementary to those found around a particular polymorphism. A good probe would latch onto only the fragment of DNA containing that polymorphism. Ultimately, of course, the probe Gusella wanted to find was one that latched onto a polymorphism inherited along with a Huntington's gene.

Collecting the probes was slow work. By 1982--when researchers had discovered only a few dozen polymorphisms--Gusella had his first batch of 13 probes ready for testing, with more waiting in the lab. He was not too hopeful; he noted that it could take 300 probes to more or less cover the entire human genome and find a marker within a reasonable distance of the Huntington's gene. But when he began to test the probes on DNA from a small Huntington's lineage in Iowa charted by Michael Conneally, a geneticist at Indiana University, he quickly hit unexpected pay dirt. The third probe in the batch seemed to grab onto a marker that showed up consistently in family members with Huntington's--but not in those who did not have the disease. Conneally remembers phoning Wexler with the news. She let out a scream, he says.

It seemed inconceivable that Gusella could have gotten so lucky so soon; besides, the Iowa family was far too small to clinch the case. So he turned to Wexler and the Venezuelans. She had been giving him samples of blood since her first collecting trip in 1981--she'd send them along with any researcher heading toward the Boston area--so Gusella had plenty to work with. Conneally ran the statistical checks on his computer. One after the other, the samples confirmed the early finding. Through an extraordinary stroke of luck, they had found a marker for the Huntington's gene.

As it turned out, the polymorphism used as a marker came from the short arm of chromosome 4, which meant that the Huntington's gene was there as well. And 96 percent of the time, in each and every lineage, some version of the marker--there are 20 in all--traveled with the Huntington's gene. That meant that if you could determine which version traveled with the gene in each family, you could tell with 96 percent accuracy whether or not a person would develop the disease.

The finding unleashed an ethical storm. In effect it constituted a predictive test for a disease for which there was still no treatment, much less a cure. And it could be used only on people who had living relatives who were both sick and healthy so that the marker could be traced. Before the marker was discovered, 70 percent of people at risk said they would want to be tested for the disease, if such a test were available. Yet in the decade since the marker test became available, only

about 13 percent of the at-risk population has been tested.

Ironically, the Wexlers chose as a family not to use the technology. Both Alice and Nancy said that a positive result for either one would devastate all of them. If you take the test, you have to be prepared to be really depressed, said Nancy. I've been depressed. I don't like it.

With the marker found, Wexler set her sights on the gene itself. She continued to organize workshops and seek out researchers willing to work on the gene hunt. She traveled from coast to coast, from the United States to the United Kingdom--in between forays to Venezuela, of course-- and in early 1984 she and Tobin pulled together the formal Huntington's Disease Collaborative Research Group. At their suggestion, participants in the group agreed to share information during the search and to share the glory when it came to an end. The collaboration pitted a socialist model--their group--against several independent laboratories pursuing the gene on their own. They had two main competitors: a lab that had been invited to join but declined, and another that had personality conflicts with people already in the group.

Even within the group, collaboration on this large a scale often created tensions and resentment. Sometimes someone would charge that one group was withholding a particularly useful marker, or that another was not sharing data as quickly, says Tobin. Whenever there was a question about whether someone was sharing, I would call the person and say, 'Gee, it would be awfully nice if you brought that material to the meeting.' So it was typical for people to come with little tubes of recombinant DNA that contained a new marker, a new piece of DNA from the suspect region of the chromosome where we thought the Huntington's gene was located. They would distribute it at the beginning of the meeting, and of course then everybody felt better.

Wexler traveled from lab to lab through the 1980s, soothing bruised egos, seeking new talent, and cheering on anyone who was losing momentum. Conneally remembers when he was having trouble finding a place to store cell cultures from the Iowa family on whom the first marker tests had been performed. The only available mutant cell bank, in Camden, New Jersey, took a maximum of three samples from any family. They were persuaded and cajoled by Nancy to take more, he recalls. She got it up to 15 individuals. We collected blood from 30 and sent it to them. They couldn't simply throw it away, so they stored it.

Through it all, Wexler returned to Venezuela each year, collecting blood and data until the pedigree spread like polka-dot wallpaper along the corridors outside her office at Columbia University Medical Center in New York, where she had begun lecturing and doing research in 1985. She began to pick up some peculiar patterns, patterns similar to ones Conneally had told her he'd seen in the Iowa family and others. For instance, in some families the gene did not wait until middle age to strike but hit children as young as two. In these juvenile-onset cases, it struck with particular intensity, causing stiffness in addition to the chorea, and death within a decade. And in most of the juvenile cases, the affected parent was the father: indeed, as a Huntington's father continued having children, often successive children would develop the disease earlier and earlier in life.

Wexler particularly remembers one woman in Venezuela who had been sick from the time she was 14. Now she was 21 and she lay dying in a clinic. Her body had wasted away and stiffened to immobility. When Wexler, sitting at her bedside, enfolded the woman in one of her trademark hugs, the woman smiled up at her. It took a while to develop, but it was a radiant smile, Wexler says. Yet I knew that chances were, the next time we returned she would be dead. She remembers the frustration. I felt like I was staring at the answer, she says with quiet intensity. I knew that locked in that woman's body was the answer to how Huntington's disease works.

Nothing could slow her down in the pursuit of that answer. When she wasn't in Venezuela, or touring labs, or hunting for talent, Wexler joined her father to raise money for the foundation. One of her more successful forays came when she spoke at a dinner hosted by Dennis Shea, a prominent Wall Street financier whose former wife has Huntington's and whose children are thus at risk. In that one night $1 million was raised for the Huntington's fight.

It would take more than money to win this battle, however, and the gene hunters were working with several handicaps. Although Huntington's was the first gene mapped to a specific chromosome through markers, the markers didn't lead directly to the gene, as the researchers had hoped. Indeed, researchers looking for other genes--those searching for the cystic fibrosis gene, for example--were able to use the marker techniques to capture their quarry much more quickly. The Huntington's hunters, though, had trouble finding markers on both sides of the gene, which would help them narrow their search. Furthermore, they had no biochemical clues as to what the gene might actually be doing, so they couldn't search for it in any logical, function-based way. Then, in 1990, they realized they had been looking in the wrong place altogether.

Their efforts had been aimed at a portion of chromosome 4 near the very tip, a confusing region of about 150,000 base

pairs that Wexler calls the Twilight Zone of genetics. As they continued to rule out chunks of DNA, the gene, like a tantalizing mirage, always seemed to lie just out of reach. Finally Gillian Bates of the Imperial Cancer Research Fund in London managed to clone the entire tip of the chromosome--a step that would ensure rapid isolation of the gene. But any optimism generated by that development was quickly squelched when the gene hunters realized the gene wasn't there. Instead, Marcy MacDonald, a senior researcher working with Gusella at Mass General, found evidence that the gene lay 2 million nucleotide links down the DNA chain in the opposite direction. The group had already suspected that this region might hold the gene but had avoided it because it contained about 2.2 million nucleotide pairs and might require a decade or more to sequence. The frustration was enormous, yet they had no choice but to take a breath, switch their focus, and press on.

The end came in far less than a decade. In 1992 several laboratories--including the two not in the collaboration--began focusing on a fairly small portion of the target area and isolating a number of genes, though they had no way of knowing which was the Huntington's culprit. In January of this year MacDonald began sequencing one very large gene that had caught her attention. Close to the spot at which its protein-building instructions begin, MacDonald found a trinucleotide repeat, a kind of broken-record repetition of the three nucleotide bases--cytosine, adenine, guanine--that make up the genetic instruction for the amino acid glutamine. When she and Gusella compared genes from normal and Huntington's chromosomes, it seemed that the number of repeats was always higher in the Huntington's genes. We said, 'This can't be true. It can't be this easy,' MacDonald recalls gleefully.

Drawing on a vast pool of DNA samples from 75 different Huntington's families--including some from the United States, Canada, Mexico, China, Japan, Africa, Germany, Italy, France, and, of course, Venezuela--MacDonald and two co-workers went into a frenzied two-week period of lab work. Over and over, they spread fragments of DNA onto treated plates, then ran an electric charge through them. The fragments, showing up as black bands on a white plate, separated by weight as they moved across the plate in response to the charge. The heaviest fragments, which contained the most repeats, traveled the shortest distance; the lightest fragments, containing the fewest repeats, traveled farthest. The researchers were able to tell the number of repeats in each fragment by determining precisely how far it had moved.

In every instance the genes fell into line. In normal individuals there were between 11 and 34 repeats of the glutamine code; Huntington's patients had 37 to 86 repeats. There was no ambiguity, no overlap. In addition, some of Wexler's most perplexing puzzles began to come clear. The researchers found that the youngest victims carried the most repeats, and that the number of repeats tended to expand as the gene was passed from generation to generation. They also found that sperm cells from a man with Huntington's could range wildly in the number of repeats they carried, though the rest of the man's tissues had but one consistent number. Researchers are now looking into whether, as these men age, they have a higher proportion of sperm with lots of repeats.

After the final experiment, on February 26, the triumphal report was sent to the journal Cell. As promised, it was signed simply the Huntington's Disease Collaborative Research Group. And when the participants' names were listed at the bottom, Nancy Wexler's was among them.

The press conferences and parties followed quickly. A month after the paper was published, everyone flew to Dennis Shea's estate in the Florida Keys for a communal sigh of relief. They had been going there once a year since 1987, spending their days working on the beach, and their nights at a bar called Woody's, listening to the hard-driving rock and roll of the house band, Big Dick and the Extenders. This time was no exception. When Big Dick saw the scientists at the bar, he stopped in midsong. The 6- foot-6 singer called to David Housman, who was wearing a T-shirt printed with the likeness of a tuxedo, Hey, Doc, why don't you come up here and tell the folks what you did! Self-consciously but with pride, Housman jumped onto the stage and said, We're molecular biologists, and we've just found the gene for Huntington's disease. Nancy Wexler sat in the back and grinned.

Within days, Wexler was bouncing from Los Angeles to New York to Texas, planning more workshops--first the Philadelphia conference, then a conference on the riddle of the trinucleotide repeats. It is the repeating sequence that now consumes her. How does the stuttery repeat change the normal gene into a killer? After the gene was pinned down, the researchers quickly found that it was expressed in every tissue--yet it seems to devastate only a few cells found deep in the brain. Why? There are some signs that mitochondria, the energy factories in cells, might be harmed by the altered gene product, but how?

The answers to these questions may come from yet another collaborative group Wexler began to put together a few years ago. This one will share the precious supply of several hundred brains and other pathological specimens from Huntington's victims around the world-- including the 21-year-old woman from Venezuela, who died just months after Wexler last saw her. The hope is that this tissue might hold new clues to the workings of the disease, and that it might

divulge its secrets to researchers willing to work together.

Wexler returned to Venezuela late last winter, just before the Cell paper was published. She went mainly to gather new data--the work is never truly done--but also to spread the good news. She walked through a maze of alleys in a shantytown near Maracaibo and saw old friends from her years of research, many of whom were showing signs of chorea. As she waved at them and smiled, she says, she couldn't help but visualize on their faces the broken-record repeat from the plates back in the laboratories, like the shadows cast by a venetian blind. Everywhere she looked, the trinucleotide stutter looked back, dizzying in its persistent mystery.

I had spent so many years being so curious about what it was, studying all these people whose bodies contained the mystery, Wexler says. And suddenly it was superimposed on them, almost like a silk screen. It was an image without words, saying, 'Here's the answer. And here's another question.'

