Pennsylvania State University

Annual Progress Report: 2010 Formula Grant

Reporting Period

January 1, 2011 – June 30, 2011

Formula Grant Overview

The Pennsylvania State University received $7,001,127 in formula funds for the grant award period January 1, 2011 through December 31, 2014. Accomplishments for the reporting period are described below.

Research Infrastructure Project 1: Project Title and Purpose

Research Infrastructure Renovation to Create the Penn State Center for Translational Informatics - The purpose of this project is to create a Center for Translational Informatics that will provide the necessary software tools for researchers to collect and manage patient data, analyze genomic information, and mine the electronic medical record for data to support research into comparative effectiveness and personalized medicine. To create the Center, approximately 600 sq ft of existing space will be renovated to establish a collaborative workspace to house informaticists, IT analysts, and data analysts who will consult with basic and clinical researchers, develop software, and manage databases. Software and hardware will be purchased to allow the collection, management, analysis and transfer of large datasets.

Anticipated Duration of Project

1/1/2011 – 6/30/2013

Project Overview

With advances in understanding the genetic basis of disease, translational research has relied more heavily on biomedical informatics tools, and new technologies are being exploited to make clinical research and healthcare delivery more efficient and effective. In this project we are creating a new Center for Translational Informatics (CTI) that will transcend departmental, college, and campus boundaries to implement a systematic, multi-thematic effort toward sustainable pathways for translational research. The specific aims of this project are to (1) Renovate existing space to create the bioinformatics support infrastructure for the CTI; (2) Support clinical research by assisting researchers in the management and analysis of complex biological and clinical data.

The CTI will house the Director and support staff who will consult with faculty to identify informatics needs and develop solutions, and will have space for training faculty, staff, and students on the use of software. The CTI will purchase software that will allow researchers to

conduct large-clinical trials more efficiently, analyze genomic data, keep clinical data secure and private, move large amounts of data more easily, and access the electronic medical records of patients to promote research in comparative effectiveness and personalized medicine.

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Expected Research Outcomes and Benefits

Penn State has been engaged in a decade-long mission of removing barriers to translational research by creating targeted pathways to unify traditional information technology (IT) and biomedical informatics through systems and services that merge scholarly activity with production delivery, and by forming interdisciplinary centers that transcend departmental, college, and campus boundaries to seed new collaborative research directions and provide shared research infrastructure and instrumentation. The proposed Center for Translational Informatics (CTI) will forge a systematic, multi-thematic effort toward sustainable pathways for translational research by leveraging existing strengths and latent synergies between basic science and translational research to reach new milestones at the confluence of (1) genomics and biomedical research; and (2) informatics and clinical best practices. Targeted collaborations and additional investment and development of human capital and shared research infrastructure by Penn State will lead to new breakthroughs in translational research. Innovative projects supported through the CTI will use informatics to answer biological questions and to accelerate how laboratory findings and best practices are best implemented in the clinical setting, for example, using genomics data to support personalized medicine. Electronic medical records (EMRs) are one of the most exciting potential resources for research data. The CTI will allow researchers to access patients' EMRs to conduct complex genomic analyses of disease susceptibility and therapeutic outcomes across diverse patient populations and to identify best practices for Institutional Review Board interactions, participant consent, data sharing, returning research results, etc.

Summary of Research Completed

The Center for Translational Informatics has been established and has been working in temporary space until the proposed renovations have been completed. A call for proposals was sent out to architectural firms, and the firm of Kaylor Architects in Lebanon, PA has been

selected. They have submitted a number of designs, and a configuration for the new space has been selected. Renovations should begin in September, 2011.

The purchase of a clinical trial data management system to enable the collection and administration of clinical research information is underway. A number of vendors were contacted, and they presented demonstrations of the functionality of the systems to interested faculty and staff. The decision was made to purchase the Click Portal clinical trial data management system from Huron Consulting Group. This software will help track study data and ensure compliance with federal regulatory guidelines. It is anticipated that this system will be available for researchers to use in the spring/summer 2012. The Cerner Health Facts system is also being implemented, and should be available to researchers in early 2012. This platform provides deidentified electronic health record information to researchers to use when designing personalized treatments or to conduct comparative effectiveness research. RedCAP, a secure web-based application that supports data capture and management for research studies, and was developed with funding from the national Clinical and Translational Science Award Consortium, will also be made available to researchers this fall. The Bioinformatics Consulting Group of the Center for Translational Informatics has provided consultation to researchers needing expertise in analysis of genomic, proteomic, and epigenetic analyses of data. Dr. Istvan Albert also taught a course in Spring 2011 entitled "Practical Data Analysis for Life Scientists" that focused on practical data processing an analysis. Once renovations for the new consultation space are completed, an expanded consultation service with additional faculty will be made available.

