Pennsylvania State University

Annual Progress Report: 2005 Formula Grant

Reporting Period

July 1, 2009 – December 31, 2009

Formula Grant Overview

The Pennsylvania State University received $7,845,194 in formula funds for the grant award period January 1, 2006 through December 31, 2009. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Role of Transient Receptor Potential (TRP) Channels in Cardiac Ischemia - The purpose of the project is to evaluate the role of TRP ion channels in mediating heart protection during low blood flow states (ischemia) and increased heart cell growth (hypertrophy) of the surviving heart cells after a heart attack (myocardial infarction). Virtually nothing is known about the function of TRP channels in the heart. We have preliminary evidence that TRPC4, TRPC5 and TRPC6 channels in the heart are increased after a heart attack, and that TRPC channels mediate calcium ion entry into cells. Understanding how TRP channels function in the heart, both in healthy and diseased states, will offer novel and rational therapeutic strategies for the treatment of both acute and chronic heart ischemia.

Duration of Project

1/1/2006 – 6/30/2009

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 2: Project Title and Purpose

Research Center on the Molecular Aspects of Obesity and the Metabolic Syndrome - Improvements in obesity-related sleep apnea, satiety/appetite, and diabetes occur rapidly after gastric bypass (GBP) surgery and are not explained by significant weight loss or decreased food intake alone. The goals of this project are to: 1) Identify animal models of obesity that mimic the improvements in appetite, sleep and metabolic syndrome observed after GBP surgery; 2) Investigate the mechanisms by which appetite, satiety, sleep, and diabetes are improved after GBP; 3) Collect and bank tissue before and after GBP surgery in patients for

proteomic/functional genomic analysis, and 4) Apply this information to test specific hypotheses in knockout/ transgenic mice and obese patients. We believe that understanding how GBP surgery improves appetite, sleep, and diabetes will elucidate new targets, develop less invasive treatments for obesity, and provide important information on the pathogenesis of obesity and obesity-related medical co-morbidities.

Duration of Project

1/1/2006 - 6/30/2009

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 3: Project Title and Purpose

Functional Elements in the Genomes of Human and Mouse, Phase 2 - The DNA sequences of virtually all the genetic material in humans, mice and other species have been determined, but only a small fraction appears to be needed for proper function (e.g., growth, development, cognition). Computational and experimental approaches developed in the PSU Center for Comparative Genomics and Bioinformatics are used to find these critical sequences. This project will continue support of this Center to (a) improve bioinformatic servers so that they facilitate translation of the data to improve understanding of human development and disease and (b) apply new sequencing technology to find functional sequences, such as the DNA sequences bound by particular proteins in the nucleus.

Duration of Project

1/1/2006 - 6/30/2008

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 4: Project Title and Purpose

Biochemical Mechanisms that Contribute to HIV Transcriptional Latency - The goals of this project are to understand the biochemical mechanisms that permit HIV to establish latency. Latently infected cells do not actively express HIV and, because they are not sensitive to current anti-viral treatments, they are important reservoirs for virus late in AIDS or when treatments are interrupted. The project will use novel experimental systems to study how latency is established,

including what proteins are associated with the HIV DNA. These studies will provide insights into ways to eradicate HIV from all infected cell populations.

Duration of Project

1/1/2006 - 6/30/2007

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 5: Project Title and Purpose

Ceramide Liposomes for Combinatorial Drug Delivery of Chemotherapeutics and siRNA - The purpose of the project is to utilize nanotechnology to encapsulate pharmacological and molecular agents to synergistically treat cancers. The project will optimize and validate ceramide-incorporated nanoliposomes as a treatment modality for breast cancers, melanomas and leukemias. Our nanoliposomes (70-80nm in size) have the potential to target cancerous lesions without activating the body's defense mechanisms.

Duration of Project

1/1/2006 - 12/31/2008

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 6: Project Title and Purpose

Mouse Metabolic Phenotyping Center for Diabetes - Type 2 diabetes is characterized by altered metabolism and insulin resistance and leads to complications affecting organs including heart, kidney, and eyes. To determine the underlying mechanism, gene targeting techniques have been applied to develop transgenic mouse models of diabetes and obesity. The goal of this project is to develop an interdepartmental investigator-initiated Pennsylvania State Mouse Metabolic Phenotyping Center (MMPC) that will perform an array of novel and sophisticated experiments using the state-of-the-art equipment for the purpose of investigating transgenic mice potentially useful for understanding diabetes and its complications. The MMPC consisting of multiple Cores will provide important insights into identifying the candidate genes of diabetes and potential therapeutic targets in the treatment of diabetes and its complications.

Duration of Project

1/1/2006 - 6/30/2009

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 7: Project Title and Purpose

Center for Molecular and Cellular Neuroscience - Pennsylvania State is in the midst of a significant expansion in its Neuroscience research and education enterprise. This expansion includes the establishment of a Center for Molecular and Cellular Neuroscience. The different research groups of the Center share the use of molecular, genetic, electrophysiological, immunohistochemical and animal behavior techniques to investigate different aspects of synaptic transmission and animal models of neurological disorders. As part of this joint effort, a pilot research program will use gene targeting of GABA-A receptor subunit genes in mice. Our laboratory has developed an animal model of anxiety-driven depression that replicates the hallmarks of anxiety-driven depressive disorders including i) selective vulnerability in early life, ii) behavior indicative of anxiety-driven depression, iii) and an adult deficit in hippocampal neurogenesis. The project hypothesizes that these mice are suitable to further analyze the molecular and cellular substrate of anxiety-driven depression.

Duration of Project

1/1/2006 – 12/31/2009

Project Overview

Neuroscience at Pennsylvania State University, unlike at most medical schools, is best described as a loose association of scientists that are part of five different colleges spread across campus. Along with the recent effort at Pennsylvania State University to upgrade the research facilities in the neurosciences comes an urgent need to establish new organizational structures. In particular, to further develop into an internationally recognized center of competence, neuroscientists at Pennsylvania State must increase their competitiveness in obtaining investigator-initiated extramural research grants, NIH sponsored Program Project Grants, Training Grants, and NIH Center Grants. An increase in research funding will not only benefit the science of individual laboratories; more importantly it will enhance the attractiveness and competitiveness of our campus-wide research program in recruiting students, coworkers and faculty, and enhance the quality of undergraduate and graduate level teaching in the neurosciences. This effort will therefore greatly strengthen the sustainability of our effort in developing neuroscience into a major area of strength in the life sciences at Pennsylvania State.

Towards the goal of establishing a Center of Competence in Neuroscience we will use a pilot project to develop an area of neuroscience that is thematically at the core of this Center. Using gene targeting of GABA-A receptor subunit genes in mice, our laboratory has developed an animal model of anxiety-driven depression that replicates the hallmarks of anxiety-driven depressive disorders including i) selective vulnerability in early life, ii) behavior indicative of anxiety-driven depression, iii) and an adult deficit in hippocampal neurogenesis. The project hypothesizes that these mice are suitable to further analyze the molecular and cellular substrate of anxiety-driven depression and proposes the following Specific Aims:

1) To determine whether 'depressed' mice exhibit a deficit in cortical neurogenesis in the embryonic and/or newborn brain, to narrow the time during development when such deficits can first be seen, to determine the type of neurons (glutamatergic, GABAergic) that fail to develop in cortex of gamma2 subunit heterozygous mice, and to determine whether deficits in neurogenesis are associated with a deficit in migration of neurons during brain development.
2) To determine whether GABA-A receptor deficits induce epigenetic changes in gene expression.

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Expected Research Outcomes and Benefits

The described pilot studies will elucidate the molecular and cellular underpinnings of anxiety and depression disorders. Anxiety and mood disorders show frequent comorbidity and represent some of the most common and serious mental disorders of modern society. A better knowledge of the molecular and cellular underpinnings leading to anxiety and mood disorders is essential for the development of new strategies of prevention and drug therapies. Our preliminary experiments indicate that deficits in gamma-aminobutyric acid (GABA-A) receptors during early life are detrimental to emotional health in adults. Such deficits in $GABA_A$ receptors might have genetic origins in $GABA_A$ receptor subunit genes or in genes that affect $GABA_A$ receptor expression indirectly. In addition, experiments in rodents indicate that environmental stress during early life can lead to deficits in GABA-A receptor and anxiety- and depression-like behavior in adults. Thus, pharmacological enhancement of GABA transmission (i.e., by benzodiazepines such as diazepam) given during pregnancy or in early postnatal life might alleviate stress induced or genetic deficits in $GABA_A$ receptors, and thereby alleviate deficits in brain development associated with anxiety and mood disorders later in life. Indeed, while benzodiazepines are ineffective as antidepressants in adults they might act as antidepressants

when given during development. The establishment of such drug therapies requires knowledge of the temporal window of brain development that is most vulnerable to GABA-A receptor deficits. In addition, knowledge about GABA-A receptor deficit induced changes in gene expression might lead to the identification of novel therapeutic targets that could be used to block the downstream effects of the receptor deficit that lead to deficits in neurogenesis and/or depressive like behavior in mice.

Summary of Research Completed

Determine whether GABA-AR deficits of $\gamma2^{+/-}$ mice are associated with reduced survival or altered migration of embryonic neurons. Subtle $GABA_AR$ deficits in $\gamma2^{+/-}$ mice or conditional heterozygous KO mice (Emx1Cre x fγ2/+) that extend to immature neurons of the subgranule cell layer of the hippocampus result in marked deficits in maturation and long-term (>4 week) survival of these neurons. By contrast similar $GABA_AR$ deficits that are induced in adolescence and limited to mature neurons (as seen in CaMKIICre x fγ2/+ mice) lack such cellular deficits and are behaviorally normal, suggesting that anxious depressive-like behavior is associated with GABAergic deficits in immature neurons of the hippocampus. Analysis of brain volumes by MRI indicated that the hippocampal volume of $\gamma2^{+/-}$ mice was unaltered. Nevertheless, reduced hippocampal neurogenesis of $\gamma2^{+/-}$ vs. WT mice was seen not only in adulthood but also when neurons were labeled at 3 weeks of age and analyzed at 7 weeks of age.

Importantly, these experiments did not exclude a contribution to the behavioral phenotype by altered survival or migration of embryo derived neurons that populate other brain regions implicated in anxiety and depression. In particular, the pathology of depression in patients is known to include altered gene expression and neural activity in the frontal cortex. To test whether subtle $GABA_AR$ deficits of $\gamma2^{+/-}$ mice result in altered neurogenesis or migration of embryonic neurons we metabolically labeled embryonic neurons of $\gamma2^{+/-}$ and WT mice by injection of BrdU into pregnant dams at either 12.5 or 15.5 days of gestation and analyzed the final destination of BrdU labeled neurons at postnatal ages of 18.5 and 21 days, respectively. Brain regions analyzed included the cortical subplate and layer II/III area of the neocortex, as well as the CA1, CA3 and DG areas of the hippocampus. While the number of BrdU positive neurons varied dramatically between different brain regions, we found no genotype dependent differences in the number of BrdU positive cells or BrdU/NeuN positive neurons (Fig. 1). Thus, deficits in neurogenesis of $\gamma2^{+/-}$ mice appear to be limited to the postnatal hippocampus where immature neurons compete with mature neurons for integration into already established neural networks. This competitive environment appears to contribute to the increased vulnerability of the hippocampus to genetic and environmental challenges that affect the maturation of neurons.

Determine whether GABA-AR deficits of $\gamma2^{+/-}$ mice results in changes of gene expression implicated in anxiety and mood disorders. Deficits in signaling by the neurotrophic factor BDNF are implicated in the etiology of depression. Moreover, BDNF is essential for antidepressant drug mediated changes in behavior. In the context of $\gamma2^{+/-}$ mice, BDNF is critically important for normal maturation of GABAergic neurons and normal formation of GABAergic synapses, which are impaired in $\gamma2^{+/-}$ mice, at least in adult born hippocampal neurons. We therefore first compared BDNF protein levels in different brain regions of $\gamma2^{+/-}$ and WT mice at both 3- and 9-weeks of age. The level of BDNF recovered from brain extracts by ELISA was significantly

increased in cortex of 9-week compared to 3-week-old mice, as expected. By contrast, the expression of BDNF in $\gamma2^{+/-}$ vs. WT was unaltered in all three brain regions analyzed (hippocampus, cortex, brain stem) (Fig. 2).

A recent genome wide analysis of depression related transcript changes in patients and in a chronic stress-induced mouse model of depression led to the identification of 40 transcripts that were similarly changed in amygdala of patients and mice in depressed vs. normal brains. Altered expression of these genes therefore provides a molecular signature of depression that is conserved in mice vs. man (Sibille et al 2009, Surget et al 2008). We wondered whether the $\gamma2^{+/-}$ mouse model could replicate the same transcript changes. As part of a collaboration with the Sibille lab we have conduced quantitative PCR analyses for 24 of the signature transcripts in amygdala of $\gamma2^{+/-}$ and WT littermates (for transcripts analyzed and PCR primer used see Table 1). Interestingly, the transcript changes measured in amygdala of male $\gamma2^{+/-}$ vs. WT mice closely follow the patterns seen in male patients and male UCMS treated mice (Pearson quotient of 0.58 and 0.62, i.e. both correlations are highly significant). Similarly, gene expression changes seen in female $\gamma2^{+/-}$ mice appear to follow a pattern that is similar to that seen in female patients although this apparent correlation has not reached significance. Moreover, depression related changes of gene expression seen in males appear to run in opposite direction from those seen in females, and this sexually dimorphic pattern is seen both in patients and $\gamma2^{+/-}$ mice. These data firmly establish $\gamma2^{+/-}$ mice as a genetically defined animal model of depression. They further establish $GABA_AR$ deficits as likely causal factor in the pathology of this disorder, or a critical sequela of stress and other causes that leads to depression and this represents a core feature of the disease.

