Drexel University

Annual Progress Report: 2007 Formula Grant

Reporting Period

July 1, 2009 – December 31, 2009

Formula Grant Overview

The Drexel University received $1,138,730 in formula funds for the grant award period January 1, 2008 through December 31, 2009. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Imaging Amyloid Therapy In Vivo Using Two-Photon Microscopy - The goal of this project is to develop a drug specifically targeted to the amyloid-*beta* plaques involved in Alzheimer's disease (AD). AD is the most common age-related neurodegenerative disorder and cause of dementia amongst the elderly. AD was discovered exactly 100 years ago. At that time autopsy of patients showed a characteristic biochemical hallmark in the diseased brain: the amyloid-*beta* (or simply "Aβ") plaques. Surprisingly, the exact relationship between neuronal loss, dementia and plaque deposition is not yet understood. However, it is well established that the Aβ plaques destroy neurons. The goal of this project is to use synthetic organic chemistry to make an Aβ-specific drug by linking a plaque-targeting fluorescent tag to an artificial peptidase which will then destroy the harmful plaques in the diseased brain.

Duration of Project

1/1/2008 – 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

Genetic and Immunological Analysis of Resistance to HSV-1 Infections - The purpose of this study is to understand the host genetic components associated with restricting the entry of the neurotropic enveloped viral pathogen, herpes simplex virus type 1 (HSV-1), into the peripheral and central nervous systems from peripheral sites of infection. The importance of these studies lies in determining how the body limits neuronal infection, which, in humans, is related to the frequency of reactivation from latent infection, the frequency and severity of recrudescent

disease, the frequency and duration of viral shedding for transmission to other susceptible individuals, and the potential for the development of life-threatening herpes encephalitis. The identification of immune-associated genes involved in this process will facilitate the design of more effective anti-HSV-1 vaccines and therapeutics in the future.

Duration of Project

1/1/2008 – 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

Identification of Biomarkers and Therapeutic Targets in 3D Breast Cancer Model - Breast cancer is the most common female cancer in the Western world, affecting 1 of 8 women over their lifetime. One critical prognostic indicator for many tumor types including breast cancer is decreased oxygen levels or hypoxia. Hypoxic cancers are generally associated with aggressive growth, metastasis, and poor response to radiation treatment and chemotherapy. Understanding the molecular mechanisms and signaling pathways driving breast cancer development is critical for developing diagnostic and therapeutic agents. The purpose of this project is to use molecular techniques to analyze the role of hypoxia in regulating gene expression during breast cancer development and to utilize this information to identify novel diagnostic markers and therapeutic targets that can be used to detect and treat breast cancer.

Duration of Project

1/1/2008 – 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 4: Project Title and Purpose

Therapeutic Magnetic Nanoparticles for Targeted Delivery to Stents - Stent angioplasty, including use of drug eluting stents (DES), has led to a major paradigm shift in the care of coronary disease. Although the efficacy of the DES platform has been well validated in large clinical trials across various population and lesion subsets, there are still remaining concerns regarding the long term safety and efficacy of these devices. Under this program, a novel method of magnetically-mediated drug delivery to stents will be developed. This approach will allow re-

dosing of drug by additional delivery if needed and will enable us to tailor the drug dose specifically for the patient, which will result in a more effective treatment of a coronary disease.

Duration of Project

1/1/2008 - 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 5: Project Title and Purpose

Modeling L-Selectin Mediated Attachment Strength During Embryo Implantation - The main challenge in understanding the fundamental mechanisms associated with primary implantation lies in an understanding of the mechanism of "braking" required to stop the blastocyst rolling and to begin the complex biochemical pathways that result in embryo attachment. Our strategy is to develop an *in vitro* cell model that can be used to study the attachment of trophoblast-like cells and a human endometrial epithelial 3D model. Through this work, we will for the first time, quantify attachment strength providing a functional assessment of the L-selectin adhesion system. The functional assay will bring us closer to understanding the importance of L-selectin expression in achieving adequate strength to slow and stop the blastocyst in the uterus as the key initial step toward implantation.

Duration of Project

1/1/2008 - 12/31/2009

Project Overview

The *long-term goal* of this research is to understand the role of the L-selectin adhesion system in the initial events necessary for blastocyst attachment to the endometrial epithelium. The *objective* of this project, which is a first step toward that goal, is to examine L-selectin expression in model cell lines for trophoblasts, to investigate interactions with endometrial epithelial cells, and to quantify the attachment strength mediated by the functional interaction of L-selectin and L-selectin ligands using a custom designed parallel plate flow apparatus. We *hypothesize* that the attachment strength due to the L-selectin adhesion system is modulated by the local environment of steroid hormones, cytokines and inflammatory mediators, and mechanical shear forces due to flow within the uterus. Our *rationale* for this project is that its successful completion is expected to provide compelling evidence toward the critical L-selectin adhesion system expression necessary to provide primary attachment and for the first time, to quantify this attachment strength as a function of expression. This research will be accomplished through two specific aims. In Specific Aim 1, the effects of endocrine mediators on trophoblast-like cell L-selectin expression, shedding, capping and attachment mechanics under static and

fluid flow conditions, using a parallel plate flow apparatus will be determined. In Specific Aim 2, a 3D endometrial epithelial *in vitro* cell culture model will be developed from endometrial epithelial cells (EECs) isolated from human uterine biopsies under an approved IRB protocol. Attachment mechanics between the 3D EEC culture and rolling trophoblast-like cells will be quantified, enabling a functional cell culture model for examining the effects of different mediators on initial attachment mechanisms. This quantitative approach is *significant* because it will facilitate functional understanding of expression levels in the L-selectin system and regulation by endogenous or therapeutic hormones as well as fluid flow, and allow further investigation into regulation by inflammatory mediators and cytokines. In addition, the development of a novel 3D human EEC system will advance our understanding of primary interactions with a highly relevant culture model and provide a tool for assessing numerous effects on human EECs (hEECs).