Research Project 2: Project Title and Purpose

A Trial to Evaluate the Safety and Tolerability of a Novel Oral Iron Supplement for the Treatment of Iron Deficiency Anemia - The purpose of this study is to conduct a Phase 1 clinical trial designed to evaluate the safety of a novel oral iron supplement utilizing a nutritional strain of yeast. Iron deficiency anemia (IDA) is a problem of world-wide importance that affects approximately 1.3 billion people. IDA has been identified by the World Health Organization as the number one nutritional disorder in the world. The elderly, infants, children, and women of reproductive age are particularly affected by iron deficiency and iron deficiency anemia. In the US, IDA is prevalent in lower socioeconomic groups. The impact on health, mother-child interactions, and days lost from the workplace is widely published. In many countries and among the poor in the US, the primary driver for iron deficiency is the lack of available iron rich foods. Current treatment strategies for IDA are inadequate because of poor absorption rates for iron in oral tablets and significant side-effects leading to non-compliance. The primary objective of this project is to demonstrate the safety and tolerability of a novel oral iron supplement. The secondary objective is to begin to evaluate efficacy and identify a potential therapeutic dose of this novel oral iron supplement.

Anticipated Duration of Project

7/1/2011 – 6/30/2012

Project Overview

Iron deficiency is the most prevalent nutritional disorder in the world and constitutes a public health epidemic that affects more than 3.5 billion people in developing as well as developed countries. Iron deficiency impairs overall health and work productivity, delays motor and mental development in infants, increases maternal hemorrhaging, increases the risk of premature delivery, and is associated with postpartum fatigue and depression. Iron deficiency affects more than a quarter of women following child delivery and in the most severe situation is associated with maternal or fetal death.

Current approaches to the treatment of iron deficiency are not universally available and contain basic flaws in concept and utilization. The primary objective of this project is to demonstrate the safety and tolerability of a novel oral iron supplement. The secondary objective is to begin to evaluate efficacy and identify a potential therapeutic dose of this novel oral iron supplement.

Aim: Perform a pilot study on iron deficient human subjects to determine safety, tolerability, and iron absorption rates using our oral iron supplement.
The study will begin with 3 patients meeting inclusion criteria. These individuals will receive 300 mg tablets of the novel oral iron supplement. The iron content of these tablets is 30 mg. This dose was chosen as the initial starting dose because it contains the RDA for iron for pregnant women.

This study will utilize the standard "3+3" rule-based dose-escalation scheme, which uses predetermined dose levels and cohorts of three patients. In this study, the following dosages will be used: 300 mg per day (Level 1), 600 mg once a day (Level 2); 600 mg 2X per day (Level 3), 600 mg 3X per day (Level 4-target therapeutic dose equal to current standard of care at 180 mg iron per day). The final dose (Level 5) is 600 mg 4X per day and would be used in extreme cases of iron deficiency. Given that the iron content of the tablets is 10%, individuals receiving the maximum dose in our study are ingesting 240 mg of iron; well below the 1000 mg of iron given therapeutically for conditions of iron deficiency that require intravenous intervention. Since there are 5 different doses, the minimum number of subjects is 3 and the maximum number is 5 times 6 for a total of 30 subjects, each will be involved with the study for 3 months.

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Expected Research Outcomes and Benefits

Essential Data Generated:

We will have safety, tolerability and hematologic data on 30 subjects. Specifically we will collect blood samples to obtain the following measurements:

- CBC including hemoglobin and hematocrit,
- Plasma iron, TIBC, and serum ferritin.
- Liver Tests: AST (SGOT), ALT (SGPT), ALP, GGT (GGTP).
- Kidney Tests: Analytes Albumin, BUN/creatinine, chloride, CO2 content, creatinine clearance, glucose, K+, total protein, Na+, protein.

At the end of the funding period we expect to have data that demonstrate we have a safe oral iron supplement. Within the proposed doses that will be examined, we expect to find a therapeutic dose from which we will develop a Phase II/III clinical trial.

The long term benefits to the population are expected to be a safe, efficient and economical method to treat iron deficiency anemia. Furthermore, our mode of iron delivery is a nutritional staple in most cultures of the world so there should be little cultural resistance to consuming our product.

Summary of Research Completed

The project has not yet started. No monies have been spent. Changes to the IRB protocol are awaiting approval.

Research Infrastructure Project 3: Project Title and Purpose

Research Infrastructure Renovation to Create a Biomedical Research Imaging Core Facility - The overall goal of the project is to establish an imaging core to meet existing and rapidly growing needs for an equipped biomedical research imaging facility dedicated to experimental approaches in the life sciences. We will do this by renovating 2300 sq ft of space to optimally place existing and/or acquired instruments that include (i) confocal, (ii) deconvolution (iii) electron microscopes with support equipment and (iv) a facility director (to be named) to provide expert guidance in optimal sample preparation, supervision of instrument use, data collection and training for researchers and students in imaging and image processing.