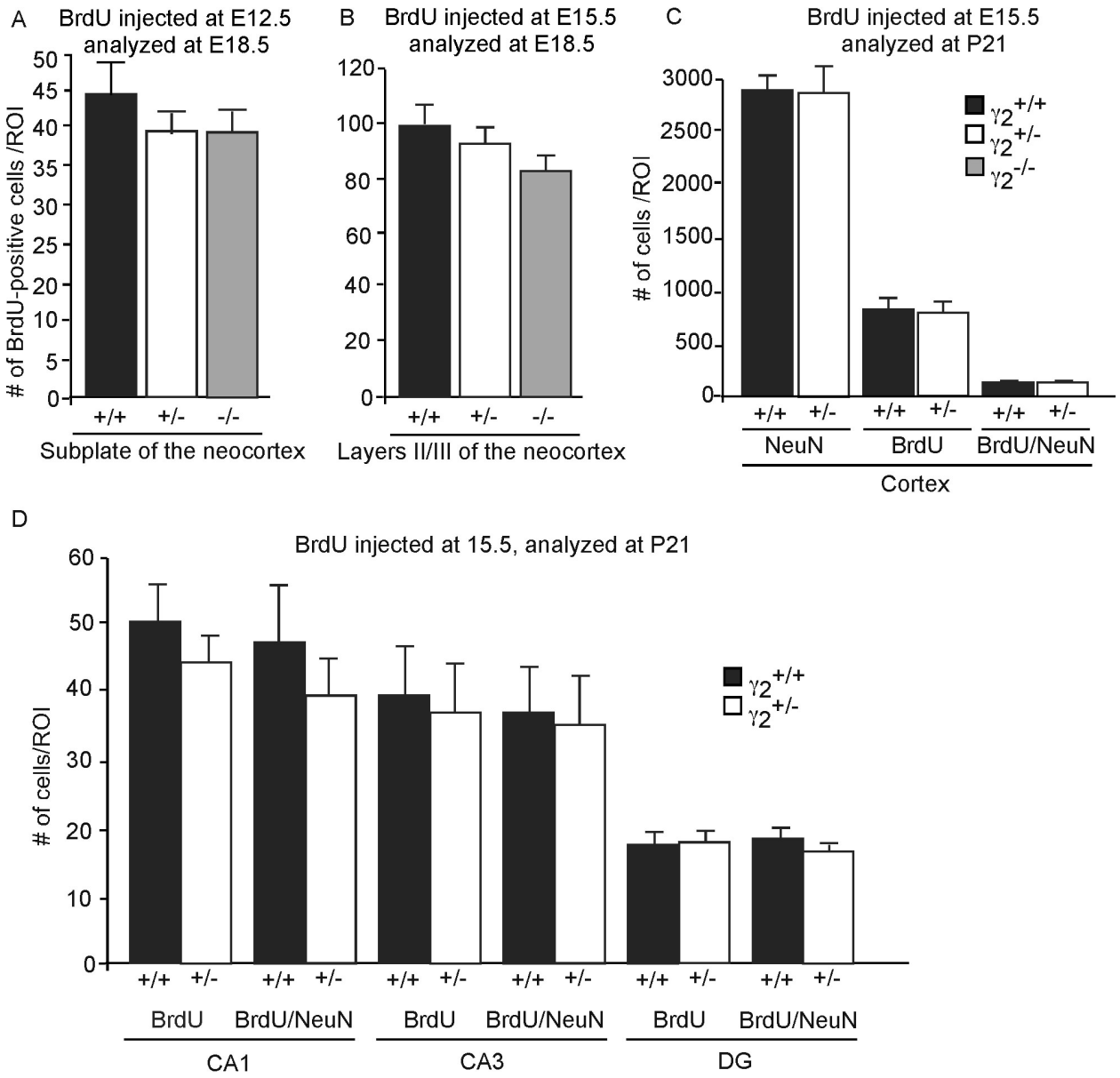

Figure 1

Quantitative assessment of embryo derived neurons in the postnatal cortex of $\gamma2^{+/-}$ mice. A, B) Pregnant dams of $\gamma2^{+/-}$ x $\gamma2^{+/-}$ matings were injected with BrdU at embryonic day (E) 12.5 (A) or 15.5 (B) and the density of BrdU positive neurons in the subplate (A) or layers II/III (B) of the developing neocortex was analyzed at E18.5. The number of BrdU labeled cells that had migrated to these regions of interest was unaltered in both homozygous and heterozygous mutants compared to WT controls. C, D) in order to analyze the embryonic cells that had properly migrated as well as differentiated into and survived as neurons, mice that had been labeled at E15.5 were analyzed at postnatal day (P) 21. Due to the perinatally lethal phenotype of $\gamma2^{-/-}$ mice these experiments were limited to $\gamma2^{+/-}$ and WT mice. The number of BrdU-positive cells and BrdU/NeuN double positive neurons in the cortex (C) and hippocampus (D) was unchanged in $\gamma2^{+/-}$ vs. WT mice. Data indicate Means +/- SEM (t-tests, n = 5/genotype, $p > 0.05$ for all comparisons).

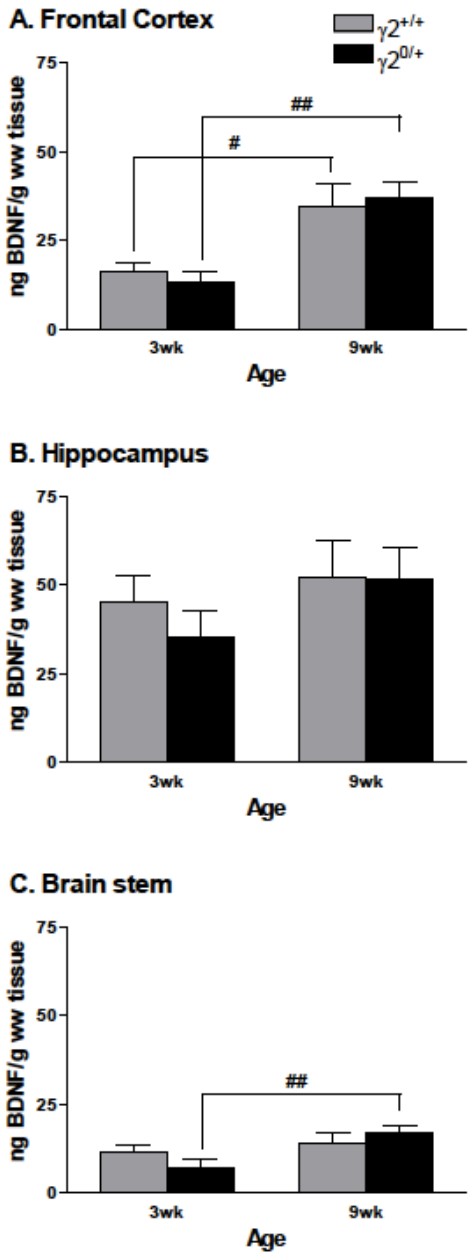

Figure 2. Quantitation of BDNF protein levels in g2+/- and WT mouse littermates in frontal cortex (A), Hippocampus (B) and brain stem (C). No genotype dependent differences were evident for any of the three brain areas analyzed, in both 3 and 9 week old mice. Comparison of samples of 3 and 9 week old brains revealed developmental increases in BDNF in Frontal cortex and brain stem as expected. # $p < 0.05$, ## $p < 0.01$

	Gene ID	Forward Primer sequence	Reverse Primer sequence	Product Size
Studied Genes	AGA	ACACACTCCTGCGCTTTC	TATGGCTGGGTCATCTCC	71
	ANKRD43	CCTAATGAATGTAACTAATCCCAAT	CTCACAAGCCCAACCATAC	77
	ARHGAP6	GGAAACCTTGCCTATGGAC	GACTTTCTTTTGTGTTGCTTTTT	87
	BCAS1	AAAGATTTGTTTCTGCACTATTTC	GACATTACAGACAGGCGTGA	105
	CACNB2	GGGAAGTAATATCCAAGAGAAGG	GGTCATTGGCGGATGTAT	70
	CAMK2D	ACTCACAGAACCCTCTTGCT	CTCCCTTTGTGGCTCTCA	160
	CDH13	TGGATCAAGACCCTAAAGGA	ACTGGCCCTTCCAGAGTT	148
	CHSY1	GAGTGCGAGCTAAACAGACA	AGCCATCTCCATCCACAG	89
	CNP	CCACATGCTAGGGACAAAC	GAGCCCTCTCTAAGCTACCA	60
	CPNE9	CGTTCAGTTTGTCCCATTC	GGAATCTCAGCTAGTACATCCTT	96
	DGKG	CCACCTGCAAGAAACTCC	GGAAGATGTTGCTCAGATCC	75
	EDG2	GTGTGTGCTCTCCAAACG	AGGGTCTGGCACTGAGAA	91
	EGR1	GGTCTCCAGAATGTAAGAAGAAA	CAACACTCCAACTCCTGAATAG	112
	ENPP2	GGCTTACGTGACATTGAGG	GTAGTGGGTAGGGACAGGAA	72
	GPR37	GTGACGACAACGACAACG	CATCTCCCTGCGGATAGT	71
	JUN	CAAGAACTGCATGGACCTAA	GTTAAGGAGCACTACAGAAGCA	108
	KCTD12	TACAAGGCCCATTAAACAAA	CAACTGGTCAACCCTCCTA	89
	MATN2	AGCCAACAGAACCTGAGC	GTGTAGACCGTGAAAGATTGTC	114
	MBP	ACTCACACACGAGAACTACCC	GAAGAAATGGACTACTGGGTTT	92
	MOBP	GCCTACGGCTCCTACACA	CCTGGAAGACAATGATGGA	88
	NPTX1	GTGGCTCGAAGTGGTCAT	GCAATACATTTCCCATTGTTAG	76
	PLLP	TGCCTTGTCATCAGAGAACT	AGGCTGAGGGTCTGTTAGTG	83
	PPP1R16A	GAGGCGTCTGTGGAGAAG	TGAACCCTGCCATACTGA	72
	RPH3A	CCATTCCCAAGTCTCACAG	CTCAGGAACAGCACGAGTT	86
Housekeeping Genes	ACTIN	CCTAGCACCATGAAGATCAA	GGAAGGTGGACAGTGAGG	
	GAPDH	AACTCCCACTCTTCCACCT	CACCACCCTGTTGCTGTA	
	CYCLO	AAAGCCGATGACAAGGAG	AAGAATCGTCGCTGGTATG	

Table 1 : Selected Genes and primers'sequence for qPCR analysis.

Table 1: list of genes analyzed by qPCR. See text for description.

Glial/Neuronal Gene	Gene Name	Human		Mouse Models			
		Male MD1 qPCR	Female MD3 qPCR	Male UCMS qPCR	Male UCMS-Flx qPCR	Male γ2 +/- qPCR	Female γ2 +/- qPCR
G	AGA	-0.60	0.19	-0.09	-0.04	-0.07	0.81
N	ANKRD43	0.16	0.09	0.16	-1.42	-0.08	-0.11
N	ARHGAP6	0.44	-0.18	0.34	-0.24	0.45	-1.39
G	BCAS1	-0.64	0.71	-0.40	-4.03	-0.11	0.07
N	CACNB2	0.27	0.02	0.06	-0.60	-0.01	-0.19
N	CAMK2D	0.49	-0.37	0.20	0.27	0.47	0.11
N	CDH13	-0.24	-0.28	0.02	-0.64	-0.19	-0.09
G	CHSY1	0.05	0.30	-0.03	-0.01	-0.15	0.01
G	CNP	-0.25	0.40	-0.29	-0.14	-0.21	0.07
G	CPNE9	-1.00	0.25	-0.32	-0.24	-0.42	0.65
N	DGKG	0.25	-0.13	0.28	-0.11	0.70	-0.38
G	EDG2	-0.39	0.64	-0.32	-0.31	-0.15	0.02
N	EGR1	-0.14	0.09	-0.13	-0.61	-0.74	0.20
G	ENPP2	-0.26	0.40	-0.33	-0.12	0.17	1.77
G	GPR37	-0.23	0.60	-0.25	-1.63	-0.11	0.07
N	JUN	0.04	0.36	0.50	0.41	0.37	0.06
N	KCTD12	0.21	0.02	0.36	0.13	-0.09	0.01
N	MATN2	0.49	0.17	0.55	-0.14	0.93	0.23
G	MBP	-0.49	0.65	-0.38	1.06	-0.26	-0.11
G	MOBP	-0.60	0.77	-0.14	-0.52	-0.99	0.29
N	NPTX1	0.39	-0.08	0.26	-0.32	-0.02	-0.13
G	PLLP	-0.56	0.43	-0.03	0.30	0.06	-0.03
N	RPH3A	-0.54	-0.11	-0.14	0.13	0.26	0.30
N	PPP1R16A		0.43	0.20	1.18	0.33	0.19

Table 2: ΔΔCT values in Human Depressed and Animal Models

Table 2: Summary of transcript changes analyzed by qPCR of total RNA isolated from amygdala of familial male depressed patients (MD1) vs. normal controls, non familial female depressed patients (MD3) vs. normal controls (from Sibille et al. 2009 and unpublished data from the Sibille lab), male UCMS mice vs. unstressed controls chronic fluoxetine treated male UCMS mice vs. unstressed controls (from Surget et al 2008), male $\gamma2^{+/-}$ vs. WT littermates, and female $\gamma2^{+/-}$ vs. WT littermates (this study). Note the transcript changes in male $\gamma2^{+/-}$ vs. WT mice closely follow the patterns seen in male patients and male UCMS treated mice compared to the respective controls (Pearson quotient of 0.58 and 0.62, respectively, with values > 0.48 being significant, 0 = no correlation and 1 = perfect correlation, Pearson value significance i.e. both correlations are highly significant). Similarly, gene expression changes seen in female $\gamma2^{+/-}$ mice appear to follow a pattern that is similar to that seen in female patients although this apparent correlation has not reached significance. Moreover, depression related transcript changes seen in males appear to run in opposite direction from those seen in females, and this sexually dimorphic pattern is seen both in patients and $\gamma2^{+/-}$ mice.