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Expected Research Outcomes and Benefits

We expect this work to introduce the novel concept of targeting attachment mechanisms associated with primary adhesion to address issues of infertility. Without further understanding of the embryo attachment mechanisms, little can be done to address this critical clinical issue for fertility.

The expected results from this project will allow us to begin to understand the role of the L-selectin adhesion system as a regulator of the attachment process during implantation and as a possible novel target for treating infertility and improving success rates of *in vitro* fertilization. Cutting edge engineering technology used in this project for identification and quantification of specific cellular interactions will also provide a basis for further understanding of these initial attachment mechanisms.

Summary of Research Completed

In this reporting period we further optimized a three-dimensional uterine epithelial model using the Ishikawa cell line in preparation for human EEC explants cultures. Previously, we had

optimized conditions for culture initiation in regular growth medium supplemented with 10% fetal bovine serum, based on morphology, as examined by immunofluorescent staining of actin skeleton. While cells cultured in three-dimensional matrices can more closely recapitulate the in vivo microenvironment, it is not known whether ECM proteins, such as those present in matrigel, have an effect on expression of L-selectin carbohydrate ligands. Therefore, during the current reporting period, we focused on defining and modulating expression of L-selectin ligands in 3D culture with the ultimate goal of being able to quantify binding strength of trophoblast to these cultures under different conditions of surface availability of ligands.

Our previous studies have shown that we can modulate expression of L-selectin ligands by hormones or dexamethasone in 2D Ishikawa cultures. We therefore generated 3D cultures of Ishikawa cells treated with similar hormone conditions to examine whether this modulation translates into the 3D system. Based on our results in 2D cultures, 3D cultures were treated in medium void of hormone-mimetics, as those can increase basal expression levels and, thus, reduce the overall extent of modulation. For initiation of cultures on matrigel-layers, phenol-red-free medium was supplemented with charcoal-stripped fetal bovine serum, as this resulted in higher recovery (not shown). Once 3D cultures were established, after about 3-4 days, this medium was replaced with defined phenol-red and serum-free medium, and cultures were treated with hormones for 48 hours. Expression levels of L-selectin ligands were assessed using a very sensitive three-step immunofluorescent procedure with the MECA-79 antibody. Due to the strong fluorescence of the actin stain phalloidin-FITC, which caused significant spillover into the red channel, we only used DAPI as nuclear counterstain and the Texas-red conjugated MECA-staining system, after confirming that morphology was maintained in serum-free medium (not shown). While controls treated with rat IgM showed a non-sepcific staining pattern (figure 1D), MECA-79 stained 3D cultures in a distinct punctate staining pattern (figure 1B), suggesting membrane-bound ligands; we are currently confirming this by performing confocal microscopy. Staining intensity can also be (semi-)quantified by immunofluorescent staining; however, the MECA-79 staining was very weak overall, therefore it was not possible to determine differences of expression between the different hormone treatments. In addition, matrigel itself showed some background staining, which interferes with the analysis. Overall, we could confirm maintenance of L-selectin ligand expression in three-dimensional culture under appropriate culture conditions; we therefore expect to be able to translate this to human EEC explant cultures.

Thus, we have established both components of the blastocyst adhesion system, the three-dimensional blastocyst, and the 3D uterine counterpart, with functional L-selectin adhesion system, to be able to quantify its contribution to the early blastocyst adhesion process.

During this reporting period, we initiated a collaboration with Dr. Dan Carson to include MUC1 as another target molecule contributing to human blastocyst attachment. MUC1 is a large, type I membrane mucin expressed in all uterine epithelia examined to date. Its ectodomain is composed of a large number of 20-22 amino acid tandem repeat motifs enriched in serine, threonine and glycine residues and carries hundreds of O-linked, i.e., mucin type, oligosaccharides. Normal uterine epithelia abundantly express MUC1 in a polarized fashion at the apical cell surface. These properties not only help hydrate the apical surface, but also make MUC1 an effective barrier molecule that protects mucosa from microbial infection, enzymatic

attack and cell or embryo attachment. It has been observed that MUC1 carries selectin ligands in human uterine epithelia and human uterine epithelial cell lines. Since MUC1 has a highly conserved 70 amino acid cytoplasmic tail (CT) that participates in a remarkable number of intracellular signal transduction events , we hypothesize that MUC1 engagement with selectins triggers some signaling events in humans that ultimately may contribute to adhesion of the blastocyst. Ishikawa cells do not express MUC-1, but our collaborator provided a MUC-1 positive cell line, hTERT-EEC, which we will use to determine the role of MUC-1 in L-selectin-mediated adhesion events. Figure 2 confirms expression of L-selectin ligands in hTERT-EEC, as shown by western blot using the MECA-79 antibody. Since we have antibodies that can block MUC-1 function, this cell line will allow us to dissect the contribution of MUC-1 dependent and independent L-selectin ligands for blastocyst binding.