Anticipated Duration of Project

6/1/2011 - 6/30/2013

Project Overview

The objective of this application is to create a Core facility dedicated to biomedical research imaging in the Penn State Hershey College of Medicine. The project aims to: 1) create a newly renovated 2300 sq ft research infrastructure to house existing imaging equipment; 2) centralize

the existing Core Facilities shared equipment, which includes confocal and transmission electron microscopes; 3) create a desirable workspace to recruit an experienced Imaging Core Director able to aid investigators in a wide variety of complex imaging projects. The confocal microscopes are configured specifically for live-cell and regular histology studies to meet the rapidly growing need for a variety of sophisticated imaging techniques at the tissue, cell and sub-cellular levels, including time lapse studies, fluorescence resonance energy transmission (FRET) and fluorescent recovery after photobleaching (FRAP). The electron microscopes collect images at the sub-cellular and molecular level, and include a cryo transmission electron microscope (cryoEM) - a JEOL model JEM-2100 that is a 200 kV Transmission Electron Microscope (TEM), with a Lanthanum Boride (LaB6) emitter, a full Cryo package, CCD camera and a stage-tilting capability for tomography. This particular cryo-TEM is one of the easiest to operate and maintain, making it the choice of many cryo TEM facilities as a first instrument to acquire. It functions better than comparable instruments to fulfill the basic needs of a general research environment and is often retained as a training instrument when facilities expand because it has a reputation for versatility in a multi-user environment and is relatively inexpensive to repair and maintain.

Broader Impacts: A wide variety of structural and functional studies will be made possible in a centralized location which can be supervised by an expert Core Director. The instrumentation placed in the optimized space will offer the powerful tools of direct visualization that can guide and complement other research approaches focusing on a variety of health issues including viral infections (including HIV and herpes), cancer, diabetes and its complications (primarily diabetic retinopathy, muscle protein synthesis, kidney and brain complications), heart disease, and degenerative diseases of the central and peripheral nervous system (including Parkinson's disease, Alzheimer's disease and restless leg syndrome).

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Expected Research Outcomes and Benefits

Core Facilities shared equipment has become a major strength of the Penn State Hershey College of Medicine since its inception in1987. Since then the facilities, services and associated expertise have grown dramatically. Expanding and optimizing the Imaging Core will enable the continuation and enhancement of ongoing research. Equipment will be placed in an adequate

facility and infrastructure to function optimally. Microscopes will image rapid events in live cells or explant tissue transfected with fluorescent protein markers, or labeled with metabolic dyes, to determine changes in protein expression, trafficking and redistribution in response to pharmacological, environmental or genetic manipulations. Transmission electron microscopy (TEM) is suitable for simple negatively stained biological sample viewing and data collection for reconstruction as well as thin sectioning, trimming & semi-thin sectioning, polymer and materials science applications. Cryo electron microscopy (cryoEM) provides two additional resources not currently available to Penn State COM: cryoEM single particle reconstruction techniques and tomography.

Research enhanced by live imaging will have broad relevance to human health because it will enable us to study rapid interactions between proteins and other molecules in living cells, in real time. Determining the location and movement of any protein is essential to understanding its function in health and disease. TEM and CryoEM allow three-dimensional (3D) imaging at the subcellular level, filling a gap between the atomic resolution provided by NMR and X-ray, and visualization by light microscopy. Imaging instrumentation will enhance a wide variety of public health projects being conducted at the Milton S. Hershey Medical Center, including cancer, diabetes, heart disease, viral infection and neurodegenerative diseases.

Summary of Research Completed

The project is in a very early stage. No monies have yet been spent. Several meetings have been held with the architect to discuss design and all parties have visited the facility at the University of Pittsburgh. The PI has received a very high score on her application to the NIH for the CryoEM.

Research Project 4: Project Title and Purpose

Sphingolipid-based Therapeutics for AML - The long term goal is to develop new therapies for the treatment of acute myeloid leukemia (AML). These funds will support three specific aims that provide additional/ new/ critical data that are essential for submission of an integrated interdisciplinary NIH P01 application in 2011 that will investigate the role of dysfunctional sphingolipid metabolism in acute myeloid leukemia. The funds will support three team projects that will benefit each of the eventual P01 projects.

Anticipated Duration of Project

5/1/2011 – 6/30/2012

Project Overview

We will be gathering preliminary data to strengthen the projects and cores that will be included in a P01 application. We will conduct three team based projects: Project 1will carry out gram scale synthesis of and purification of compounds; Project 2 will complete proteomic analysis of lipid metabolic proteins, and lipidomic analysis of cellular samples and plasma samples; Project

3 will establish and examine NOD/SCID/IL2rγ^{null} animal model using primary human acute myeloid leukemia samples.

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Expected Research Outcomes and Benefits

Gathering this data collected through the three team based projects will strengthen our P01 application which will hopefully result in new therapies for a fatal disease, AML.

Summary of Research Completed

Over the last two months, we have begun to generate team-centric data that will support a P01 application to be submitted to the NIH/NCI. Specifically, we are generating NSG mice that have the unique ability to propagate human AML cells with defined cytogenetic properties. We have shown that these mice are a useful and unique tool to evaluate therapeutic efficacy of agents for treatment of AML. At the present time, we are evaluating proprietary sphingosine-kinase inhibitors with this model.

We have also made progress in utilizing combinatorial MS-lipidomic and MRM-based proteomic approaches to evaluate AML cells after treatment with agents that alter ceramide metabolism. This data is crucial in defining the role of altered ceramide metabolism in AML.