Research Project 8: Project Title and Purpose

Hemispheric Lateralization for Unilateral Reaching Movements - The purpose of this project is to test a hypothesis that the left and right brain hemispheres are differentially specialized in controlling different features of movement. To test this hypothesis, we will examine the effect of unilateral brain damage on the performance of the ipsilesional arm of stroke patients with hemiparesis during simple reaching movements made toward multiple target directions, and also during adaptation to novel dynamic conditions. Findings from this project will lead to a better understanding of the functional deficits of the ipsilesional arm in stroke patients, which may be useful to improve rehabilitation techniques for stroke patients.

Duration of Project

1/1/2006 - 6/30/2008

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 9: Project Title and Purpose

Assembly of Nipah Virus-Like Particles - Nipah virus is a recently emerged, highly pathogenic paramyxovirus. Like H5N1 avian influenza virus, Nipah virus spreads poorly between humans, and the disease is spread to humans primarily via contact with infected livestock. In the case of Nipah virus, transmission results from contact with infected pigs, and the mortality rate in humans is about 40%. The purpose of the research project is to develop an experimental system by which Nipah virus biology can be more easily studied. An additional purpose of the research project is to develop a vaccine that can protect pig-handlers and their livestock from Nipah virus infection.

Duration of Project

1/1/2006 – 12/31/2009

Project Overview

For many viruses, including the paramyxoviruses, it has been possible to generate virus-like particles (VLPs) by expressing only a small subset of "key" viral proteins in cells. The resulting particles resemble bona fide virus particles, but in reality are harmless mimics, lacking genetic material that is required for virus infectivity. The first aim of the research project is to define this "key" subset of proteins for Nipah virus that will allow VLP production. This will allow the process of particle formation, an important step in the normal life cycle of the virus, to be easily studied while avoiding the need for elaborate and costly biocontainment facilities that are required when studying the "live" virus. Various combinations of Nipah virus proteins will be expressed in cultured cells, and release of VLPs into the culture medium will be measured using biochemical assays such as western blotting. The second aim of the research project is to assess whether Nipah VLPs might be potentially useful as a vaccine to protect humans and pigs from Nipah virus infection. VLPs are often quite effective as vaccines, because they resemble authentic virus particles. In addition, VLPs lack viral genomes and are completely non-infectious, avoiding safety concerns associated with live-attenuated virus vaccines and other vaccination strategies. VLPs will be used to inoculate mice, and immune responses induced in the mice will be characterized. If VLPs are successful at inducing immune responses in mice, then challenge experiments will be carried out in collaboration with researchers at the CDC to test whether the VLPs are capable of protecting from an otherwise lethal Nipah virus infection.

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Expected Research Outcomes and Benefits

It is anticipated that on completion of the research project, a greater understanding of the Nipah virus assembly process will be achieved. This should provide a foundation for future studies that seek to block this step of the virus life cycle through development of antiviral drugs. In addition, it is anticipated that a new vaccine candidate for Nipah virus will be developed as a result of this project. This would have the potential to protect pig handlers and pig populations from Nipah virus infection in the event of either a natural outbreak, or of an incident in which the virus is released intentionally.

Summary of Research Completed

<u>Progress on the role of Nipah virus C protein in VLP production</u>

1. Nipah VLP production, driven by M protein, is enhanced by C protein.

To define the set of Nipah virus proteins important for VLP production, different combinations of viral proteins were expressed in 293T cells via transient transfection. After metabolic labeling, VLPs were purified from the culture medium using sucrose flotation gradients, and loaded directly onto SDS gels for quantification. As anticipated from earlier studies, the viral M protein expressed by itself led to significant VLP production (Figs. 1A and 1B). Various Nipah virus structural proteins, including N, F, G, and C, were co-expressed in cells together with M protein. The most significant effect on VLP production was observed when M protein was co-expressed together with C protein (Figs. 1A, 1B, and data not shown). The increase in VLP production upon C protein co-expression was approximately 3-fold, on average. These results suggest that the Nipah virus proteins most important for particle production are M and C.

Many retroviruses and other enveloped viruses recruit host machinery from the Class E pathway, also known as the Vps (vacuolar protein sorting) pathway, for budding. This pathway can be disrupted through expression of the dominant-negative (DN) form of the Vps4 ATPase. When DN Vps4 protein was expressed together with Nipah virus M protein, production of M-alone VLPs was unaffected (Figs. 1A and 1B). This result is in agreement with those obtained by Dr. Chris Broder's group, and suggests that any host machinery recruited by Nipah virus M protein for budding is unlikely to be part of the Class E pathway. On the other hand, VLP production

after M+C protein expression clearly was affected by expression of DN Vps4 protein (Figs. 1A and 1B). In this case, VLP production was reduced to a level similar to that observed when M protein is expressed by itself. Thus, disabling the Class E protein network seems to prevent C protein from enhancing VLP production. The most straightforward interpretation of this result is that C protein could directly or indirectly recruit proteins from the Class E protein network to virus assembly sites to assist Nipah virus budding. This scenario is consistent with earlier studies with another paramyxovirus, Sendai virus, in which Dr. Takemasa Sakaguchi's group found that C protein recuits the Class E protein Alix to virus assembly sites to facilitate VLP production. Although we have not detected interaction between Nipah virus C protein and Alix using co-immunoprecipitation assays (data not shown), it is possible that Nipah virus C protein recruits other Class E proteins to facilitate particle budding.

2. Nipah virus C protein binds cellular membranes via an N-terminal domain.

The possibility that Nipah virus C protein has an intrinsic membrane binding capability was investigated. Such an activity would reinforce the notion that C protein recruits host factors to virus assembly sites on cellular membranes for budding, as suggested from Figs 1A and 1B. Although C proteins are internal proteins and lack transmembrane domains, the N-terminal portion of Nipah virus C protein contains a cluster of six positively-charged amino acid residues (Fig. 1C). This could potentially allow electrostatic interactions with the phospholipid head groups of cellular membranes. To assess membrane binding, Nipah virus proteins were expressed in 293T cells via transient transfection, and cell lysates were prepared in the absence of detergent by dounce-homogenization. Samples were applied to the bottoms of sucrose flotation gradients, and fractions were collected after ultracentrifugation. Membrane-bound proteins float to the top three fractions of the gradient. 29% of Nipah virus C protein was found in the membrane-bound fraction (Fig. 1D). By comparison, 30% of Nipah virus M protein (known to be the predominant protein at paramyxovirus asembly sites) was membrane-bound. Removal of the N-terminal basic cluster of amino acid residues prevented membrane-binding by Nipah virus C protein (Fig. 1D). C protein lacking it's membrane-binding function also lost much of its ability to enhance VLP production (Fig. 1E), as VLP production increased by only 1.8-fold in the presence of mutant C protein, but increased by 3.4-fold in the presence of wt C protein. These results are consistent with the possibility that Nipah virus C protein functions to re-route host proteins such as Class E proteins to cellular membranes, where they can potentially act to facilitate the budding of virus particles.

To further explore the membrane binding characteristics of Nipah virus C protein, the N-terminal 20 amino acid residues were appended to the N-terminus of GFP, and the modified GFP protein was tested for the ability to bind cellular membranes, judged by flotation on a sucrose gradient. The modified GFP protein was able to bind with membranes with an efficiency similar to that of wt Nipah virus C protein, while unmodified GFP did not bind membranes (Fig. 2). Thus, we have identified a membrane-targeting sequence near the N-terminus of the Nipah virus C protein that is important for the ability of C protein to enhance production of Nipah VLPs.

Progress on the role of host proteins in paramyxovirus particle production

3. Inhibition of paramyxovirus budding using polypeptides derived from host protein angiomotin-like 1 (AmotL1).

In a separate but related project, we used yeast two-hybrid screening with PIV5 M protein as bait to identify PIV5 M-interacting host factors. Host protein AmotL1 was identified in this screen. Interaction between AmotL1 and PIV5 M protein was confirmed in mammalian cells by co-immunoprecipitation (data not shown). The region of AmotL1 that binds PIV5 M protein was mapped to a 83 amino acid-long fragment (AmotL1-m), and expression of AmotL1-m in cells very effectively blocked the production of PIV5 VLPs (Figs. 3A and 3B). This finding is consistent with the general notion that short fragments of budding relevant host proteins that bind to M proteins can act as potent inhibitors of paramyxovirus budding, likely because they act as competitive inhibitors and prevent binding by the endogenous host proteins, or otherwise disrupt viral M protein function on binding.

To investigate the possibility that AmotL1-derived polypeptides might inhibit the budding of a broad range of paramyxoviruses in addition to PIV5, these polypeptides were expressed in cells transfected to produce Nipah VLPs or mumps VLPs (Fig. 3C). This had no effect on Nipah VLP production, but partially inhibited the production of mumps VLPs. We conclude that Nipah virus likely recruits host factors for budding that are distinct from those recruited by PIV5 and mumps virus.

Publications resulting from this project

Pei, Z., Bai, Y., and Schmitt, A.P. (2010). PIV5 M protein interaction with host protein angiomotin-like 1. *Virology* 397, 155-166.

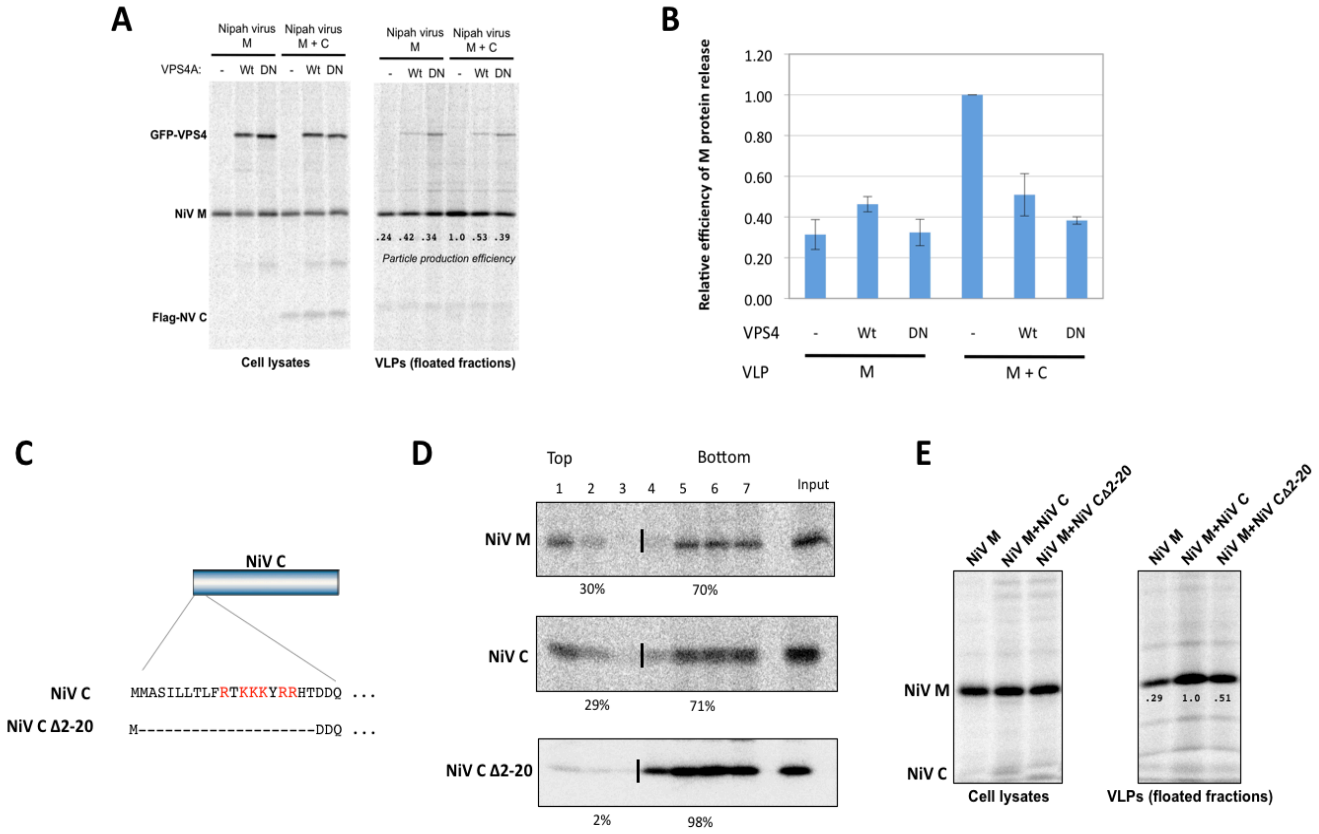

Figure 1 Enhancement of Nipah VLP production by the membrane-binding C protein. (A) 293T cells were transfected to express the indicated proteins for VLP production using pCAGGS vectors. After metabolic labeling, VLPs released into culture supernatants were purified on sucrose flotation gradients and loaded directly on SDS gels. Viral proteins in cell lysates were detected by immunoprecipitation. Particle production efficiency was calculated as the amount of M protein detected in VLPs divided by the amount detected in cell lysates, and was normalized to the M+C particle production efficiency. (B) VLP production was measured from three experiments performed as in (A), with standard deviations indicated by error bars. (C) Schematic diagram of Nipah virus C protein, with basic residues colored red. (D) 293T cells were transfected to produce the indicated proteins. Membranes from Dounce-homogenized cell lysates were fractionated using sucrose gradient flotation. Membrane-bound proteins are present in fractions 1-3 of the gradient. (E) 293T cells were transfected to express the indicated proteins, and VLP production was measured as described in (A). Experiment conducted by T.M. McCrory.