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THE DNA AGE

Taking a Peek at the Experts' Genetic Secrets

By AMY HARMON

BOSTON — Is Esther Dyson, the technology venture capitalist who is training to be an astronaut, genetically predisposed to a major heart attack?

Does Steven Pinker, the prominent psychologist and author, have a gene variant that raises his risk of Alzheimer's, which his grandmother suffered from, to greater than 50 percent?

Did Misha Angrist, an assistant professor at Duke University, inherit a high risk of breast cancer, which he may have passed on to his young daughters?

On Monday, they may learn the answers to these and other questions — and, if all goes according to plan, so will everyone else who cares to visit a public Web site, www.personalgenomes.org. The three are among the first 10 volunteers in the Personal Genome Project, a study at Harvard University Medical School aimed at challenging the conventional wisdom that the secrets of our genes are best kept to ourselves.

The goal of the project, which hopes to expand to 100,000 participants, is to speed medical research by dispensing with the elaborate precautions traditionally taken to protect the privacy of human subjects. The more genetic information can be made open and publicly available, nearly everyone agrees, the faster research will progress.

In exchange for the decoding of their DNA, participants agree to make it available to all — along with photographs, their disease histories, allergies, medications, ethnic backgrounds and a trove of other traits, called phenotypes, from food preferences to television viewing habits.

Including phenotypes, which most other public genetic databases have avoided in deference to privacy concerns, should allow researchers to more easily discover how genes and traits are linked. Because the "PGP 10," as they call themselves, agreed to forfeit their privacy, any researcher will have a chance to mine the data, rather than just a small group with clearance.

The project is as much a social experiment as a scientific one. "We don't yet know the consequences of having one's genome out in the open," said George M. Church, a human geneticist at Harvard who is the project's leader and one of its subjects. "But it's worth exploring."

A new federal law prohibits health insurers and employers from discriminating against individuals on the basis of their genetic profile. But any one of the PGP 10 could be denied life insurance, long-term care insurance or disability insurance, with no legal penalty. And no law can bar colleagues from raising an

annoyed eyebrow at a PGP participant who, say, indulges in a brownie after disclosing on the Internet that she is genetically predisposed to diabetes.

Then there is the matter of potential recrimination — from siblings, parents and children who share half of the participants' genes and did not necessarily agree to display them in public. Prospective participants are advised to consult with first-degree relatives, but except for identical twins, their consent is not required. Some volunteers are worried about their hurting their teenagers' dating prospects.

"A potential boyfriend could look at my genome and say, 'I don't know if this relationship is meant to be,' " said John Halamka, a participant and the chief information officer of Harvard Medical School, who has a 15-year-old daughter. (His daughter, he said, told him that if a suitor did that, "I wouldn't want them as a boyfriend anyway.")

Because of the known and unknown risks, Dr. Church required the first 10 participants to demonstrate the equivalent of a master's degree in genetics. Most are either investors or executives in the biomedical industry, or else teach or write about it, so they may have a financial interest in encouraging people to part with their genetic privacy.

The project has drawn criticism from scientists and bioethicists who caution that even its highly educated volunteers cannot understand the practical and psychological risks of disclosing information long regarded as quintessentially private.

"I'm concerned that this could make it seem easy and cool to put your information out there when there is still a lot of stigma associated with certain genetic traits," said Kathy Hudson, director of the Genetics and Public Policy Center at Johns Hopkins University. "There will be new uses of this data that people can't anticipate — and they can't do anything to get it back."

For now, the PGP, which is privately funded, is sequencing only the fraction of participants' genomes thought to have the most influence over disease, behavior and physical traits. But the question of how much value to place on genetic privacy has taken on more urgency as the technology for sequencing an entire human genome accelerated and the price has plummeted to as low as $5,000, so that it may soon be possible for everyone to possess their own genetic readout.

Sequencing a human genome — the six billion letters of genetic code containing the complete inventory of the traits we inherited from our parents — cost over $1 million just two years ago.

The two scientists whose full genomes were sequenced in the name of research both made them public. But they differ on whether the practice should be widely recommended.

"I put mine out there, but I'm 80," said James D. Watson, the chancellor emeritus of Cold Spring Harbor Laboratory and co-discoverer of the structure of DNA. "Randomly putting up young people's genomes could cause individual harm, simply because there will be so many mistakes. We don't know enough yet to interpret them."

J. Craig Venter, a pioneer in human genome sequencing, said his nonprofit institute planned to sequence several dozen human genomes by the end of next year and to deposit the information in the public domain

along with phenotype information in a model similar to that of the PGP. He said he had already heard from thousands of volunteers.

"If they want privacy we tell them to go somewhere else," Dr. Venter said. "To truly understand humans we need a huge data set of 10,000 complete genomes, and the data needs to be open to everyone for interpretation."

Besides, promises of privacy may be impossible to keep, given the extraordinary identifying properties of DNA. Over the last three years, more than a half-million people who participated in over 100 publicly financed genetic studies on traits like schizophrenia and drug addiction were promised that their anonymity would be protected. But last month, after a paper in a scientific journal described how an individual's profile could be identified even when it was aggregated with hundreds of others, the National Institutes of Health abruptly restricted access to the data.

There are some signs that the reflex to protect genetic privacy may be shifting. On the Web site of 23 and Me, a company that markets a $400 minisnapshot of traits from risk of heart disease to ear wax type, some customers use pseudonyms to discuss their results, while others include links with their contact information.

And Sergey Brin, the co-founder of Google, recently revealed on his blog that he learned he has a considerably higher than average risk of developing Parkinson's disease, which was diagnosed in his mother several years ago. (Mr. Brin is the husband of Anne Wojcicki, a co-founder of 23 and Me.)

"There are costs to keeping things secret," Mr. Brin said in an interview. "There's a much better chance that you will learn something useful if you are not trying to hide it."

Still, it may depend on what "it" is.

As the PGP 10 gathered Sunday at Harvard Medical School in Boston to receive the first batch of their genetic data, many said they were motivated by a desire to demystify genetics, which is often wrongly viewed as determining a person's fate.

As the hour approached when they would be asked to reveal their data to the world, Dr. Pinker said he was still considering whether he wanted to learn of his Alzheimer's risk, or if he would ask the researchers to withhold the data from himself and the public. Everyone, Dr. Church said, is given a chance to change their mind about going public up until the last minute, "but we try very hard in our screening process to choose the people who understand that it is better to have it all out there."

Only about 1,300 of the 20,000 human genes have been so far linked to a particular trait, PGP researchers said.

Thus, even if Dr. Pinker chooses to remove from public view the chunk of DNA currently associated with Alzheimer's risk, he is not necessarily protecting himself from future associations scientists may make about genetic data that may now seem innocuous enough to put on the Web.

Dr. Halamka, a PGP volunteer who found out Sunday afternoon that he has a gene variant that has been

associated with childhood blindness, said he had no qualms about putting that, and all of his other information, online. Since he is not blind, and neither is his 15-year-old daughter, the project's researchers told him it seemed likely that something in his genetic makeup was compensating for the defect.

Still, he asked whether it was associated with multiple sclerosis, which his father has. "My daughter," Dr. Halamka said, "will be asking questions."

What happens to the PGP, Dr. Church said, may serve as a litmus test for the fears of sharing genetic data, in an era when everyone's inborn imperfections are becoming more identifiable. If this group is tracked "like major league baseball players, everyone will want to be like them," he said. "If it runs into social hassles and financial hassles, then no one will."

The volunteers will be given more information as the data is analyzed, and they may be asked to answer questions that might help researchers. But the only requirement is that they notify the project if they suffer any adverse effects from their participation.

Dr. Church said that information, too, will be made public.

personal genetics education project

curriculum about the social and ethical issues in personal genetics

Contents:

All materials available online at www.pged.org
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personal genetics education project

Social, Legal and Ethical Issues in Personal Genetics

Lesson One: Social, Legal, and Ethical Issues in Personal Genetics:

Genome sequencing and ethical issues: Who, what, why, and when?

Introduction:

Ask students to read " Life with a Lethal Gene" in advance of the lesson.

High school and college age students are likely to become independent health care consumers at about the time that personal genome sequencing becomes an affordable and accessible option. This lesson will introduces them to the recent advances in genetics, genetic testing, and personal genome sequencing, and present some of the decisions and ethical challenges an individual may face regarding the use of this technology. It also introduces some of the likely benefits of personal genomes, such as gaining the ability to act on one's genetics risks, tailoring medicines and interventions, and becoming more active and engaged healthcare consumers. Via class discussion and the readings, students will be able to generate ideas about the possible risks, in addition to the potential benefits.

Guiding Questions:

How does one decide to get sequenced? What are the benefits and risks?

Learning Objectives:

After completing this lesson, students will be able to:

Define a personal genome sequence

Outline the likely risks and benefits of personal sequencing

The Lesson:

The lesson presented below is intended for a lecture/discussion format, with a short small group discussion activity.

1. Begin with a question and answer about the NYT article " Life with a Lethal Gene". Ask students what they would have done Katharine's shoes – would they have gotten tested? Would they tell their mother, against her wishes?

Note some of the more powerful aspects of the story:

- The difficulty of deciding to get tested for a single disease
- The emotional impact
- Her actions (planning ahead, activism) as a result of a positive test
- How it changes her outlook about marriage, kids, career, and her identity
- Getting tested impacts not just her, but her family and friends

2. The NYT article can segue into talking about the types of genetic testing available, and also can frame this discuss in terms of its timeliness and relevance to their lives. Example:

Human Genome Project took 12 years and cost 3 billion dollars, and it is widely predicted that the cost of a doing a "Human Genome Project" on individuals will be reduced to $1,000 within the next five years and take only a few days to complete.

Imagine the "Life with a Lethal Gene" story with the added dimension of having learned about a number of other genetic risks she or her relatives might have…

3. Ask students to name some genetics tests they have heard of.

Examples might include the "predictive" type, which are done before symptoms are present, such those for increased risk of breast or colon cancer.

Prenatal tests, performed in the early weeks of pregnancy, often include cystic fibrosis and Down's syndrome.

Explain that some of the more common types of genetic tests examine the pattern of nucleotides (A, G, T, C) in a specific gene or set of genes, looking for differences from the typical sequence that would cause the gene to

malfunction. Resources about the technology and process behind genetic testing can be found at Human Genome Project Information.

Genetic tests can be obtained from one's physician, online retailers such as DNA Direct or Consumer Genetics, and recently in some drugstores. The costs of genetic tests are sometimes covered by health insurance, but fear persists that the results will be used to discriminate in health insurance and employment (See "Insurance fears leads many to shun DNA tests" from the NYT for examples that could be used in the discussion).

Part of the appeal of the " direct to consumer" approach of selling tests via the web or at pharmacies is the belief that this approach will give consumers more control over their privacy and genetic data. Others argue that more oversight and regulation is needed to ensure the quality and accuracy of genetic tests. The Genetics and Public Policy Center and the Personalized Medicine Coalition have a number of articles on this subject, available free on their websites.

4. New to the marketplace in 2007 and 2008, private companies are offering a form of whole-genome analysis. The services differs from a traditional "genetic test" as they that do not directly sequence specific genes but determine the presence or absence of small DNA changes, called SNPs (single nucleotide polymorphisms). SNPs are statistically indicative of the presence or absence of a nearby piece of DNA associated with a trait or disease.

These so called " genome scans" are generally believed to be an early pre-cursor to full personal genome sequences, and signal the start of "consumer genomics". It is expected that this particular technology will be obsolete in the new few years, as low cost full sequencing becomes technically possible.

