Graduate
Partnerships
Program

Graduate Student Research Symposium

Program

Second Annual
NIH Graduate Student Research Symposium

"The Faces of Tomorrow's Science"

Graduate Partnerships Program
Lipsett Auditorium
April 22, 2005

8:40 – 8:45	Opening – Dr. Rick McGee, Director of Student Affairs, Graduate Partnerships Program
8:45 – 9:45	Student Oral Presentations – Session 1
	Refreshments available 9:30 – 11
9:45 – 10:45	Student Oral Presentations – Session 2
10:45 – 11:00	BREAK
11:00 – 11:10	Welcome – Dr. Michael Gottesman, Deputy Director for Intramural Research
11:10 – 11:25	Presentation of Outstanding Mentor Awards – Graduate Student Council
11:25 – 11:30	Introduction of the Speaker – Dr. Mary DeLong, Director, Graduate Partnerships Program
11:30 – 12:15	Plenary Presentation – Allan I. Levey, PhD, MD, Chair, Department of Neurology
	Emory University School of Medicine
12:15 – 2:00	Poster Sessions and Buffet Lunch
12:30 – 1:15	Poster Session I – Authors of odd-numbered posters present
1:15 – 2:00	Poster Session II – Authors of even-numbered posters present
2:00 – 3:00	*Pathways Conversation* – An informal conversation between graduate students
	and Dr. Allan I. Levey
3:00 – 4:00	Student Oral Presentations – Session 3
4:30 – 6:30	Reception at The Cloisters

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Abstracts

Analysis of Clonal Contributions to Lymphoid and Myeloid Lineages during Early Hematopoiesis following Autologous Transplantation in the Rhesus Macaque

Rima L. Adler, Peiman Hematti, Boris Calmels, Colin Wu, Dean Follmann, Karin Lore, and Cynthia E. Dunbar

Graduate Student:
Rima L. Adler, NHLBI
NIH Research Advisor:
Cynthia E. Dunbar
University:
The George Washington University Genetics

Hematopoietic stem cells are defined by their ability to both self-renew and differentiate into cells that repopulate all hematopoietic lineages for the animal's life span. Lineage-restricted progenitors have been shown to only transiently repopulate hematopoietic cells and in a lineage specific manner in the mouse model. Using rhesus macaque as a model for human hematopoiesis, we asked whether lineage-restricted repopulating cells contribute to hematopoiesis and for how long. In order to follow progeny of progenitor or stem cell clones *in vivo*, retroviral vectors were used to mark primitive hematopoietic cells before reinfusion into lethally irradiated autologous recipients. In the current study, detailed characterization of clones contributing to granulocytes and lymphocytes post-engraftment was performed, via Linear Amplification Mediated–Polymerase Chain Reaction and subsequent sequence analysis of individual clones at various time points following transplantation of CD34+ cells. Most clones detected uniquely contribute to only the lineage from which they were identified, suggesting lineage-restricted cells are responsible for hematopoietic reconstitution during both early and potentially more stable hematopoiesis in this model. **Poster 1**

Cytokine Dependence of CD8α Coreceptor Expression and CD8$^+$ T Cell Lineage Choice

Stanley Adoro, Batu Erman, and Alfred Singer

Graduate Student:
Stanley Adoro, NCI
NIH Research Advisor:
Alfred Singer
University:
University of Pennsylvania Immunology Graduate Group

T cells that express αβ T cell receptors (TCR) recognize peptides presented by Major Histocompatibility Complex (MHC) molecules and develop from a common double positive thymocyte precursor that express CD4 and CD8 coreceptors, surface proteins that enhance the TCR-MHC interaction and participate in TCR-dependent signaling. During thymic development, TCR specificity is matched to coreceptor expression such that CD4 single positive (CD4$^+$ SP) and CD8$^+$ SP T cells recognize peptides presented by MHC class II and MHC class I respectively. Here we have tested the hypothesis that CD8 transcriptional regulatory elements respond to the cytokine IL-7 using transgenic mice expressing CD4 under control of the CD8α promoter and a 7.6 kb CD8 cis transcriptional enhancer. Expression of reporter CD4 was MHC class-I-dependent and restricted to mature CD8$^+$ SP thymocytes and peripheral CD8$^+$ T cells. Interestingly, potential binding sites for Stat5a/b, transcription factors activated by IL-7, were identified within the enhancer. Reporter CD4 expression, but not endogenous CD4, was significantly enhanced by IL-7 in *in vitro* cultures and paralleled the modulation of endogenous CD8α expression by Stat5-dependent cytokines. These preliminary data support a role for IL-7 in CD8α expression and CD8$^+$ T cell lineage decision. **Poster 2**

Polymorphism of Genes in the Lipid Metabolism Pathway and the Risk of Biliary Tract Cancers: A Population-based Study in Shanghai, China

Gabriella Andreotti, Yu-Tang Gao, Lori Sakoda, Asif Rashid, Jie Deng, Ming-Chang Sheng, Bin-Sheng Wang, Tian-Quan Han, Bai-He Zhang, Hope Cohen-Webb, Shelley Niwa, Meredith Yeager, Robert Welch, Stephen Chanock, Joseph F. Fraumeni, Jr., and Ann W. Hsing

Graduate Student:
Gabriella Andreotti, NCI
NIH Research Advisor:
Ann W. Hsing
University:
George Washington University Epidemiology
University Research Advisor:
Paul Levine

Biliary tract cancers, encompassing the gallbladder, extrahepatic bile duct, and ampulla of Vater, are rare, but often fatal. Gallstones, a known risk factor for biliary tract cancers, involve cholesterol supersaturation of the bile. In a population-based case-control study conducted in Shanghai, China, we examined the role of 12 SNPs in five genes (*ALOX5, ApoB, ApoE, LDLR, LPL*) in lipid metabolism with biliary tract stone and cancer etiology. The study included 406 incident biliary tract cancer cases, 880 biliary tract stone cases, and 779 healthy controls. Relative to individuals with CC genotype of *ApoE* IVS1+69, those with G allele had 2-fold (95%CI 1.3-3.1) and 2.5-fold (95%CI 1.2-5.1) risk for bile duct and ampullary cancers, respectively. Risks were more evident among men and individuals without diabetes or with a lower BMI. Among men, *ApoE* IVS1+69 G variant was associated with significant excess risk of gallstones and gallbladder cancer. Men who carried high-risk alleles for ApoE IVS1+69 and LDLR IVS9-30 had a 5-fold risk of bile duct cancer (OR=5.34, 95%CI 2.1-13.8). Results from our study suggest that variants of the *ApoE* gene may confer susceptibility to gallstones and biliary cancers, especially bile duct cancer. **Poster 3**

Streamlining Znta: Strategic Truncation of a P Type Pump for Crystalography

Benjamin B. Bartelle, William J. Rice, and David L. Stokes

Graduate Student:
Ben Bartelle, OD
University:
New York University
Structural Biology
University Research Advisor:
David Stokes

The P type ATPases are large family of proteins that transport metals across membranes. Attempts to crystallize these proteins are particularly difficult due to their hydrophobic transmembrane domains and their complex and flexible cytosolic domains. Using molecular modeling, heavily informed by proteolysis experiments, we explored the structure of the P type ATPase ZntA. These data were used to construct a "streamlined" ZntA that has its highly flexible regions removed, but theoretically retains activity. Efforts to crystallize the new construct are ongoing. **Poster 4**

Mutations in PINK1 Associated with Recessive Parkinsonism Have Differential Effects on Protein Stability

Alexandra Beilina, Marcel Van Der Brug, Rili Ahmad, Sashi Kesavapany, David W. Miller, Gregory A. Petsko, and Mark R. Cookson

Graduate Student:
Alexandra Beilina, NIA
NIH Research Advisor:
Mark R. Cookson
University:
University of California–Davis
Biochemistry and Molecular Biology

Several mutations in the PINK1 gene have been associated with recessive parkinsonism. The encoded protein is predicted to be a Ser/Thr protein kinase targeted to mitochondria. In the current study, we have investigated the effects of mutations on PINK1 kinase activity *in vitro* and on expression levels and localization in mammalian cells. We chose to examine two point mutations; G309D, which was originally reported to be stable and properly localized in cells; and L347P, which is of interest because it is present at an appreciable carrier frequency in the Philippines. We were able to confirm kinase activity and produce artificial "kinase-dead" mutants that are stable but lack activity. The L347P mutation destabilizes PINK1 and drastically reduces kinase activity, whereas G309D has much more modest effects on these parameters *in vitro*. We also examined the localization of PINK1 in transfected mammalian cells using constructs that were tagged with myc or GFP at either end of the protein. These results show that PINK1 is processed at the N-terminus in a manner consistent with mitochondrial import, but that the mature protein also exists in the cytosol. The physiological relevance of this observation is not yet clear, but it implies that a portion of PINK1 may be exported after processing in the mitochondria. **Poster 5**

Cellular Expression of PINK1, Implicated in Parkinson's Disease, in Human, Rat and Mouse Tissue

J.G. Blackinton, D. Galter, A. Carmine, M.R. Cookson, and L. Olson

Graduate Student:
Jeff Blackinton, NIA
NIH Research Advisor:
Mark Cookson
University:
Karolinska Institutet
Neuroscience
University Research Advisor:
Lars Olson

Parkinson's disease (PD) affects about 2 percent of people over age 65, making it the second most common neurodegenerative disorder. While degeneration of the dopamine neurons in substantia nigra is the hallmark of PD and leads to the cardinal symptoms of the syndrome, other neuron systems are also often affected. It is clear from the spectrum of symptoms, variety in age of onset and rate of progression and neuropathological findings that PD is a complex disorder, a contention supported by the fact that many genes with widely different functions have been implicated in monogenic forms of parkinsonism. Most recently, mutations in PINK1 (PTEN-induced putative kinase) have been found in Italian and Spanish families previously linked to the PARK6 locus on chromosome 1p35-36. The gene contains eight exons encoding a 581 amino acid protein with a mitochondrial targeting peptide and a serine/threonine kinase domain. Here, we report in situ hybridization data demonstrating the expression of PINK1 mRNA in various human, rat and mouse tissues. Although expressed widely throughout the body and in a variety of brain structures, expression is particularly strong in substantia nigra neurons. Work in progress includes immunohistochemstry to localize PINK1 protein in human tissues and genetic analysis of PINK1 from Swedish early onset PD cases. **Poster 6**

Mechanism of Protection against Severe Malaria by Haemoglobin C

Nathaniel Brittain, Rick Fairhurst, Grace Ostera, and Thomas Wellems

Graduate Student:
Nathaniel Brittain, NIAID
NIH Research Advisor:
Thomas Wellems
University:
George Washington University
Genetics

Plasmodium falciparum (Pf), the causative agent of malaria, infects up to 500 million people each year of which about 2 million die. The close association of Pf with humans for a long period of time has resulted in the natural selection of various protective polymorphisms. One of these is haemoglobin C (HbC) which has a single amino acid substitution (6: glu-lys) resulting in protection against severe, life-threatening malaria. Both homozygous (CC) and heterozygous (AC) erythrocytes support *Pf* growth, so this does not account for protection. Instead, we have found that pathogenic characteristics of parasitised erythrocytes are dramatically altered in HbC compared to normal erythrocytes. These characteristics all involve the membrane of the infected cell and one protein in particular, PfEMP-1, which is known to be critical to the pathology of *Pf*. This mechanism may account for protection in young children prior to the acquisition of immunity. **Poster 7**

Sprouty1 Differentially Modulates T Cell Receptor Signaling Depending on the Activation State of the T cell

Heonsik Choi, Sung-Yup Cho, Ronald H. Schwartz, and Kyungho Choi

Graduate Student:
Heonsik Choi, NIAID
NIH Research Advisors:
Ronald H. Schwartz and Kyungho Choi
University:
Seoul National University
Molecular Immunology
University Research Advisor:
RH Seong

Sprouty(Spry) is known to be a negative feedback inhibitor of growth factor receptor signaling through inhibition of the Ras/MAPK pathway. Several groups, however, have reported a positive role for Spry involving sequestration of the inhibitory protein c-Cbl. Thus, Spry may have various functions in the regulation of receptor-mediated signaling depending on the context. In this study, we investigated the role of Spry1 in TCR signaling. Spry1 was specifically turned on among the 4 mammalian homologues by TCR signaling of $CD4^+$ murine T cells. In fully differentiated Th1 clones, overexpressed Spry1, as a transducible TAT-fusion protein, inhibited TCR signaling, leading to decreased IL-2 production. In contrast, in naive T cells, Spry1 enhanced TCR signaling, resulting in increased proliferation and cytokine production. This enhancing effect was abrogated by preactivation of naive T cells with antigen and APC, indicating that the history of exposure to antigen is correlated with a responsiveness of T cell to Spry1. Biochemically the NFAT as well as the MAPK pathway was influenced by Spry1 in both a negative and positive way. Thus, Spry1 uses a novel mechanism to bring about differential effects on TCR signaling through the same receptor, depending on the differentiation state of the T cell. **Poster 8**