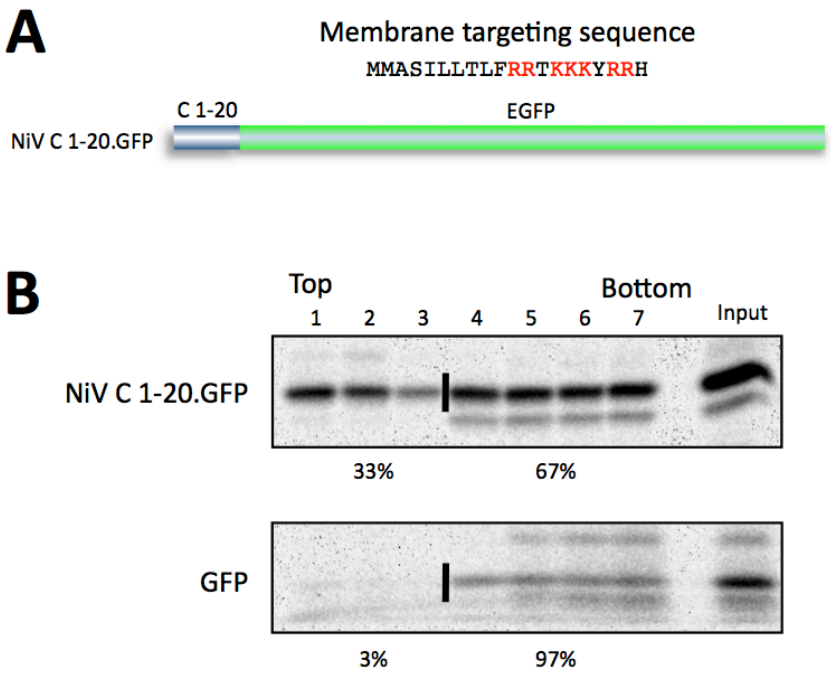

Figure 2 The N-terminal portion of Nipah virus C protein acts as a membrane-targeting sequence, capable of directing a heterologous protein to membranes. (A) Schematic illustrating fusion of the N-terminal 20 amino acid residues of Nipah virus C protein to EGFP. (B) 293T cells were transfected to produce the indicated proteins. Membranes from Dounce-homogenized cell lysates were fractionated using sucrose gradient flotation. Membrane-bound proteins are present in fractions 1-3 of the gradient. Experiment conducted by T.M. McCrory.

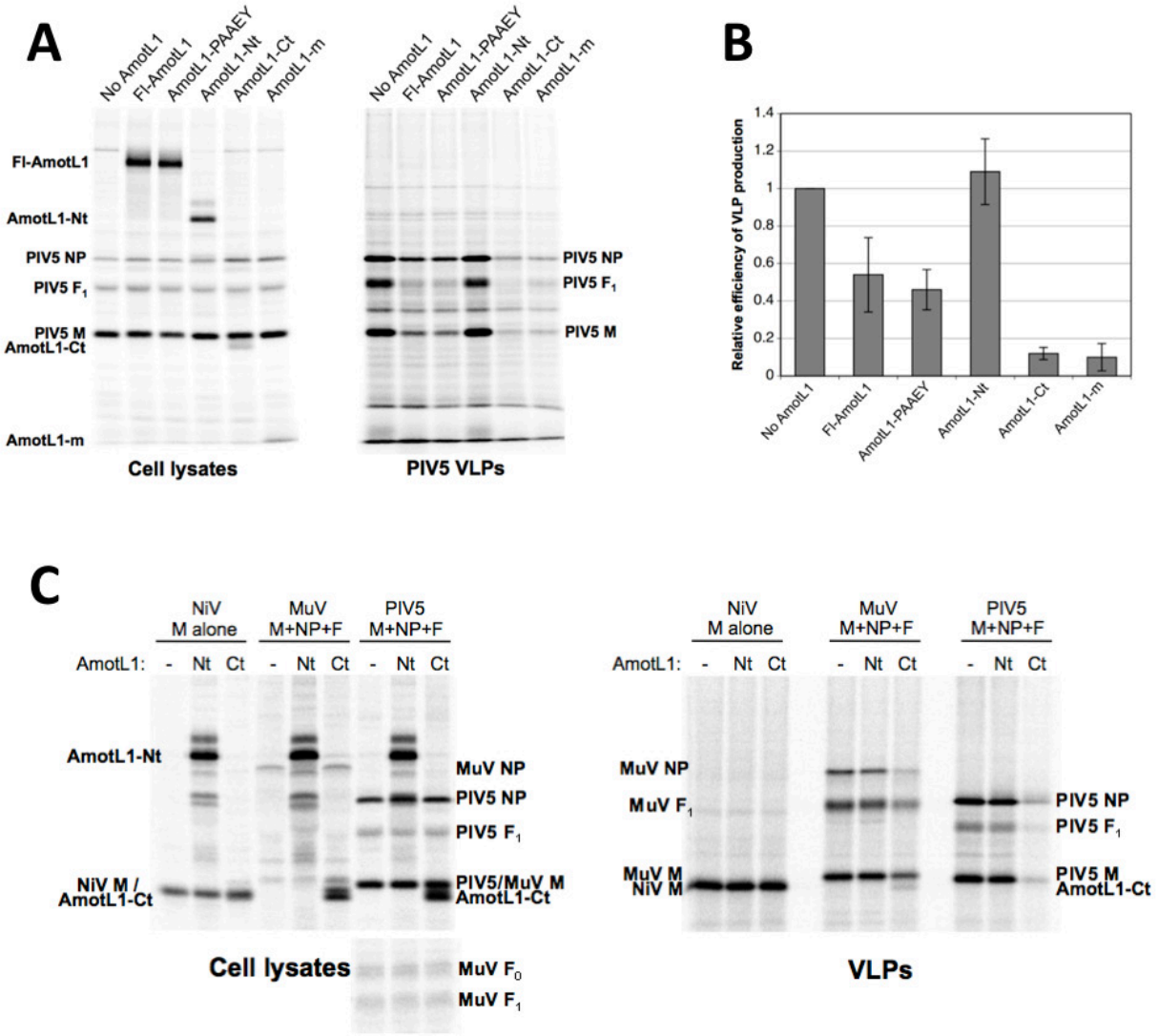

Figure 3 AmotL1-derived polypeptides inhibit budding of a subset of paramyxoviruses. (A) 293T cells were transfected to express the PIV5 M, NP, and F proteins for VLP production, together with the indicated AmotL1-derived polypeptides. AmotL1-m and AmotL1-Ct both bind to PIV5 M protein, and both inhibited VLP production. AmotL1-Nt lacks the M-binding region and failed to affect VLP production. (B) Results were quantified from four independent experiments performed as in (A), with standard deviations indicated by error bars. (C) AmotL1-derived polypeptides were expressed together with Nipah virus, mumps virus, or PIV5 proteins, and effects on VLP production were measured. The PIV5 M-binding polypeptide AmotL1-Ct blocked production of PIV5 VLPs, partially inhibited the production of mumps VLPs, and had no effect on production of Nipah VLPs. AmotL1-Nt fails to bind PIV5 M protein and had no effect on production of any of the VLPs. Modified from Pei, et al., Virology (2010), 397, 155-166. Experiment conducted by Z. Pei.

Research Project 10: Project Title and Purpose

DNA Repair and Susceptibility to Tobacco-Induced Cancer - The long-term goal of this research is to identify high-risk subgroups that are susceptible to cancer and might benefit

disproportionately from screening or chemoprevention interventions. We hypothesize that reduced DNA repair capacity increases susceptibility of tobacco-induced lung cancer. The current protocols that test DNA repair capacity of an individual are not adequate to determine whether an individual is at risk.

Duration of Project

1/1/2006 - 12/31/2009

Project Overview

It has long been recognized that people differ in their susceptibility to cancer. Some of these differences are attributed to heritable traits that can modify the effects of environmental exposures. More than 80% of lung cancers are attributed to tobacco. However, only a fraction of smokers (~15%) will develop lung cancer in their lifetime. One of the several cellular processes that could explain this inter-individual variation in risk is DNA repair capacity. DNA repair plays a central role in defending cells against the carcinogenic assault of tobacco. There is a wide inter-individual difference in DNA repair capacity within a population. At the extreme end are patients with deficient DNA repair, which is a direct cause of cancer. There may also be a larger subgroup of patients with reduced DNA repair capacity that are at risk for cancer but are phenotypically normal.

This study will attack this problem with three interdisciplinary projects led by three project leaders. (1) Using biochemical techniques, we will examine the DNA repair of two different polycyclic aromatic hydrocarbons that are repaired at different rates. We will determine whether a transcription-coupled or global genomic DNA repair assay correlates best with carcinogenesis. (2) Using analytical chemistry techniques, we will determine whether decreased DNA repair capacity correlates with decreased levels of DNA adducts. (3) Using genetic techniques, we will determine whether the decreased DNA repair capacity is associated with a specific haplotype of the ERCC1 (a helicase) and ERCC2 (also know as XPD, an endonuclease) DNA repair genes that are located within 70 Kb on the 19th chromosome. This combined biochemical and genetic approach should enable us to better identify sub-groups within the population that exhibit deficient DNA repair and are at increased risk for cancer.

Principal Investigators

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Other Participating Researchers

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Expected Research Outcomes and Benefits

The identification of risk factors associated with tobacco-induced lung cancer has important health related benefits. The successful outcome of this program project will result in (1) increased knowledge about how reduced DNA repair capacity impacts carcinogenesis, (2) a better laboratory test to measure the DNA repair phenotype of individuals, and (3) the first haplotype test to identify individuals with reduced DNA repair capacity.

Summary of Research Completed

This work is a continuation of work described in the Annual Report submitted in 2007.
In that work, we used the host cell reactivation assay to examine the DNA damaged by the (+/-)-*anti*-diol epoxides (DE) of benzo[a]pyrene (B[a]P), benzo[c]phenanthrene (B[c]Ph), dibenzo[a,l]pyrene (DB[a,l]P), and benzo[g]chrysene (B[g]Ch) in cell lines. In the Annual Report in 2008 we described that we were unable to translate this work to peripheral blood lymphocytes so that the assay can be used with human samples.

This past half-year we quantitate the amount of damage produced by the PAH-diol epoxides using HPLC with fluorescence detection.

Methods and Materials
The carcinogen-damaged plasmid containing the firefly luciferase gene was prepared by reacting the pGL3 plasmid (0.5 mg/mL) with various concentrations of the (+/-)-*anti*-diol epoxides (DE) of benzo[a]pyrene (B[a]P), benzo[c]phenanthrene (B[c]Ph), dibenzo[a,l]pyrene (DB[a,l]P), and benzo[g]chrysene (B[g]Ch) in 10 mM Tris-HCl (pH 7.4), 1 mM EDTA. See Figure 1 for the structures. pGL3 (500 μg/mL) was incubated with the PAH-DEs at various concentrations for 3 h at 37°C in the dark. Un-reacted PAH-DE and the tetrols derived from their hydrolysis were removed by ethyl acetate (3×) and ether (1×) extraction, followed by nitrogen bubbled through the solution for 5 min to evaporate the ether. The DNA was diluted with 10 mM Tris-HCl (pH 7.4), 1 mM.