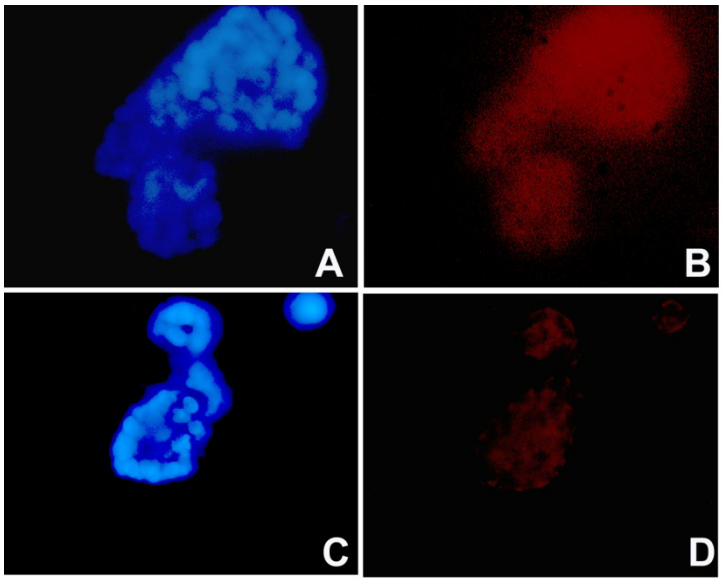

Figure 1: Ishikawa cells at $5x10^5$ cells/ml were mixed with phenol-red-free media containing 2% Matrigel (BD Biosystems) and 10% charcoal-stripped FBS and seeded onto chamberslides coated with thin (<1mm) layers of matrigel. Cultures were allowed to grow at 37°C, 5% CO2, air, for 3-4 days. Medium was then replaced with phenol-red free DMEM/F12 supplemented with 0.2% fatty-acid free BSA and hormones as described earlier (estrogen, progesterone and both); controls were kept in medium without hormones. After 48h of hormone treatment, cultures were fixed in 4% paraformaldehyde in PBS. For immunofluorescent staining, a three-step procedure was used to maximize sensitivity, consisting of primary rat antibodies (non-specific IgM or MECA-79), biotinylated secondary goat-anti-rat IgM antibody (Jackson laboratories) and Stretavidin-Texas-Red (Vector laboratories). Endogenous biotin was inactivated using a streptavidin-biotin kit (Vector labs). DAPI was used to counterstain nuclei (A,C). Images were taken at 200x magnification. A representative experiment of controls, i.e. Ishikawa in hormone-free medium, is shown (C, D stained with rat-IgM, and A, B with MECA-79).

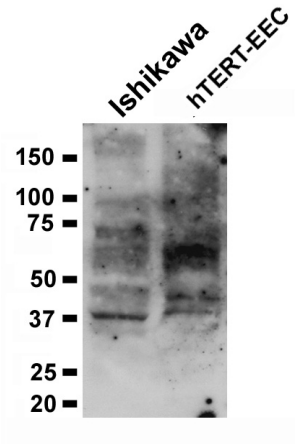

Figure 2 Whole cell extracts of Ishikawa and hTERT-EEC cells were subjected to Western blots using the MECA-79 primary and goat-anti-rat secondary antibody. A representative blot is shown.

Research Project 6: Project Title and Purpose

Small Molecule Inhibitors of Parasite Invasion - Malaria is a disease caused by parasites that take huge toll on human populations: estimated 500,000,000 cases and 1-3 million deaths per year. The parasites that cause malaria have become resistant to most widely used drugs, and our arsenal against this disease is becoming limited. Thus, there is an urgent need to find alternative drugs to treat malaria. The malaria parasites live within human red blood cells and to do this, they have evolved sophisticated machinery to invade our cells. We have discovered components of this invasion machinery and have derived a detailed structure of some of these components. Our purpose is to use this detailed structure to discover small molecules that can inhibit the invasion machinery. We have already identified a few drug-like molecules that appear to have this property, and our purpose is to develop these into potential drugs.

Duration of Project

1/1/2008 – 9/30/2009

Project Overview

Over the last few years, it has become apparent that malaria parasites, as well as other related parasites belonging to the Phylum Apicomplexa, possess a conserved and unusual means of locomotion that is used for invading host cells. This locomotion is powered by parasite-encoded myosin motors moving along short actin cables. An atypical type XIV myosin heavy chain, called MyoA, is a central component of this machinery, which interacts with a myosin light chain discovered at Drexel called MTIP. We have shown that a short 15-amino acid peptide from the carboxy terminal tail of MyoA is capable of disrupting this interaction and also has the ability to inhibit the growth of human malaria parasite *Plasmodium falciparum*. An X-ray crystal structure of MTIP-MyoA peptide has been derived, identifying critical interaction points between these molecules. Based on this structural information as well as on biophysical properties of MTIP-MyoA interaction, we have identified several small drug-like molecules with potential to interfere with this interaction. We have screened over 100 such molecules in whole

cell assays for parasite growth inhibition, and identified four different chemical scaffolds with mid-point effective inhibition concentrations (EC_{50}) in low nanomolar range. We now wish to explore this system further to have a deeper understanding of the target of these compounds with a view to develop anti-parasitic drugs targeting a novel and critical pathway of a large group of medically and economically important parasites. Our Specific Aims will be: 1. Definitive identification of target(s) hit by compounds with low nM EC_{50} through a combination of cell biological and molecular genetic approaches; 2. Developing animal malaria models for testing these compounds through modifications of the animal parasite genes encoding motor components to resemble those of human parasites; 3. Assessing the activity of the identified compounds for their activity against other Apicomplexan parasites such as *Toxoplasma*, *Cryptosporidium* and *Eimeria*; 4. Conducting exploration of the identified chemical scaffolds through synthetic medicinal chemistry to establish structure-activity relationship (SAR); and 5. Co-crystallizing the active compounds with MTIP for crystal structure determination, followed by design, synthesis and testing of new compounds. We expect to identify several lead compounds through these approaches which will then be subjected to further pre-clinical development analyses such as toxicity evaluation, bioavailability assessment and pharmacodynamics.

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Expected Research Outcomes and Benefits

We have identified and validated a target in malaria parasites, and by implication in other important parasites, for development of novel drugs. These studies will identify and optimize compounds that can be further developed as anti-parasitic drugs. Parasites against which such drugs would work take a major toll on human health and development, and because many parasites are becoming resistant to the existing drugs, there is a clear need to develop new drugs targeting essential aspects of parasites in a way that will be toxic to the parasites without harming the hosts. In addition to providing leads for drug development, these studies will also provide much needed insights into basic aspects of parasite biology. Such insights are critical in our goals of devising strategies to control parasitic diseases.

Summary of Research Completed

The HSB method integrates both ligand-based and structure-based methods of virtual screening of chemical databases; hence, it is particularly well suited for the discovery of small-molecule inhibitors of protein-protein interactions. The HSB method was developed as an internal tool to integrate the various ligand-based and receptor-based programs and to customize it to every specific target. Several published articles that utilize commercially available docking and scoring functions have shown that no one docking program or a scoring function can provide docking solutions that can be utilized to design inhibitors. Thus to develop a practical application for drug discovery, the essential docking and scoring function should be customized to the target protein. The HSB scheme of virtual screening was used to identify compounds from an in-house database of 300,000 structurally diverse chemicals. Using a 4-point pharmacophore the small molecule database was queried to identify 40 compounds, which were docked using the GOLD program and the protein-ligand complexes were scored with the customized scoring function as described elsewhere.