SNP testing does not examine a specific gene associated with a trait or disease, but looks to nearby areas in the genome where a difference from the average is strongly associated with certain traits. Examples of companies offering genome scans include 23andMe, Navigenics, and DeCodeMe. 23andme has a very accessible series about the so-called " SNP chip" analysis that is appropriate for high school and college students.

5. What, why, and how it works:

Explain that a personal sequence is essentially a genetic analysis. performed on most or all the genes in an individual's body, all at the same time. A useful way to frame this might be as a " Human Genome Project performed on YOU" or as taking every known and future genetic test

simultaneously. Many of the genes sequenced are not well understood, and it is unclear what roles they play in the development or function of the body. The ability to sequence a genome will dramatically outpace the ability to understand or interpret a genome.

Genome sequencing is thought to have enormous potential to link genetic mutations and changes to diseases, physical characteristics, and even some complex human traits and behaviors. If thousands of people were to be sequenced, and then share some of that information with the research and medical community, many believe it will be possible to find connections that are significant, translating into better and more personalized health care. For more on the concept of the link between genotype and phenotype, please see pgEd's summary.

6. Ask students for a show of hands as to who would consider getting their genome sequenced. Why or why not?

From here, a discussion of the risks and benefits will likely ensue.
The risks and benefits are outlined in the slides and also at pgEd.org

Often students respond to this question as if it is still a theoretical one. It is not. Knome, based in Cambridge MA, will sequence a human genome from $350,000 (**now 100,000K as of early 2009) – and the price is expected to fall over the next few years.

The Personal Genome Project (PGP) is a unique research study at Harvard Medical School that is currently enrolling volunteers. They are currently sequencing the first ten volunteers, as well as collecting detailed medical histories, health records, and 3-D facial photographs. The first 10 volunteers have agreed to public disclosures of their genotypes and phenotypes. Future participants (they hope to have 100,000 volunteers) will have the option to make all the their data publicly available. One of the volunteers, renowned psychologist Steven Pinker recently published " My Genome My Self" in the New York Times Magazine.

The first ten volunteers have been carefully chosen in part because they are taking on unknown risks by making their information available to all. Many believe such openness about one's genetic profile and medical history will:

A. Lead to individuals playing a more active role in their own health
B. Encourage people to become active and engaged stakeholders in scientific research through their participation.
C. Aid in the discovery of cures and treatments that could benefit many people.

D. Encourage discussion as to whether or not genetic information requires special privacy considerations.

Future PGP volunteers may be interested in public information sharing, but it thought to be likely that the majority of volunteers in this project and others would prefer a more measured level of disclosure, if at all.

Many organizations and scholars have stated concerns about personal genomics and the approaches some of these projects have taken. These include:

A. Promises of privacy and anonymity are misleading – people should be aware that it may not be possible to have their data truly protected.

B. The service and interpretation of direct to consumer genome scans are ahead of true clinical usefulness, and may be unfairly taking advantage of consumer's enthusiasm for this new product.

C. The emphasis on the genetic basis of disease and complex social traits is at the expense of a more nuanced understanding of the multifactorial nature of human health and experiences.

D. Although the technical capability is just around the corner – the legal, social, moral, political, religious, and medical (to name just a few) infrastructure needed to use and regulate genomic information are not yet in place.

See the Council for Responsible Genetics and the Biopolitical Times for news and opinions in this area.

Classroom activity:

Begin by describing a vision of a possible future, where humans have learned to control their genetic profile at very high level. For example, the average human life span might be 100 years, cancer may be unheard of, and all medication may be tailored specifically to an individual's genetic profile. Genetic technology may also allow people to pass only their " best" traits to their children, traits such as good vision, a strong immune system, and freedom from allergies.

Break students into small discussion groups to share what they think some other desirable attributes might be. (Common answers are intelligence, athletic ability, good looks, and nice personality).

Students can then debate the feasibility and wisdom of such a scenario. Is it even possible? Appealing?

Other suggestion: Use the accompanying " sequencing scenarios", by dividing the group into sections and giving each a scenario to debate. A group leader should be chosen to report to the class the content of the discussion and the areas of agreement and disagreement.

Other classroom tools:

The PowerPoint slides located at www.pged.org (Lesson 1) can be used to supplement some of the discussion. Feel free to modify and reorganize as needed. Brief notes accompany some of the slides, please leave the " personal genetics education project" label in the lower right hand corner.

Discussion questions:

A. What are the major considerations in the decision to get sequenced? How do you decide if the benefits outweigh the risks?

B. How might the information you learn from sequencing come to have different kinds of meaning or import over the years?

C. If you don't want to be sequenced now, what might be some of the times in my your future that you would want to (Answers you may hear are marriage, planning a family, getting older)? What are best arguments for waiting, and for acting now?

Assessment:

Students can complete a short paragraph or essay explaining what personal genome sequencing is, and why or why not they would like having their genome sequenced.

They should outline and support what they see as the single biggest benefit, and also the greatest risk of sequencing.

Essay can also include 3 things they might learn from their personal sequencing, and 3 things that likely will not be revealed by sequencing.

If time permits, students can discuss their ideas in a debate format.

Additional resources

More on genetic tests and their common uses can be found at University of Washington's excellent site, www.genetests.org.

For more on biological determinism, and writings on social vs. biological traits, and the limits of genetic inheritance, please see:

Human Genome Project: Behavioral Genetics unit

The Mismeasure of Man. SJ Gould. New York, W.W. Norton, 1996

A daily round-up of genomic research developments and interesting posts from some of the well-respected science blogs can be found at Genome Technology Online

MIT's Technology Review has an excellent "Biotech" section with in-depth stories and news items that are challenging but appropriate for high school and college students.

Scenario A :

You have just received a copy of your personal genome sequence. Included in the results is this piece of information:

You have tested positive for Huntington's Disease – a degenerative neurological condition that can strike as early as in one's 30s or 40s. If you have the mutation, it is 100% certain you will get this disease. You relieved to have a answer, but are also very upset and distressed to have these fears confirmed. You are seeking support from friends and family.

Your mother knows that you were planning to get your genome sequenced. Your maternal grandfather had Huntington's, and it was a source of confusion and secrecy in your family. She has decided she would rather not know. She feels that since there is no treatment or cure for Huntington's, what's the point of learning if she carries the mutation? She is also concerned about discrimination.

Because Huntington's is an autosomal dominant condition, your positive status for the gene mutation implicates your mother: It means she will definitely have the disease. You are 21 years old, she is 47.

<u>Discussion points:</u>

How do you handle this piece of information with your mother?

If you tell her that you have the mutation for Huntington's, you are also telling her that she has it as well. You know she doesn't want to know – but you need her emotional and financial support. Do you tell her? What are the ethical issues at play?

How might this information change your life, and the lives of the other members of your family? So you share this information with friends and the person you are dating?

Scenario B:

You have just received a copy of your personal genome sequence. Included in the results is the following piece of information:

You carry a genetic mutation that places you at a much higher risk than average for colon cancer. This is a surprise, as nobody in your family on either side has ever had colon cancer. You read that colon cancer runs in families, so you suggest your two siblings to also get sequenced as they may be at risk too.

The results of the sequencing prove to be very confusing. It appears that you and your siblings may not share the same mother and father. Your DNA does not match your siblings as might have been expected, and there are too many differences for you all to be full, biological siblings.

<u>Discussion points:</u>

What could be the explanation?

Learning about your genome has proven to be more than you bargained for. How might this sudden question of your genetic lineage change things in your family?

Early and frequent screening for colon cancer is an effective prevention tool. Knowing you carry the mutation is a potentially a life-saving piece of information. Despite the unexpected and possibly distressing information, was it worth it to learn about the colon cancer risk?

How might you seek answers about the fact that you and your siblings appear to not be full biological siblings?

What could be some of the other unexpected outcomes of personal genome sequencing?

Scenario C:

You have just received a copy of your personal genome sequence. Your personal history was a significant part of why you decided to learn about your genome:

You have always known your mother used an anonymous sperm donor when you were conceived. Shortly after you were born, your mother married and had two more children. You are a close knit family of five.

Since you don't know anything about the medical history of your biological father or his family, you decide to get your genome sequenced, as the resulting information could inform you of your possible risks for certain diseases and conditions.

You receive your sequence and learn you have a lower than average risk for certain types of heart disease, have dry (as opposed to wet) earwax, can't taste the bitterness of certain foods like Brussels sprouts, and have a much higher than average risk for diabetes and that you are a carrier for Cystic Fibrosis.

Sequencing has been around a few years at this point, so the cost of sequencing your genome was covered as one of your health insurance benefits.

<u>Discussion points:</u>

Your curiosity is piqued and you decide you want to learn more about your biological father and his personal and medical history. How might you go about finding him? Do you want to meet your half-siblings if it turns out you have them?

Consider how this might impact you and your family – put yourself in the shoes of your mother, your father who has raised you, and your siblings.

Now, consider the perspective of the sperm donor – at the time he was a 19 year old college student and frequently donated at the local clinic. He hasn't really thought about this time in his life for decades. You have learned he is 51, married with 2 children, and lives approximately 100 miles from you. Is it fair to seek him out?

Scenario D:

You have just received a copy of your personal genome sequence. Included in the results is the following piece of information:

You are a "fast metabolizer" for a prescription drug that you take to prevent a potentially fatal stroke. This piece of information will help you choose the right dosage, making it much more effective. You also learned you are at above average risk for a heritable form of eye disease that can lead to blindness – macular degeneration. This is particularly distressing as you are paying for college via ROTC and plan to enter the Air Force as a pilot.

After receiving your results, you notice a "personal genomes" group on Facebook. People have openly identified themselves as "fast-metabolizers" as well as "macular degenerators" and are donating their DNA to various research projects at a number of hospitals and labs. There are groups such as
"BRCA 1 and 2 genetic mutation group" (last accessed 3/17/09, 455 members) and many others related to supporting people with diseases that have a known genetic component. Some people share information and treatment plans, and review doctors and hospitals – a sort of www.ratemyprofessor.com for the medical world.

Discussion points:

Would you join a Facebook group for "fast metabolizers" or "macular degenerators"? Do you have concerns about the impact of sharing your sequence data for each of these conditions? Why or why not?

How do you balance the importance of social networking, research and activism with the need for personal privacy?

A few people have posted their full genetic sequence online, along with their names, medical history, health records, and photographs (www.personalgenomes.org). They hope to contribute to basic research and feel that making their information freely available will enable the scientific community to make new connections between genes and traits. Would you consider this option for yourself?

Scenario E:

You have just received a copy of your personal genome sequence. Included in the results is following piece of information:

You have a mutation on your APOE gene that is strongly associated with an elevated risk of Alzheimer's Disease. It is a progressive, degenerative disorder of the nervous system, and there are no clinically accepted treatments or cures. Carrying the APOE mutation does not 100% guarantee you will actually develop this typically late-onset (50's or older) disease.

You watched your grandmother suffer for many years with Alzheimer's, and also a great aunt, and your family struggled emotionally and financially to make sure they had the right medical attention and a safe and comfortable living situation.

Discussion points:

Will knowing about your risk for Alzheimer's change how you think about your future? How might it impact your plans for career, family, or travel?

You have decided to get sequenced while in college – you are 19 years old. Are you glad to have learned this information so young? How might it have been different if you were 59 when you learned about your genome sequence, as opposed to being a young adult?

Knowing this information now, do you have an obligation to share this information with your relatives? What about someone you are dating? When is the right time to mention something like this?

personal genetics education project bibliography

Articles highlighting some of the social, legal and ethical questions related to personal genomics.