The amount of damage to the plasmid was determined by acid hydrolysis followed by HPLC separation with fluorescence detection. Damaged DNA (50 μL, 0.1 mg/mL) was acidified with 6 μL 1 N HCl and heated at 80°C for 30 min. The solution was cooled on ice and neutralized with 6 μL 3 N NaOAc. DNA was precipitated by the addition of 150 μL ethanol and pelleted by centrifugation. The decanted solution was analyzed by reverse-phase HPLC with a 150 x 4.6 mm Gemini-NX 5u NX C18 column. The column was eluted with a linear gradient from 80% 10 mM sodium phosphate (pH 7.0) 20% methanol to 100% methanol over 24 min at 1 mL/min at 40 C. The effluent was monitored by UV at 260 nm and by fluorescence (B[a]P tetrols , λ_{ex} = 344, λ_{em} = 398 nm; DB[al]P tetrols, λ_{ex} = 340 nm, λ_{em} = 410 n; B[*g*]Ch and B[*c*]Ph tetrols, λ_{ex} = 292 nm, λ_{em} = 375 nm). The chromatograms are illustrated in Figure 2.

Total amount of DNA was determined by enzymatic hydrolysis followed by HPLC analysis. The DNA (10 µL of 0.5 mg/mL) was treated with DNase I (100 units) for 4 h followed by phosphodiesterase I (2 units) for 4h followed by alkaline phosphatase (100 units) for 4h in 50 mM-Tris-HCl (pH 8.0, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$ at 37 C. The enzymes were removed by ultrafiltration and the dG quantitated by reverse-phase HPLC.

Results and Discussion

The quantification allowed the data to be plotted as shown in Figure 3. Figure 3D shows the amount of luciferase produced by the XP-A cells. The first important point is that all damage affects the production of luciferase equally. This indicates that all of the damage is equally effective at inhibiting RNA polymerase II transcription. The second point is that one damage per transcribed strand of the luciferase gene can block transcription. Figure 3A shows that the repair proficient cells exhibit different degrees of luciferase production. This indicates that the various damage are repaired with different efficiencies in the order B[*a*]P, DB[*a,l*]P > B[*g*]Ch > B[*c*]Ph. The data in Figure 3B and C are consistent with B[*c*] Ph being repaired less efficiently than the other damage.

These results show that the identity of the PAH plays a role in the rate of transcription-coupled nucleotide excision repair.

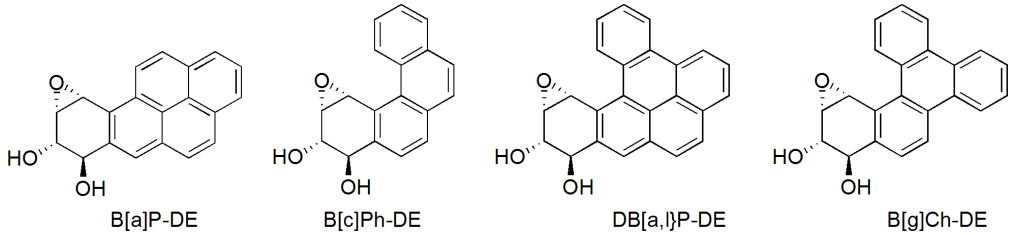

Figure 1. Structures of the anti-diol epoxides used in this study.

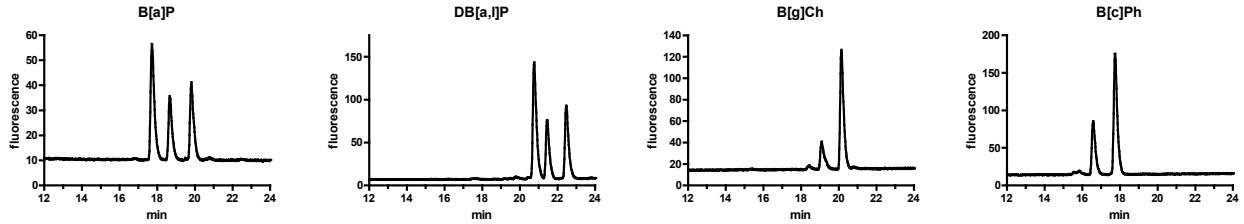

Figure 2. HPLC chromatogram of acid hydrosylate of pGL3 damaged with the diol epoxides of B[*a*]P, DB[*a,l*]P, B[*g*]Ch, and B[*c*]Ph.

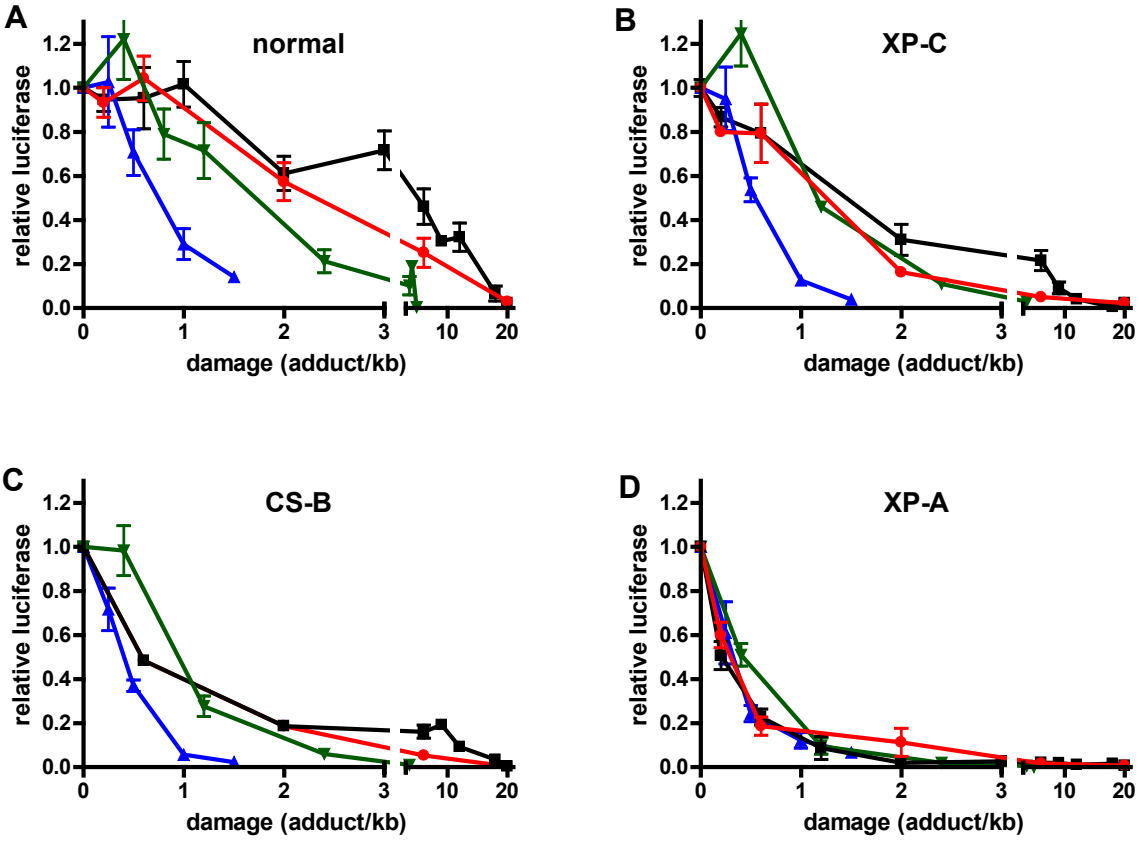

Figure 3. Dose response for the repair of DNA damaged with PAH-DE. pGL3 control plasmid was damaged at varying extents with (■) B[*a*]P-DE, (●) DB[*a,l*]P-DE,d (▼) B[*g*]Ch-DE, and (▲) B[*c*]Ph-DE. The damaged pGL3 was mixed with undamaged phRL (49:1 ratio). The DNA (2 μg) was transected into (A) DNA repair proficient (B) XP-C, (C) CS-B, and (D) XP-A B-lymphocyte cell lines. After 24h, the luciferase production was determined. The Y-axis is the relative luciferase production as described in equation 1 in which the undamaged pGL3 is the value at 0 adducts/kb. There values are the mean of 3 to 5 determinations and the error bars are the standard deviations.

Research Project 11: Project Title and Purpose

Human Retinal Pigment Epithelial Cell (hRPEC) Mediated Ex-vivo Gene Therapy in Parkinson's Disease - Parkinson's Disease (PD) is a devastating neurological illness that affects 1 million Americans and the health of a large population of senior citizens in Pennsylvania. hRPEC transplants are a novel and newly emerging technique that has enormous promise in helping PD patients. hRPEC transplants into the brain of parkinsonian animals and in 6 PD patients have shown significant amelioration of parkinsonism with minimal side effects. In this project we will test the mechanisms through which hRPEC mediates its effects, explore methods to enhance such beneficial effects and use hRPEC as a tool to "prevent" or "retard" the progression of PD. Results from these studies would further elucidate the biology of hRPEC transplants and its use in the PD patients and also in other major medical illnesses that could benefit from cell therapy, such as diabetes and macular degeneration.

Duration of Project

1/1/2006 - 12/31/2009

Project Overview

Human Retinal Pigment Epithelial Cells (hRPEC) xenotransplants into the striatum of non-immunosuppressed parkinsonian animals and hRPEC allografts in Parkinson's disease (PD) patients have shown significant amelioration of parkinsonism with no evidence of a host immune response or graft rejection. hRPEC striatal transplantation in PD patients ameliorates drug-induced dyskinesias, increases total "on" time and does not cause "runaway dyskinesias". In this project we will test the mechanisms through which hRPEC mediate their anti-parkinsonian and anti-dyskinetic effects. hRPEC will be labeled with enhanced green florescent protein (EGFP). Preliminary studies indicate that hRPEC secrete dopa and that they do not express the enzyme aromatic aminoacid decarboxylase (AADC), which is required for efficient conversion of dopa to dopamine. hRPEC can be genetically engineered using recombinant lentiviral vectors to introduce the AADC gene, and such modified cells remain stable and express the transgene when transplanted into the brain. In specific aim 1, we will examine the hypothesis that introduction of the AADC gene into hRPEC will enhance the production and secretion of dopamine and that intrastriatal transplantation of hRPEC-AADC-EGFP is superior in ameliorating parkinsonism when compared to striatal transplants of hRPEC-EGFP alone. In specific aim 2, we will test the hypothesis that intrastriatal transplantation of hRPEC-AADC-EGFP is superior to intrastriatal transplantation of hRPEC-EGFP alone to ameliorate drug-induced dyskinesias in parkinsonian animals "primed" to develop dyskinesias. In specific aim 3 we will introduce glial derived neurotrophic factor (GDNF) into hRPEC cells and use them to test the notion that GDNF secreting hRPEC cell transplants into the striatum will slow down the progression of parkinsonism . We will use behavioral tests, immunohistochemistry and in vivo microdialysis as assessment tools to test our hypothesis and to delineate the pathophysiology of graft function. These studies will provide important insight into the pathophysiology of drug-induced dyskinesias. Results from these studies would further elucidate the biology of hRPEC striatal transplants, pathophysiology of drug induced dyskinesias and the potential neuroprotective effects of hRPEC-GDNF-EGFP, and provide preclinical data for hRPEC mediated ex-vivo gene therapy in advanced PD patients to ameliorate drug-induced dyskinesias and to provide continuous transplant mediated symptomatic dopaminergic therapy. These studies, using an immune privileged hRPEC, will also have broad implications for the use of ex-vivo gene therapy for diabetes and macular degeneration.

Principal Investigator

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Other Participating Researchers

Kala Venkiteswaran, PhD - employed by Pennsylvania State University

Expected Research Outcomes and Benefits

Three major outcomes are expected. 1. Genetic modification of hRPEC cells will enhance their ability to help patients with PD. If this outcome is achieved, the implications are enormous to over 1 million PD patients in the United States in that they could potentially have this cell carefully placed into their brain for symptomatic control. Such a result will also throw open the use of hRPEC in helping patients with other devastating illnesses like diabetes and macular degeneration, disorders that effect a large number of elderly individuals. 2. hRPEC transplants prevent the occurrence of disabling side effects associated with current modalities of treatment in PD. This result will imply that hRPEC transplants can be used to prevent a number of complications that plague patients with advanced PD at the current time. Such a benefit will reduce morbidity and health care costs by avoiding these side effects. 3. Neuroprotection can be achieved by hRPEC mediated release of GDNF. Such a result will revolutionize the field. For the first time we will have an agent that will slow down the progression of a neurodegenerative disorder. Implications for similar therapy for other neurodegenerative disorders like Alzhiemer's disease (AD) and Amyotrophic Lateral Sclerosis (ALS) will be huge. All three outcomes taken together will move the field of cell transplantation dramatically forward with positive implications for the treatment of a number of disorders that contribute to human suffering including PD, diabetes mellitus, macular degeneration, AD and ALS.

Summary of Research Completed

We have made progress in all Specific Aims: *These data are shown in the following pages and have led to the following peer reviewed publications or presentations.* 2 NIH RO1 grants have been submitted as a result of this work and are in the process of getting reviewed.