The 15 top-ranking hits that passed drug-like property filters were selected for *in vitro P. falciparum* growth inhibition. All the 15 compounds were tested at 25µM and 12.5µM, six of them exhibited growth inhibitory activity against *P. falciparum* exceeding 50% at 25 µM (Figure 1). Further, a clustering of all six compounds along with other antimalarial compounds chosen from PubChem database suggested that these six compounds belonged to unique chemical classes and, hence, each might represent a novel antimalarial lead compound. Analysis of their docking mode in the MTIP revealed that all six compounds occupied the same binding site as that of the MyoA peptide in the crystal structure. The compounds in their docked conformation interact with Leu169, Ile203, Phe199, Ala184, Leu176, Leu145, Phe149 and also with Asp174 and Trp172 in the binding site of MTIP. However, there was no hydrogen bonding interactions with MTIP residues for any of the six inhibitor molecules which leads us to speculate that the interactions were mainly hydrophobic in nature. The six hit molecules appear to mimic the hydrophobic alpha helix of the MyoA tail in the binding site. Among the six compounds, C416 was the most potent inhibitor (EC_{50} = 145nM) and, hence, was chosen as the lead compound to derive analogs using iterative HSB method.

Structure-Activity Relationship (SAR) of Pyrazole Urea compounds

Using the pyrazole-urea compound C416 as the query, 51 analogs were identified to develop the structure activity relationship (SAR) of this class of compounds. Docking studies predicted that all these molecules bind to MTIP at the same site as the parent compound C416. Structural analysis of the docking mode of compound the pyrazole urea compounds in MTIP binding site suggested that the R1 group has interactions with Phe149, Met148 and Leu145; R2 interacts with Gly173, Leu176 and Ala175; and R3 interacts with Asn167, Ile168, Leu169 and Thr171. The urea core interacts with Trp172 and Asp174 within a distance of 4.5 to 5 Å. Based on the docking mode of C416 in MTIP, positions R2 and R3 were modified conservatively to retain their hydrophobicity and aromatic features. The methyl group at position R1 in C416 was sampled systematically replaced by a proton and or aromatic groups. R3 was sampled with either a hydrogen atom or aromatic groups at either C3 or C4 carbon atoms of the pyrazole ring. Changing the urea core to an acetamide did not affect the activity of the compounds; however, an increase or a decrease to the length of the linker group between the pyrazole and R2 rings

drastically reduced the activity irrespective of the other substitutions. Compound C2-1 had the best inhibitory profile against the parasite growth with an EC_{50} of 47nM. C2-1 was very similar to C416 with a pyrazole acetamide core instead of the pyrazole urea. Among the 52 pyrazole compounds that were tested, eight compounds had an $EC_{50} < 400$nM. Among these active compounds, C2-1, C21-12, C416, C2-19, C2-13 and C2-21 shared similar structural elements while their EC_{50} values ranged from 45nM to 342nM. The SAR of compounds C416, C2-12, C2-13 and C416-r showed that changing the hydrophobic bromine group at the para position on R3 ring attached to C4 carbon of pyrazole ring (in C2-12) to the more hydrophilic hydroxyl group (in C416-r) reduced the affinity by ~30 fold. Thus this position preferred a more hydrophobic group with activity pattern –Br > OMe > F > OH. Similarly, at position R2, removing fluorine atoms from the ortho and para positions led to a minor improvement in the EC_{50} values from 336nM (C2-13) to 282nM for C2-19. In other compounds such as C3-18, C3-19, C3-20, C3-21 and C3-23, the methyl group at R1 was substituted with an aromatic group, and the aromatic R3 group was bonded to C4 atom of the pyrazole ring instead of the C3 atom. These compounds were moderately active with EC_{50} ranging from 300nM to 10μM. Major modification to the structure by shifting the urea linker to the C4 carbon on the pyrazole ring yielded 3-25, a weakly active compound with an EC_{50} of ~13μM. Apart from these highly active compounds, the screening has also led to the identification of 10 new compounds with EC_{50} values between 1 and 10μM (C2-2, C2-3, C2-4, C2-15, C2-16, C2-17, C2-18, C2-22, C3-4 and C3-24).