<u>Research Project 5: Project Title and Purpose</u>

Genome-wide Mapping of Transcriptional Factors Involved in Pathogenesis of LGL Leukemia – Large Granular Lymphocite (LGL) leukemia is characterized by a clonal proliferation of antigen-primed terminally differentiated effector memory cytotoxic T cells (CTL). The overall objective of this project is to understand the pathogenesis of LGL leukemia by identifying the key genes which promote leukemic LGL survival. This project capitalizes on strengths of Penn State University by investigating the pathogenesis of a leukemia discovered by the PI utilizing a

genomic approach in collaboration with colleagues at Penn State-University Park. T-bet is considered the master switch for the generation of antigen primed effector CTL. We previously showed that leukemic LGL are characterized by T-bet upregulation. Thus, LGL leukemia provides us with a unique model to study the functional role of T-bet in generalizing and maintaining CTL response.

Anticipated Duration of Project

7/1/2011 – 12/31/2013

Project Overview

Leukemic LGL represent a pure population of terminally differentiated effector memory (TEMRA) cells. Our *experimental approach* will create a genome-wide map of T-bet bound loci in CD8+ TEMRA cells from healthy volunteers and T-LGL leukemia patients. Five samples each will be acquired from healthy volunteers and T-LGL leukemia patients. T_{EMRA} will then be isolated by negative selection techniques including immunorosette and immunomagnetic separation. Genome-wide T-bet binding sequences will be identified using ChIP-Seq technique. Binding of a transcription factor to the promoter binding site of a target gene does not necessarily result in expression or repression of a transcript, however, the strength of our project comes from the fact that we have created an expression profile of leukemic T-LGL using gene profiling studies, allowing us to correlate T-bet binding of its putative target gene promoter sites with the expression of these putative targets. Finally, the T-bet binding partner network will be constructed using putative translational products of its binding partners as nodes. Edges will represent both direct and indirect interactions among the translational products documented in the protein-protein interaction database or the signaling pathway database.

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Expected Research Outcomes and Benefits

This project will provide further understanding on the molecular pathways responsible for survival of leukemic LGL. There is no current curative therapy for this disease. Results of this study will provide key information for identifying new targets for therapy.

Summary of Research Completed

The project has not yet started. No monies have been spent.

Research Project 6: Project Title and Purpose

Gut Microbial Metabolism and Receptor-Mediated Regulation of the Host Environment - As a whole, the gut microbiota contains nearly 100 times more genes than that in the human genome of which some are used to tightly control and regulate the host environment. In spite of this, little is known about specific gut microbial metabolism, their positive or negative effects on the host, or how microbes use specific diet-derived substrates to modulate the host environment (e.g., receptor-mediated regulation of intestinal inflammation). This project seeks to understand and characterize—using advanced metabolomic approaches, mouse models, and high throughput sequencing—the gut microbiota metabolome and how specific diet-derived substrates (e.g., tryptophan) are used to influence and modulate the host environment.

Anticipated Duration of Project

7/1/2011 - 12/31/2014

Project Overview

Broad objective: Metabolomics has generated overwhelming amounts of evidence that the gut microbiota contribute significantly to the host's metabolic phenotype. The studies described herein would generate a detailed view of the host-gut microbiota interaction (through the aryl hydrocarbon receptor [AHR]) and lay groundwork for future studies.

Specific Aim 1: Optimize anaerobic microbial culture and develop high throughput culture systems, along with biofluid isolation, and metabolomics analyses.

Experimental approach: Microbes will be cultured with and without various dietary substrates such as tryptophan. Advanced chromatography-mass spectrometry (LC-MS, GC-MS) coupled with chemometrics and multivariate data analysis will be used to metabolically phenotype each microbial strain.

Specific Aim 2: Generate and identify probable associations between metabolites and gut microbiota changes, utilizing dextran sodium sulfate (DSS) and *C. rodentium* to modulate the gut microbiota.

Experimental approach: Urine metabolomics of control wild-type, control *AHR*-null, DSS-treated (or *C. rodentium*) wild-type, and DSS-treated (or *C. rodentium*) *AHR*-null mice will be conducted to assess any changes in the urinary metabolome that are indicative of changes in the gut microbiota. The cecal contents will be typed for microbes and the inflammation assessed.

Specific Aim 3: Develop analytical and data analysis pipelines that provide a metabolomics platform for the biomedical research community at Penn State University.

Experimental approach: Build a robust pipeline for sample preparation, data generation, and data analysis built around three robust analytical platforms that together allow for the comprehensive analysis of the metabolome of simple and higher organisms. A combination of GC-MS and LC-MS methodologies will be developed that allow for automated data reduction and compound annotation.

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Expected Research Outcomes and Benefits

The studies outlined in this project will impact human health and disease. These studies may open new insights into antibiotic design and development, and provide unique views of inflammatory bowel disease, particularly from the vantage point of the gut microbiota.

This project will focus specifically on the AHR, given that microbial-derived metabolites of tryptophan, including kynurenic acid, xanthurenic acid, indoxyl sulfate, tryptamine, and indoleacetic acid, are known ligands for this receptor. Furthermore, since the gut has the highest concentration of T-cells and AHR activates potent inflammatory and anti-inflammatory pathways, it seems logical to conclude that AHR may be a primary target of the gut microbiota to modulate the host environment. Interestingly, AHR recently has been shown to be important for the generation of regulatory T cells (Tregs) as well as a specific subset of CD4+ T cells known as T helper 17 (Th17) cells. While Tregs decrease immune reactivity and Th17 cells promote the immune response, it is thought that AHR is important for altering the balance between these two T cell populations. These observations may help to explain the immuno-suppressive effect of the AHR ligand TCDD. Interestingly, the indoleamine 2,3-dioxygenase (IDO) pathway, the rate-limiting step in tryptophan degradation, may be essential for the development of human Tregs at least in culture.