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Personalized Medicine in the Era of Genomics. W Burke, B Psaty. JAMA October 10, 2007 298 V. 14

Research Ethics Recommendations for Whole-Genome Research: Consensus Statement. Caufield, et al. Plos Biol 2008 vol. 6 (3) pp. e73

Good for Cops, Bad for NIH. Couzin ScienceNOW 29 August 2008: 1

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Research ethics and the challenge of whole-genome sequencing. McGuire et al. Nat Rev Gen 2008 vol. 9 (2) pp. 152-156

Interpreting the Genome. Emily Singer. Technology Review. Jan/Feb 2009. http://www.technologyreview.com/biomedicine/21805/

Impact of Genetic Testing for Huntington Disease on the Family System. S Sobel, D Cowan. American Journal of Medical Genetics 90:49–59 (2000)

Lost in Translation: Challenges in the Expanding Field of Adult Genetics" MR Taylor et al. Amer J Adult Gen 2006 Nov 15;142C(4):294-303.

Genetic Testing For Alzheimer's Disease And Its Impact On Insurance Purchasing Behavior. Zick et al. Health Affairs Mar/Apr2005, Vol. 24 Issue 2, p483-490, 8p

Medical School Application Tips

Pre-requisite Courses

The minimum science course work necessary for preparing for medical school and the MCAT includes two semesters each of general biology, general chemistry, organic chemistry, and physics. These courses should be completed no later than the end of your junior year so that you will be prepared for the MCAT exam. The MCAT is now offered 22 times each year which allows students more flexibility as they prepare to apply to medical school.

Medical school admission's committees focus attention on overall GPA, Science GPA, MCAT scores and exposure to the clinical environment achieved through volunteer work, internships, shadowing or employment.

Each medical school determines its specific requirements for admissions. These requirements can be found in the Medical School Admissions Requirements (MSAR), an official guide published by the Association of American Medical Colleges. Additional recommended electives for medical school are Biochemistry, Molecular Biology, Microbiology, courses that expose students to Multi-cultural Diversity, Biomedical Ethics and Medical Terminology.

Medical College Admission Test (MCAT) & The Application Process

The MCAT is required by allopathic, osteopathic and podiatric medical schools. This standardized test is directed at an introductory level of knowledge for science courses and comprehensive skills in reading and writing. To do well on these tests students must have analytic skills and problem solving ability. Adequate preparation for these tests is essential. A student should aim to take the test only once. Although some professional schools will consider only the highest score or the most recent score, other schools will average all test scores. To adequately prepare for these tests, students must not only be knowledgeable about content, but must also be familiar with the test format and develop the resilience and stamina needed to concentrate for these marathon length exams. Most standardized tests have a deadline for applying that is about 6 weeks prior to the scheduled test date. Students must allow another 6 to 8 week for the test scores to be forwarded to the professional schools when determining when to take the test and apply to professional school.

The MCAT is offered only in a computerized format and is offered 22 times during the year. Applicants must register for this exam on line at their local Prometrics Testing Center. The verbal reasoning, biological reasoning and physical science sections are graded out of 15 points and the writing section is graded on a letter scale from J to T. The highest total score one can achieve on the MCAT is a 45; the national average is currently at 30.

Application Service (AMCAS)

Centralized application services are used to apply to most allopathic, osteopathic, and podiatric programs. Students who apply to any of the professional programs submit just one application through the service. The centralized service verifies the information provided on the application and submits the application to the

professional schools designated by the applicant. The verification process may take up to 6 weeks and this time should be factored in when trying to meet application deadlines. Most professional schools require additional information – called a secondary or supplemental application – from the applicant once they receive the student's application from the centralized service.

Design a Plan!

FIRST YEAR

Fall Semester:
• Explore various majors and declare as early as possible. Because you will need to complete the 32 credit hours of sciences before you can take the MCAT it will be necessary to get your science courses underway as quickly as possible.
• Make an appointment with your advisor to discuss professional goals and determine an academic game plan to ensure you will have taken all courses needed to prepare for standardized admissions tests in your field of interest by the time you take the exam (usually at the end of your junior year).
• As soon as you get settled into your classes, contact the Pre-professional Health Advisor, to make an advising appointment to discuss professional goals and discuss what the professional will want you to have completed by the time you're ready to apply.

Spring Semester:
• Meet with your academic advisor to discuss your progress.
• Search for shadowing and volunteer opportunities in your field of interest.
• Explore areas of community outreach to establish your humanitarian interests.

First Summer:
• Shadowing, working, or volunteering to gain insight into your career choice.

SECOND YEAR

- Fall Semester: Meet with your advisor to discuss your Spring schedule.
- Learn about the MCAT
- Continue volunteer work in your field to whatever degree manageable during the academic year (Grades should always take priority)

Spring Semester:
- Discuss your academic progress with your advisor and adjust your academic plan, as needed. Meet with Pre-Health Professions Advisor to make review your progress and the next phase of preparation
- Explore career options and alternatives, if your GPA is not adequate or your career interests have changed.
- Sign up to take a free practice MCAT through Kaplan, if available

Second Summer:
- Work/volunteer to gain insight of your career choice.
- Explore available internship and research opportunities
- Get involved in the community

THIRD YEAR

Fall Semester:

- Meet with your advisor to discuss spring schedule.
- Get organized, order review booklets and practice tests to prepare for standardized exams.
- Explore various professional schools and determine to which ones you will apply.

Spring Semester:

- Discuss your academic progress with your advisor. Determine if your GPA is competitive and whether or not this is the year you should apply to professional school.
- Establish a file with the College's Health Professions Committee, if available at your institution.
- Start worksheets for on-line centralized application services and/or request applications from schools that do not participate in the centralized application process. (Online AMCAS application is available early in May of each year)
- Collect materials needed to fill in application and start working on application essay.
- Apply and study for standardized admissions tests. Check the deadlines and do not miss them.
- Practice, practice, practice taking the standardized admissions test.
- Take the standardized admissions test and request that scores be released to your college or university, schools to which you are applying and the application service (if appropriate).
- Request letters of evaluation from faculty and health professionals who know you well.

Third Summer:

- Continue to work/volunteer in your field of interest and community outreach projects.
- Complete your applications and submit early. Early decision program applications must be submitted to the schools by August 1st.
- Retake standardized admissions tests, if necessary.

FOURTH YEAR

Fall Semester:

- Meet with your advisor and prepare for graduation.
- Respond promptly to requests for secondary applications from each professional school.
- Prepare for interviews.
- Interview and wait.
- Search for sources of financial aid.

Spring Semester:

- Send updated transcripts directly to the professional schools to which you have applied.
- Wait for decisions. Be sure to let your institution know the final outcome.
- Discuss alternatives with your advisor. Meet with the chair of the Health Professions Committee to develop a strategy for reapplying, if necessary.

Research Fellowships for Summer Research, Graduate School, and Medical School

One of the most important skills you can learn as a developing scientist is how to convince other people that your research questions are really interesting. So interesting in fact, that they will give you money to pursue the answers to those questions. If you are lucky you will spend the rest of your life trying to answer them. I am not sure you ever perfect the art of grant writing, but practice makes almost perfect. And there is no time like the present to start working on it. Over time, you will develop a track record of applying for and, hopefully, attaining funding for your work. One more thing, this looks great on your resume as you begin looking for that future position as an independent researcher. So let's look at the options...

Undergraduate Science and Engineering Scholarships

Goldwater Science Scholars (Rising Juniors and Seniors): http://www.act.org/goldwater/

- Provides up to $7500 dollars towards tuition, room and board, books etc. Renewable for a second year if a junior. ~300 awarded each year.

NIH Undergraduate Scholarship Program: http://ugsp.info.nih.gov/

- The program offers competitive scholarships to students from disadvantaged backgrounds who are committed to careers in biomedical, behavioral, and social science health-related research. The program offers scholarship support ($20,000), paid summer research training at NIH, and employment/training after graduation.

Summer Undergraduate Research Experiences

These are full-time summer research programs that typically provide a stipend (~$3500), travel funds and housing at the research site. Some institutions provide summer fellowships for their own students to continue working on their research during the summer. Other schools run nationally competitive summer research programs. Several undergraduate institutions keep up to date lists of these programs. Two good sites are:

http://www.psych.westminster.edu/psybio/internops.htm
http://www.swarthmore.edu/Admin/health_sciences/summer_opportunities.html

National Science Foundation Research Experiences for Undergraduates

- You can search for experiences in particular scientific fields or for programs at particular institutions at the NSF website: http://www.nsf.gov/crssprgm/reu/reu_search.cfm

National Institutes of Health Summer Internship Program in Biomedical Research (SIPS)

- This is a 10 week summer program at the NIH: http://www.training.nih.gov/student/sip/index.asp

Application deadlines are generally between January and March for the following summer.

Still want to spend some time doing research before committing to graduate school? The NIH has a post-baccalaureate year-long research program that you might be interested in:

NIH Post baccalaureate Intramural Research Training Award (IRTA):
http://www.training.nih.gov/student/Pre-IRTA/previewpostbac.asp?AppType=Postbac

So you made it to graduate school…now it is time to apply for pre-doctoral fellowships:

NSF Graduate Research Fellowship Program

- http://www.nsf.gov/funding/pgm_summ.jsp?pims_id=6201
- The Graduate Research Fellowship provides three years of support for graduate study leading to research-based masters or doctoral degrees and is intended for students who are at the early stages of their graduate study. Apply during your senior year or in the first year of graduate study. ~1000 are awarded each year.

NIH Ruth L. Kirschstein National Research Service Award (an F31 grant)

- http://grants.nih.gov/training/nrsa.htm#F31
- This is the format for grant proposals that you will write as a post-doctoral fellow (F32) and principal investigator (R15 and R01). The F31 is the predoctoral award. There are separate awards for minority students and for students with disabilities. These grants are usually written in conjunction with your advisor once you have begun your thesis research. You request a certain period of funding (~2-3 years) based on the length of the project. There are also F30 awards for predoctoral students studying for an MD/PhD

Hertz Foundation Fellowships

- http://www.hertzfoundation.org/dx/Fellowships/
- The fellowship is a five year award to those pursuing a Ph.D. in "applied sciences" and consists of a $28,000/9-month personal stipend, full tuition equivalent, and is renewable for up to 5 years.

Finally, if you work on a specific human disease or disorder there are usually private foundations that support the research. Often they will offer a limited number of pre- and post-doctoral fellowships.

But wait, I want to be a doctor or a dentist and I am still interested in research…

Howard Hughes Medical Institute (HHMI) Research Training Fellowships for Medical Students

- http://www.hhmi.org/grants/individuals/medfellows.html
- The Medical Fellows Program supports a year of full-time biomedical research training for medical and dental students. Applicants must be enrolled in a U.S. medical school or dental school and the fellowship research may be conducted at any academic or nonprofit institution in the United States, except the National Institutes of Health. For the 2006 competition: An annual stipend of $25,000, an annual fellow's allowance of $5,500, and an annual research allowance of $5,500.

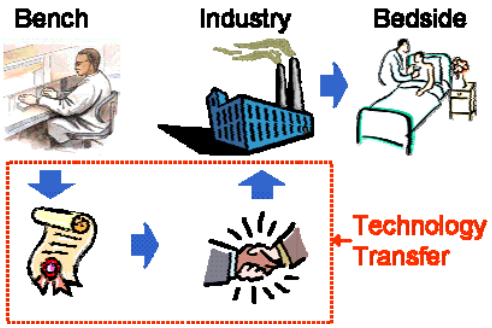
NIH Medical and Dental Student Research Programs:

- http://www.training.nih.gov/student/index.asp
- Look in the Medical/Dental Section of this website to learn about the different opportunities available at the NIH

Careers in Technology Transfer

What is technology transfer?