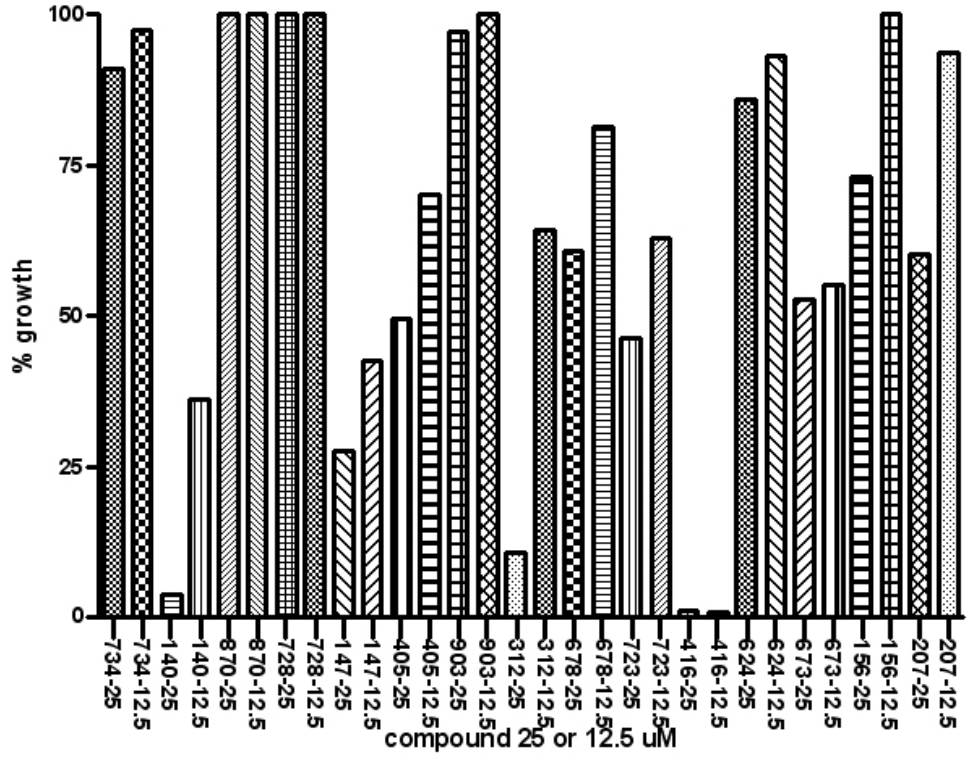

Figure 1: Growth inhibition assay as judged by ^{3}H-hypoxanthine incorporation for the initial 15 compounds tested in concentrations of 25 and 12.5µM is shown. Compound C416 was used as a lead for further investigation to derive SAR of pyrazole ureas.

Research Project 7: Project Title and Purpose

Pharmacology, Physiology and Behavioral Testing of Drugs Used to Treat Cognitive and Attentional Disorders - The purpose of this project is to develop a novel set of neurophysiological and behavioral assays that will rigorously and quantitatively measure the effects of attention deficit, hyperactivity disorder (ADHD)-medications on brain information processing in laboratory rats and then use that information to compare and interpret drug efficacy in patient populations. The central idea is that, once developed, a standard set of sophisticated assays will establish screening criteria for new ADHD medications that are based upon clinically relevant information and specific drug attributes. Such analyses are proposed for ADHD medications as prototypes but similar principles of evaluation and comparable assays can be extended to many additional classes of psychotropic compounds and neuropsychiatric disorders.

Duration of Project

1/1/2008 – 9/30/2009

Project Overview

Aim 1 To establish the effective dose range for ADHD-drug-induced improvement of rodent performance in a sensory-guided sustained attention task.
Methods: We will evaluate rat performance on a sensory-guided sustained attention task following administration of four different ADHD medications (e.g., methylphenidate, amphetamine, atomoxetine, modafonil). Dose-response curves will be constructed to dissociate doses that enhance sustained attention in our operant task from those that affect locomotor activity.

Aim 2 To examine the effects of ADHD medications on sensory and cognitive circuit function during a sensory-guided sustained attention task.
Methods: We will record task related activity from ensembles of single neurons (n = 20-30) in prefrontal cortex and visual sensory cortex of animals engaged in a sustained attention task (see Aim 1), before and after administration of ADHD medications. Task related discharge of individual neurons will be subjected to quantitative computer-based analyses to establish the magnitude and time course of drug-induced changes in neuronal response properties.

Aim 3 To develop a novel rat model that measures the effects of ADHD medications on brain information processing.
Methods: We will record activity from ensembles of single neurons in the somatosensory cortex of awake, quietly resting rats during touch stimulation of different body regions, before and after administration of ADHD medications (see list in Aims 1&2). Next we will measure the amount of information a population of neurons can represent by using the single-trial responses of neuronal ensembles to identify the location on the body where each stimulus is delivered.

Aim 4 To examine the effects of catecholamine neurotransmitters, norepinephrine and dopamine, on synaptic communication within sensory and prefrontal cortical circuitry.
Methods: Multiple whole cell patch-clamp recordings will be employed to record and evaluate the strength of monosynaptic connections between neighboring cortical neurons; both before and after application of catecholamine neurotransmitters, separately and in combination.

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Expected Research Outcomes and Benefits

The project is designed to develop methodology for a comprehensive analysis of the effects of therapeutically-relevant doses of ADHD medications on: 1) performance measures in a sustained attention task, 2) vigilance task-related discharge of neurons in sensory and cognitive circuits, 3) sensory stimulus coding in neural networks, and 4) catecholamine neurotransmitter modulation of synaptic transmission in sensory and cognitive regions of the mammalian brain. As such, these studies will provide a base of information and a standard set of assays that can be extended to investigations of ADHD drug actions (both acute and chronic) in developing animals and animal models of ADHD.

Ultimately, a better understanding of the molecular, cellular and network mechanisms underlying the therapeutic efficacy of these drugs is necessary for development of pharmacological treatments that do not possess the negative properties of this class of compounds (e.g., abuse potential, cardiac arrythmias, neurotoxicity). The experimental tests and drug profiles developed here will provide pharmaceutical companies with a better rationale and a more powerful set of tools for identifying and categorizing new candidate ADHD medications. Furthermore, such studies hold the promise of providing much new information regarding the underlying causes of ADHD.

Summary of Research Completed

Catecholamine Receptor Blockade MPH Effects on Vigilance Task Performance: In order to begin to investigate the neurotransmitter/receptor mechanisms through which MPH enhances attention, we attempted to block the acute effect of MPH on vigilance index with a systemic dose of the α-1 antagonist prazosin (ip), administered 30 min prior to MPH administration. The dose of prazosin (0.5 mg/kg) was selected on the basis of its ability to block other behavioral effects of MPH. Six well trained animals all exhibited increased vigilance index in response to 2.0 mg/kg MPH and did not respond to prazosin alone. However, this dose of prazosin significantly (*$p<0.05$) blocked the ability of MPH to enhance vigilance index (Fig 1A). Data are presented as difference scores from average baseline performance. These animals remained responsive to a subsequent dose of MPH, indicating no lasting effects of the α1 receptor blockade. We repeated this study in a separate group of rats and demonstrated that the enhancement of vigilance elicited by an oral dose of MPH (8.0 mg/kg) could be blocked by an oral dose of prazosin (1.0 mg/kg). These preliminary results (Shumsky, et al., 2009) clearly implicate α1 receptor mechanisms in MPH facilitation of attention task performance and further demonstrate the feasibility of using the oral mode of drug delivery.