Additionally, the methodologies and pipelines produced in the course of the present study will be instantly available and applicable to numerous other existing and planned biomedical research projects that would benefit from the generation of metabolomic data.

Summary of Research Completed

The project has not yet started. No monies have been spent.

Research Project 7: Project Title and Purpose

Neurogenic Actions of Dietary Salt on Sympathetic-Cardiovascular Function: The purpose of this project is to identify how dietary salt acts within the central nervous system to adversely affect cardiovascular function and morbidity. Compelling evidence from clinical studies indicates excess dietary salt intake contributes to the pathogenesis of several cardiovascular diseases and increases morbidity. Recent observations from our research team indicate the central nervous system contributes to the adverse cardiovascular effects of dietary salt. Therefore, this project will (1) identify how the central nervous system detects changes in dietary salt intake, (2) determine how dietary salt affects sympathetic-cardiovascular circuits within the central nervous system, and (3) determine whether dietary salt intake affects sympathetic-cardiovascular regulation differently in males versus females.

Anticipated Duration of Project

1/1/2011 – 6/30/2012

Project Overview

Our long term goal is to identify the neural pathways and cellular mechanisms that increase sympathetic nerve activity (SNA) and total peripheral resistance in salt-sensitive hypertension. Both preliminary and recently published data from our laboratories demonstrate that the ingestion of excess dietary salt enhances sympathetic-cardiovascular function, increases arterial blood pressure (ABP) variability, and contributes to the development of neurogenic salt-sensitive hypertension. The overall goal of this project is to determine how the brain senses changes in dietary salt intake to result in exaggerated sympathetic-cardiovascular regulation and ultimately contribute to the pathogenesis of salt-sensitive hypertension in humans.

Specific Aim 1 will determine whether dietary salt intake elevates plasma sodium concentration to activate "sodium-sensing" neurons in the forebrain hypothalamus via transient receptor potential vanilloid (TRPV) and/or benzamil-sensitive channels and subsequently enhance sympathetic-cardiovascular function, increase ABP variability, and salt-sensitive hypertension. These experiments will use "state-of-the-art" electrophysiological and molecular approaches in rodents to investigate this hypothesis.

Specific Aim 2 will determine whether excess dietary salt intake alters the excitability of catecholaminergic neurons in the rostral ventrolateral medulla (RVLM) through activation of angiotensin II type 1 receptors, the mammalian target of rapamycin signaling, and oxidative stress. These experiments will use electrophysiology, RT-PCR, and Western blot analysis in rodents.

Specific Aim 3 will determine (A) the clinical populations (i.e. men versus women) that excess

dietary salt intake affects sympathetic-cardiovascular regulation, and (B) determine whether these changes are correlated with circulating cytokines and oxidative stress. These experiments will place humans on low and high salt diets for 2 weeks and then measure sympathetic nerve activity, blood pressure, and renal blood flow at rest and in response to a variety of physiological stressors.

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Expected Research Outcomes and Benefits

Specific Aim 1 will identify the cellular mechanisms by which the central nervous system detects changes in dietary salt and/or plasma sodium concentration. Findings from this project will identify a specific ion channel that is the putative link between dietary salt intake and brain function. Moreover, this ion channel may represent a novel therapeutic treatment target for salt-sensitive hypertension.

Specific Aim 2 will determine specific signaling mechanisms within the rostral ventrolateral medulla that contributed to altered sympathetic-cardiovascular regulation in salt-sensitive hypertension. Findings from this project will identify specific cellular signaling pathways (angiotensin receptors, mammalian target of rapamycin, oxidative stress, catecholamines) that contribute to altered cardiovascular regulation in salt-sensitive hypertension.

Specific Aim 3 will define the clinical populations and mechanisms affected by dietary salt intake. These translational studies in humans will determine which specific population of humans (male versus female) is affected by dietary salt intake. These findings should provide the foundation for new health recommendations of dietary salt intake for specific populations.

Summary of Research Completed

Specific Aim 1
In the past 6 months, we have established an in vitro neuronal slice preparation of neurons from the organum vasculosum of the lamina terminalis (OVLT) – the principle set of osmosensitive neurons in the central nervous system. Using this neuronal slice preparation, we have performed *in vitro* patch clamp recordings to address this specific aim. First, the majority of OVLT neurons (16/23, 70%) responded to an increase in bath osmolality produced by mannitol (Figure 1) and

displayed dose-dependent changes in membrane depolarization and cell discharge (Figure 1A, B). Second, bath application of the broad spectrum TRPV antagonist ruthenium red and benzamil (Figure 1D, E) significantly attenuated the increase in OVLT discharge and membrane depolarization. These changes were restored when ruthenium red and benzamil were washed out. Using single-cell RT-PCR with "nested primers", we have found that osmosensitive OVLT neurons (n=2) express ENaC, a subset of ASIC channels, and TRPV4 but not TRPV1 mRNA (Figure 1E).