1. Gilmour, T., Fang, J., Guan, Z., Subramanian, T., Manual rat sleep classification in principal component space. *J Neuroscience Letters, 469 (2010) 97–101.* PMID: 19944737
2. Subramanian, T., Lieu, C. A., Guttalu, K, Berg, D., Detection of MPTP-induced substantia nigra hyperechogenicity in rhesus monkeys by transcranial ultrasound, *Ultrasound in Medicine and Biology, in Press, (2010)* doi:10.1016/j.ultrasmedbio.2009.12.001

<u>Methods</u>:
E 13.5 mFVM were dissected and prepared as single cell suspension and plated at a density of 100,000 cells per well as previously described by Hou et. al. into 8 wells of a 12 well plate until a designated day *in vitro* (DIV). A set of 3 wells (group 1) were designated as "growth factor before MPP+ group", a second set of 3 wells (group 2) were designated as "growth factor with MPP+ group". One well remained untreated for the entire experiment and one well received MPP+ only and no other treatment except for medium change. Each experiment was done in triplicate. On DIV 0**,** three group 1 wells were fed with growth factors containing medium:

GDNF (1ng/ml), PEDF (100ng/ml) or hRPEC-CM (1:1 with medium) and 4th well. On DIV 1, 8 wells were treated with MPP+ (100uM for 1 hour at 37°C) following which, the designated wells in both groups of wells received either GDNF (1ng/ml), PEDF (100ng/ml) or hRPEC-CM (1:1) each day for 21 days. We chose to keep cultures until day 21 based on recent findings by Jakobsen et. al., that suggest longer cultures to detect the effects of growth factors on MPP+ induced neurotoxicity. On DIV21 the cultures were fixed and stained for TH by immunochemistry. The positive dopaminergic neurons were scored by Petrimetrics unbiased stereology method using Stereo Investigator software by MBF Biosciences. From the counts obtained, the total number of neurons was calculated using the formula:

$$N_{total} = \sum Q \times 1/asf$$

Where N_{total} is the total number of TH positive neurons, Q = the number of objects captured by unbiased counting frame, $\sum Q$ = the sum of counts obtained from all the counting frames and 'asf' is the area of sampling fraction which is obtained by the equation asf = counting frame area / sampling grid area. The counting frame area was set as 300 x 300um and sampling grid area was set as 900x900 microns.

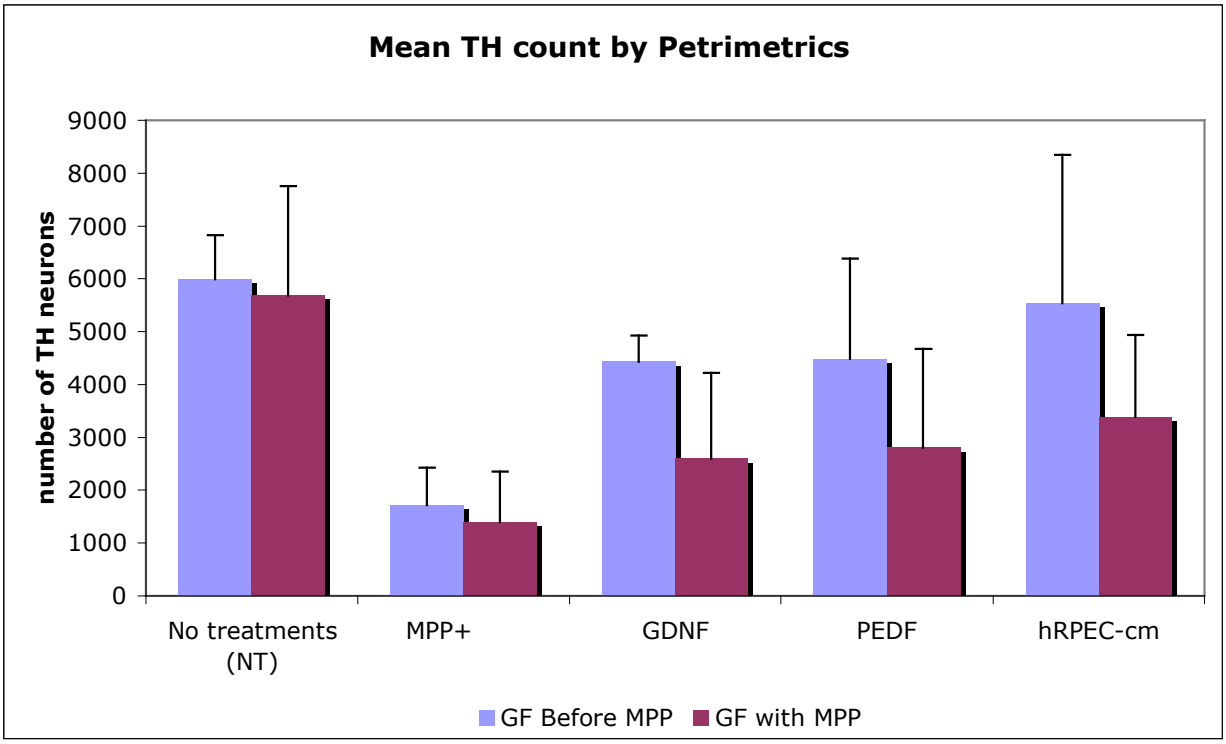

Figure 1: Results from 3 experiments. This graph shows the effects of treatments with PEDF, GDNF, hRPEC-cm (collectively termed, GF) on TH cell counts in mFVM cultures exposed to MPP+ treatments. The first two bars labeled "no treatment" (NT) are mFVM cell cultures that were not treated with MPP+ (positive control). The second set of bars are negative controls that were treated with MPP+ alone (no growth factors were added).The blue bars represent cultures that were pretreated with growth factors. The maroon bars indicate results from cultures that were simultaneously treated with MPP+ and growth factors. All growth factor treatments were statistically significant versus negative control (MPP+) using student t-test, 2 sample assuming unequal variances with the following p values for pretreatment *, $p<0.01$ and for simultaneous treatments with MPP+ **, $p<0.05$. *These findings confirm the notion that PEDF has potent neurotrophic/neuroprotective effects on FVM cells when applied prior to or simultaneously with MPP+ exposure. The effects of PEDF treatments appear to be statistically not different from the effects of GDNF and hRPEC-cm treatments.* Additional quantitative analysis of TH axonal length (neurolucida) and total fiber density (petrimetrics) are ongoing.

Cell Culture Study Design

No treatment	GDNF before MPP	PEDF before MPP+	hRPEC-CM before MPP+
MPP+	GDNF with MPP+	PEDF with MPP+	hRPEC-CM with MPP+

Research Project 12: Project Title and Purpose

Center of Excellence in Nutrigenomics: BOLD Effects on Established and Emerging Cardiovascular Disease Risk Factors - The field of nutrition, as well as many others, is moving in a direction where global analytical methodologies such as genomics, proteomics, and metabonomics are providing important new insights into health and disease processes. "Nutrigenomics" describes the use of cutting-edge molecular technologies to understand diet-gene interactions and human or animal health. The present project will provide continued support for the Center of Excellence in Nutrigenomics (CEN) at Pennsylvania State University with the ultimate goal of obtaining multi-investigator extramural support. As part of this effort, a pilot research program that fully utilizes the CEN's infrastructure and expertise will be initiated where the hypothesis being tested is that a heart-healthy diet, including lean beef (the BOLD diet), will reduce Cardiovascular Disease (CVD) risk.

Duration of Project

1/1/2006 - 6/30/2006

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Infrastructure Project 13: Project Title and Purpose

Research Infrastructure for a Center for the Treatment, Prevention, and Cure of Cancer - To design and construct a new building for the Pennsylvania State Cancer Institute that will bring together patient care, basic and translational research under one roof to enhance our ability to provide comprehensive, coordinated cancer care. The new facility will allow top researchers to work together to create new therapies and to bring them quickly into state-of-the-art clinical care for the patients of central Pennsylvania.

Duration of Project

7/1/2007 – 12/31/2009

Project Overview

The long-range goal of this project is to continue to work towards developing the Pennsylvania State Cancer Institute (PSCI) as a world-class center for the treatment, prevention, and cure of cancer in Central Pennsylvania, and achieving designation by the National Cancer Institute as a Comprehensive Cancer Center. Cancer has been designated as the primary priority area for growth by the Pennsylvania State College of Medicine/Hershey Medical Center, and a new 159,000 square foot cancer center building is proposed that will combine clinical cancer care with basic and translational research. The aims of this project are: (1) Integrate cancer patient

services at the Hershey Medical Center into one building designed specifically for cancer care; (2) To develop the research infrastructure to bring together patient care, basic, and translational research under one roof to enhance the ability of PSCI to provide comprehensive, coordinated cancer care to Central Pennsylvania; (3) To develop the resources, infrastructure and leadership to apply for designation from NCI as a Comprehensive Cancer Center. Centralizing patient care, basic and translational research in one location will greatly enhance the ability of PSCI to provide comprehensive, coordinated care. This will allow top researchers to work together to create new therapies and bring them quickly into state-of-the-art clinical care. However, the greatest impact of the new building will be on the lives of cancer patients in Central Pennsylvania and their families, who will benefit from the increased efficiency in translating advanced medical research into increased survival and a better quality of life.

Principal Investigator

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Other Participating Researchers

Philip Lazarus, PhD, Karam El-Bayoumy, PhD, Tony Pegg, PhD, Harriet Isom, PhD, Witold Rybka, MD – employed by Pennsylvania State University

Expected Research Outcomes and Benefits

There is a need for academic health centers, in the nation, state, and Central Pennsylvania to increase the knowledge base related to cancer, and to aggressively prevent, treat and cure the many forms of this disease in diverse populations. The construction of a new facility at Pennsylvania State Cancer Institute (PSCI) combining clinical care and basic science research under one roof will allow the recruitment and retention of outstanding cancer researchers in Central Pennsylvania, and enable State funds to be leveraged for federal and industrial funds to enhance innovative research discoveries and effective treatments. It will increase interactions between basic and clinical scientists, thus translating promising scientific discoveries into new and better options for the prevention, diagnosis, and treatment of cancer. The benefits will be improved care for cancer patients, and, through interactive community education programs, improved knowledge of diverse individuals in the communities in Central Pennsylvania about all aspects of cancer, including prevention and control. Recognition of the PSCI as a Comprehensive Cancer Center by the National Cancer Institute will increase the recognition and competitiveness of Pennsylvania State researchers and colleagues for national funding. It will also allow PSCI to recruit the best trainees and new scientists and clinicians, and encourage the establishment of new high technology "spin offs" as an economic engine for Central Pennsylvania. Meanwhile, cancer patients in Central Pennsylvania and their families will benefit from the increased efficiency in translating advanced medical research into increased survival and a better quality of life.

Summary of Research Completed

Aim (1) Construction progress:
The construction of the Penn State Cancer Institute is now complete. The Certificate of Occupancy was awarded on June 8, 2009 and Clinical operations opened to the public on July 13, 2009.

Aim (2) Development of Research Infrastructure:
The PSCI continued to focus on improving the infrastructure of the organization, building collaborative relationships between researchers and physicians that span disciplines and campuses, and striving to bring a greater cancer focus to ongoing research.

In 2009 PSCI completed the recruitment of Dr. Wafik S. El-Deiry, M.D., Ph.D. to serve as the Chief of the Division of Hematology Oncology and the Associate Director for Translational Research. Dr. El-Deiry is the Principal Investigator on several National Cancer Institute funded research projects. His research lab will be located on the 3rd floor of the new building. Dr. El-Deiry will be recruiting several additional clinical and research faculty. Also in 2009, a new clinical faculty member was added to the Division of Hematology Oncology. In addition, the PSCI recruited Thomas J. O'Leary, Ph.D. as the Associate Director for Research Administration. Dr. O'Leary has over 20 years experience in the administration of NCI designated cancer centers.

Aim (3) Progress towards NCI designation:
The progress of the Cancer Institute and researchers are evaluated by a number of internal and external committees. The PSCI continues to be evaluated on a monthly basis by the governing internal committees (Executive Committee, Scientific Leadership Committee, Research Team, and Departmental Chairs and Section Chiefs) in place at PSU COM/HMC. The external bodies responsible for reviewing the PSCI include the NCI Cancer Center's Branch and the PSCI External Advisory Board (EAB). Each of these reviews will be expanded upon below. In September of 2009, the External Advisory Board again visited PSU. For this review, the Cancer Institute leadership prepared a draft Cancer Center Support Grant (CCSG) application. The focus of the EAB session was to critique the draft application. Overall, the EAB had a very favorable review as summarized by their statement: "*We were uniformly impressed that the PSCI has made enormous progress and now seems poised to submit its initial application with an excellent chance of success.*"

The EAB felt that important progress was made by PSU toward each of the six CCSG Essential Characteristics as detailed below. Cancer Focus: The EAB believes that Cancer Focus has improved. Faculty membership in the PSCI has been more clearly defined, with scientific members limited to those with cancer-related peer-reviewed funding. Cancer Director Authority: Dr. Loughran was judged to be a qualified clinical scientist who has all the attributes needed to lead the cancer center. He has the appropriate institutional authority to assign administrative and research space; to oversee all PSCI budgets and designated philanthropic gifts/endowments; and to make cancer center appointments and to jointly control faculty appointments. As well, all outpatient cancer services are under the direction of Dr. Loughran. The fact that under his

leadership a dedicated cancer center building has opened and more than 75 faculty members have been recruited is testimony to Dr. Loughran's broad and exceptional authorities. Institutional Commitment: The opening of the new building demonstrates outstanding institutional commitment for the cancer center. Facilities: The research and clinical facilities of PSCI have expanded since the last review. The new 178,000 square foot facility is impressive and clearly designed to foster translational research. Organizational Capabilities: In large part, the lines of authority are becoming clearer with this draft application. However, the organizational chart presented in the application was difficult to read. The recruitment of Tom O'Leary as Associate Director of Research Administration is an outstanding choice, and a good bulk of the narrative dedicated to his background and recent accomplishments in his short tenure at PSCI are important highlights of this section. Interdisciplinary Interactions: There is seed funding from the College and the Cancer Institute to enhance interactions for grant submissions. The EAB applauded the research Program seminars and retreats that are held on a very regular basis sponsored by each of the Programs. The number of intra-programmatic publications is very good and should continue to increase. The challenge will be to convert these ongoing meetings to a greater number of inter-programmatic publications and grants.