Relationship of Maternal Methadone Dose and Meconium Concentration to the Severity of Neonatal Abstinence Syndrome and Other Birth Outcomes

Robin E. Choo, Hendree A. Jones, Jennifer E. Schroeder, Constance M. Murphy, and Marilyn A. Huestis

Graduate Student:
Robin E. Choo, NIDA
NIH Research Advisor:
Marilyn A. Huestis
University:
University of Maryland–Baltimore
Toxicology

The objectives of this study were to relate severity of neonatal withdrawal (NAS) and other birth outcomes to total and trimester cumulative maternal methadone dose and methadone/metabolite concentrations in meconium. Methadone-maintained pregnant women were enrolled and followed throughout gestation. Tri-weekly urine specimens were obtained and analyzed for drugs of abuse. Meconium specimens were analyzed for methadone/metabolites by LC-MS/MS. Cumulative maternal methadone doses ranged from 4190 – 21270 mg (10167.1 ± 4499.6) (mean ± SD). Methadone and EDDP concentrations in meconium were 95 -17268 ng/g (5819.1 ± 5191.8) and 6375-80503 ng/g (37968.3 ± 19312.9), respectively. Infants displayed NAS peak scores of 3 to 19 (9 ± 5) and duration of 3 to 24 days (8.1 ± 7.0). Increased birth weight and head circumference were found in infants whose mothers were maintained on higher cumulative methadone doses during the 3rd trimester of gestation. There were no significant relationships between total maternal or cumulative trimester methadone doses, methadone and metabolite meconium concentrations and severity of NAS. We conclude that maternal total or cumulative trimester methadone dose does not correlate to the severity of neonatal withdrawal or the concentrations of methadone and metabolites in meconium. **Poster 9**

Expression of the Norepinepherine Transporter (NET) and Phenylethanolamine N-Methyltransferase (PNMT) in Normal Human Chromaffin Cells and n Pheochromocytoma

S. Cleary, F.M. Brouwers, G. Eisenhofer, K. Pacak, D.L. Christie, J. Lipski, A.R. McNeill, and J.K. Phillips

Graduate Student:
Susannah Cleary, NINDS
NIH Research Advisors:
Graeme Eiesenhofer and David Goldstein
University:
Murdoch University (Perth, Western Australia), Biomedical Sciences
University Research Advisor:
Jacqueline Phillips

Chromaffin cells of the adrenal medulla synthesize and secrete the catecholamines epinephrine and norepinepherine into the bloodstream. Two populations of chromaffin cells exist, based on the presence of the enzyme phenylethanolamine N-methyltransferase (PNMT), which mediates the conversion of norepinepherine to epinephrine. Those expressing PNMT are termed 'adrenergic', while those lacking PNMT are termed 'noradrenergic'. Pheochromocytoma represents the neoplastic transformation of chromaffin cells, characterized by increased production and secretion of catecholamines. In this study, NET and PNMT expression was examined immunohistochemically in normal adrenal chromaffin cells (n=5), and in pheochromocytomas (n=18). In normal tissue, NET was colocalised with PNMT and displayed a cytoplamic and punctate appearance. This contrasts with pheochromocytoma tissue, where NET was absent from three samples, and ranged from moderate (n=11) to strong (n=3). PNMT was detected in the majority of pheochromocytoma samples, but staining intensity did not correlate with tissue adrenaline content evaluated by HPLC. Unlike normal cells, in pheochromocytoma NET expression was not consistently colocalised with PNMT, suggesting a significant disruption to catecholaminergic synthsis pathways and uptake proteins in these tumors. **Poster 10**

Myosin VI in Fish Ears

Allison B. Coffin, Matthew W. Kelley, and Arthur N. Popper

Graduate Student:
Allison Coffin, NIDCD
NIH Research Advisor:
Matthew Kelley
University:
University of Maryland, Biology
University Research Advisor:
Arthur N. Popper

Myosin VI is an important hair cell protein, with myosin VI mutations resulting in hereditary deafness in humans and mice. Recent work in other labs has identified zebrafish (*Danio rerio*) myosin VI mutants that show physiological defects in mechanotransduction. These studies also show that there are two myosin VI paralogs expressed in zebrafish, *myo6a* and *myo6b*. Here, we characterize myosin VI expression and distribution in the ears of zebrafish and other fishes such as American shad (*Alosa sapidissima*) and oscar (*Astronotus ocellatus*). RT-PCR analysis of inner ear cDNA indicates that both myo6 paralogs are expressed in teleost inner ears. Indirect immunofluorescence shows that myo6 is found in at least some hair bundles of all species examined in this study. Myosin VI is distributed throughout the cytoplasm and hair bundles in all zebrafish inner ear hair cells, while it is not found in utricular hair bundles in other species. Our work adds depth to current studies of myo6-associated hereditary deafness and suggests that comparative studies between zebrafish and other fishes such as shad that differ in myo6 protein distribution will help elucidate the function of this critical hair cell protein. Comparisons between the two *myo6* paralogs will further aid in functional studies and shed light on hair cell evolution. **Poster 11**

Development of a Packaging System for rep+ Adeno-Associated Virus Gene Therapy Vectors

Anthony Craig and Roland Owens

Graduate Student:
Anthony Craig, NIDDK
NIH Research Advisor:
Roland Owens
University:
Howard University, Genetics
University Research Advisor:
Verle Headings

Adeno-associated virus serotype 2 (AAV2) is a human parvovirus. The AAV2 genome includes two inverted terminal repeats, the *rep* gene, and the *cap* gene. AAV2 integrates site-specifically into chromosome 19. However, to achieve site-specific integration the AAV2 *rep* gene is required. The *rep* gene encodes Rep proteins that form a bridge between the AAV2 genome and chromosome 19. The Rep proteins are also important for AAV2 replication. Current packaging systems for AAV2 vectors include a helper plasmid harboring the AAV2 *rep* and *cap* genes. The *cap* gene encodes the AAV2 capsid proteins. Most AAV2 vectors do not include the *rep* gene, because of the possibility of recombination with the helper plasmid that would lead to the production of wild-type AAV2. Removing the *rep* gene from the helper plasmid does not prevent recombination, because there is an overlap between the *rep* and *cap* genes that includes the p40 promoter, which drives expression of the *cap* gene. In order to package *rep*+ vectors, we mutated the region of the *rep* gene that includes the p40 promoter, rendering the promoter non-functional. We also placed the *cap* gene on a separate plasmid under the control of a CMV promoter. We have produced 10^{12} particles of a *rep*+ AAV2 vector. This vector should be safer than current randomly integrating vectors. **Poster 12**

Whole Body Biodistribution and Estimation of Radiation-Absorbed Doses of a Dopamine D1 Receptor Ligand [^{11}C]NNC-112 in Humans

Vanessa L. Cropley, Masahiro Fujita, Anil Ramachandran, Subroto Ghose, Janet Sangare, Jinsoo Hong, John L. Musachio, Victor W. Pike, and Robert B. Innis

Graduate Student:
Vanessa Cropley, NIMH
NIH Research Advisors:
Robert B. Innis and Masahiro Fujita
University:
Swinburne University
University Research Advisor:
Pradeep Nathan

Objectives: To perform kinetic whole body positron emission tomography (PET) imaging studies of [^{11}C]NNC-112, a dopamine D1 receptor radioligand, in human subjects to estimate radiation-absorbed doses of [^{11}C]NNC-112.

Methods: Seven healthy subjects underwent whole-body PET scans after injection of 19 ±2 mCi [^{11}C]NNC-112. 2D dynamic scans were acquired in seven segments of the body in frames of increasing duration, for a total scan time of 120 minutes. Regions of interest were drawn on compressed planar images of source organs to generate organ time-activity curves and residence times. Residence time of the urinary bladder was calculated from bladder activity using a dynamic bladder model. The organ residence times were used in OLINDA to obtain radiation dose estimates.

Results: Peak uptake in all source organs occurred within 5 minutes post [^{11}C]NNC-112 injection. Peak uptake was lungs: 28 percent, liver: 22 percent, brain: 7 percent, heart: 5 percent injected dose. Organs with the highest radiation-absorbed doses (rem/mCi) were the gall bladder (0.120), followed by the liver (0.0821), lungs (0.0626), kidneys (0.0614). Biexponential fitting of bladder activity showed that an average of 26 percent of activity was excreted via urine. The effective dose was 0.021 rem/mCi.

Conclusions: The estimated radiation-absorbed doses of [^{11}C]NNC-112 is quite low and would permit several PET scans in the same subject per year. **Poster 13**

Proteomic Analysis of Mouse Melanoma Tumor Progression

W. David Culp, Rachel Neal, Robert Massey, Pavel Pisa, and Donita Garland

Graduate Student:
W. David Culp, NEI
NIH Research Advisor:
Donita Garland
University:
Karolinska Institutet
Department of Oncology and Pathology
University Research Advisor:
Pavel Pisa

The ability of a tumor to evade an elicited immune response involves malignant cells, host infiltrating cells and complex biological processes such as angiogenesis. In a mouse melanoma model, immunotherapy that is successful 3 and 4 days post-tumor challenge, fails after 1 week of established tumor growth. To address the role of the tumor environment in tumor immune evasion, a comparative proteome analysis was performed on B16-F10 derived tumors in C57BL/6 mice from day 3 to day 10. To begin to identify the cellular origin of proteins that are differentially expressed in mature tumors, comparisons were made between tumors in C57BL/6 mice and B6 *RAG2*$^{-/-}$ mice (T and B cell deficient). At each time point multiple mice were used, and when possible, sample replicates were performed. Proteins were separated using 2D gel electrophoresis, and stained with colloidal Coomassie. Using Ludesi 2D Analyzer and Interpreter imaging software there were statistically significant differences in spot expression between experimental groups. Spots of interest were excised and identified by peptide mass finger printing and tandem mass spectrometry. This work identifies proteins involved in melanoma tumor progression and potential proteins associated with tumor immune evasion. **Poster 14**

Homodimer and Preferential Heterodimer Formation of the "Zinc Clasp" Domain in T-cell Specific Proteins CD4, CD8α, and Lck

Alisa M. Davis and Jeremy M. Berg

Graduate Student:
Alisa Davis, NIDDK
NIH Research Advisor:
Jeremy M. Berg
University:
Johns Hopkins University
Molecular and Computational Biophysics

The number of characterized protein domains that utilize zinc(II) for structural purposes has grown tremendously since the discovery of the "zinc finger" domain over two decades ago. Recently, a novel zinc motif, dubbed a "zinc clasp," was described (Kim, PW et al., *Science*, 301(5640), 19 Sep 2003, pp. 1725-1728). In this motif, it is not a single polypeptide chain utilizing the metal for structural stability, but two distinct proteins coordinating the metal. The C-terminal cytosolic domains of the T-cell coreceptors CD4 and CD8α have a *C-X-C* motif that interacts with the N-terminal *C-X-X-C* motif of the T-cell specific Src-family kinase, Lck. We have used cobalt(II) as a spectroscopic probe to study these interactions. Despite the lack of evidence for homodimerization of these domains from published NMR and ITC data (Kim PW et al., *Science*), we use visible spectroscopy to show that homodimerization of each domain does occur. Using principal component analysis, we have constructed visible absorption spectra for each of the homodimer species. In addition, heterodimer formation exhibits a unique spectroscopic feature, a 100-nanometer red-shift of one of the d-orbital transitions, distinguishing this species from the homodimer. **Poster 15**

Association in the HLA Region for Ulcerative Colitis

Betty Q. Doan, Lisa W. Datta, Joan E. Bailey-Wilson, Yin Y. Shugart, Steven R. Brant, and the NIDDK IBD Genetics Consortium

Graduate Student:
Betty Q. Doan, NHGRI
NIH Research Advisor:
Joan E. Bailey-Wilson
University:
Johns Hopkins Bloomberg School of Public Health, Epidemiology
University Research Advisor:
Yin Yao (Shugart)

Previous linkage studies have been identified a peak within the MHC/HLA region on chromosome 6p, named IBD3, for inflammatory bowel disease, and association studies have inconsistently shown various HLA alleles to be associated with inflammatory bowel disease. Because IBD3 consists of a dense cluster of immune genes, not only the HLA genes, we focused on the fine mapping of the IBD3 locus within the MHC/HLA complex on chromosome 6p to narrow the region of identified linkage. The study was limited to ulcerative colitis (UC), a subtype of IBD, to minimize disease heterogeneity. Forty microsatellite markers spanning the IBD3 region (with an average distance of about 0.08 cM) were genotyped in 450 UC father-mother-affected child and father-mother-affected children tetrads. Approximately 1,500 individuals were genotyped, and single point family-based association analysis was performed to test for the significant over transmission of particular alleles to affected offspring. Potential associated haplotypes that may confer an increased risk of developing UC were also assessed using haplotype reconstruction and family-based association tests. **Poster 16**