Specific Aim 2

Thus far, we have examined neuronal responses of spinally-projecting RVLM neurons during various physiological perturbations including intracarotid infusion of 250 mM NaCl (100 µL, 20 s) to increase forebrain osmolality by 3-4%. These initial experiments have been performed in control, normotensive animals. Figure 2 demonstrates the RVLM unit projected to the spinal cord and was barosensitive. Intracarotid injection of hypertonic saline (250 mM NaCl, 100 uL over 10 s) significantly increased RVLM discharge in 83% (5/6) of neurons (4.1±1.4 to 9.6±2.9 Hz, $P<0.01$), elevated renal SNA (38±10%, $P<0.01$), and increased arterial blood pressure (14±2 mmHg, $P<0.01$). These neurons were barosensitive as inflation of an aortic cuff increased arterial blood pressure (31±2 mmHg) but decreased RVLM neuronal discharge (4.2±1.4 to 1.5±0.5 Hz, $P<0.01$). All neurons were antidromically activated from the spinal cord and had an average conduction velocity of 3.5±0.8 m/s. Intracarotid injection of hypertonic saline failed to increase discharge in 1 barosensitive RVLM neuron with a baseline discharge of 19.4 Hz and conduction velocity of 3.5 m/s. Intracarotid injection of isotonic saline had no effect on any variable. These findings suggest that acute increases in plasma sodium concentration activate sympathetic-regulatory neurons in the RVLM. This work was submitted to the American Heart Association Council for High Blood Pressure Research Conference and will be presented in September.

In a subset of cells, we determined whether OVLT neurons mediate this effect. At 10 s before the intracarotid infusion of hypertonic NaCl, one of various drugs was microinjected (20 nL) into OVLT via a multi-barrel glass pipette. Intracarotid infusion of hypertonic NaCl increased RVLM discharge, splanchnic SNA and ABP. This response was prevented by OVLT infusion of GABA (0.5M). Preliminary data suggests that OVLT infusion of either ruthenium red (20µM) or benzamil (100µM) may also attenuate this response (Figure 2). Drug infusions outside the OVLT had no effect (n=3).

Specific Aim 3

We have recruited 6 male and 5 female subjects. These subjects will begin the study in the next several weeks.

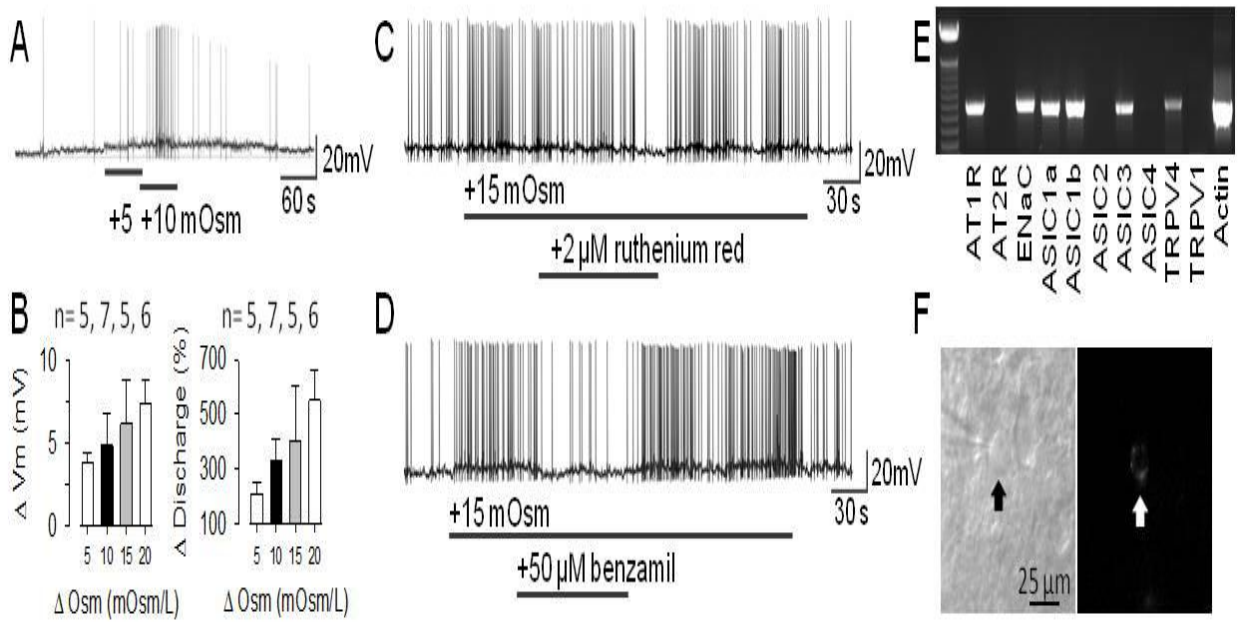

Figure 1. Examples of *in vitro* patch-clamp (current-clamp) recording of an OVLT neuron. (A) Increase in bath osmolality of +5 or +10 mOsm dose-dependently changes in membrane voltage and increases in cell discharge. (B) Summary data (C and D) Bath application of the TRPV antagonist ruthenium red or the ASIC and ENaC antagonist benzamil significantly attenuated increase in cell discharge to increase in bath osmolaity – the response was reversible. (E) Single-cell RT-PCR of an osmotically-responsive OVLT neuron – note the presence of AT1 but not AT2 receptor mRNA, mRNA for ENaC and various ASIC channels, and TRPV4 but not TRPV1 mRNA. (F) IR-DIC image (left) of OVLT neuron retrogradely labeled from PVH with rhodamine microspheres (right).