In accordance with the Cancer Center Support Grant (CCSG-P30), guidelines, a three hour NCI pre-application consultation was held December of 2009 with the leadership of PSCI and Dr. Linda K. Weiss, Chief of the Cancer Centers Branch. As was the case in the August of 2008 visit between PSU leadership and NCI Cancer Center's staff, a written document containing the types of information that will be included in the CCSG application was provided to Dr. Weiss and her staff in advance of the meeting. This document included databases for membership, funding and publications for each of the four Scientific Research Programs, a written overview for each program defining the specific aims and progress made to achieve those aims for each program, a discussion of how PSCI has addressed each of the six essential characteristics of a Cancer Center, a Scientific Overview, and data on PSCI Shared Resources Core Facilities utilized for research by members of each of the four Scientific Programs. Presentations regarding PSCI progress to date were made by the Dr. Loughran.

Overall, this review was favorable and the NCI provided a letter granting PSU permission to apply for CCSG designation in May of 2010. Strengths included the successful recruitment and hiring of individuals to fill key leadership positions and the establishment and growth of the four Scientific Programs. Regarding cancer focus, the NCI staff was pleased to see the large increase in peer reviewed cancer funding but recommended that we should increase NCI funding in one of the 4 research programs. The NCI staff recognized that both the interprogrammatic and intraprogrammatic collaborations had been initiated and fostered but demonstration of these collaborations by coauthored publications and collaborative funding needs to increase. As a new Cancer Center, the number of such publications will be lower than is seen in an established NCI designated center. The PSCI was recognized as having in place mechanisms for promoting interactions between the basic scientists and physician scientists to move translational research forward, and it was anticipated that this process would result in many additional collaborations. The NCI staff felt that the opening of the new PSCI building was very important to the success of our upcoming application.

Research Project 14: Project Title and Purpose

Longitudinal Examination of the Structural, Metabolic and Functional Brain Changes Associated with Moderate and Severe TBI - There are few investigations using advanced MRI techniques to examine both short-term and long-term consequences of moderate and severe traumatic brain injury (TBI). This project will examine recovery following brain trauma at several weeks after injury and several months after injury. At both time points, data will be collected about the patient's recovery as it is observed by physicians and through the use of advanced MRI techniques. By integrating information about changes in the brain (using MRI) with information about changes in patient behavior (as observed by physicians), recovery from TBI can be better characterized. With an improved understanding of brain changes after TBI, future medical treatments and interventions can be tested to treat not only changes that can be observed, but also those changes in the brain that are not immediately apparent to clinicians.

Duration of Project

6/5/2006 – 6/30/2009

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 15: Project Title and Purpose

Center for Magnetic Resonance Imaging and Spectroscopy - Pennsylvania State University (PSU) has recently established a Center for Magnetic Resonance on the University Park campus. The purpose of the center is to develop new magnetic resonance measurement and imaging techniques which can be used by numerous animal researchers on campus. As part of this effort, a pilot research program will develop new hardware, software and imaging methods for very rapid imaging of cardiac function and structure in mouse models of disease. This will result in significant advances in the amount of information that is available from such studies, and will be applicable to many other studies at PSU.

Duration of Project

6/5/2006 – 6/30/2007

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 16: Project Title and Purpose

Function of PAD4 and Histone Arg Methylation in Cell Differentiation and Cancer - The overall goal of this project is to understand how PAD4 activity and histone citrullination is regulated during transcription by other protein factors, how PAD4 complex efficiently regulates gene expression by incorporating multiple histone/chromatin modifying activities, and how PAD4 and histone Arg methylation regulates a fundamental cell differentiation process, the X chromosome inactivation in mouse female embryonic stem cells.

Duration of Project

7/12/2006 - 6/30/2007

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 17: Project Title and Purpose

Identification of Ubiquitination Events in CL Leukemia as Targets for Therapy **-** Chronic Lymphocytic leukemia (CLL) is a common form of lymphoid cancer, accounting for approximately 25% of all leukemias. Although some effective treatments are available, the disease remains incurable with the occurrence of relapses and drug resistance. Thus, there is a need for novel therapies. The purpose of this project is to identify ubiquitin-mediated proteolysis events (UMPEs) in CLL cells, which, when interrupted, could be clinically beneficial. An UMPE is a signal that instructs the cell to degrade certain proteins that perhaps, may be considered targets for therapeutic intervention. An important objective of this project is to identify novel targets that could lead to the development of new drugs for CLL.

Duration of Project

12/26/2006 – 1/31/2009

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 18: Project Title and Purpose

The Role of Osteopontin in the Alteration of Tight Junction Complex in Metastatic Colon Cancer The objective of this project is to identify pathways through which the tight junction proteins and osteopontin intersect which affect the metastatic potential of colon cancer cells. Osteopontin is a

protein in cancer cells that becomes more active as colon cancer progresses, possibly aiding in the spread to other organs, or metastasis. One way it aids in metastasis is that it causes cells not to stick together. Since cells must release their attachment to the parent tumor if they are to metastasize, it is important to recognize the changes in proteins that normally exist between the cells that maintain the integrity of the tissue. The tight junction complex is located between epithelial cells and has a role in cell-cell adhesiveness and permeability. A study of the tight junction protein complex will help in the understanding of how cells become metastatic.

Duration of Project

12/26/2006 - 6/30/2008

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 19: Project Title and Purpose

Adoptive Cellular Immunotherapy for EBV and CMV Disease - The purpose of this study is to investigate new ways to present viral and tumor antigens to the immune system. We will study the use of B-lymphoblastoid cell lines (BLCL) derived from bone marrow donors to present viral proteins to immune cells (T cells) derived from that same donor. We will stimulate these donor T cells to become specific for cytomegalovirus and Epstein Barr virus (CMV and EBV), two common viruses that can cause complications following a stem cell transplant. In this study we will be infusing stem cell transplant patients with these virus specific T cells post-transplant for the prevention of viral reactivation.

Duration of Project

12/26/2006 - 12/31/2007

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement Annual C.U.R.E. Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 20: Project Title and Purpose

What Makes Fatty Food "Irresistible"? Relating Food Structure and Eating Behavior - Heart disease and diabetes accounted for about 31.5% of all cause mortality in the United States in 2003. Dietary fat intake is a key factor thought to increase risk for both of these diseases. Yet in spite of repeated health messages to reduce fat in the diet, per capita fat intake has increased. Fat brings many qualities to food (e.g., texture, flavor, calories) and there must be some combination

of these that stimulates consumption and makes fatty foods "irresistible". In this project fat emulsions will be created in which the flavor and caloric content vary. Rats will be used to identify which emulsions: (i) promote the largest intakes, (ii) promote binge-eating, and (iii) have rewarding qualities. If the "irresistible" properties that trigger excessive fat intake can be identified, then appealing low-fat foods can be developed that will help people stick to a healthy diet.

Duration of Project

12/26/2006 – 12/31/2009

Project Overview

The objective of this project is to identify if the eating response to fatty foods is stimulated by (i) the caloric content of the meal, (ii) the flavors naturally present in the fat (volatile components), or (iii) the flavors derived from the fat by the salivary enzymes. The overall hypothesis is that both flavor and calories contribute to the rewarding qualities of fats, i.e., fats with both taste and calories will be the most acceptable, will promote binge-type eating, and will have the highest reward value. Three model oils will be used: 1) tricaprylin plus linoleic acid (i.e., caloric, flavored oil), 2) pure tricaprylin (i.e., caloric, flavor-free oil) and 3) mineral oil plus linoleic acid (i.e., non-caloric, flavored oil). The different oil emulsions will be developed in Aim 1. The oils will be blended with a liquid mineral oil (Mo) to maintain a constant total lipid concentration while varying the concentration of "active lipid" present. The blended fat will be homogenized with a sodium caseinate solution (3%) to produce a narrow distribution of droplet sizes (median ~ 0.5 μm) and a final total droplet concentration of 56%. Because the droplet concentration and interfacial protein is the same in all products, we expect their rheological properties to be similar. Acceptance and the ability to induce bingeing will be assessed in Aim 2. In Aim 2, Experiment 1 (short-term acceptance), 45 rats will be divided into 3 groups of 15 rats each, and assigned one of the 3 emulsion products (calories + flavor, calories + no flavor, no calories + flavor). One concentration of each emulsion will be presented for 5 min each day for 5 days, and then a different concentration will be presented and so on until all 3 concentrations have been tested. Intakes will be measured. In Aim 2, Experiment 2 (long-term acceptance), the same rats, emulsions, and approach will be used except that the emulsions will be presented for 1 hour each day instead of only for 5 min. Three equally acceptable emulsions from each type will be used in Aim 2, Experiment 3. 90 naive rats will be matched and assigned to 6 groups of 15 rats each: 3 different emulsions X MWF (binge) or Daily (control) 1-h access. Intakes will be measured for 8 weeks. In Aim 3 anticipatory contrast will be used to assess the reward value of the different fat products in naïve (Experiment 1) and bingeing (Experiment 2) rats. In Aim 3, Experiment 1, 144 rats will be divided into 3 groups and assigned one of the fat products. Half the rats in each group will be tested under a zero delay condition and half under a 5-min delay. Rats will be tested under free-feeding and food-deprived conditions. In Aim 3, Experiment 2, similar tests will be done in 72 of the rats from Aim 2, Experiment 3.

Principal Investigator

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Other Participating Researchers

Rebecca L. Corwin. PhD, Patricia S. Grigson, PhD – employed by Pennsylvania State University

Expected Research Outcomes and Benefits

Fat can promote consumption for a variety of reasons, including its caloric density and ability to reduce satiety signaling. Evidence also exists that dietary fats have rewarding effects in the mouth as well as in the intestinal tract, i.e., they provide "good" tastes and textures to the food. The properties of fat that trigger eating behavior must be better understood in order to design healthier alternatives that people will enjoy. A key premise of this project is that the rewarding effects of fats are critical to why they are so irresistible. The specific chemical and physical properties of fats that make them so rewarding are not known and the goal of this project is to begin to figure them out. Finding ways to reduce the energy density of fats, while still maintaining their pleasurable qualities, would be one way to help people reduce energy intake and lose weight. This alone would help reduce diabetes and heart disease risk. The approach used in this project is unique. While others have used fat emulsions, texture and viscosity were not systematically controlled. Similarly, others have used nutritive and non-nutritive oils, but flavor compounds were not systematically controlled. In short, the physical and chemical properties of fats that make them so appealing have never been identified. The collaborative effort represented in this project offers the distinct advantage of being able to systematically manipulate the chemical and physical properties of the fat, and then assess how those manipulations affect intake and reward. Such an approach provides a unique opportunity to find out what makes fatty food so irresistible. The goal of the proposed research is to identify which properties of a fatty meal will trigger different eating responses in animal protocols of intake and reward. By finding the triggers to eating behavior in animals, insights may be gained that will assist the development of either (i) full-fat foods that do not trigger unhealthy (binge) eating, or (ii) low-fat foods more appealing to the consumer.

Summary of Research Completed

Over the last funding period, Dr. Nu-Chu Liang completed the writing of three manuscripts that examine underlying neural substrates that contribute to the processing of oil reward. Dr. Grigson was a co-mentor for Dr. Liang and, as such, was able to contribute to the design of the related thesis studies and to the writing of the manuscripts for publication. Funds from the Coupland project award were not used to support this student or her research but, as said, Dr. Grigson contributed intellectually to the shaping of this somewhat extensive and important project on the study of oil. As such, the PA Dept. of Health was listed in the acknowledgement sections of

these papers. The three manuscripts will be submitted for publication in the next few weeks and they are as follows:

Liang, N.C., Freet, C.S., Grigson, P.S., & Norgren, R. Pontine and Thalamic Influences on Fluid Rewards: I. Operant Responding for Sucrose and Corn Oil.
Liang, N.C., Grigson, P.S., & Norgren, R. Pontine and Thalamic Influences on Fluid Rewards: II. Sucrose and Corn Oil Conditioned Aversions.
Liang, N.C., Norgren, R., & Grigson, P.S. Pontine and Thalamic Influences on Fluid Rewards: III. Parabrachial but Not Ventroposteromedial Nucleus Destruction Eliminates Anticipatory Contrast for Sucrose and Corn Oil.