<u>Development of a novel rat model that measures the effects of ADHD medications on brain information processing</u>

To develop a novel rat model that measures the effects of ADHD medications on brain information processing we are using two approaches: electrophysiological and electrochemical. Since drugs that alleviate the symptoms of ADHD have been shown to increase the extracellular

concentration of norepinephrine (NE) and modulate the activity of neurons in the primary somatosensory cortex, it has been suggested that MPH can alter the processing of sensory information in the brain by activating NE synapses.

Impact of MPH on the release and re-uptake of NE in the primary somatosensory cortex: To assess the effects of MPH electrochemically, we assessed its effects on the release and reuptake of NE in the SI cortex by measuring the effect of MPH on the concentration of NE recorded from the SI cortex after stimulation of the locus coerlueus as shown in Figure 2. In general, the pharmacological response of the signal recorded after the administration of MPH was consistent with the electrophysiological actions of MPH and studies using microdialysis. Moreover, these studies suggested novel finding of the effect of MPH on the release and uptake of NE in the SI.

NE was recorded in the rat SI after high frequency (60Hz) electrical stimulation of the LC (n = 5 rats) before and after administration of MPH. The oxidation current of NE was recorded and the release (NEpp) and uptake (VMAX) of NE were evaluated. Normalized values of VMAX and NEpp post administration of MPH were compared to the normalized values before administration of MPH (One way ANOVA; $p < 0.05$). At 105 minutes post administration of MPH, the uptake and release were at the lowest compared to pre administration of MPH. The release decreased sooner than the uptake and recovered faster. The decrease in the release of NE was significant after 15 minutes and continued to be significantly less than the normalized pre-MPH value until 135 minutes post MPH. At 180 minutes post MPH, the release returned to 53% (+/- 7%, SEM) of the pre MPH value and was no longer significantly different. Uptake also decreased, but the percent decrease did not become significant until 45 minutes post MPH. The decrease in the uptake remained significant until 165 minutes post MPH administration. The uptake returned to 70% (+/- 14%) after 180 minutes.

To further understand the effect of MPH on NE in SI cortex, the ratio of NEpp to Vmax was evaluated throughout the time course of the MPH. For the stimulations starting at 15 minutes through 60 minutes post MPH administration, this ratio was .49 (+/- .09, SEM) meaning that the uptake term was twice as much as the amount of NE release. However, for stimulations starting at 135 minutes through 180 minutes, the ratio averaged 1.16 (+/- 0.28, SEM) with a peak value of 1.91 (+/- 0.12, SEM) at 135 minutes post injection. This suggests that for 135 to 180 minutes post administration of MPH, the amount of NE released per pulse was almost twice as much as the uptake of NE in the SI. These results are consistent with voltammetry studies using more specific NE uptake blockers. The effect of MPH on the release of NE is likely due to activation of NE alpha-2 receptors that regulate the release of NE. These results suggest that the regulation of NE in the extracellular space may allow for NE to remain in the extracellular space longer but does not allow the concentration to reach high concentrations.

In summary, our results indicated that, as expected, MPH decreased the rate of uptake (Vmax). However, MPH also decreased the amount of NE released per stimulation pulse (NEpp). The impact of these two competing mechanisms was that, on the one hand, MPH did not increase the peak concentration (Cmax) of NE post stimulation as might be expected from blocking uptake but MPH did increase the time to reach the maximum concentration, Tm. Since sensory stimulation transiently modulates the firing rate of LC neurons, these results suggest that MPH may have an important impact on the stimulus evoked release of NE. This impact is likely to be

directly responsible, in part, for the effect of MPH on the sensory stimulus evoked activity of neurons in sensory networks.

Application of the information theoretic measures to population of cortical neurons before and after administration of MPH: Seven animals were used to test the effect of MPH on the information represented by populations of single neurons to whisker stimuli of different magnitudes (based on duration of whisker stimulator: 60 us, 120 us and 240 us) as shown in Figure 3. Briefly, animals were chronically implanted with arrays of microwires into the barrel cortex (whisker cortex) and a whisker pad stimulator into the whisker pad. Animals were allowed one week to recover and the impact of the different magnitudes of stimulation on the response properties of single neurons was assessed while the animals were awake and freely moving on a treadmill. Once it was determined that the whisker pad stimulation modulated the response of the single neurons, the effect of MPH on the information conveyed by the population was assessed. Since the effect of MPH on neuronal activity and the release and re-uptake of NE occurred within 45 minutes after administration of drug we assessed the impact of the drug on the information conveyed by the population of neurons within the first 45 minutes after drug administration (0.5 mg/Kg, 1.0 mg/Kg and 2.0 mg/Kg). The procedure was as follows: the animal was given an i.p. injection of saline and patterns of stimulate were applied to the whisker pad stimulator for two hours using an iso-flex stimulus isolator (AMPI Jerusalem, Israel) connected to a Master-8 unit stimulator (AMPI Jerusalem, Israel). The Master-8 stimulator was connected to a computer and controlled using a custom built MATLAB algorithm (Mathworks Inc., Natick MA). The algorithm was designed to deliver a selected number of square pulses with a intensity chosen at random between 60, 120 and 240 microseconds and an inter-stimulus interval of 2 secs plus a random interval of 200 micro-seconds. Current intensity of the stimulus was chosen so that the medium intensity generates a receptive field similar to that obtained with mechanical stimulation. This process took approximately 12 minutes to collect 100 stimuli for each magnitude (60, 120 or 240). Then the animal was given an injection of drug and the protocol repeated. To control for behavior, animals were videotaped and the video was time-locked to the neural data acquisition so periods when the animals was sitting quietly could be discriminated from periods when the animal was whisking. Information conveyed by the population about the stimulus magnitude (60, 120 or 240 us) was assessed for each 12 minute period and data were presented for each 15 minute interval.