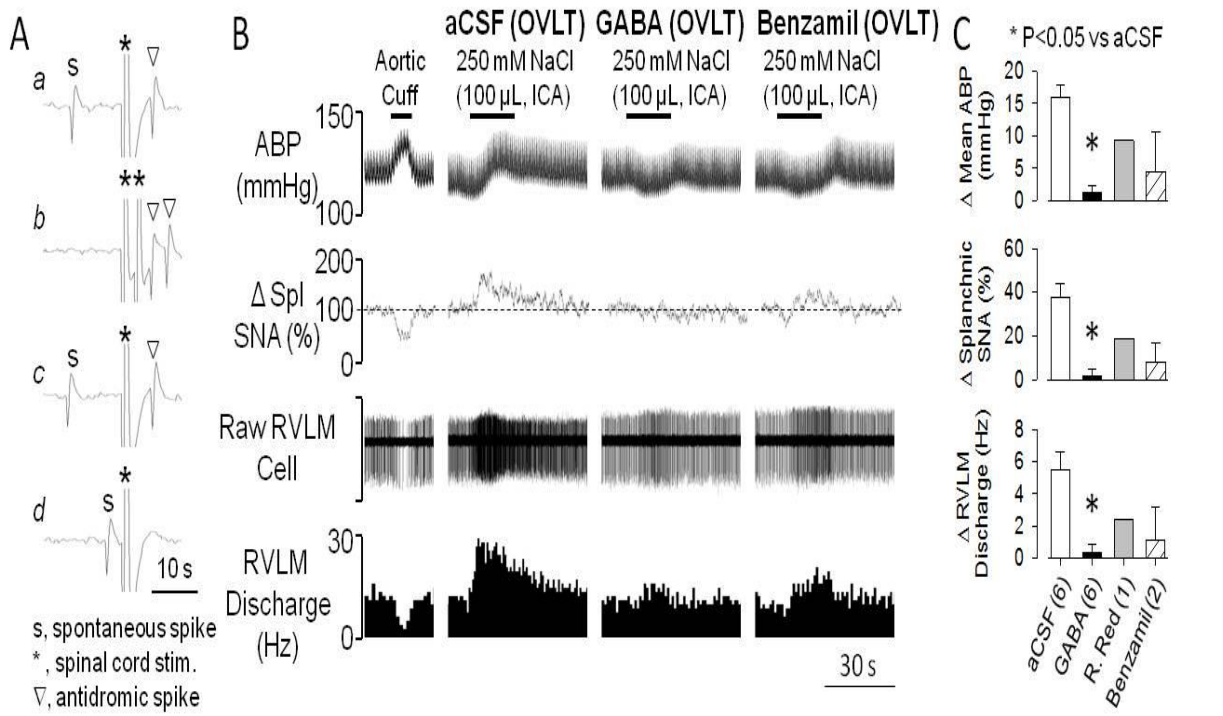

Figure 2. (A) Antidromic activation of spinally-projecting RVLM neurons. Note constant onset latency (a-c), ability to follow high frequency stimulation (b), and collision with a spontaneous spike (c, d). (B) Intracarotid infusion of hypertonic NaCl increases ABP, splanchnic SNA, and RVLM discharge. These sympathoexcitatory effects were abolished by infusion of GABA into the OVLT (0.5 M, 30 nL over 15 s) and attenuated by benazmil (100 µM, 30 nL over 15 s). OVLT infusions began 5 s before the ICA injection. (C) Summary data illustrating ability of GABA-mediated inhibition to eliminate sympathoexcitatory response to acute hypernatermia. Infusion of Ruthenium Red (n=1) or benzamil (n=2) appears to blunt the effect.

Research Project 8: Project Title and Purpose

Resolving the Structure and Function of Proteomes - The project proposes to: (i) develop/refine computational methods capable of accurate assessment of domain boundaries, conservation profiles for predicting mutants, and fold-recognition and atomic-resolution structural models for proteins from large screens, (ii) integrate/synergize these computational endeavors with the updated mass-spectrometry (MS) core-center for high-throughput identification and computational characterization of proteins obtained from wet-lab experiments and (iii) build the infrastructure for interdisciplinary training for investigators/students involved in these endeavors. This is the next step towards an interactive, high-throughput, collaborative, and interdisciplinary Center for Computational Proteomics.

Anticipated Duration of Project

1/1/2011 – 12/31/2012

Project Overview

The overall goal of this project is to develop a world-class proteomics center at Penn State. By bridging the current strengths at PSU in computational biology (The Center for Computational Proteomics (CCP)) and the existing mass-spectrometry (MS) core-facility, an interdisciplinary center will be formed capable of high-throughput computational proteomics.