Inclusion of a Propensity Score as a Single Covariate in a Genome-wide Mega-Linkage Analysis Identifies New Significant Regions on 5q, 9p, 20p for Ulcerative Colitis and on 19q for Crohn's Disease

Betty Q. Doan, Constantine E. Frangakis, Joan E. Bailey-Wilson, Steven R. Brant, Yin Y. Shugart, and the NIDDK IBD Genetics Consortium

Graduate Student:
Betty Q. Doan, NHGRI
NIH Research Advisor:
Joan E. Bailey-Wilson
University:
Johns Hopkins Bloomberg School of Public Health, Epidemiology
University Research Advisor:
Yin Yao

A covariate-based linkage analysis was performed on a dataset of five published genome wide scans for inflammatory bowel disease (IBD), consisting of Crohn's disease (CD) and ulcerative colitis (UC) subtypes. Age at diagnosis, gender, and smoking status at exposure were analyzed as covariates as they are known risk factors. Using multiple covariates, however, may dampen the power gained or increase the type I error rate, so a subtype-specific propensity score (PS), which collapses multiple covariates into one, was also evaluated. Analytical methods included no covariates, each individual covariate, multiple PS definitions, and multiple covariates analyzed simultaneously. The IBD3 locus on chromosome 6p resulted in the greatest evidence of linkage ($p<0.005$) for IBD and CD, while the 2p locus was the most significant for UC ($p<0.005$). The incorporation of smoking status suggest an interaction with the 5p locus, and a PS using age at diagnosis and gender identified a new locus on 19q for CD, and loci on 5q, 9p, and 20p for UC. These regions have been previously identified, but not consistently. The results suggest that the use of covariates can increase the power to detect linkage for complex diseases, especially when important covariates can be taken into account to model disease heterogeneity. **Poster 17**

Towards A Better Understanding of Immune Correlates of Protection: An Analysis of CD4+ T Cell Function in HIV-1- and HIV-2-Infected Gambians

Melody G. Duvall, Assan Jaye, Tao Dong, Jason M. Brenchley, Marianne van der Sande, Samuel J. McConkey, Hilton C. Whittle, Richard A. Koup, and Sarah L. Rowland-Jones

Graduate Student:
Melody G. Duvall, NIAID/VRC
NIH Research Advisor:
Richard A. Koup
University:
University of Oxford, Biomedical Sciences
University Research Advisor:
Sarah L. Rowland-Jones

Virus-specific CD4+ T cells play a key role in immunity to chronic viral infection, but HIV-specific responses are characteristically absent in chronic HIV-1 infection. We conducted a study in The Gambia to investigate whether preserved HIV-2-specific T-cell responses could be linked with the better clinical outcome described in HIV-2 infection. HIV-specific CD4+ T-cell responses were measured in 40 HIV-1+, 26 HIV-2+ and 23 HIV seronegative donors using stimulation with overlapping HIV-1 or HIV-2 gag peptides and intracytoplasmic staining for IFN-γ and IL-2. Stimulation of PBMC from asymptomatic HIV-2+ individuals (CD4 count >28 percent) induced the production of both IFN-γ and IL-2 by a significantly greater proportion of CD4+ T cells compared to asymptomatic HIV-1+ individuals. At CD4 counts <28 percent, this difference was no longer seen. We also detected a population of HIV-2-specific CD4+ T cells that produced both IFN-γ and IL-2, which was absent in most HIV-1 infected individuals studied ($p<0.0001$). Thus, HIV-2 infected individuals with relatively preserved immune status maintain a CD4+ T-cell response that is greater in both magnitude and breadth than CD4-matched HIV-1 infected individuals, supporting the hypothesis that control of viral replication is due to a more efficient host immune response to HIV-2. **Poster 18**

The Biglycan/fibromodulin Double Knockout Mice Develop Accelerated Temporomandibular Joint Osteoarthritis

Mildred C. Embree, Sunil Wadhwa, Laurent G. Ameye, and Marian F. Young

Graduate Student:
Millie Embree, NIDCR
NIH Research Advisor:
Marian Young
University:
Medical University of South Carolina Dental Medical Scientist Training Program
University Research Advisor:
Steve London

Biglycan (BGN) and fibromodulin (FBN) are proteoglycans highly expressed in cartilage and bone. Our goal was to analyze the temporomandibular joint (TMJ) in mice doubly deficient in BGN and FBN to determine whether they acquire TMJ osteoarthritis (OA). Immunohistochemistry was used to localize BGN and FBM in mouse WT TMJ. TMJ Histological sections from WT and DKO mice at 3, 6, 9, and 18 months were compared. The proliferation of condylar cartilage cells was analyzed using proliferating cellular nuclear antigen (PCNA) staining. BGN and FBM were co-ordinately expressed in the articular cartilage of the WT mouse TMJ at 3 months. At this age, PCNA staining showed a decrease in condylar chondrocyte proliferation in the DKO when compared to the WT mice, but no histological differences in the tissue structure integrity were noted. At 6 months, formation of vertical clefts, acellular regions, and cellular clusters in the cartilage were observed in the DKO mice. By 9 months, vertical clefts extended deeper in the cartilage and nearly all cartilage cells formed clusters in the DKO. By 18 months, condylar cartilage integrity was lost in the DKO. Mice deficient in BGN and FBM develop accelerated TMJ OA. The DKO mouse line could be used to understand the underlying molecular mechanisms of the disease. **Poster 19**

Functional Anatomical Network Involved in Habituation of Hemodynamic Responses to Facially Expressed Emotion

T.A. Ferguson, C. Gautier, W. Bogers, T. Lowry, J.L. Price, P. Greer, D.J. Kupfer, and W.C. Drevets

Graduate Student:
Teresa Ferguson, NIMH
NIH Research Advisor:
Wayne Drevets
University:
Karolinska Institute Department of Clinical Neuroscience
University Research Advisor:
Arne Öhman

Functional imaging studies have shown the hemodynamic response of the amygdala to fearful or happy face stimuli rapidly habituates to repeated exposures of the same stimuli. Habituation of neurophysiological responses to facially expressed emotion has not been sensitively assessed in the extended anatomical network of visual association areas and limbic structures. This study investigated these processes through PET imaging of cerebral blood flow in 16 healthy humans as they viewed two blocks each of face stimuli with fearful, sad, happy, or neutral expressions.

CBF responses in the amygdala showed rapid habituation during re-exposure to the same fearful, happy, sad, or neutral face stimuli. In addition, habituation of amygdala CBF response to all expressions occurred most prominently in the left dorsal amygdala. These data showed rapid habituation of CBF responses to all expressions in visual and polymodal association areas of inferotemporal, lateral orbital, temporopolar and prefrontal cortices that share extensive anatomical connections with the amygdala. These regions play roles in modulating behavioral and autonomic responses to threatening stimuli in experimental animals, suggesting they are recruited during repeated exposure to nonreinforced, potentially threatening stimuli in humans. **Poster 20**

Antibodies to Outer Membrane Proteins of Both Infectious Types of Vaccinia Virus are Necessary to Protect Mice Against a Lethal Intransasal Challenge

Christiana Fogg, Shlomo Lustig, J. Charles Whitbeck, Roselyn J. Eisenberg, Gary H. Cohen, and Bernard Moss

Graduate Student:
Christiana Fogg, NIAID
NIH Research Advisor:
Bernard Moss
University:
University of Maryland–College Park Cell Biology and Molecular Genetics
University Research Advisor:
Anne Simon

The use of the licensed live vaccinia virus vaccine during recent smallpox vaccination of healthcare workers and members of the military has brought attention to the complications associated with vaccination. Vaccinia virus immune gamma globulin (VIG) from recently vaccinated human donors has been used to treat patients suffering from complications, but a safer and inexhaustible source of VIG is more desirable. The selection of viral antigens for the development of an alternative VIG has been aided by recent immunization studies with recombinant proteins or DNA encoding proteins of two infectious forms of virus, intracellular mature virus (IMV) and extracellular virus (EV). We studied the protection of BALB/c passively immunized with polyclonal (PAbs) or monoclonal antibodies (MAbs) to the EV proteins, A33 and B5, and the IMV protein L1. Mice that were passively immunized IP and challenged IN with the WR strain of vaccinia virus were partially protected from weight loss and death. However, mice receiving a combination of A33+L1, B5+L1, or A33+B5+L1 showed superior protection after challenge. Mice receiving PAb to A33, B5 or L1 were protected from weight loss and death following challenge, and mice given A33+L1, B5+L1, or all three PAbs were better protected than those given any of the individual PAbs. These results confirm previous observations that enhanced protection is associated with immunity to both forms of infectious virus, and, mice can be protected from vaccinia virus infection by a combination of well-characterized Abs. **Poster 21**

The Role of Regulated Degradation of Chemoattractants during Directed Cell Migration

Gene L. Garcia and Carole A. Parent

Graduate Student:
Gene Garcia, NCI-CCR
NIH Research Advisor:
Carole Parent
University:
Johns Hopkins University
Biology

The social amoebae *Dictyostelium discoidium* live as individual cells under favorable conditions. Upon starvation these cells signal to each other and migrate to secreted cyclic adenosine monophosphate (cAMP), leading to their aggregation and differentiation into spore and stalk cells. Regulated degradation of the cAMP allows chemotaxing cells to respond quickly and persistently to the signal by preventing the accumulation of the cAMP attractant that leads to receptor saturation and loss of sensitivity. The molecule responsible for this breakdown is an extracellular cyclic nucleotide phosphodiesterase called PdsA. It has been shown that cells lacking PdsA exhibit defects in aggregation and differentiation. We now show that cell density as well as plating media influences these defects. Moreover, western blot analysis has established that key signaling molecules have lower protein levels in cells lacking PdsA than in wild type cells. In order to gain more insight into the role of PdsA during chemotaxis we have fused GFP to PdsA and are currently studying the distribution of the fusion protein in live cells. This study on the regulation of cAMP by PdsA will undoubtedly help decipher the complex signaling machinery that is involved in regulating chemotaxis. **Poster 22**

Duke Nukem 3-D: Video Games, Virtual Reality, and Spatial Memory in Patients with Mood Disorders

N. F. Gould, M. K. Holmes, D.S. Pine, N. Burgess, H. K. Manji, and C.A. Zarate Jr.

Graduate Student:
Neda F. Gould, NIMH
NIH Research Advisors:
Carlos A. Zarate and Husseini K. Manji
University:
George Washington University
Clinical Psychology
University Research Advisor:
Rolf Peterson

Spatial and other memory deficits have been observed in patients with mood disorders through neuropsychological testing. Traditional spatial memory neuropsychological tasks do not provide scenarios similar to what patients would encounter in their daily lives. Virtual reality-based navigation tasks may provide a more useful measure of spatial memory. Subjects are administered a spatial-memory-guided navigation task (Duke Nukem 3D) using a computer generated town, and later tested on their memory of the town. Fourteen unipolar patients, 20 bipolar depressed patients, and 17 normal controls have been tested thus far. Controls found a mean of 3.82 ± 2.10 locations. Bipolars found a mean of 2.63 ± 1.37 locations. Unipolars found a mean of 2.12 ± 2.14 locations. One-way ANOVA revealed that unipolar and bipolar subjects performed significantly worse than controls ($p < .05$), as measured by number of locations found. Spatial memory performance on this task may represent a quantifiable endophenotypic measure to assess possible hippocampal deficits. This hypothesis will be further tested by correlating performance with neuroimaging measures such as hippocampal volume. In future experiments we will assess whether changes in spatial memory are state dependent as effects of medication on performance are assessed. **Poster 23**

Attenuation of Methamphetamine (METH)-Induced Monoaminergic Toxicity in Rats with Prior Exposure to Increasing METH Doses

D.L. Graham, P.-A.H. Noailles, K.G. Becker, W.H. Wood III, B. Ladenheim, T.H. Moran, and J.L. Cadet

Graduate Student:
Devon L. Graham, NIDA
NIH Research Advisor:
Jean Lud Cadet
University:
University of Maryland School of Medicine, Toxicology
University Research Advisor:
Katherine Squibb

Methamphetamine (METH) is an illicit drug with neurotoxic properties. Repeated administration of the drug causes behavioral tolerance, which leads to increases in the amount of drug self-administered by human addicts in order for them to experience euphoric effects. It is not clear if administration of the drug according to such a schedule would cause toxicity in animals. The present study was carried out to test such a possibility. Adult male Sprague-Dawley rats were injected with METH or saline according to an escalating dose (ED) schedule for 2 weeks, followed by a challenge with either saline or METH (10 mg/kg every 2 hrs X 3). Brain regions were extracted at 2 or 24 hrs following the last injection. HPLC analyses show that METH causes significant depletion of dopamine (DA) and serotonin (5-HT) in the striatum of saline-treated rats. In contrast, there was significant attenuation of the toxic effects of METH in animals pre-treated according to the ED METH schedule. These results suggested that the ED schedule causes induction of protective mechanisms or suppression of protoxic events that render the animals resistant to METH toxicity. The molecular underpinnings of these pathways are being evaluated by using large-scale gene expression techniques. **Poster 24**