Technology transfer is the process of transferring scientific findings from the laboratory to entities that can develop them into products, which can ultimately benefit the public.



Technology transfer managers liaise with scientists who may have a commercially valuable idea, assess its commercial potential, manage the patenting process (if it is something which can be patented) and then try to ensure that the idea is exploited successfully, usually through licensing or sometimes by forming a spin-out company.

Technology management usually involves legal work such as licensing, and forming an extensive network of contacts with businesses seeking to license an invention or support a spin-out company based on the technology.

Links:

General Information about Technology Transfer

Professional organization for technology managers working in *academia*, AUTM: http://www.autm.net/

The professional organization for technology managers working in *industry*, LES: http://www.usa-canada.les.org/

Technology transfer for MUSC is managed by the MUSC Foundation for Research Development: http://frd.musc.edu/

Internship opportunities in Technology Transfer

National Institutes of Health: http://www.ott.nih.gov/about_nih/intern.html

National Cancer Institute: http://ttc.nci.nih.gov/employment/

USCLA http://www.research.ucla.edu/oipa/interns/

Boston University http://www.bu.edu/otd/education/internships.html

Education programs in Technology Transfer

To learn about technology transfer education programs, click on the “Education” tab on this website: http://ttic.nal.usda.gov/

General information on alternative careers

http://sciencecareers.sciencemag.org/

http://chronicle.com/

Book

“Alternative Careers in Science: Leaving the Ivory Tower” by Cynthia Robbins-Roth http://books.google.com/books?id=qe5oyXwW6UQC Chapter 15.

Careers in Medical Writing

Christine Lauay, PhD

Scientific medical writers (those with a degree in medicine, science, pharmacy or related fields) might prepare regulatory documents (clinical trial reports, integrated summaries of efficacy and safety, investigator brochures etc.), physician speeches, posters, sales training manuals, policy documents, journal abstracts and articles, advertising copy for pharmaceuticals, internet content, medical education materials, pharmaceutical marketing and advertising.

Marketing medical writers (those with a degree in journalism or English) might prepare advertising copy, articles, internet content, magazine articles (popular or professional press), marketing materials, newsletters, patient education materials, public relations materials, training manuals, and perform editing and proofreading.

The two main pathways to a career in medical writing:

- Scientists who learn writing, and
- Writers who learn science.

In addition, courses or programs in medical writing are available (e.g., University of the Sciences in Philadelphia Masters in Biomedical Communications, University of Chicago Graham School of General Studies, Medical Writing and Editing). Medical writing associations offer certification programs (eg, American Medical Writers Association: AMWA) for medical writers. Also, taking courses in pharmacology, statistics, and life sciences is useful.

As with any career, there are pros and cons that go with a career in medical writing. Medical writing can be a lucrative career, and writers generally rate themselves as very satisfied with their career. There is great flexibility with full time, part time, remote, and freelance opportunities. Medical writers are often required to interpret data and prepare a document in a short time, must be prepared to have their work heavily edited, and should not expect to be a author when a manuscript is published.

Some qualities important in a medical writer include: ability to gather, analyze, and summarize large amounts of data, ability to express ideas succinctly, a background in science or medicine, ability to work within a team, and ability to communicate with many professionals from many functional disciplines.

<u>Tips for those interested in starting a career as a medical writer</u>

- Join AMWA or Drug Information Association (DIA).
- Read medical journals.
- Starting out can be tough – especially for regulatory writing. Try working first for a contract research organization (CRO) or small medical writing company as a freelance or part-timer, or get into pharmaceuticals via some other avenue (eg, clinical research) to gain exposure to the documents being used. If regulatory writing is not what you are interested in, then try starting in ad work or continuing medical education (CME). Start as an editor.
- Look at company websites. Find a company you're interested in, call up the human resources person and ask for an interview (you're likely to get it, even if no job openings exist at the moment), then go and talk with as many people as time allows. Be prepared with lots of questions when you go (e.g., what kind of projects do the writers do? Publications, regulatory docs? What would someone in an

entry level position do? What kind of training is available?). Also be prepared to answer questions (eg, why are you interested in the company? Why do you want to be a writer?).

- Be enthusiastic.
- Publish your own research and write reviews if completing a thesis.
- Develop relationships with more experienced writers who may give you a start.
- Write! For example, take a technical or science writing course or program (for example, Jackson Laboratory has an undergrad intern program for science writing, see http://education.jax.org/science_writer.html)

Data from the 2007 AMWA Salary Survey

Region of Primary Work

(some areas have higher population of pharma companies)

NY, PA, NJ, DE, Eastern Canada:	30%
Pacific NW, CA, HI:	15%
Southeast US, MD, DC:	13%
Midwest:	13%
MA, CT, RI, VT, NH, ME:	9%

Highest Education Level

Associate's degree or below:	2%
Bachelor's degree:	33%
Master's degree:	35%
Advanced degree:	30%

Field of Highest Degree

Science	(40%)
Liberal Arts	(11%)
Journalism	(5%)
Pharmacy	(5%)
Medicine	(4%)
Communication	(4%)
Public Health	(3%)
Technical Writing	(3%)
Nursing	(2%)
Medical Writing	(1%)
Other	(25%)

Primary Employer

Pharmaceutical company	(25%)
Communication or advertising	(11%)
Biotech company	(9%)
University or medical School	(9%)
Contract research organization	(7%)
Health care organization	(7%)
Journal or publisher	(5%)
Research or education	(4%)
Association or professional soc	(4%)

Medical device company	(4%)
Other	(12%)

Useful Websites:
Science Magazine: http://sciencecareers.sciencemag.org/career_development
(click on "more topics", then "alternative careers")
American Medical Writers Association: http://www.amwa.org/
National Association of Science Writers: http://www.nasw.org/
Drug Information Association: http://www.diahome.org/DIAHome/
US Food and Drug Administration: http://www.fda.gov/
(eg, search for 'guidance' to see outlines of content of regulatory documents)
Salary.com: http://www.salary.com/

Some factors that affect income:
Degree, location, size of company, experience
The following data are from salary.com, in the Chicago area (accessed Feb 2008)
Medical Writer I (BA/Master's, 0-2 yrs experience):

Benefit	Median Amount	% of Total
Base Salary	$57,337	70.3%
Bonuses	$64	0.1%
Social Security	$4,391	5.4%
401k / 403b	$3,559	4.4%
Disability	$918	1.1%
Healthcare	$5,328	6.5%
Pension	$2,411	3.0%
Time Off	$7,506	9.2%
Total	$81,515	100%

Medical Writer II (BA/Masters & 2-5 years experience):

Benefit	Median Amount	% of Total
Base Salary	$67,194	70.7%
Bonuses	$368	0.4%
Social Security	$5,168	5.4%
401k / 403b	$4,189	4.4%
Disability	$1,081	1.1%
Healthcare	$5,328	5.6%
Pension	$2,838	3.0%
Time Off	$8,835	9.3%
Total	$95,001	100

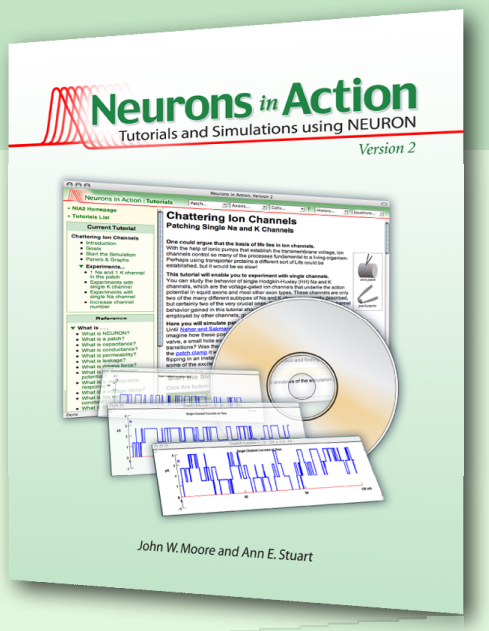
Medical Writer III (BA/Masters & 2-5 years experience):

Benefit	Median Amount	% of Total
Base Salary	$78,460	70.8%
Bonuses	$979	0.9%
Social Security	$6,077	5.5%
401k / 403b	$4,925	4.4%
Disability	$1,271	1.1%
Healthcare	$5,328	4.8%
Pension	$3,336	3.0%
Time Off	$10,388	9.4%
Total	$110,765	100%

Neurons in Action 2

Tutorials and Simulations Using NEURON

John W. Moore and Ann E. Stuart



Neurons in Action 2 is the second version of a unique CD-ROM-based learning tool that combines hyperlinked text with NEURON simulations of laboratory experiments in neurophysiology. Neurons in Action's moving graphs provide insight into nerve function that is simply not possible with conventional, static text and figure presentations. Students discover how changing parameters such as neuronal geometry, ion concentrations, ion channel densities, temperature or degree of myelination affects the generation of action potentials, synaptic potentials, and the spread or propagation of voltages within a neuron. For instructors, minimovies of NEURON simulations are provided for use in lectures.

CONTENTS (* New to this version)

On the web:
neuronsinaction.com

Order from
Sinauer Associates
www.sinauer.com

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Neurons in Action in Action

Educational settings for simulations and tutorials using NEURON

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urn:nbn:de:0009-3-14016

Abstract. *Neurons in Action* (*NIA1*, 2000; *NIA1.5*, 2004; *NIA2*, 2007), a set of tutorials and linked simulations, is designed to acquaint students with neuronal physiology through interactive, virtual laboratory experiments. Here we explore the uses of *NIA* in lecture, both interactive and didactic, as well as in the undergraduate laboratory, in the graduate seminar course, and as an examination tool through homework and problem set assignments. *NIA*, made with the simulator NEURON (http://www.neuron.yale.edu/neuron/), displays voltages, currents, and conductances in a membrane patch or signals moving within the dendrites, soma and/or axon of a neuron. Customized simulations start with the plain lipid bilayer and progress through equilibrium potentials; currents through single Na and K channels; Na and Ca action potentials; voltage clamp of a patch or a whole neuron; voltage spread and propagation in axons, motoneurons and nerve terminals; synaptic excitation and inhibition; and advanced topics such as channel kinetics and coincidence detection. The user asks and answers "what if" questions by specifying neuronal parameters, ion concentrations, and temperature, and the experimental results are then plotted as conductances, currents, and voltage changes. Such exercises provide immediate confirmation or refutation of the student's ideas to guide their learning. The tutorials are hyperlinked to explanatory information and to original research papers. Although the *NIA* tutorials were designed as a sequence to empower a student with a working knowledge of fundamental neuronal principles, we find that faculty are using the individual tutorials in a variety of educational situations, some of which are described here. Here we offer ideas to colleagues using interactive software, whether *NIA* or another tool, for educating students of differing backgrounds in the subject of neurophysiology.

Keywords: Tutorial, simulation, action potential, synaptic potential, ion channel, ion channel kinetics

Citation: Stuart A (2008). *Neurons in Action* in Action - Educational settings for simulations and tutorials using NEURON. Brains, Minds & Media, Vol.3, bmm1401, *in: Lorenz S, Egelhaaf M (eds): Interactive Educational Media for the Neural and Cognitive Sciences,* Brains, Minds & Media, 2008.