Specific Aim 1: Develop/Refine high-throughput computational approaches capable of accurate assessment of protein characteristics. Results obtained from the recent Critical Assessment of Techniques for Protein Structure Prediction (CASP) competition by CCP indicate that the Adaptive-BLAST algorithm developed by CCP is capable of performing high-throughput structural homology modeling; overcoming a major hurdle to proteomics research. However, proteins that have less than 30% identity with known protein folds are still extremely difficult to accurately predict and model. Our preliminary data demonstrates that with sufficiently large and divergent position specific scoring matrix (PSSM) libraries, that protein folds can be predicted with >90% accuracy. To obtain acceptable accuracy levels for all structural folds, structural libraries comprised of divergent fold-PSSMs will be generated and benchmarked. Computational and wet lab experiments will be performed to determine the accuracy of the libraries. CCP will also interact with the MS Center to develop all the necessary tools and infrastructure (i.e. website, wiki-base, etc) such that the resources developed during the course of this project will be open access.

Specific Aim 2: Outfit the current MS core-facility with cutting-edge instrumentation/improved data collection services, and synergize the pipeline with our computational protein models. To excel at proteomics research, the technology within the current MS center must be updated. We plan to purchase a Thermo LTQ Orbitrap Velos (ETD) mass spectrometer, a Dionex Ultimate 3000 nanoflow LC, and a Proxeon ion source; all of which are currently top-of-the-line instruments that are capable of identifying thousands of proteins from a single sample. *Most importantly, we will work closely with the experimental biologists using the MS facility to translate our predictions to the benchtop. In these experiments we will critically evaluate our assessment of soluble protein fragments, ligand-binding pockets (e.g. protein-protein, protein-lipid, and/or protein-pharmacophore binding), and putative loss-of-function or gain-of-misfunction mutants. These combined aims will result in a pipeline which provides researchers with protein identification and cutting edge computational models*.

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Expected Research Outcomes and Benefits

It is expected that developing a high-throughput MS facility and coupling it to high-throughput computational analysis of proteins at the sequence level, will result in a world-class center capable of asking research questions of the highest quality. Typical questions asked of MS include: (i) What protein(s) bind to my ligand of interest, and/or (ii) What changes in expression/localization result from a particular cellular condition? *The output of MS is the identification of a set of protein coding sequences. If the sequences are not annotated sufficiently, the biochemical characterization of these proteins can be a slow arduous process.* Typical outputs of our computational algorithm include: (i) domain boundaries for protein sequences, (ii) conservation profiles for predictions of putative mutants, (iii) quantitative statistics for putative functions and structural fold(s) and isolation of templates for structural modeling. Together, these approaches give added value to each other and help inform the experimental scientist. During the course of this project we will: (i) determine the best methods for MS sample preparation, collection, and measurements; (ii) refine, evaluate, and improve the accuracy of our method for protein homology modeling; (iii) create transparent protocols for all processes and computational databases/interfaces to inter-relate these data. The potential benefits from this project are broad and have immediate impact. From pharmacophore development to gene therapy and beyond, the tools and initiatives being developed during this project will assuredly promote Penn State as a leader in computational proteomics. At the same time, this project will provide our faculty and students with state-of-the art tools and computational support. We expect that this will also translate to both Center grants and increased R01s for our faculty. Importantly, without these improvements, obtaining Proteomic Center grants are simply outside our reach. Upon completion of this project, we expect to be highly competitive for NSF Training and Center grants, as well as Keck grants and other awards.

Summary of Research Completed

In the few months since this project was initiated we have made progress towards our specific aims. Specifically, the Thermo LTQ Orbitrap Velos mass spectrometer system was installed in June 2011. The system comprises a high resolution hybrid mass spectrometer (the Orbitrap) with electron transfer dissociation option, along with the Dionex Ultimate 3000 nano LC front end. The system was installed in the Proteomics and Mass Spectrometry Core facility, based in 3 Althouse Laboratory, a facility with around 60 regular faculty users. Basic operator training was received by Drs. Tania Laremore and Phil Smith, who are currently working on the development of several standard operating procedures. Advanced operator training is scheduled for later in the year. Residing within a University Core Facility, we have calculated use rates that are compliant with Federal requirements for recharge centers at Universities; these are now approved by the

University's Corporate Controllers Office, and as such the equipment is ready to commence work.

With respect to the computational aims, we are actively (i) refining our Fold-Specific Libraries to include additional structural representatives not in our previous version. In our previous work we built family based (SCOP)-fold specific libraries that consist of 1086 different structural folds and now there are 1195 in the newest SCOP 1.75 release; (ii) adding Superfamily-, and Family-Specific hierarchies for those structures that share structural and functional similarity to infer a divergent evolutionary relationship but not necessarily detectable sequence homology. Together these libraries are necessary for us to make advances in domain detection, fold-recognition, and homology modeling; and (iii) interfacing with refinement algorithms such as Rosetta and extrapolated motion to generate more energetic models and discriminate between models, respectively.