Delayed Onset of a Wild Type Electrical Potential in the Retina of Ames Waltzer Pcdh15$^{av\text{-}5J}$ Mice

Ricky J.L. Haywood-Watson II, Zubair M. Ahmed, Sten Kjellstrom, Ronald A. Bush, Yuichiro Takada, Kumar Alagramam, Lori L. Hampton, James F. Battey, Paul A. Sieving, and Thomas B. Friedman

Graduate Student:
Ricky J. L. Haywood-Watson II, NIDCD
NIH Research Advisor:
Thomas B. Friedman
University:
Tulane University
Molecular and Cellular Biology
University Research Advisor:
Meredith M. Garcia

Usher syndrome (USH) affects an estimated 1/16,000 to 1/50,000 people worldwide and has been reported to underlie a majority of deaf-blindness. There are three clinical subtypes of Usher syndrome, which is a recessively inherited neurosensory disorder. Usher syndrome type 1 is characterized by profound congenital deafness, retinitis pigmentosa, and vestibular abnormalities in humans. Protein truncating mutations in the gene encoding protocadherin 15 *(PCDH15)* cause Usher syndrome type 1 (USH1F) while less disabling mutant alleles of *PCDH15* are associated with nonsyndromic recessive hearing loss (DFNB23). This suggests that the retina is somehow resistant to the effects of altered or reduced levels of protocadherin 15 due to particular missense mutations, which nevertheless cause profound deafness. In the Ames waltzer *(av)* mouse, recessive mutations of *Pcdh15* are associated with profound deafness and circling behavior. Pcdh15^{nmf19} was initially identified through complementation testing at the Jackson Laboratory and found to be an allele of Ames waltzer and recently characterized at the molecular level. In this study of Pcdh15^{nmf19} we extend the characterization of the auditory phenotype and initiate a study of the retinal phenotype of these mice. **Poster 25**

siRNA Technology Modulates the Expression of T Cell Receptors from Clones Specific to Myelin Basic Protein

Brenna J. Hill, Laura E. Ruff, Daniel M. Altmann, and Daniel C. Douek

Graduate Student:
Brenna J. Hill, NIAID/VRC
NIH Research Advisor:
Daniel Douek
University:
George Washington University
Genetics Program

Multiple sclerosis (MS), an autoimmune disease affecting over 1 million people worldwide, is characterized by demyelination of the central nervous system (CNS). MS begins with an acute inflammatory phase responsive to immune therapy and progresses into a chronic neurodegenerative phase less responsive to immune modulation. The inflammatory phase of MS occurs when activated, oligoclonal myelin-specific T cells present in healthy non-affected individuals enter the CNS, destroying large portions of the myelin sheath mainly targeting myelin basic protein (MBP) and proteolipid protein (PLP).

In 1998, the phenomenon of RNA interference was described and is now a powerful tool for molecular biologists. In our present study, we aim to utilize siRNA specific to T cell receptors (TCRs) reactive to $MBP_{83\text{-}99}$ peptide antigen. We will knock down expression of myelin specific TCR and measure T cell responsiveness by multiparameter flow cytometry.

Results from initial tests of our siRNAs show specific knockdown of TCR mRNA. We have cloned our siRNA constructs into lentiviral vectors, transduced them into a T cell hybridoma clonally expressing the $MBP_{83\text{-}99}$-specific TCR $\alpha\beta$ heterodimer. We are currently characterizing constitutive knock down of TCR mRNA and T cell function by real-time polymerase chain reaction and flow cytometry. **Poster 26**

Regional Decreases in Brain Volume in Schizophrenia: A Meta-Analysis of Voxel-Based Morphometry Studies

Robyn Honea, Tim Crow, Dick Passingham, and Clare Mackay

Graduate Student:
Robyn Allyson Honea, NIMH
NIH Research Advisors:
Daniel Weinberger and Joe Callicott
University:
Oxford University
Psychiatry and Experimental Psychology
University Research Advisors:
Dick Passingham and Tim Crow

Voxel-based morphometry (VBM) is a method for detecting group differences in the density or volume of brain matter. We investigated its capabilities for clearly identifying specific structural differences in patients compared with controls by reviewing all VBM studies published up to May 2004. The study looked for consistently reported results of gray and white matter decreases in schizophrenia and evaluated VBM to propose a future strategy for using VBM in schizophrenia research. We reviewed 15 studies, which included 390 patients diagnosed with schizophrenia and 364 normal volunteers. We identified the regions that were reported to be reduced in patients relative to controls. Fifty regions were reported decreased in patients with schizophrenia, and the most consistent results were the left superior temporal gyrus (STG), and the left medial temporal lobe (MTL). Use of a lower smoothing kernel (4–8 mm) lead to detection of a greater number of regions implicated in schizophrenia. This review implicates the left STG and the left MTL as key regions of structural difference in patients with schizophrenia. The diversity of regions reported is in part due to choice of variables in the automated process, such as smoothing kernel size, linear versus affine transformation, as well as differing patient groups. **Poster 27**

Effect of Surface Electrical Stimulation on Hyo-laryngeal Movement in Healthy Individuals at Rest and during Swallowing

I. Humbert, C. Ludlow, W. Wright-Harp, J. Payne, and O. Harris

Graduate Student:
Ianessa A. Humbert, NINDS
NIH Research Advisor:
Christy Ludlow
University:
Howard University
Communication Sciences and Disorders
University Research Advisor:
Wilhelmina Wright-Harp

Dysphagia, or swallowing dysfunction, is commonly associated with neurologic diseases and disorders. Approximately 50 percent of patients with dysphagia present with reduced laryngeal elevation and intrinsic closure (Lundy, 1999), which may result in aspiration pneumonia if ingested substances enter the lungs (Kahrilas PJ, 1997). Surface electrical stimulation (SES) to therapeutically aid swallowing has been gaining attention, but few studies have been conducted to understand the physiological bases of this new treatment modality. This study aims to determine if SES causes hyo-laryngeal elevation or vocal fold movement at rest or reduced pharyngeal transit time during swallowing in healthy individuals. Twenty-eight healthy volunteers between 20 and 60 will undergo SES during videofluoroscopy and nasoendoscopy. SES to the laryngeal region and/or submental region is not expected to not cause elevation of the larynx and/or hyoid bone or movement of the vocal folds. If the hyo-laryngeal complex is pulled down with stimulation, stimulation concurrent with swallowing might cause hyoid and laryngeal depression that could be initially detrimental or unsafe during swallowing. Preliminary data in patients has shown SES over the larynx lowered the hyoid bone by 20 mm from the resting position (Ludlow, in progress). **Poster 28**

Determining Mutation Profiles of the NCI-60 Human Cancer Cell Lines for Correlation with Gene Expression and Drug Activity

O. Ikediobi, R. Wooster, U. Shankavaram, A. Futreal, M. Stratton, J.N. Weinstein, and D. Bentley

Graduate Student:
Ogechi Ikediobi, NCI
NIH Research Advisor:
John Weinstein
University:
Cambridge University
Health Sciences
University Research Advisor:
Richard Wooster

The NCI-60, a diverse set of human cancer cell lines comprising nine different cancer types, has been used by the Developmental Therapeutics Program of the NCI to screen >100,000 chemical compounds for anti-cancer activity. The Weinstein Laboratory and its collaborators have profiled the NCI-60 cells at the protein, RNA, and DNA levels, then correlated their gene expression patterns with chemosensitivity and resistance to >100,000 potential anti-cancer compounds. The DNA studies have included array CGH for copy number, bisulfite sequencing for methylation silencing, and SKY for chromosomal aberrations. What is lacking is genetic characterization of the cell at the level of DNA sequence. Therefore, we are analyzing the NCI-60 for the mutation profiles of 100 genes causally implicated in tumorigenesis or the efficacy or metabolism of anti-cancer drugs. All exons and exon-intron boundaries are being amplified and sequenced. Sequence changes are detected using the Mutation Surveyor™ software. In the initial phase of the project, we have analyzed the following genes: APC, BRCA1, CDH1, FGFR2, MAP2K4, PDGFRA, RB1, STK11, VHL, BMPR1A, BRCA2, CTNNB1, MADH4, MET, PTEN, RET, and TP53. Their mutational state is being correlated with the drug activity patterns and gene expression profiles of the cell lines. **Poster 29**

Classification of Splice Sites Using Adaboost with Decision Trees

Rezarta Islamaj and W. John Wilbur

Graduate Student:
Rezarta Islamaj, NLM/NCBI
NIH Research Advisor:
W. John Wilbur
University:
University of Maryland–College Park
Computer Science
University Research Advisor:
Lise Getoor

Splice sites have been modeled by a variety of methods over the past twenty years. Still the search for improvement continues as splice site detection is a key ingredient for accurate gene finding. In this work, we improve on Adaboost with decision trees splice site model by Kim and Wilbur.

Observing that on a random training set the Adaboost model had only a slight improvement over other methods in literature, we changed the training medium to emphasize the most confusable data and we retrained Kim and Wilbur's model. This model uses 23 nucleotides from the non-coding part of the splice site, the consensus nucleotides AG and 25 nucleotides from the coding part. We compared test results to those from GeneSplicer by Pertea et al. on the same data set. Both methods performed similarly. Finally, we modified the Adaboost model to use a site model of 80-AG-80 as in GeneSplicer. This model outperformed both previous methods significantly.

Our results show that combining boosted decision trees as a modeling framework with information from a larger non-coding and coding region results in the best performance. Currently, we are continuing our experiments to put our results in perspective and examining possibilities of improvement. **Poster 30**

Reverse and Rational Engineering of Mammalian Cells: A Microarray Approach

Pratik Jaluria, Nicole Bleckwenn, Renee Rubio, Konstantinos Konstantopoulos, Michael Betenbaugh, and Joseph Shiloach

Graduate Student:
Pratik Jaluria, NIDDK
NIH Research Advisor:
Joseph Shiloach
University:
Johns Hopkins University
Chemical and Biomolecular Engineering
University Research Advisors:
Michael Betenbaugh and Konstantinos Konstantopoulos

Using both cDNA and oligonucleotide microarrays we identified variations in gene expression between similarly derived, but different phenotypic HeLa cell lines. The study concentrated on comparing attachment-dependent with attachment-independent HeLa cells; both derived from a common ancestral cell line. Initial work focused on growth characterization in controlled environments, enabling us to prepare samples with cells properly aligned in the same phase of growth; a key prerequisite for meaningful microarray analysis. The microarray data was first normalized using statistical software, and then filtered to scour the genome for likely candidates for further investigation. Using this approach, the following six genes were identified as potential targets for further study: siat7a, cdkl3, cox15, lama4, endogl1, and tsarg2. Focusing on siat7a, a Type II membrane glycosylating sialyltransferase, we were able to successfully alter the physiology of the attachment-independent HeLa cells using siRNA. We believe our strategy of applying bioinformatics techniques to characterize and manipulate phenotypic behaviors is applicable to a plethora of cellular functions with specific objectives in mind. **Poster 31**

Disposition of Cocaine and Metabolites in Human Sweat Following Controlled Cocaine Administration

Sherri L. Kacinko, Allan J. Barnes, Eugene W. Schwilke, Edward J. Cone, Eric T. Moolchan, and Marilyn A. Huestis

Graduate Student:
Sherri L. Kacinko, NIDA
NIH Research Advisor:
Marilyn Huestis
University:
University of Maryland–Baltimore
Toxicology
University Research Advisor:
Barry Levine

Fourteen volunteers received three doses subcutaneous cocaine HCl of 75 mg/70kg within 1 week and, 3 weeks later, nine received three 150 mg/70kg doses. PharmChek® sweat patches were collected during a 3-week washout period, and during and after dosing. Almost 25 percent of patches were positive for cocaine (COC) at the method's limit of quantification of 2.5 ng/patch and 7 percent were positive at the proposed Substance Abuse and Mental Health Services Administration (SAMHSA) 25 ng/patch cutoff. Ecgonine methyl ester (EME) generally was detected more often and at higher concentrations than benzoylecgonine (BE). Two weekly washout had concentrations greater than 25 ng/patch during washout week 1; one patch remained positive for washout week 2. COC and EME were detected in sweat within 1 to 2 hours. BE was not detected until 4 to 8 hours after the low dose, 2 to 4 hours after the high dose. Most drug excretion occurred within 24 hours; increased patch wear time was needed to detect drug after day 1. Over 70 percent of patches worn for 1 week during low doses were positive for COC at the proposed SAMHSA cutoff, increasing to 100 percent during high doses. The week after the last low dose, a single patch had COC and BE concentrations above 25 ng/patch. These data will improve the interpretation of sweat test results. **Poster 32**