Published: May 19th, 2008

Introduction

The subject of neurophysiology can be intimidating to students who may fear electrical circuitry and cannot easily relate concepts such as conductance and capacitance to their general understanding of biology and physiology. Yet, with the subject of neuroscience expanding in so many directions, it seems essential that the neurobiologist have a grasp of the fundamental principles of neuronal function and an appreciation of how the field can be aided by computational tools. To this end, we (John W. Moore and I) authored a set of tutorials with interactive simulations on a CD, *Neurons in Action (NIA)* (Moore and Stuart, 2000; 2007). Here, we detail the various ways in which this software

has been used by us and by others. We offer these ideas to colleagues using interactive software, whether *NIA* or another tool, in hopes of increasing students' understanding of neurophysiology.

NIA exploits the power of its browser to run customized simulations using the simulator NEURON (Hines and Carnevale 1997, 2001; Carnevale and Hines 2006) under the direction of tutorials written in HTML. In 2000 we published *NIA* Version 1 (*NIA*1; Moore and Stuart 2000). We hoped that being able to ask "what if" questions, to change parameters, and then receive instant feedback from the simulations would make neurophysiology more approachable, even engaging for undergraduate and graduate students and fun and instructive even for the seasoned neuroscientist. In response to requests from colleagues, we later added a set of "minimovies" to this software (Moore and Stuart 2004) for faculty who did not have the time available in their course to explore action potentials or synaptic potentials beyond one or two lectures. The minimovies illustrate fundamental aspects of neuronal and synaptic function.

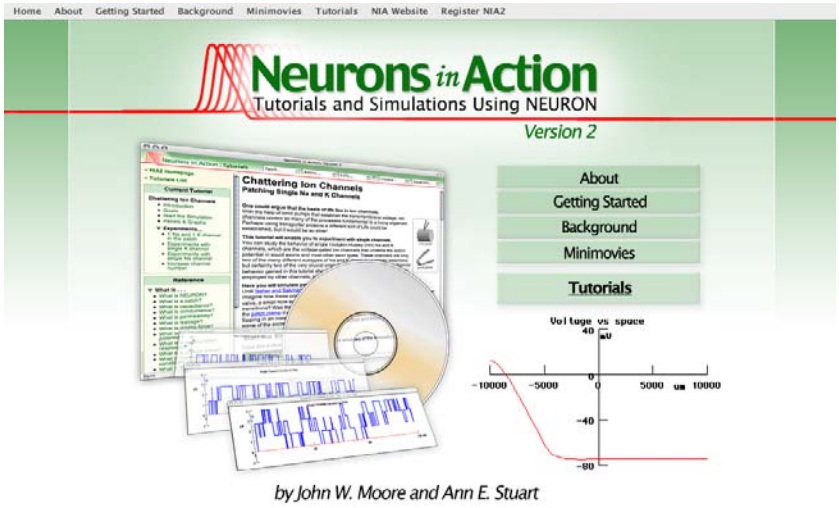

Figure 1: The Home Page of *NIA2*. Selecting Background allows the user then to choose History, an extensive exposition of the events and experiments culminating in the elucidation of the action potential, or PDFs, a selection of classic papers on the CD, including the Hodgkin-Huxley papers. Selecting Minimovies takes the user to the three categories of 19 minimovies detailed below. Selecting Tutorials brings up the list shown in Figure 2.

BASIC Level Tutorials

Patch: Stationary Signals

Introduction to Neurons in Action
The Membrane Tutorial
Equilibrium Potentials
The Na Action Potential
Threshold: To Fire or Not to Fire
Voltage Clamping a Patch
Chattering Ion Channels
The Ca Action Potential
The Neuromuscular Junction
Postsynaptic Inhibition
Interactions of Synaptic Potentials

Axons: Signals That Move

The Passive Axon
The Unmyelinated Axon
The Myelinated Axon
Partial Demyelination

ADVANCED Level Tutorials

Patch: Stationary Signals

Extracellular Ca Sensitivity of the Na Channel
A Dynamical View of Threshold
Na and K Channel Kinetics

Axons: Signals That Navigate

Axon Diameter Change
Non-uniform Channel Density

Cells

Site of Impulse Initiation
Synaptic Integration
Impulse Invasion of the Presynaptic Terminal
Coincidence Detection
"Voltage Clamping" Intact Cells

Figure 2: The set of 25 tutorials in *NIA2*. Tutorials are grouped into Basic and Advanced categories; within each category they are grouped by geometry.

In 2007, working with colleagues who use the program, we redesigned the interface and navigation, added new tutorials and revised others, linked the minimovie voltages to sound, and published these changes as *NIA* Version 2 (*NIA2*; Fig 1)(Moore and Stuart 2007). This effort was generously supported by the USA National Science Foundation. In all versions we link the tutorial text to explanations, answers to questions, historical context, and background material to aid both students and faculty in the interpretation of the simulations.

Using *Neurons in Action* in lecture

Didactic lecture: making lecture lively with movies and sound

Many faculty teaching in psychology, physiology, and medical school courses find that they have only one lecture in which to convey to students the nature of that miniature, propagating electrical explosion - the action potential. Typically a second lecture is devoted to synaptic transmission. Then the course material moves quickly on from these rather difficult topics to subjects such as memory or aggression, to which a student can more quickly relate. Faced with the problem of making the action and synaptic potentials more exciting, so to speak, we designed *NIA* minimovies for our colleagues to introduce into their PowerPoint lectures. In *NIA2* we have attempted to make the minimovies still more lively by linking the voltage to sound.

Movies: Action Potential	Movies: Axons
Increasing Stimulus Amplitude	Passive Spread of a Depolarizing Stimulus
Very Near Threshold Stimuli	AP Traveling in a 500 µm diam Unmyelinated Axon
Changing External Na Concentration	AP Traveling in a 50 µm diam Unmyelinated Axon
Blocking the K Channels	AP Traveling in a Myelinated Axon
Ion Conductances Underlying the Action Potential	AP Traveling in an Axon with 5 Myelin Wraps
Blocking the K Channels: View the Conductances	AP Traveling in a Partially Demyelinated Axon
Effects of Cooling	AP Initiation in a Motoneuron
	Synaptic Integration

Figure 3. Lists of minimovies in the Action Potential and Axons categories. Minimovies of aspects of excitatory and inhibitory transmission are also available. The instructor can preview each minimovie before importing it into PowerPoint.

The minimovies (available since *NIA1.5*) are Quicktime movie files captured by the authors from *NIA* simulations. They are grouped into three categories: (1) the basic properties of the action potential in a patch, (2) the action potential as it propagates along unmyelinated, myelinated, or partially demyelinated nerve (Fig 3), and (3) the salient features of excitatory and inhibitory postsynaptic potentials. Action potential minimovies demonstrate topics ranging from increasing the stimulus amplitude to the effect of cooling. In *NIA2*, each voltage trace in the first group is also converted to frequency for an accompanying sound track.

Beyond making lectures more engaging, the use of such movies dispels the inaccurate notions of the propagation of neuronal signals that seem to be difficult to eliminate from textbook material. For example, when students see that the action potential actually spreads out in myelinated nerve, its peak covering many nodes at once, they are usually at first bewildered, for did the text not say that it "jumped" from node to node? Soon the student has the correct notion of the breadth of the voltage change in centimeters as it propagates actively along an axon.

One particularly popular minimovie with our colleagues, derived from the Partial Demyelination tutorial, compares an action potential in unmyelinated and myelinated axon by showing the action potential as it travels along a bare length of axon and then invades a myelinated portion (Fig 4).

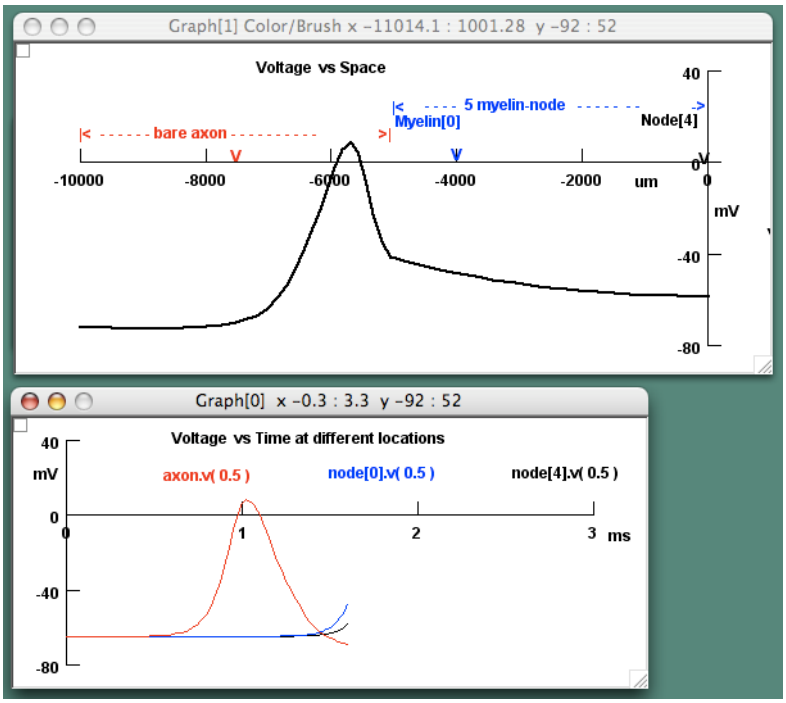

Figure 4. Arrested minimovie of the propagation of an action potential in a partially demyelinated nerve of 10 μm diameter. The upper, voltage-versus-space graph diagrams the axon as bare on the left half, with a red recording electrode in the center of the bare portion, and myelinated on the right half (5 nodes and 5 internodes each 1000 μm long), with a blue recording electrode in the first node and a black recording electrode in the last node. The movie was stopped when the peak of the action potential, triggered at the left end, was just about to invade the myelinated half, the depolarization spreading far in front of the impulse. The lower, voltage-versus-time graph shows the complete action potential, since it has passed the red electrode, and the arrested depolarization at the blue and black electrodes.

At first, students find it almost shocking to see the change in the action potential from a rather narrow event in space, its entire overshoot extending over less than 0.5 mm in the bare axon, to a voltage change that remains at peak amplitude over the whole 5 mm of myelinated axon in the simulation! As the action potential traveling in the bare axon approaches the myelinated portion, the depolarization spreading far in front of itself makes absolutely clear how propagation occurs. A separate minimovie of only myelinated nerve shows a stretch that is long enough so the student can see the tiny bumps and dips on the voltage trace at the nodes as the ionic currents surge in on the rising phase and out on the falling phase (Fig 5). Such detail captivates students, in our experience, surprising them and helping them to form a correct picture of what happens to the currents and the voltage at the nodes and internodes. A voltage-versus-time graph associated with this movie that plots the action potential as it passes two locations in the axon is useful in showing medical students, in particular, how conduction velocity is calculated, an essential technique in neurology.

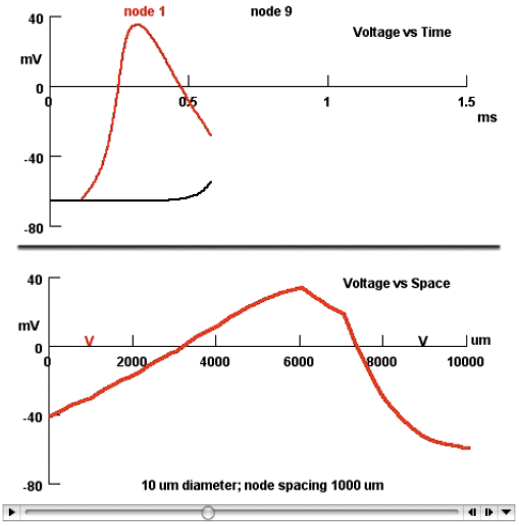

Figure 5. Arrested minimovie of an action potential in a myelinated nerve recorded by electrodes at node 1 and node 9 of 10 nodes 1000 μm apart. The movie has been stopped when the peak of the action potential is at the 6000 μm node. The upper, voltage-versus-time graph shows a complete trace recorded by the red electrode at node 1 and the beginning of the depolarization as the impulse approaches the black electrode at node 9. The lower, voltage-versus-space graph shows the electrode positions. The action potential was started at the left end of the axon.