We assessed traditional neurophysiological parameters that measure the response magnitude to the stimuli as well as information theoretic measure. As previously reported (Drouin et al., 2006), at doses below 5 mg/Kg, our results show that MPH had no effect on the short latency excitatory response of single neurons to whisker deflection. This is the response that conveys most of the information about stimulus location. Next, we used a two-way analysis of variance (bin size x behavior) to determine if drug influenced the information carried by the population of cortical neurons and if the timing of individual spikes (bin size) impacted the amount of information conveyed. There was a significant impact of bin size on the information conveyed ($F(7,23)= 7.09$, $p<0.0001$), which is consistent with our previous work (Foffani et al., 2004). There was also a significant impact of drug ($F(2,23)=24.6$, $p<0.0001$) with no significant interaction between the two, which was expected. Post-hoc analysis (Tukey) showed, as expected, that drug carried significantly more information (0.319 +/- 0.217) compared to saline (.245 +/- 0.163) or baseline (.239+/- 0.158). However, the drug also had a significant impact on

the animal's behavior, as expected (Drouin et al., 2006). Therefore, to complete our analysis, it is necessary to complete videotaped analysis of the animal's behavior to separate out those time periods when the animals is sitting quietly to complete our assessment of the effect of MPH on information. Once this is completed, we will continue with our analysis of the way information is represented by neurons using the GPEB approach to assess the impact.

To apply information theory measures to populations of single neurons responding to sensory stimuli or behavioral events represents a methodological challenge. The problem can be approached in classification terms, i.e., given the response of a population of neurons to a set of stimuli/events, which event most likely generated the response on a single-trial basis? We have developed an efficient algorithm to solve this problem, referred to as the "post-stimulus time histogram (PSTH)-based classification method". This approach consists of creating a set of templates based on the average neural responses to stimuli and classifying each single-trial by assigning it to the stimulus with the 'closest' template in the Euclidean distance sense. Classification performance is rigorously quantified in bits of mutual information between predicted stimuli and actual stimuli. Being based on first-order statistics, the PSTH-based classification method is computationally efficient and is particularly suitable for comparing the informational contribution of the spatial component of the neural code, given by spike-count, with the information contribution of the temporal component of the code, given by spike-timing in large populations of neurons. Using the PSTH-based classification method, changes in performance as a function of bin size can be intuitively interpreted in relation to the PSTH profiles. Therefore, the PSTH-based classification method is an ideal tool to study drug-induced changes in PFC information representation.

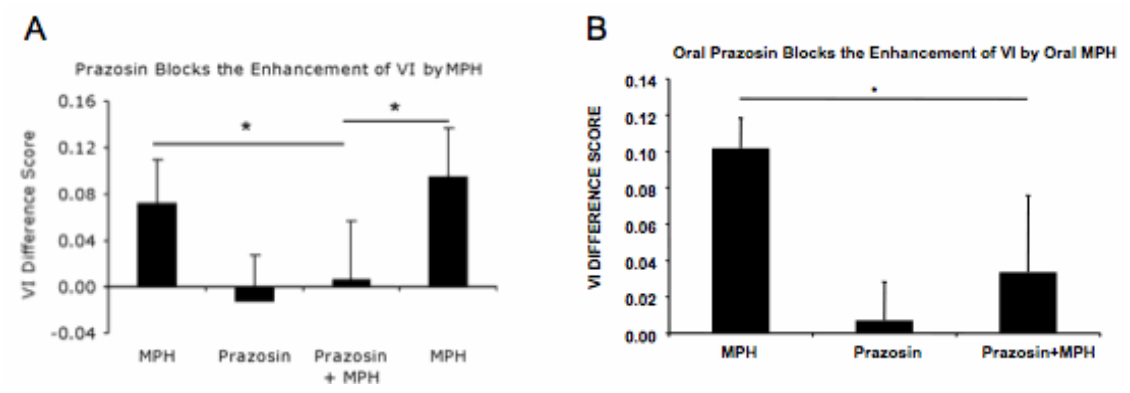

Figure 1. *Effects of IP and oral prazosin on MPH-induced enhancement of sustained attention*. A) Male SD rats (n=6) were trained to stable baseline performance on the sustained attention task (as above). 2.0 mg/kg IP MPH enhanced Vigilance Index (*$p<0.05$). Prazosin (0.5 mg/kg IP) had no effect on its own and completely blocked the ability of MPH to enhance attentional performance (*$p<0.05$). B) Another set of male SD rats (n=5) was trained as above. Oral MPH (8.0 mg/kg) enhanced Vigilance Index (*$p<0.05$). Oral prazosin (1.0 mg/kg) had no effect on its own and completely blocked the ability of MPH to enhance attentional performance (*$p<0.05$).

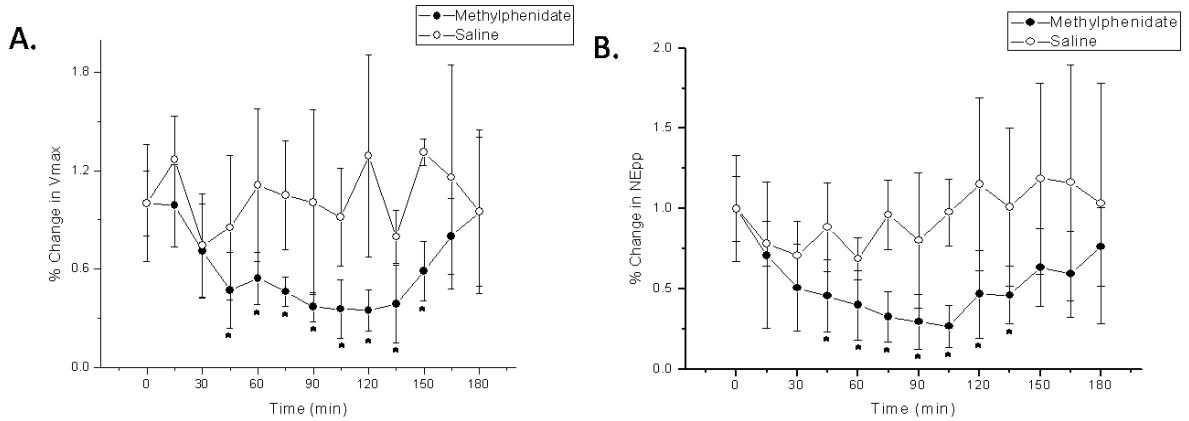

Figure 2. *Impact of MPH on the release and reuptake of NE in the somatosensory cortex***.** A. MPH significantly reduced the clearance of NE from the extracellular space. Solid circles represent the relative change in Vmax following the administration of MPH. Hollow circles represent percent change in Vmax following saline administration. A significant decrease in the voltammetric signal occurred between 45 and 150 min following MPH administration (*p< 0.01). A. MPH significantly reduced the amount of NE released per stimulation pulse. Solid circles represent the relative change in NEpp following the administration of MPH. Hollow circles are percent change in NEpp following saline administration. A significant decrease occurred between 45 and 150 min following MPH administration (*p< 0.01).