Osteoblasts Inhibit Osteogenesis in Mesenchymal Stem Cells *in vitro*

Catherine M. Kolf, Lin Song, and Rocky S. Tuan

Graduate Student:
Catherine M. Kolf, NIAMS
NIH Research Advisor:
Rocky Tuan
University:
Johns Hopkins University
Biology

A fundamental question in developmental biology concerns the mechanism of cellular differentiation in an organism as a function of the cellular composition of surrounding tissue. It is thought that direct contact between stem cells and their neighboring cells may be crucial to the maturation program of the former. Mesenchymal stem cells (MSCs), isolated from adult bone marrow, differentiate into mature bone, cartilage, tendon, and fat cells. These processes of differentiation can be mimicked *in vitro* using soluble growth factors. Since osteoblasts (OBs) make up a large portion of the bone marrow, it is probable that MSCs are frequently in contact with them. To test their effect(s) on the differentiation potential of MSCs, the two cell types were cultured together for 14 days. Differentiation of MSCs to OBs was monitored every 2 days by measuring osteocalcin levels through luciferase reporter assays, RT-PCR, and immunocytochemistry. Our preliminary data indicate an overall trend of OB inhibition of MSC osteogenesis. This is logical considering the oncogenic consequences of over-stimulating these cells with bone-forming potential. Future work will focus on determining what factors are engaged in this cell-cell contact and how they function to mediate MSC plasticity. **Poster 33**

Laminins are Involved in Neural Stem Cell Maintenance

J.D. Lathia, Z. Wang, S.J. Gossage, Y. Luo, M.A. Caldwell, C. ffrench-Constant, and M.S. Rao

Graduate Student:
Justin D. Lathia, NIA
NIH Research Advisor:
M. S. Rao
University:
University of Cambridge, Department of Pathology and Centre for Brain Repair
University Research Advisors:
M.A. Caldwell and C. ffrench-Constant

During the development of the nervous system neural stem cells reside in distinct microenvironments, or niches, such as the subventricular zone (SVZ). Extracellular matrix (ECM) molecules are important regulators in other stem cell niches such as the mammalian testes, ovaries, and skin. We hypothesize that ECM molecules are therefore involved in neural stem cell maintenance and focused on the role of laminins. Laminin alpha 2 is expressed in the embryonic SVZ while fibronectin is expressed widely and laminin-1 is restricted to the blood vessels.

We assessed the ability of these ECM molecules in the media to enhance neurosphere formation. Laminin-2 increased neurosphere formation by fourfold, while laminin-1 inhibited neurosphere formation. We evaluated the gene expression profile of neurospheres exposed to laminin-2 or fibronectin using cDNA microarrays. The gene expression profile on fibronectin suggested differentiation with the upregulation of differentiation markers and the downregulation of stem and precursor markers. However, the gene expression profile on laminin-2 showed an upregulation of stem and precursor markers such. The location of laminins in the SVZ and the ability of laminin-2 to promote neural stem cell maintenance suggests that laminins are involved in neural stem cell maintenance. **Poster 34**

Effects of Co-Culture with Micro- and Macrovascular Endothelial Cells on Mesenchymal Stem Cell Differentiation

Thomas Lozito, Catherine K. Kuo, Juan M. Taboas, Anna Tickler, Cait MacPhee, Christopher Dobson, and Rocky S. Tuan

Graduate Student:
Thomas Lozito, NIAMS
NIH Research Advisor:
Rocky S. Tuan
University:
University of Cambridge Biological Physics
University Research Advisors:
Christopher Dobson and Cait MacPhee

Vascular tissue engineering requires the generation of vessel-like structures that can mimic both macro- and microvascular blood vessels. The former consists of an inner layer of endothelial cells surrounded by smooth muscle cells, while the latter lacks a smooth muscle layer. Furthermore, the endothelial cells themselves express different markers depending on the type of blood vessel in which they reside. It has been shown previously that mesenchymal stem cells (MSCs) can be differentiated into endothelial cells and smooth muscle cells through soluble factors. Our studies focus on the potential influence of cell-cell contact on the differentiation of MSCs into these cell types. We hypothesize that cell-cell contact between MSCs and microvascular endothelial cells will push the MSCs to differentiate into endothelial cells, while cell-cell contact with macrovascular endothelial cells will induce the MSCs to differentiate into smooth muscle cells. Adult human MSCs were co-cultured with either micro- or macrovascular endothelial cells and analyzed for both endothelial and smooth muscle cell markers. Elucidating the contribution of MSCs in vasculargenesis should facilitate the fabrication of improved engineered blood vessels. **Poster 35**

Can a Single Scale Factor Be Used to Scale Femur Bone Models?

Weidong Luo, Frances Sheehan, and Zohara Cohen

Graduate Student:
Weidong Luo, CC
NIH Research Advisor:
Frances Sheehan
University:
The Catholic University of America Physics
University Research Advisor:
Robin Selinger

This study aimed to determine the relationship between femur dimensions in order to determine the number of scale factors that are necessary to match a template to an individual. Six femur lengths were obtained for each of 62 femurs. Significant (p-values <0.0001) correlation values (R) relating each of the five dependent measures to the palpable length ranged from 0.50 for (depth) to 0.96 (shaft length). The 95 percent confidence intervals were narrow and the lower bounds found for R were never lower than 0.29 and the upper bounds were as high as 0.98. The strong correlations for the three length measurements indicate individual femoral sections change size proportionally to changes in femoral length. The width and depth correlations indicate the changes in the two orthogonal dimensions are also proportional to the superior-inferior femoral dimension. The linear regression model proved valid as all models demonstrated significance (t-value $>>2$ and significance $<<0.01$). The important finding was that the regression coefficients for the normalized data were all the same, indicating all five parameters vary with the palpable length at the same rate. Thus, when scaling a model femur to match an individual femur, a single scale factor is sufficient to match the three sections and three dimensions of the bone. **Poster 36**

Sildenafil Citrate (Viagra®) As A Treatment for Neurodegeneration in the Mouse Model of Multiple Sclerosis

Stephanie C. Manson, Elaine K. Jordan, Paul M. Matthews, and Joseph A. Frank

Graduate Student:
Stephanie Manson, CC
NIH Research Advisor:
Joe Frank
University:
Oxford University
Biomedical Sciences
University Research Advisor:
Paul Matthews

Evidence from neurological disorders, in particular stroke, indicates that sildenafil citrate (Viagra®) has potential as a neuroprotective agent. Neurodegeneration plays a significant role in multiple sclerosis (MS). Therefore, using the mouse model of MS (Experimental Allergic Encephalomyelitis, EAE), we tested whether sildenafil might have a neuroprotective effect in this disease model. Eighty mice were induced with EAE, and treatment with sildenafil or placebo was started at day 7 in concurrence with the start of neurological symptoms. For the first 80 days during the relapsing-remitting phase of the disease, there was no statistical difference in disease course between the placebo and treatment mice. However, during the progressive phase of the disease when the placebo mice started to accumulate disability, the mice treated with sildenafil continued with a relapsing remitting disease course with minimal disability during periods of remission. This indicates that sildenafil potentially provides neuroprotection in MS and could be indicated as a treatment for patients with progressive MS. **Poster 37**

Regulation of Dictyostelium Development by Presenilin and GSK3 Signaling

V. C. McMains and A.R. Kimmel

Graduate Student:
Vanessa McMains, NIDDK
NIH Research Advisor:
Alan Kimmel
University:
Johns Hopkins University
Biology

Dictyostelium grow as single-cells that initiate multicellular development upon starvation. During development, single cells form multicellular aggregates, which ultimately differentiate into spore and stalk cells. Aggregation and cell differentiation are regulated by receptor-mediated responses to secreted cAMP. The cAMP receptors CAR3 and CAR4 respectively direct prespore or prestalk fate choice by antagonistically regulating GSK3, a serine/threonine protein kinase. GSK3 has been shown to physically interact with Presenilin (PS) in other organisms, and we have shown that Presenilin 2 (PS2) regulates *Dictyostelium* development. However, it is not clear if PS- and GSK3-signaling pathways intersect. To determine if GSK3 and PS2 interact genetically to regulate cell fate choice in *Dictyostelium*, I created two double mutant strains, car3/ps2- and car4/ps2-nulls. We are particularly interested if loss of PS2 rescues (suppresses) either the car3- or car4-null phenotype or further impairs (enhances) their phenotypes, reciprocally. This would provide initial evidence that PS2 and the CARs cooperate during cell fate determination. I have begun comparative analyses of the developmental phenotypes and gene expression patterns of these mutants, which will clarify how PS2 controls prespore/prestalk selection. **Poster 38**

Fluorescence Correlation Spectroscopy Study of Probe Diffusion in Poly (Vinyl Alcohol) Solutions and Gels

A. Michelman-Ribeiro, H. Boukari, R. Nossal, and F. Horkay

Graduate Student:
Ariel Michelman-Ribeiro, NICHD
NIH Research Advisor:
Ralph Nossal
University:
Boston University, Physics
University Research Advisor:
Rama Bansil

Transport properties of small particles in polymer systems are important in designing tissue scaffolds and drug delivery devices. In this study, we have applied fluorescence correlation spectroscopy (FCS) to measure the diffusion of fluorescent probe particles (TAMRA (Mw = 430), TAMRA-labeled dextran (Mw = 10k)) in non-fluorescent semi-dilute PVA solutions and PVA gels over a range of polymer concentrations (1 to 8.6 percent w/v) and cross-link densities (1/400 to 1/50 monomers per cross-link units). We find a linear dependence of the diffusion time on the concentration of polymer, with the slope depending on particle size. The measurements indicate that for a fixed polymer concentration, the diffusion of the particles decreases when the polymer solution is cross-linked. The more the polymer chains are cross-linked, the slower the probe particles diffuse. We attribute this effect to the formation of large-scale structural changes caused by cross-linking of the PVA chains. These results suggest that the cross-link density is an important parameter when assessing and analyzing probe diffusion data in gels. Measurements of the elastic modulus support this conclusion, as indicated by the linear correlation between the diffusion time of the particles and the elastic modulus of the weakly cross-linked gels. **Poster 39**

A PH Domain-containing Protein Complex Is Formed at the Plasma Membrane in Response to Chemoattractant Stimulation

Vassil A. Mihaylov, Frank I. Comer, and Carole A. Parent

Graduate Student:
Vassil A. Mihaylov, NCI-CCR
NIH Research Advisor:
Carole A Parent
University:
Medical University of Sofia, Bulgaria
Chemistry and Biochemistry

Chemotaxis, the ability of cells to sense external chemical signals and respond by migrating towards them, is fundamental to many biological functions. Investigations in *Dictyostelium discoideum* and neutrophils have established that PH domain-containing proteins, such as CRAC (Cytosolic Regulator of Adenylyl Cyclase) and Akt/PKB, translocate specifically to the leading edge of chemotaxing cells. CRAC is involved in regulating chemotaxis and is essential for the chemoattractant-mediated activation of adenylyl cyclase in *Dictyostelium*.