In addition to completing the analysis and writing of these manuscripts, during the funding period we also conducted a final study designed to follow up on the behavioral corn oil data described in our last annual report. Specifically, in our last report we found that corn oil 'behaves' like sucrose in the anticipatory contrast paradigm (Flaherty & Checke, 1982). Thus, rats avoid intake of a lesser valued corn oil solution when it predicts access to a more preferred concentration. As with sucrose (Flaherty et al., 1991), however, we also found that rats are unwilling to forego (i.e., will not suppress intake of) the lesser valued concentration of corn oil in anticipation of access to the higher, preferred concentration if they are (a) hungry and/or (b) if they are required to wait 5 min for access to the preferred reward. These data suggest that the calories in the corn oil have a big impact on the behavior of the rats (i.e., on their willingness to wait for the preferred, more caloric reward).

Although calories appear to be important, rats also will show anticipatory contrast effects with disparate concentrations of non-caloric saccharin solutions (Flaherty & Rowan, 1986). In our final phase of funding, then, we used the same paradigm to test whether rats would exhibit anticipatory contrast using low and high concentrations of another fat that is preferred, but is non-caloric, - mineral oil.

Anticipatory contrast with mineral oil concentration pairs tested under non-deprived and food-deprived conditions.

Methods. Forty-eight male Sprague-Dawley rats were used in the experiment. Phase I: Non-deprived. All rats were initially tested in a non-deprived state (i.e., free-feeding). During testing, they were given 3 min access to a low concentration of mineral oil (5%) followed by 3 min access to either the same low 5% mineral oil solution, a medium concentration (15%), or a high concentration (30%) of mineral oil. For half of the rats, zero seconds elapsed between retraction of the first bottle solution and presentation of the second bottle solution. For the other half of the rats 5 min elapsed between retraction of bottle 1 and the advance of bottle 2. After the 12 days of testing, the concentration of the low solution was increased to 8%, the medium concentration was increased to 18%, and the high concentration was reduced slightly to 28%. There were 10 such pairings. Phase II: Food-deprived. The rats were then reduced to 85% body weight, concentrations remained the same, and testing continued for 14 more days.

Results.

Figure 1. Bottle 1 licks: Free-feeding: Zero sec ISI. The data are shown in 2-day blocks. Rats in the Low-Low condition tended to make fewer licks for the Low concentration presented in Bottle 1 than rats in the Low-Medium or Low-High condition. This is the opposite of anticipatory contrast and opposite to the pattern obtained with corn oil where rats readily avoided intake of a lesser valued corn oil cue in anticipation of access to a more palatable, higher concentration.

Figure 2. Bottle 1 licks: Free-feeding: 5 min ISI. Use of a 5 min interval between access to the two solutions served to increase licking of the first bottle Low concentration by rats in the Low-Low control condition, particularly when using the 8% mineral oil concentration (right panel). Despite this increase in bottle 1 intake by the Low-Low controls, rats in the Low-Medium and Low-High groups failed to exhibit contrast. Mineral oil, then, fails to support the development of an anticipatory contrast effect in non-deprived rats when zero sec or 5 min elapse between access to the lesser valued cue and the more valued consequence.

Figure 3. Bottle 2 licks: Free-feeding: Zero sec ISI. Although intake was relatively low (compared to sucrose or corn oil, for example), rats exhibited a magnitude of reward effect by making more licks for the 2nd bottle medium and the high concentration of mineral oil than the 2nd bottle low concentration.

Figure 4. Bottle 2 licks: Free-feeding: 5 min ISI. This pattern was even more evident when tested using the 5 min ISI. Thus, while the rats failed to exhibit an anticipatory contrast effect in bottle 1 intake, they clearly exhibited a magnitude of reward effect by making more licks for the higher than for the lower concentrations of mineral oil.

Figure 5 and 6. Bottle 1 licks: Food-Deprived: Zero sec and 5 min ISI, respectively. When tested in a food-deprived state, the rats failed to exhibit an anticipatory contrast effect in bottle 1 licks whether zero sec (Figure 5) or 5 min (Figure 6) elapsed between access to the lesser valued cue and the more preferred consequence. Once again, rats tended to make more licks in the zero sec ISI condition (Figure 5) when 5% mineral oil served as the cue (left panel) and a similar tend was evident in Figure 6 right panel. Thus, rather than exhibiting contrast, rats tend to make more, rather than less, licks for a lesser valued mineral oil cue when it predicts access to a preferred reward.

Figure 7. Bottle 2 licks: Food-Deprived: Zero sec ISI. Once again, as in the non-deprived condition, food-deprived rats clearly demonstrated a magnitude of reward effect by making more licks for 2nd bottle medium and high concentrations of mineral oil (see left and right panels).

In sum, while corn oil supported both a magnitude of reward effect and a contrast effect, mineral oil supported only a magnitude of reward effect. Specifically, rats made more licks for higher than for lower concentrations of mineral oil, but they failed to avoid intake of a lesser valued mineral oil cue when it predicted subsequent access to a more valued higher concentration of mineral oil. Indeed, they exhibited the opposite behavior (i.e., a reinforcement effect) by making more licks for the first bottle Low concentration of mineral oil when it predicted subsequent access to higher concentrations of mineral oil. Rats, then, appear to attribute the rewarding properties of the preferred mineral oil concentrations to the lesser valued mineral oil cue. As

such, they treat this non-caloric cue as though it were a simple sensory cue rather than a reward. In this light, it would be interesting to determine whether mineral oil, like corn oil (Liang et al., 2006), also would elicit the release of dopamine from the nucleus accumbens. Future studies will investigate this more fully.

Figure 1

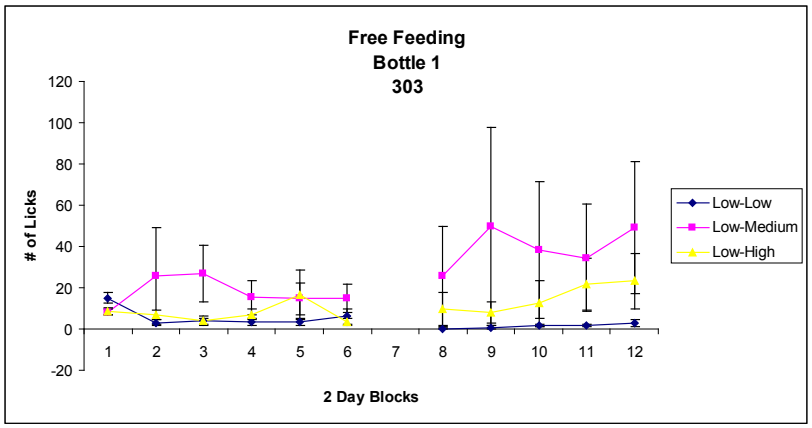


Figure 2

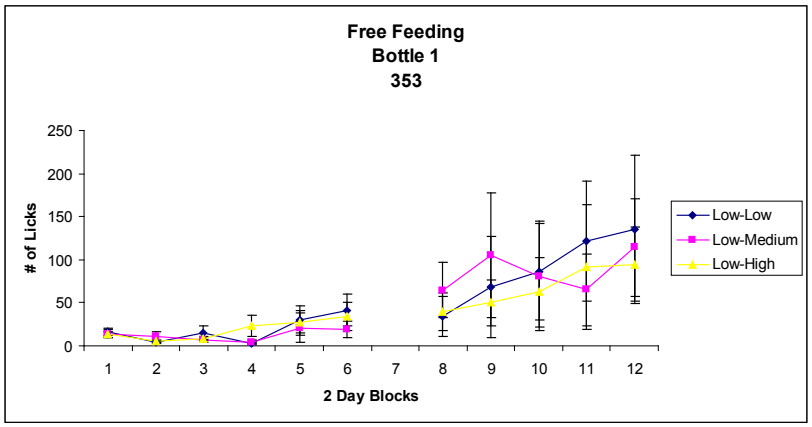


Figure 3

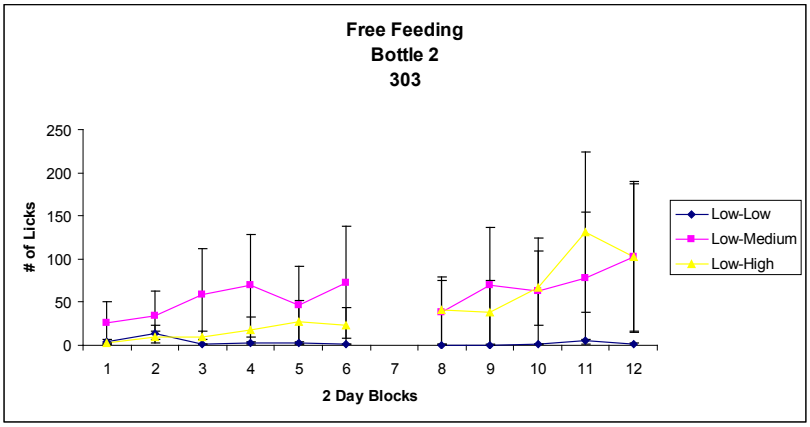


Figure 4

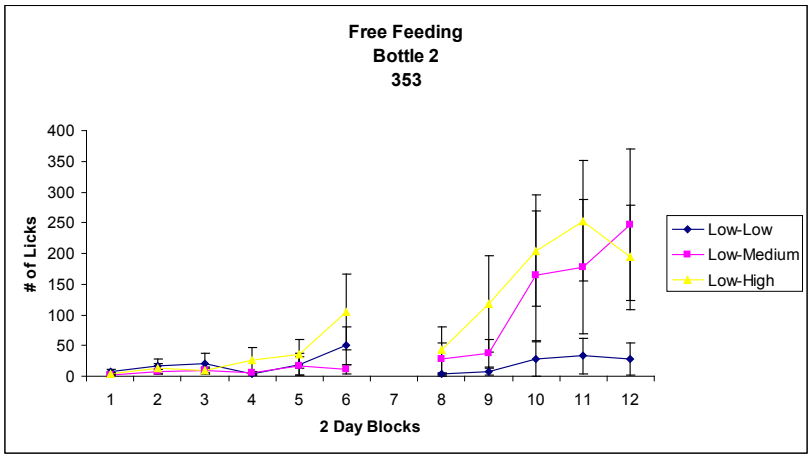


Figure 5

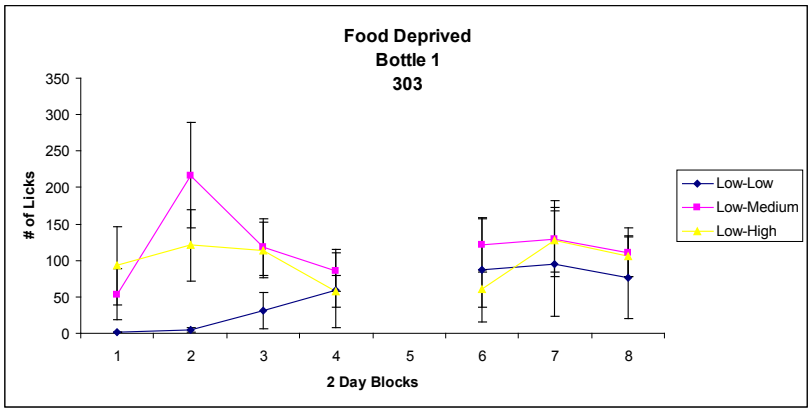


Figure 6

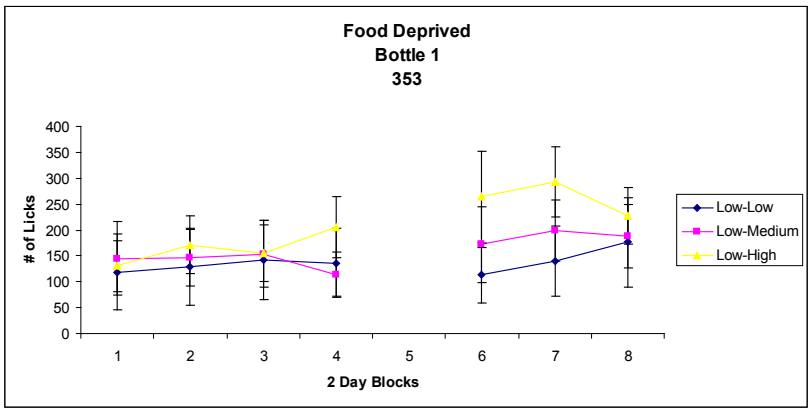
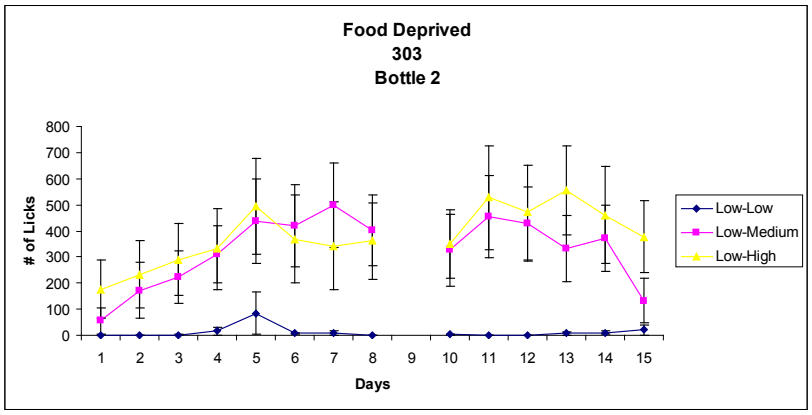


Figure 7


Food Deprived
303
Bottle 2
# of Licks
800
700
600
500
400
300
200
100
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
Days
Low-Low
Low-Medium
Low-High