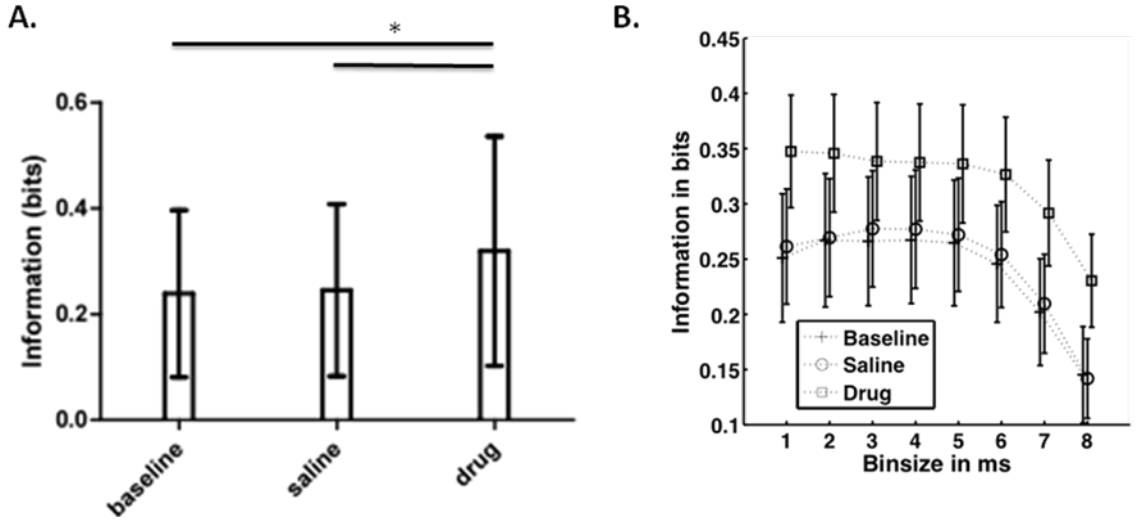

Figure 3. *Effect of MPH on the information encoded by neurons recorded from the somatosensory cortex of the rat in response to stimulation at different intensities.*
A) Mean information conveyed before and after administration of the drug in three different conditions: baseline, saline, drug. The y-axis represents information in bits and the x-axis represents the three conditions. The figure shows that the mean information during the drug condition is significantly higher compared to both baseline and saline conditions. * represents significance based on the Tukey's post-hoc test. Error bars represent 95 % confidence interval of the mean. B) Information measured during baseline (crosses), saline (circles), drug (squares) conditions at 8 bin sizes: 1 ms, 2 ms, 4 ms, 5 ms, 8 ms, 10 ms, 40 ms. The y-axis represents information in bits and the x-axis represents bin size in milliseconds. The figure shows that the information conveyed after administration of the drug is higher at every bin size tested. Error bars represent 95 % confidence interval of the mean.

Research Project 8: Project Title and Purpose

A Novel Microfluidic-Platform Liver Model System for Testing Drug Toxicity - Drug-induced liver injury (DILI) is a common reason for stopping drug development, failure to acquire governmental drug approval and withdrawal of a drug from the market. There is no human liver model system that can be used to test DILI. Attempts to make a human liver system have been hampered because large quantities of human liver cells are unavailable. There is a need to create a human liver model that retains normal liver architecture and uses small numbers of cells. In this project, we will use rat liver cells and microfabrication of microchambers (1cm x 100um) to produce a functional mini-liver unit to test DILI. Our long-term goal is to use experimental conditions derived from studies of the rat mini-liver model to generate a human mini-liver system. This system will be used to study human liver biology and DILI.

Duration of Project

1/1/2008 - 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 9: Project Title and Purpose

Vascular Complications in Diabetes: Effect of High Glucose Extracellular Matrix Alterations on Angiogenesis - The purpose of this project is to study how high blood sugar levels contribute to increased cardiovascular disease in people with diabetes. Angiogenesis, the growth of new blood vessels, is particularly disturbed in diabetes, which can lead to blindness, kidney failure, and poor wound healing. Angiogenesis is a tightly regulated process in which endothelial cells, which line all blood vessels, respond to chemical signals in the environment to either produce or regress blood vessels. High glucose concentrations, such as might be seen in diabetes, have been shown to alter the cell environment in ways that both enhance and reduce angiogenesis. In this project, we will determine how a combination of high glucose changes in the extracellular environment cause endothelial cells to produce or regress new blood vessels.

Duration of Project

1/1/2008 – 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 10: Project Title and Purpose

Implantable Microdevice for the Treatment of Hydrocephalus - This research project will develop an innovative implantable device for the treatment of hydrocephalus which is one of the most frequently encountered problems in Neurosurgery. An implantable microdevice that resembles the function of the native arachnoid granulations which acts as biological valves to eliminate cerebrospinal fluid will be developed using microfabrication technology. The proposed microfabricated arachnoid granulations (MAG) will make it possible to replace the malfunctioning arachnoid granulations that lead to hydrocephalus. If successful, the proposed MAG may pioneer a new era in the treatment of hydrocephalus.

Duration of Project

1/1/2008 – 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.