To investigate the mechanisms of CRAC signaling we employed *in vivo* chemical crosslinking to show that CRAC interacts with other signaling components. We demonstrate that, upon stimulation with chemoattractant, activated CRAC forms high molecular weight protein complexes at the plasma membrane. Using His-tagged CRAC we were able to purify these complexes by immobilized metal ion affinity chromatography. Mass spectrometric analysis is currently under way to identify the components of these CRAC containing complexes. We are also mapping these interactions to specific domains of CRAC using a variety of CRAC deletion mutants. These studies will provide valuable insight into the complex signals that regulate chemotaxis. **Poster 40**

Role of Noradrenergic Mechanisms in Sustained Attention, Impulse Control, and Effects of Methylphenidate in Rats

J.A. Milstein, O. Lehmann, D.E.H. Theobald, J.W. Dalley, J.R. Walters, and T.W. Robbins

Graduate Student:
Jean A. Milstein, NINDS
NIH Research Advisor:
Judith Walters
University:
University of Cambridge
Health Sciences
University Research Advisor:
Trevor Robbins

There has been renewed clinical interest in noradrenergic (NA) modulation of sustained attention and impulse control with the approval of the NA reuptake inhibitor atomoxetine for the treatment of attention deficit hyperactivity disorder. The current study examines the role of NA in the modulation of sustained attention and impulse control using the 5-choice serial reaction time task (5CSRT) in rats. Experiment 1 examined the systemic antagonism of methylphenidate (MP)-induced impulsivity with either prazosin, an α_1 antagonist, or propranolol, a β-adrenoreceptor blocker. Experiment 2 examined the comparative effects of selective serotonin (5HT) receptor blockade. Central, but not peripheral β-adrenergic blockade abolished, and α_1-adrenergic antagonism partially attenuated MP-induced premature responding. The lack of effects of 5HT1A and 5HT1B receptor antagonists indicate selective NA β-receptor effects of propranolol. Taken together, these studies provide evidence for a role of NA in the mediation of MP-induced impulsivity. **Poster 41**

Antigenic Characterization of A28, a Vaccinia Virus IMV Protein Essential for Cell Entry

Gretchen E. Nelson and Bernard Moss

Graduate Student:
Gretchen Nelson, NIAID
NIH Research Advisor:
Bernard Moss
University:
Johns Hopkins University
Biology

Vaccinia virus shares over 90 percent nucleotide identity with variola virus, the agent of smallpox, and is the virus used in the current smallpox vaccine. Vaccinia virus is able to infect a wide range of cells, but its mode of entry is not well understood. The vaccinia virus protein A28 has recently been shown to be necessary for cell entry. It is 97 percent identical to the variola protein and is located on the intracellular mature virion membrane. Virions lacking A28, assemble and exit cells normally, have normal morphology, and are able to attach to cells. However, penetration of the core into the cell is blocked. We would like to further characterize the A28 protein to understand its function in cell entry by studying the protein's antigenicity and immunogenicity. To do this we have expressed a recombinant form of A28 using the baculovirus system. Using phage display technology we were able to isolate scFv, which bind to A28. We have also used the recombinant A28 to make polyclonal rabbit antibodies and mouse monoclonal antibodies are in the works. By studying these antibodies' binding properties to both recombinant A28 and whole virions and their neutralizing capabilities to vaccinia virus, we can learn more about the regions of A28 involved in cell entry. **Poster 42**

Non-Invasive Measurement of Coronary Blood Flow at High Field Magnetic Resonance Imaging

R. Nezafat, C. Stehning, A.M. Gharib, M.Y. Desai, R.G. Weiss, R.I. Pettigrew, E.R. McVeigh, and M. Stuber

Graduate Student:
Reza Nezafat, NHLBI
NIH Research Advisor:
Elliot R. McVeigh
University:
Johns Hopkins University
Biomedical Engineering
University Research Advisor:
Elliot R. McVeigh

In native and bypass graft coronary arteries, magnetic resonance flow measurements have shown to be a successful method for the non-invasive assessment of patency. Because of the small dimensions and rapid motion of the coronary arteries, a high spatial and temporal resolution is mandatory for the assessment of flow. Improved spatial resolution will reduce partial-volume effects, which supports a better estimation of the flow rate. An improved temporal resolution will not only minimize blurring induced by rapid myocardial motion, but also lead to a significantly improved estimation of peak flow velocity. However, high spatial and temporal resolution imaging results in low SNR and prolonged acquisition time. While navigator techniques have been used as an alternative to breath-holding to remove motion constraint, early systolic flow could not be measured and the quality of the flow measurement was only adequate for time frames in close temporal proximity to the navigator. In this study, we have developed a dual-navigator technique that enables access to early systolic flow while respiratory-induced artifacts on images with a relative temporal distance to the navigator are no longer a limitation. Furthermore, we exploit the extra SNR offered by a higher magnetic field strength and short acquisition intervals to improve spatial and temporal resolution in coronary flow imaging. **Poster 43**

B_1 Insensitive T_2 Preparation Sequence for Coronary MR Imaging at High Field

R. Nezafat, R. Ouwerkerk, A. Gharib, R.I. Pettigrew, E.R. McVeigh, and M. Stuber

Graduate Student:
Reza Nezafat, NHLBI
NIH Research Advisor:
Elliot R. McVeigh
University:
Johns Hopkins University
Biomedical Engineering

We propose a B_1 insensitive T_2 preparation sequence employing the adiabatic plane rotation RF pulses for contrast generation in coronary MRA at 3T. The effective wavelength of RF field at 3T is comparable to the dimension of the human body, which causes a significant variation of the B_1 phase along the sample and B_1 standing wave effects which cause RF field inhomogeneity at higher field strengths. Non-endogenous contrast enhancement such as T_2 prep is used extensively in coronary imaging. This preparation scheme is relatively insensitive to B0 inhomogeneity, however it's not efficient in compensating for the B_1 inhomogeneity in high-field magnets. An adiabatic T_2 prep sequence in which the refocusing is achieved with two adiabatic inversion RF pulses to perform a plane rotation is proposed. Simulations and in-vivo results illustrate an excellent suppression of the artifacts originating from B_1 inhomogeneity while achieving similar T_2 contrast enhancement. **Poster 44**

Evaluation of β-Amyloid Ligands Using Isolated Human Amyloid Plaques with *In Vitro* Binding Assays

L. Nichols, L. Cai, V.W. Pike, and R.B. Innis

Graduate Student:
Lisa Nichols, NIMH
NIH Research Advisor:
Robert Innis
University:
Purdue University
Neuroscience
University Research Advisor:
Robert Meisel

Alzheimer's disease (AD) is characterized by the presence of amyloid plaques and neurofibrillary tangles (NFT's), which led to the proposal of the amyloid cascade hypothesis. This hypothesis states that environmental or genetic factors may enhance secretion or reduce clearance of the Aβ peptide. The presence of Aβ protein and plaques may lead to changes in brain which could create an imbalance of kinase and phosphatase activity, resulting in the development of NFTs, synaptic changes, transmitter deficits, and neurodegeneration. AD is definitively diagnosed at autopsy. The development of new radioligands for detection of plaques using positron emission tomography (PET) will allow for *in vivo* diagnosis, can evaluate the efficacy of drugs aimed at reducing Aβ protein, and may allow for the detection of the disease prior to the onset of clinical symptoms. We are evaluating new radioligands for amyloid using an *in vitro* assay which utilizes isolated human β-amyloid plaques. The displacement of tritiated 6-OH-BTA-1, a compound which detects amyloid plaques in humans with AD using PET, by new amyloid ligands developed in our lab will allow us to determine the efficacy of these compounds. Compounds of superior efficacy will be radiolabeled, and utilized in autoradiography and in PET studies in TgCRND8 mice. **Poster 45**

Characterization of the Stable Fraction of Mad2 at Kinetochores Using FRAP

Ryan P. O'Quinn and Edward D. Salmon

Graduate Student:
Ryan P. O'Quinn, OD
University:
University of North Carolina–Chapel Hill Biology
University Research Advisor:
Edward D. Salmon

Kinetochores lacking kinetochore microtubules bind Mad2 and Cdc20, leading to the formation of a complex that prevents the activation of the anaphase-promoting complex (APC^{Cdc20}). Mad2 is targeted to kinetochores by Mad1. Mad1, Mad2, and Cdc20 are transported from spindle poles to kinetochores along microtubules by cytoplasmic dynein. It remains unclear exactly what portion of Mad2 complex remains stably bound to Mad1 at kinetochores, what portion is transported to spindle poles, and what portion rapidly dissociates into the cytoplasm. If dynein-dependent transport is inhibited, Mad2 at kinetochores is not depleted as normal by kinetochore microtubule formation, and spindle checkpoint activity is not turned off. To examine Mad2 dynamics at kinetochores, fluorescence recovery after photobleaching (FRAP) measurements were made at kinetochores of PtK2 cells stably expressing YFP-Mad2. Various recovery levels were seen, with 10 to 70 percent of Mad2 stable at the kinetochore over 10 min. The rapid dissociation phase had a half-life of 20s or less. Variance in the amount of stable Mad2 could be due to unforeseen microtubule interactions at kinetochores. Future work will involve using nocodazole to arrest these interactions and microinjecting dynein inhibitors to explore the role of dynein in Mad2 kinetochore dynamics. **Poster 46**

Gadd34 Requirement for Normal Hemoglobin Synthesis

Andrew D. Patterson, M. Christine Hollander, Georgina F. Miller, and Albert J. Fornace, Jr.

Graduate Student:
Andrew D. Patterson, NCI-CCR
NIH Research Advisor:
Albert J. Fornace, Jr.
University:
The George Washington University Genetics

The protein encoded by the growth arrest and DNA damage-inducible transcript 34 (Gadd34) is associated with translation initiation regulation following certain stress responses. Through interaction with the protein phosphatase-1 catalytic (PP1c) subunit, Gadd34 recruits PP1c for the removal of an inhibitory phosphate group on the alpha subunit of the elongation initiation factor-2 (eIF2); thereby reversing the shutoff of protein synthesis initiated by stress-inducible kinases. In order to elucidate the functions of Gadd34, we generated *Gadd34*-null mice. Initial analysis of the *Gadd34*-null mice revealed several significant findings, including hypersplenism, decreased erythrocyte volume, increased numbers of circulating erythrocytes, and decreased hemoglobin content, resembling some thalassemia syndromes. Biochemical analysis of the hemoglobin-producing reticulocyte (an erythrocyte precursor) revealed that the decreased hemoglobin content in the *Gadd34*-null erythrocyte is due to the reduced initiation of the globin translation machinery. This evidence supports the existence of an equilibrium state between Gadd34/PP1c and the opposing heme-regulated inhibitor (HRI) kinase during hemoglobin synthesis in the reticulocyte. **Poster 47**

Chromatin Remodeling of the Mouse AHSP Gene Requires EKLF

André M. Pilon, Clara Wong, Lisa J. Garrett-Beal, Mitchell Weiss, Patrick G. Gallagher, and David M. Bodine

Graduate Student:
André M. Pilon, NHGRI
NIH Research Advisor:
David M. Bodine
University:
The George Washington University Biomedical Sciences

Alpha-Hemoglobin Stabilizing Protein (AHSP) prevents precipitation of γ-hemoglobin. We are interested in how AHSP is expressed, and have studied the roles of *cis* DNA sequences, the transcription factor EKLF, and chromatin structure on expression. The -170/+269 promoter gave position independent expression of human γ-globin in transgenic mice. Copy number and γ-globin mRNA level did not correlate and 3/5 lines were variegated. To examine the role of EKLF, we used subtractive hybridization, microarray, and RNase protection to compare AHSP mRNA levels in fetal liver cells from wild type and EKLF-/- mice. Nulls had 9-fold less AHSP mRNA than wild type. We hypothesized that EKLF was involved in remodeling chromatin at this locus, and assayed for DNase I hypersensitive sites (HS) in wild type and null mice. We found a HS between -400/-200 that was absent from EKLF-/-. To examine local histone acetylation we performed Chromatin Immune Precipitation. In wild type, two regions were hyperacetylated. One corresponds to the -400/-200 HS; the second maps 3' to the poly-A signal. Histones were acetylated between these regions, while regions up- and downstream were hypoacetylated. All EKLF-/- sites were hypoacetylated. We conclude that EKLF is required for remodeling the AHSP locus and that it may modify thalassemias. **Poster 48**

Genetic Risk Factors for Congenital Heart Disease: Investigation of Polymorphisms in Folate Pathway Genes

Reid Prentice, James Mills, Joseph Ward, Rebecca Seltzer, Faith Pangilinan, Mary Conley, Kenneth Pass, Mark Russell, Charlotte Druschel, and Lawrence Brody

Graduate Student:
Reid Prentice, NHGRI
NIH Research Advisor:
Lawrence Brody
University:
The George Washington University Genetics
University Research Advisor:
Timothy McCaffrey

Congenital heart defects (CHD) affect 1 in 100 live births. These defects are etiologically heterogeneous and affect all components of the heart. Folic acid supplementation reduces the occurrence of neural tube defects. In randomized trials, folic acid also reduced the occurrence of VSDs and conotruncal defects. We hypothesize that polymorphisms in folate pathway genes may be genetic risk factors for CHD. Using a population-based, case-control design, we tested this hypothesis by genotyping the functional single nucleotide polymorphisms MTHFR 677C>T, MTHFD1 R653Q, RFC1 R27H and TCII P259R. We studied 2100 children with CHDs (nearly complete ascertainment) and ethnically matched controls; all were born in New York state during 1998. Allele frequencies were significantly different between ethnic groups. ASDs were associated with homozygosity for MTHFD1 653R in African Americans (OR=1.7 [1.1-2.6, p=0.02]) and MTHFR 677C in Caucasians (OR=1.5 [1.1-2.2, p=0.05]). VSDs were associated with MTHFR 677C homozygosity in African Americans (OR=2.1 [1.1-4, p=0.02]). Conotruncal defects were associated with TCII 259P homozygosity in Hispanics (OR 4.3 [1.5-13, p=0.008]). RFC1 R27H was not associated with CHD. Positive findings have not been adjusted for multiple hypothesis testing and must be interpreted with caution. **Poster 49**

Exploring The Role of Rasgrp3 and Its Downstream Pathways in VEGF-induced Dysmorphogenesis