Interactive lecture: the educational value of suspense

Students often become engaged when *NIA* simulations are used interactively at the podium. Running simulations during the lecture is relatively easy, since toggling between PowerPoint slides and a simulation is straightforward on either a PC or a Mac. The effective strategy is to ask the students, for example, "What do you expect will happen to the peak of this action potential if I increase the external sodium concentration? How many of you vote that it will get bigger? How many vote for smaller? How many aren't sure?" A little practice might be necessary before the lecture, but even in a class of over one hundred students this method can work, providing a degree of suspense that is uncommon in standard lectures and causing even the tuned-out students to be curious about the outcome.

A popular *NIA* tutorial to use in this fashion is, again, Partial Demyelination, but this time stimulated at the myelinated end. When the action potential is triggered at the far right end of the myelinated portion, it propagates normally along the myelinated half but then falters at the junction between the myelinated and demyelinated halves. Hesitating, it ultimately fails to invade the bare axon. Since this condition mimics that of multiple sclerosis, it never fails to intrigue students.

The instructor might now ask, "What bare axon parameters can we change to make this action potential invade and propagate in the bare axon? If we were to set out to look for a drug to help this multiple sclerosis patient, what would we want our drug to do?" Ultimately the class can suggest a drug that partially blocks potassium channels; one that increases the density of the sodium channels in the membrane might be suggested at first but, after discussion, the students might conclude that such a drug would be difficult to design from a biological standpoint. But either a decrease in the potassium channel density or an increase in the sodium channel density in the simulation will lead to invasion of the bare axon and reinforce the students' understanding of threshold as a tug-of-war between the sodium and potassium currents. What about a change in diameter (which tests the students' understanding of capacitance as membrane area)? What about an overdose of the

potassium channel blocker, a complete block (which tests their knowledge of the mechanism of the action potential)? Would this overdose kill the patient? (Yes.)

Would a change in temperature facilitate invasion of the bare axon? Multiple sclerosis patients are known to prefer certain temperatures (Humm et al. 2004), but, based on neurophysiology, can the students predict whether the patient might prefer to be cool or warm? Changing the temperature in this tutorial is among the most suspenseful and effective of all of the interactive simulations, probably because of its immediate relation to the disease and perhaps, also, because the temperature change required to permit propagation into the bare axon is so small. When the correct change is made, the action potential hesitates for an excruciatingly long time, finally becoming regenerative and even leading to student applause in our experience! The final answer seems at first counterintuitive but effectively solidifies a student's understanding that channel transitions underlie the action potential and these transitions -- from closed to open to inactivated states -- obey Michalis-Menten kinetics and temperature sensitivity.

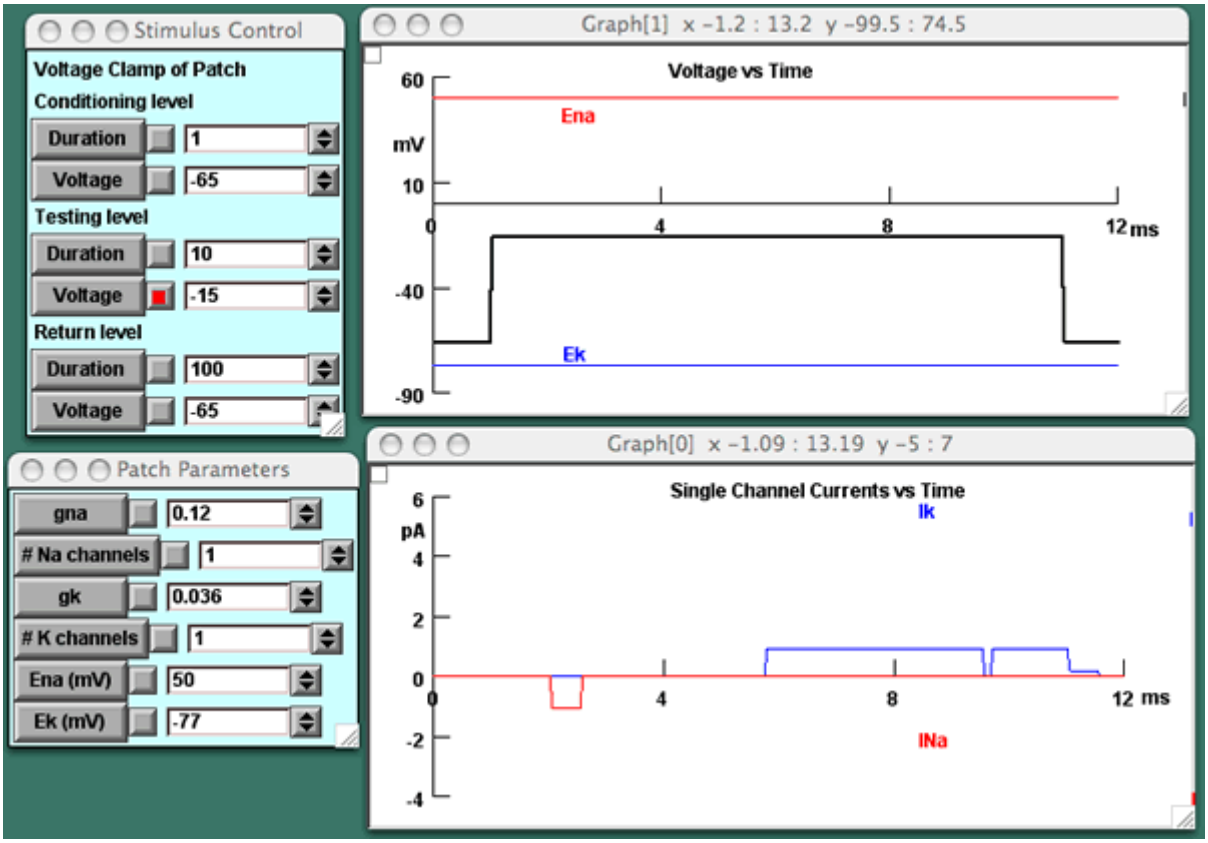

Fig 6. The results of one run of a voltage step from -65 mV to -15 mV and back (see the Stimulus control panel) in the Chattering Ion Channels tutorial. The patch in this simulation contains one Na channel and one K channel, as specified in the Patch Parameters menu. Clearly the Na channel has opened once and either closed or inactivated; the K channel has opened and closed twice, the second closing happening after the voltage step is over, leading to a smaller outward current through the channel after the step.

Within *NIA*, essentially any tutorial can be used to engage student interest through suspense. In the Chattering Ion Channels tutorial, the single channel simulations, the lecturer can specify the type of channel in the patch and its number (Fig 6). When there is a single channel, each delivery of the depolarizing pulse leads to a different result because channel opening is stochastic. Ergo the suspense: is there an opening? Does the direction of the current tell us which channel it is? When is

the opening -- early or late in the pulse? One opening, or two, or maybe more? Would so many openings be expected of this type of channel? We also find suspense when incrementally increasing the channel number in the patch from one to two, then three, then, ultimately, to hundreds, thousands, and tens of thousands to reveal how macroscopic currents are built up. The "What If? approach" in lecture leads to understanding through engaging curiosity about the outcome.

Using *Neurons in Action* in an undergraduate laboratory setting

Turning students loose with *NIA* in a computer lab

If *NIA* has been introduced in lecture and used interactively, it is relatively easy to make the transition to asking the students to do whole or abbreviated tutorials on their own or in groups in a computer lab. When the time is short, we and our colleagues have found it useful to bypass the tutorials and instead hand out a limited exercise with a set of steps to follow and questions to answer at each step (and then hand in). The instructor circulates through the groups to solve any problems with the running of the simulations, to ask questions to make sure the students are understanding what they are seeing, or to ask what other parameter changes they might make in order to test the hypothesis. "So what experiment could you do here to test if your explanation is correct?" the instructor might say, holding back on giving away answers.

A number of colleagues use *NIA* in undergraduate neuroscience courses. One colleague used *NIA1.5* for two weeks in the laboratory portion of an upper level introductory neuroscience course at Bowdoin College in Maine, USA. Each student had access to *NIA1.5* in a computer lab. In the first week, the instructor led the students through several tutorials that were chosen to complement what they learned in lecture. These sessions also gave the students a chance to become facile with the program. In an innovative twist during the second week, the students, working in groups of two, designed their own hypotheses to test. The faculty member and a laboratory instructor met with each group to decide which tutorial provided the ideal parameters to manipulate and therefore was the most appropriate for running their own experiment. One hypothesis, for example, was that unmyelinated axons would be affected at lower concentrations of tetrodotoxin than myelinated axons; the students decreased the Na channel density in equal proportions in the two axon types and measured action potential rate of rise, amplitude, and conduction velocity to come to their conclusions.

Colleagues who have undergraduates do the *NIA* tutorials in a computer lab setting usually have made individualized materials to complement the tutorials: for example, handouts with specific exercises to be done in each tutorial (separate from those in the published tutorials), questions about the results of simulations, problem sets, and homework. Colleagues have sent us such materials for *NIA1* for posting on the *NIA* website (http://neuronsinaction.com). With the publication of *NIA2* and the increasing familiarity of faculty with forums on websites, this site provides a potential mechanism for sharing novel ideas about how to use these simulations and tutorials in teaching at any level.

Using *NIA* in conjunction with a wet lab

An example of a course using *NIA* in conjunction with a wet lab is a course in "Principles of Neurophysiology" taught through the Department of Neurobiology and Behavior at Cornell University (Ithaca, NY, USA). The purpose of this upper level, undergraduate course is to introduce students to the power of neural computation as a tool in the laboratory as well as providing a sophisticated laboratory experience in neurophysiology. To this end, the *NIA* tutorials are integrated with wet lab

experiments designed primarily around the Crawdad software package. Although in previous years a textbook had been used for this course, *NIA i*s now the only text; the students refresh and deepen their understanding of neurophysiological concepts through *NIA*'s hyperlinked materials.

The students begin with an RC circuit in the lab and the Membrane Tutorial from *NIA*. From then on, the tutorials are selected to complement the wet lab preparations rather than being followed in their own order. For example, the next wet lab technique to be learned is extracellular recording; here the Unmyelinated Axon and/or Myelinated Axon tutorials from *NIA* are useful where conduction velocity can be measured by inserting two virtual electrodes into an axon. As the wet lab moves on to intracellular recording from crayfish muscle fibers and then snail neurons, the *NIA* tutorials dealing with equilibrium and resting potentials, action potentials and threshold, excitatory and inhibitory synaptic potentials, and then voltage clamp, are introduced as appropriate.

For the midterm and final "exams" in this course, the students must execute a project of their own design that blends the wet and virtual lab experiences and then write it up. In one example of such a project, students recorded the extracellular action potentials from all six axons in the third motor nerve of the crayfish, calculated the six conduction velocities from these recordings, and then estimated their diameter through simulations using *NIA*. In another project, students recorded action potentials and ionic currents from snail neuronal somata, added potassium channel blockers at increasing concentrations, recorded the changes in amplitude and duration of the action potential and amplitude of the potassium currents, and then simulated these results with the Action Potential and Voltage Clamp tutorials. The students had to design the projects themselves, including figuring out which simulations were appropriate. The instructor finds these "exams" to be an especially rewarding aspect of the course as students truly understand the value of using simulations to illuminate real experiments.