P.K. Randhawa, D. Roberts, W. Dunworth, A. Anderson, B. Stanford, and V.L. Bautch

Graduate Student:
Paramjeet Randhawa, NIH/OD
University:
University of North Carolina Biology, Cell Motility, and Cytoskeleton
University Research Advisor:
Victoria L. Bautch

RasGRP3 is a guanine exchange factor (GEF) for Ras/Rap, expressed in developing blood vessels and upregulated in response to VEGF. Ongoing studies suggest that this novel non-PKC DAG/phorbol ester receptor may play a role in angiogenesis by affecting endothelial cell proliferation, migration, and vascular permeability. Diacylglycerol (DAG) is a second messenger upstream of PKC. DAG activation affects the stability of endothelial adherens junctions and subsequently contributes to disruption of endothelial barrier function. Emerging models suggest that DAG promotes vascular permeability through both PKC and non-PKC effectors. In the ES cell model, the effects of elevated DAG are mimicked by treatment with exogenous PMA. This phorbol ester induces dysmorphogenic vascular sheet formation rather than the formation of vascular plexi and is dependent on RasGRP3. Flt-1 is a VEGF receptor that negatively regulates VEGF signaling. We have shown that flt-1 -/- vessels also form sheets. To elucidate which pathways are downstream of RasGRP3, we will utilize inhibitors individually targeting potential signaling pathways, including MEK, p38, MAPK, and JNK. We hypothesize that these two models of aberrant sheet formation perturb vascular permeability and that there will be a convergence of signaling pathways. **Poster 50**

Serotonin Transporter Polymorphism Mediates Vulnerability to Loss of Motivated Action Following Acute Tryptophan Depletion

J.P. Roiser, A.D. Blackwell, R. Cools, L. Clark, D.C. Rubinsztein, T.W. Robbins, and B.J. Sahakian

Graduate Student:
Jonathan Roiser, NIMH
NIH Research Advisor:
Wayne Drevets
University:
Cambridge University Psychiatry Department
University Research Advisors:
Barbara Sahakian and Trevor Robbins

We utilized acute tryptophan depletion (ATD) to investigate the effect of serotonin (5-HT) depletion on motivated action in humans, stratifying the results by polymorphism at the serotonin transporter gene (5-HTTLPR). Thirty young healthy volunteers participated in this double-blind, placebo-controlled crossover study, 15 ss and 15 ll at the 5-HTTLPR. Five hours following the ingestion of either a balanced or tryptophan depleted amino acid drink, participants carried out the Cued Reinforcement Response Time test (CRRT), a timed three-choice visual discrimination test on which participants were motivated to respond quickly and accurately by receiving points. TRP resulted in a 60 percent drop in plasma tryptophan with no difference between genotypes. In ss volunteers, ATD abolished the speeding on trials where participants were almost certain of receiving reward relative to those on which they were unlikely to receive reward (p=0.025). In the ll volunteers, ATD did not affect motivated action (p=0.3). These data demonstrate a differential vulnerability between genotypes at the 5-HTTLPR to loss of motivated action following 5-HT depletion. Such a loss of motivated action may play a part in mediating the association of the s allele at the 5-HTTLPR with depression, specifically with the symptom of anhedonia. **Poster 51**

Endocannabinoid-dependent Striatal LTD Induced by Moderate Frequency Activation of Cortical Afferents

Jennifer Ronesi and David M. Lovinger

Graduate Student:
Jennifer Ronesi, NIAAA
NIH Research Advisor:
David Lovinger
University:
Vanderbilt University
Pharmacology

We examined activity-dependent changes in transmission efficacy at corticostriatal synapses using whole-cell voltage clamp electrophysiology in acutely-prepared brain slices. Brief high-frequency stimulation (HFS) of cortical afferents innervating the dorsolateral striatum induces a long-term depression (LTD) in synaptic efficacy. We now report that LTD at corticostriatal synapses is induced by a 10Hz, 5min train. Like HFS-induced LTD, the decrease in synaptic efficacy during LTD is associated with a decrease in presynaptic release probability and is blocked by the CB1R antagonist AM251 and the D2 dopamine receptor antagonist sulpiride. However, 10Hz LTD is elicited when the postsynaptic neuron is voltage-clamped at -70mV and is not blocked by intracellular application of 20mM BAPTA. Furthermore CPCCOEt, a type 1 mGluR antagonist, does not block 10Hz LTD. These treatments all prevent HFS-induced LTD. Thus, while 10Hz LTD is dependent on activation of CB1Rs by an endocannabinoid (eCB), it is not clear that the postsynaptic neuron is the source of the eCB as it is in HFS-induced LTD. Our findings provide the first indication that LTD in dorsal striatum can be induced by frequencies different from those that induce LTP; a fact that might be useful in modeling striatal input/output functions. **Poster 52**

Autoinhibition of Cbl-c Ubiquitin Ligase Activity

Philip E. Ryan, Nina Sivadasan, and Stan Lipkowitz

Graduate Student:
Philip E. Ryan, NCI-CCR
NIH Research Advisor:
Stan Lipkowitz
University:
The George Washington University
Genetics

Cbl family proteins are ubiquitin ligases (E3s) involved in the regulation of receptor tyrosine kinases. There are three mammalian family members Cbl, Cbl-b and Cbl-c. All three have a highly conserved N-terminus composed of the tyrosine kinase binding (TKB) domain, the catalytic RING-Finger domain (RF) and a linker region. Cbl-c is the smallest family member, lacking the long c-terminal proline rich domains and ubiquitin binding associated domains found in the other two family members. Unlike Cbl or Cbl-b, bacterially produced GST-full length Cbl-c is not active in an *in vitro* E3 assay. In contrast, a bacterially produced protein containing only the RF and surrounding amino acids is active *in vitro*. Further analysis utilizing deletion constructs revealed that removing the N-terminal region containing the TKB domain restores Cbl-c E3 activity *in vitro*. Incubation of full length GST-Cbl-c with active Src also restores its E3 activity. Inhibition of Src kinase activity with the small molecular inhibitor, PP2, blocks activation of Cbl-c E3 activity by Src. Together, these data demonstrate negative autoregulation of Cbl-c E3 activity by the TKB domain and suggest that this can be overcome by active Src. Ongoing studies are investigating the molecular mechanisms of the autoinhibition and its reversal by Src. **Poster 53**

Towards Understanding the Transcriptional Dysregulation Associated with Huntington's Disease, a Biochemical Purification of Htt interacting Proteins

Jeffrey Savas, Christopher Parkhurst, Sanford Markey, and Naoko Tanese

Graduate Student:
Jeffrey Savas, NIMH
NIH Research Advisor:
Sanford P. Markey
University:
New York University
Structural Biology
University Research Advisor:
Naoko Tanese

Huntington's disease (HD) is a dominant autosomal neurodegenerative disorder characterized by the expansion of a CAG tri-nucleotide repeat within exon one of the huntingtin (htt) gene resulting in the expression of protein with an increased number of glutamines. The current census is that the mutant protein gains a toxic function; however, inhibition of wild type huntingtin (Htt) activity may also contribute to the disease. Many suggestive observations have been made in regard to HD pathology, but currently the molecular details responsible for the death of corpus striatum/neocortex neurons remain unknown. Htt has been shown to directly interact with various transcription factors, implicating transcriptional dysregulation in HD, but their distribution does not correlate with the pattern of degeneration observed in HD patients. We have used a retroviral vector to establish stable cell lines that express double FLAG/HA tagged derivatives of Htt in order to purify its interacting proteins. We are also performing reporter assays to examine the effect of wild type (17Q) and mutant (75Q) Htt in the regulation of transcription from SP-1 dependent promoters, as well as chromatin immunoprecipitation to investigate transcription factor occupancy at the promoters of genes known to be affected in HD. **Poster 54**

Differentiation and Enrichment of Dopaminergic Neurons Derived from Embryonal Carcinoma Stem Cells

Catherine M. Schwartz, Xianmin Zeng, William Freed, and Mahendra Rao

Graduate Student:
Catherine M. Schwartz, NIA
NIH Research Advisor:
Mahendra Rao
University:
Karolinska Institutet, Department of Medical Biochemistry and Biophysics
University Research Advisor:
Ernest Arenas

Human embryonic stem cells (hESCs) can differentiate into dopaminergic (DA) neurons by co-culture with the mouse stromal cell line PA6. Here we show that NTera2, a human embryonal carcinoma cells line, can differentiate into DA neurons when co-cultured with PA6 cells. Similar to hESCs, the onset of tyrosine hydroxolase (TH) begins around 8 days in culture and after three weeks of differentiation 80%-90% of the colonies express TH. Furthermore, differentiated NTera2 cells express several DA markers including AADC, DAT, Nurr1, TrkB, TrkC, and GFRA1. Media conditioned by PA6 cells can also generate TH positive cells, indicating secreted factors from PA6 cells might contribute to DA differentiation. Using fluorescent activated cell sorting (FACS), we show that cell surface markers, including polysialylated neural cell adhesion molecule (PSA-NCAM) might be useful for enriching neuronal cells from the heterogeneous co-culture system. We show that the sorted cells have the ability to differentiate into TH positive cells when replated onto either PA6 cells or with PA6 conditioned media. Our results suggest that this described strategy of differentiation and enrichment provides a useful model to study DA neuron generation and transplantation studies in Parkinson's disease models. **Poster 55**

Does the Frozen Approximation Increase Inaccuracy in Threading?

Natasha L. Sefcovic, Aron Marchler-Bauer, Anna R. Panchenko, and Stephen H. Bryant

Graduate Student:
Natasha L. Sefcovic, NCBI/NLM
NIH Research Advisor:
Stephen H. Bryant
University:
Johns Hopkins University
Biology

There are many more known protein sequences than known protein structures. Thus, there is great interest in predicting structure from sequence. Threading is a prediction method in which a target sequence is aligned to a possible template with known structure and the alignment is scored. Targets and templates whose alignments score well tend to be homologous proteins that share evolutionarily conserved core elements. Insertions and deletions are more apt to occur between core elements than within them. Therefore gaps are only allowed between core elements. Previously, we developed a threading program that uses this core element alignment model with a Monte Carlo (MC) algorithm. Most threading programs use dynamic programming (DP) algorithms, which are faster than MC algorithms. However, the frozen approximation must be employed to use DP for threading. To assess the inaccuracy in fold recognition and alignment accuracy caused by the frozen approximation, we developed a fast threading program that combines a core element alignment model with a DP algorithm and compared the performance of both programs. The DP algorithm is a better score optimizer than MC algorithm, but surprisingly the MC threading program is better at fold recognition probably because it has a better signal-to-noise ratio. **Poster 56**

Phosphotyrosine Proteome Analysis and Construction of Signal Transduction Pathways from SILAC LC-MS/MS Studies of EphB2

Daniel Spellman, Guoan Zhang, and Thomas Neubert

Graduate Student:
Daniel Spellman, NICHD
NIH Research Advisor:
Alfred Yergey
University:
New York University
Structural Biology
University Research Advisor:
Thomas Neubert

Eph-related receptor tyrosine kinases (RTK) have been implicated in a host of biological functions including axon guidance and morphogenesis, yet the details of the signal transduction pathways that produce these specific biological functions after ligand-receptor interaction remain unclear. Stable isotope labeling by amino acids in cell culture (SILAC) in combination with LC-MS/MS was employed for characterization of cellular signaling and protein-protein interactions following receptor stimulation. As tyrosine phosphorylation functions as a key regulatory event in RTK signaling, anti-phosphotyrosine immunoprecipitation from cell lysates was included to further isolate potential participants in this pathway. SILAC experiments identified 120 unique proteins, 35 of which demonstrated increased abundance in phosphotyrosine immunoprecipitations upon ephrinB1-Fc stimulation as compared with unstimulated cells. Four proteins demonstrated decreased abundance, and 81 did not change relative abundance but were present in phosphotyrosine I.P.s of both conditions. Pathway analysis was performed with PathwayAssist™ following generation of lists of differentially tyrosine phosphorylated proteins and their interactors. Additional analysis was performed with specific immunoprecipitation and Western blotting. **Poster 57**

A Comprehensive Study of Prokaryotic and Eukaryotic Homologs of ACh Receptor Channels

Asba Tasneem, Lakshminarayan M. Iyer, Eric Jakobsson, and L. Aravind

Graduate Student:
Asba Tasneem, NCBI/NLM
NIH Research Advisor:
L. Aravind
University:
University of Illinois at Urbana–Champaign, Biophysics and Computational Biology
University Research Advisor:
Eric Jakobsson

Acetylcholine receptor type ligand-gated ion channels (ART-LGIC; also known as Cys-loop receptors) are a superfamily of proteins that include the receptor for major neurotransmitters like acetylcholine, serotonin, glycine, GABA, glutamate and histamine, and Zn++ ions. They play a major role in fast synaptic signaling in the animal nervous systems and have thus far not been reported outside of metazoa. Other gene families that are important in neural function are present in prokaryotes. In case of voltage-gated potassium channels of the Shaker-type superfamily and voltage-gated sodium channels, several representatives are known from both non-animal eukaryotes as well as numerous prokaryotes suggesting that they were employed in signaling in other contexts well before the origin of the animal nervous system. All the currently known members of the ART-LGIC superfamily possess an all-N-terminal ligand binding domain and a C-terminal transmembrane domain comprised of 4-membrane spanning helices. We have previously identified several prokaryotic members of the ART-LGIC superfamily and discuss the general implications of these proteins for the mechanisms and origin of the Cys-loop receptors of the animal nervous system. We report here a comprehensive study of these receptors in completed eukaryotic genomes. **Poster 58**

Incorporation of Sequence Information into Vector Alignment Search Tool Structure Alignments

Kenneth Evan Thompson and Stephen Bryant

Graduate Student:
Kenneth Evan Thompson, NCBI/NLM
NIH Research Advisor:
Stephen Bryant
University:
Johns Hopkins University Biology

The theory that proteins are modular in nature is related to the concept of the protein domain, which, in the broadest sense, can be defined as a distinct region of residues in a protein. Most structural domain definitions are based on the idea that a domain is a region of a polypeptide that forms a compact, independently folding unit. On the other hand, sequence-based protein domains can be thought of as evolutionarily conserved regions between the sequences of multiple proteins. Although in many cases the regions identified by either concept coincide, there are instances when a domain by one definition only partially overlaps domain by another definition. These differences may arise due to the theory underlying the definitions or the methods used in the identification of the domains. This work focuses on the development of algorithms to reconcile structural and sequence-based domain definitions as best as possible. Since structural domains are the units used by the Vector Alignment Search Tool (VAST), adjusting structure domains based on the information contained in conserved sequences may allow for increased specificity and sensitivity when searching for similar protein domains. By combining the information contained in each type of definition, new relationships between domains may be observed. **Poster 59**

An Extension to Vector Alignment Search Tool Protein Structure Comparison

Hsinyi Tsang, Thomas Madej, and Stephen H. Bryant

Graduate Student:
Hsinyi Steve Tsang, NCBI/NLM
NIH Research Advisor:
Stephen H. Bryant, Ph.D.
University:
Johns Hopkins University Biology

With the rapid growth in the size of protein structure databases, many protein structural comparison algorithms have been developed. Structure comparison methods can reveal structural and functional similarities that are not detected by sequence information alone. Vector Alignment Search Tool (VAST), which was developed by National Center for Biotechnology Information (NCBI), offers an efficient way to perform structural similarity searches. The VAST algorithms use vectors to represent secondary structure elements (SSE) in a protein structure and the structure comparison step relies on the relative alignment of the vectors. As a result, the presence of SSE is a prerequisite in performing structure similarity searches using VAST. Many small proteins that do not have more than three SSEs are therefore non-comparable and often result in inaccurate structural alignments. My project presents a way to include other special structural elements, such as disulfide bonds, in comparing protein structures using VAST. This approach will improve VAST protein comparison by incorporating some of the currently non-comparable small proteins in the database. In the long term, this more accurate structural alignment can potentially provide insight to the relationship between structure and the function of proteins. **Poster 60**

Catechol-O-Methyltransferase Enzyme Activity and Protein Expression in Human Prefrontal Cortex during Postnatal Development

Elizabeth Tunbridge, Paul Harrison, Jeff Kowalak, Sanford Markey, Joel Kleinman, Daniel Weinberger, and Cynthia Shannon Weickert

Graduate Student:
Elizabeth Tunbridge, NIMH
NIH Research Advisor:
Daniel Weinberger
University:
University of Oxford
Psychiatry Department
University Research Advisor:
Paul Harrison

The catechol-o-methyltransferase (COMT) enzyme metabolises catecholamines and its gene contains a polymorphism (Val158Met) that affects its activity. This polymorphism is associated with prefrontal function and schizophrenia, presumably mediated by differences in prefrontal dopamine catabolism. Since schizophrenia is thought to be a developmental disorder it is of interest to examine COMT function during ontogeny. Thus, we examined COMT activity and protein expression in the prefrontal cortex (PFC) during human postnatal development. Protein was extracted from PFC (BA46) of normal individuals from 6 age groups: neonates (1 to 4 months), infants (5 to 11 months), teens (14 to 18 years), young adults (20 to 24 years), adults (31 to 43 years), and aged (68 to 86 years; n=5-8 per group). COMT enzyme activity was assayed by measuring incorporation of a radioactive methyl group into catchol substrate and COMT protein was quantified by Western blotting. There was a significant, twofold increase in COMT enzyme activity during development, and this was paralleled by increases in aspects of COMT protein expression. Furthermore, COMT protein expression was also related to Val158Met genotype. This increase in COMT activity during human development may be related to maturation of the PFC and alterations in pyramidal neuron tuning. **Poster 61**

A Catalog for the Transcripts from the Venomous Structures of the Caterpillar *Lonomia obliqua*: Identification of the Proteins Potentially Involved in the Coagulation Disorder and Hemorrhagic Syndrome

Ana B.G. Veiga, Jose M.C. Ribeiro, Jorge A. Guimaraes, and Ivo M.B. Francischetti

Graduate Student:
Ana B.G. Veiga, NIAID
NIH Research Advisor:
Jose M. C. Ribeiro
University:
Universidade Federal do Rio Grande do Sul (UFRGS), Brazil
PPGBCM – Center of Biotechnology
University Research Advisor:
Jorge A. Guimaraes

Contact with *Lonomia obliqua* caterpillars causes a coagulation disorder and hemorrhagic syndrome in humans. In the present study we report sequencing and bioinformatics analyses of cDNA libraries from two venomous structures of the caterpillar: tegument and bristle. Over one thousand cDNAs were obtained and clustered to produce a database of 538 clusters for the tegument library and 368 for the bristle library. We identified full-length cDNAs coding for proteins probably involved in envenomation, such as serine proteases similar to coagulation factors, cysteine proteases, PLA2 and C-type lectins, besides sequences matching protease inhibitors such as serpins, Kazal-type inhibitors and cystatins. Antibacterial proteins and housekeeping genes are also reported. Many sequences were devoid of database matches and their function remains unknown. We also report the N-terminus of the most abundant proteins present in venomous secretions of the caterpillar. We have created a catalog that contains the predicted molecular weight, isoelectric point, accession number and putative function for selected molecules from the venomous structures of *L. obliqua*. All sequence information is available at *http://www.ncbi.nlm.nih.gov/projects/omes*. The role of these molecules in the envenomation by *L. obliqua* is discussed. **Poster 62**

Analyses of the Immune Synapse in Itk$^{-/-}$ and Itk$^{-/-}$Rlk$^{-/-}$ T Cells

Irene C. B. Viorritto, Reiko Horai, Lisa D. Finkelstein, and Pamela L. Schwartzberg

Graduate Student:
Irene C. B. Viorritto, NHGRI
NIH Research Advisor:
Pamela L. Schwartzberg
University:
University of Pennsylvania
Immunology

The Tec kinases Itk and Rlk are important components of T cell receptor (TCR) signaling, required for Ca^{++} mobilization, actin cytoskeleton regulation and IL-2 production. T cells deficient in Rlk and Itk also show defective recruitment and polarization of signaling molecules to the contact site when stimulated with anti-TCR coated beads or antigen-presenting cells, suggesting abnormal formation of the immune synapse (IS). Additionally, cytokine patterns differ between Itk$^{-/-}$ (Th1) and Rlk$^{-/-}$Itk$^{-/-}$(Th2) cells. Interestingly, Th2 cells differ in recruitment of lipid rafts and membrane organization, also suggesting differences in IS formation in Th2 cells.

We are using antigen-specific systems to stimulate Itk$^{-/-}$ and Rlk$^{-/-}$Itk$^{-/-}$ T cells under more physiological conditions *in vitro* to examine cell polarization, including IS formation and TCR downregulation, a proposed function of the IS. Although mutant cells have decreased polarization of signaling molecules, initial experiments show little differences in TCR downregulation between WT and mutant cells. We are using these systems to compare cytokine production with IS formation and TCR downregulation. Together, we hope these experiments will provide insight into the regulation of events at the immune synapse and how they affect T cell function. **Poster 63**

Experience-dependent Trafficking of NMDA Receptors in Primary Visual Cortex

Philip Y. Wang, Robert J. Wenthold, and Elizabeth M. Quinlan

Graduate Student:
Philip Y. Wang, NIDCD
NIH Research Advisor:
Robert J. Wenthold
University:
University of Maryland–College Park Neuroscience and Cognitive Sciences
University Research Advisor:
Elizabeth M. Quinlan

NMDA receptors (NMDARs) play a critical role in the initiation of many forms of synaptic plasticity. At birth, NMDARs in neonatal cortex largely consist of NR1/NR2B subunits. Over the first 5 postnatal weeks, there is an increase in the levels of NR1/NR2A-containing receptors. When animals are dark-reared (DR) from birth, the expression of synaptic NR2A in primary visual cortex (VCtx) is inhibited. When these DR animals are subsequently given 2 hours of light exposure, the levels of synaptic NR2A in VCtx increases dramatically. Further investigation of this issue is necessary to determine the source of this rapidly expressed synaptic NR2A protein. To address this question, we dark-reared mice from birth and gave them 2 hours of visual experience at postnatal day 21-35. VCtx was then dissected and analyzed to determine if there was a change in total NR2A protein levels. We then immunoprecipitated NR2A from DR and DR+2 mice and compared their various solubility characteristics and protein-protein interactions. Through this dark-rearing paradigm, we have a unique ability to investigate various experience-dependent cell-biological properties of NMDA receptors in vivo. Experiments are currently underway to further our understanding of the trafficking, subunit assembly, and molecular interactions of NMDARs. **Poster 64**

Movement Related Activation of Parietal and Temporal Cortex Revealed by Epicortical Recordings in Humans

Lewis A. Wheaton, Susumu Sato, and Mark Hallett

Graduate Student:
Lewis A. Wheaton, NINDS
NIH Research Advisor:
Mark Hallett
University:
University of Maryland–College Park Neuroscience and Cognitive Science
University Research Advisor:
Avis Cohen

Planning different types of movements may involve different brain areas. We recorded directly from the cerebral cortex of epilepsy patients undergoing electrocorticographic monitoring prior to surgical treatment. Patients were asked to perform simple thumb flexion and complex praxis pantomime (ex. using a hammer) in a self paced manner. Analysis was made on the slow rising negativity seen before and during self paced hand movements (movement related cortical potential, or MRCP), which occurs over active brain areas. We expect to see MRCP in the premotor and motor cortex for the simple thumb flexion, but parietal and temporal activation additionally for the more complex praxis pantomimes. Analysis revealed MRCP present in the dorsal premotor (PMd) and motor cortex for the thumb movements beginning as early as 2.3s before movement with no MRCP in the ventral premotor (PMv), inferotemporal (IT), or parietal areas. For the praxis pantomimes, MRCP was seen as early as 3.41s before movement in the PMd, 3.60s before movement in the IT cortex, and 3.51s in the PMv. The parietal cortex also showed MRCP for praxis movements. This illustrates that higher level brain areas are recruited in more meaningful motor tasks. Praxis MRCP is earlier than simple movement MRCP, indicating earlier processing for these tasks. **Poster 65**

The Mechanisms Involved in Ebola Virus Glycoprotein Induced Cytotoxicity

Carisa A. Zampieri, Nancy J. Sullivan, and Gary J. Nabel

Graduate Student:
Carisa Zampieri, NIAID/VRC
NIH Research Advisor:
Gary Nabel
University:
The George Washington University Genetics
University Research Advisor:
Linda Werling

Ebola virus is a highly lethal pathogen that causes hemorrhagic fever in humans and non-human primates. Among the seven known viral gene products, the envelope glycoprotein (GP) induces a cell rounding and detachment phenotype that ultimately leads to cell death. Deletion of the serine-threonine rich mucin like domain (GPDMuc) dramatically lessens the cytotoxic effects of the protein without affecting virus entry. The molecular mechanisms by which the envelope glycoprotein mediates cell detachment are not well understood. Initial experiments have shown that wild-type GP downregulates several membrane proteins that are important in cell adherence and immune function. This downregulation has been shown to be dependent on dynamin, a GTPase involved in membrane vesicle trafficking. In addition to the downregulation of adherence proteins from the cell surface, we now show that there is a reorganization of actin filaments and an alteration in signaling pathways in cells expressing Ebola GP. We hypothesize that Ebola virus GP inflicts cell damage by affecting the normal trafficking and signaling pathways within the cell through it's interactions with dynamin and other cellular proteins. **Poster 66**