Using *Neurons in Action* in a graduate student course

At the graduate level, there is typically more time to go into depth with the *NIA* tutorials. Intrigued neuroscience students can venture into the Advanced tutorials. We also know of colleagues who have assigned *NIA* to physics students, biomedical engineering students, and computer science students interested in neurons; as well, some colleagues who use simulations in their research have told us that they assign *NIA* to students joining their labs as an introduction to computational neuroscience and to NEURON.

One of the authors and her immediate colleagues have used *NIA* for several years in four very different graduate student courses offered through the Cell and Molecular Physiology Department or the Neurobiology Curriculum at the University of North Carolina at Chapel Hill, detailed below. In each case, the tutorials are primarily done by individual students or groups of students working together rather than by the faculty member.

(1) The individual student

An individual student did the entire set of tutorials as a one-person course, meeting with the author once a week to clear up questions, to be "tested" on what he had learned, and to suggest experiments he had designed beyond those outlined in the tutorial. *NIA* was originally designed to be used in this way by the individual graduate student who might wish to test his or her knowledge of neurophysiology and to have a different learning experience from that provided by textbooks. Certainly this approach can lead to an in-depth use of the software.

(2) An introductory physiology course

In the Cell and Molecular Physiology Department, first-year graduate students taking an introductory course in human physiology use *NIA* each year as their introduction not only to neurons but to concepts like membrane potentials, equilibrium potentials, and driving force, concepts that apply to all cells of the body. The students are typically assigned problem sets that they are expected to solve after becoming familiar with particular tutorials. Such problem sets (and exam problems) are easily constructed by the instructor using graphs and panels captured from an *NIA* simulation; the student must explain what is being illustrated by the traces.

As an example of a problem, the student is given voltage-vs-time and current-vs-time graphs from a voltage clamp experiment in the Voltage Clamping a Patch tutorial. They see a family of 17 Na currents where the voltage was stepped to the reversal potential for Na ion for a long enough time to inactivate the Na channels (5 ms), returned to the resting level (-65 mV) for increasing lengths of time (for a total of 17 traces), then stepped again to zero mV to open any available Na channels. The student must figure out that this experiment tests the time course of recovery of the Na channel population from inactivation.

For a slightly more advanced problem, the students are asked to calculate the first order rate constant that applies to this problem and then to calculate it as a function of voltage by choosing different voltages for the recovery step. The problem can also be put in reverse: The instructor can give the students the answer and ask them to come up with the simulation, as in "Using *NIA*, demonstrate the time course of recovery from inactivation and compare the time courses at three different voltages."

(3) A course in biophysics and ion channels

NIA is used in a 6-week block devoted to biophysics and ion channels within a year-long Cellular and Molecular Neurobiology course in the Neurobiology Curriculum. The students do individual tutorials in groups of 4 outside of class and then present them as a group to the rest of the class. Since this class is also focused on reading original papers, it is particularly useful when a tutorial such as the Chattering Ion Channels tutorial (*NIA2*) can be paired with papers describing single channel behavior. As another example, the simulations in the Site of Impulse Initiation and Synaptic Integration tutorials are useful in discussing the current literature on this problem (Palmer and Stuart 2006; Stuart and Palmer 2006). In this vein, the tutorial on Impulse Invasion of the Presynaptic Terminal is one that arises from a paper published by one of the authors, in which simulations were used to interpret experimental data. The inclusion of this tutorial in *NIA* has the double purpose of educating the student first about the presynaptic terminal and, second, about the use of computational approaches in research.

(4) 18 hours of *NIA* in a week!

NIA is the core tool for introducing basic neuronal function to graduate students each summer in a course at the Marine Biological Laboratory (Woods Hole, MA, USA; the Summer Program in Neuroscience, Ethics, and Behavior). The students in this course come from a variety of backgrounds -- from physics to neuroscience to psychology. They used *NIA1*, and now *NIA2*, in groups every morning (3 hours) for 6 sequential days, everyone doing the same Basic Patch tutorials at the beginning of the week after an introduction by the instructor but then specializing in different tutorials as the week progresses. The students present their particular tutorial to the other students after they have mastered it; typically they also master the hypothesis-testing style of the tutorials in their presentations. For example, they ask their peers if they can reason out what they think will happen before running a simulation. The students take on tutorials appropriate to their backgrounds; those

who come to the course trained in more quantitative areas, such as math and physics, tend to take on tutorials from the Advanced section such as A Dynamical View of Threshold or Na and K Channel Kinetics and explain them to their colleagues.

Using *Neurons in Action* for premed or medical students

Throughout *NIA*, we have accompanied the simulations with translational examples that colleagues might use in capturing the interest of premed students or in teaching medical students. The Partial Demyelination tutorial, detailed above, gives the lecturer the chance to explain many basic properties of neurons in the context of multiple sclerosis, from the "war" between the Na and the K currents that constitutes threshold to the forward spread of the depolarization that enables propagation to the refractory period that prevents the action potential from propagating backwards. At a more sophisticated level, the Na and K Channel Kinetics tutorial in the Advanced Patch section (Version 2) relates to chronic pain and other issues of neuronal hyperexcitability. Recent research has revealed that a particular Na channel subtype is upregulated in damaged neurons, leading to increased excitability; this tutorial explores how different channel subtypes, with differing kinetics, can determine the excitability of neurons (Fig 7). As well, the tutorial shows how certain rather well-known toxins, such as brevetoxin from one type of red tide or maculotoxin from the blue-ringed octopus, can disrupt normal neuronal function not by blocking the channel but by altering its kinetics.

Other translational examples in *NIA2* include:

- In the Equilibrium Potentials tutorial, a discussion of the clinical consequences of changing extracellular ion concentrations;
- In the Na Action Potential tutorial, a comparison of the effects on the action potential of anesthetics that block both the Na and the K channel with poisons like tetrodotoxin that block only one channel type;
- In the Threshold: To Fire or Not To Fire tutorial, the neurophysiological basis of treating myasthenia gravis and its relation to threshold;
- In The Ca Action Potential tutorial, the generation of a cardiac-like action potential;
- In the Postsynaptic Inhibition tutorial, an understanding of "The GABAergic Synapse, Target of Psychoactive Drugs;"
- In the Extracellular Ca Sensitivity of the Na Channel tutorial, how abnormal serum Ca concentration might lead to clinical problems through its effects on the voltage-gated Na channel;
- In the Non-uniform Channel Density tutorial, propagation of an action potential through a region of axon that is partially blocked with an anesthetic.

These various clinical examples can be used to spice up a lecture on basic neuronal properties. In one instance, a practicing neurologist became excited by the clinical implications of the Partial Demyelination simulations and told us of the Uhthoff phenomenon (Uhthoff, 1890) that describes the temperature sensitivity of multiple sclerosis patients. Thus the medical student who will go on in neurology might be attracted to pursue basic neurophysiology in greater detail through the *NIA* tutorials.

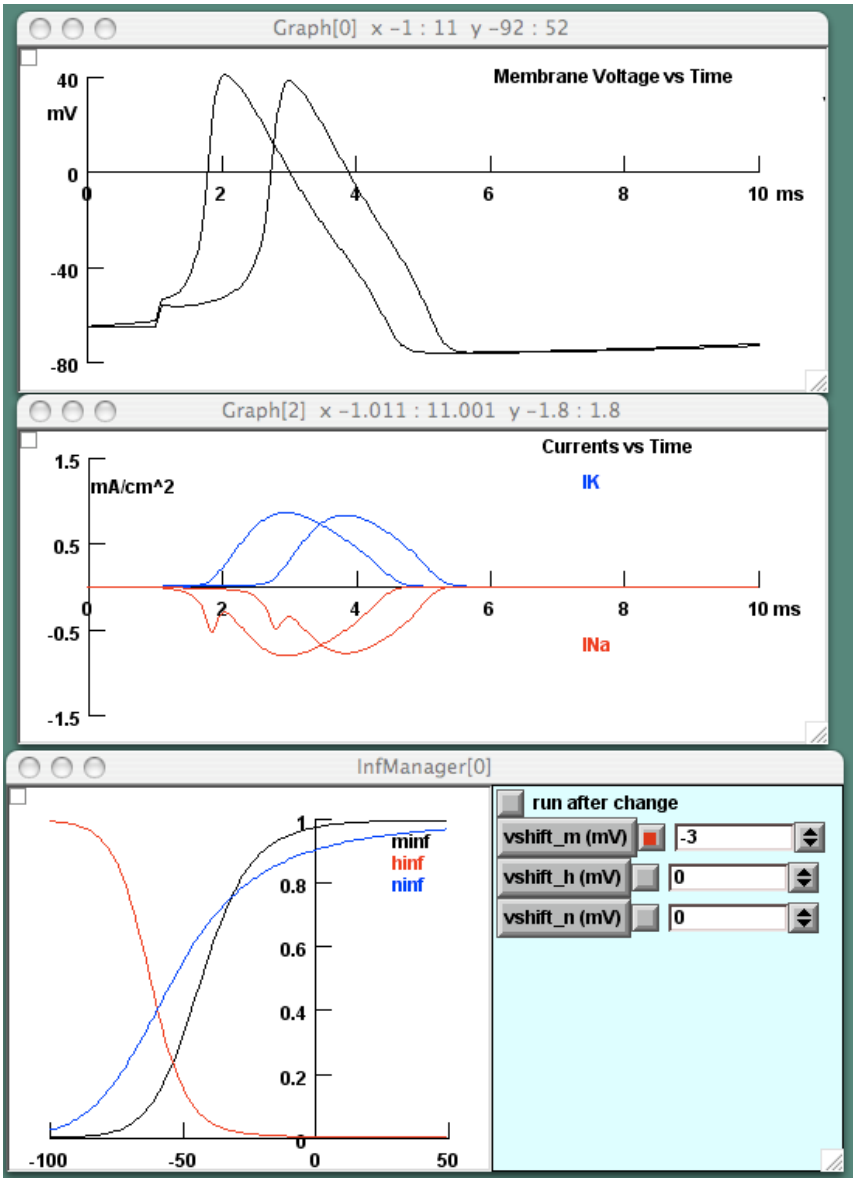

Fig 7. Panels from the Na and K Channel Kinetics tutorial. In the lowermost panel, the user can shift the steady-state values of the Hodgkin-Huxley parameters that describe the activation "m" and inactivation "h" of the Na channel and the activation "n" of the K channel. The uppermost panel shows the effect of this shift on the excitability of the neuron; in this case, a negative shift of 3 mV in the "m" curve causes the cell to become more excitable so that the action potential arises earlier in the depolarizing pulse than it did when "m" was set to zero. The middle graph shows the Na (red) and K (blue) currents during each action potential.

Summary

We have illustrated how *Neurons in Action* is used "in action" in a variety of teaching situations: in didactic and interactive lectures to undergraduates or medical students; in undergraduate laboratories, either dry or in conjunction with a wet lab; in graduate courses where the simulations can be explored extensively; or as a one-person course with an individual student. In doing so, we hope to have offered a new idea or two to colleagues using interactive software, whether *NIA* or another tool, to educate students of differing backgrounds in the possibly intimidating, certainly intricate, subject of

neurophysiology. We are convinced that real understanding comes from the immediate feedback students receive when they can formulate and test questions arising from their progressive understanding. Such feedback is obtained readily from virtual experimentation but not so easily from textbooks or lectures.

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