

# Temple University

## Annual Progress Report: 2010 Formula Grant

### Reporting Period

July 1, 2013 – June 30, 2014

### Formula Grant Overview

Temple University received \$2,050,596 in formula funds for the grant award period January 1, 2011 through December 31, 2014. Accomplishments for the reporting period are described below.

### **Research Project 1: Project Title and Purpose**

*The Effects of Music Therapy Entrainment on Pain, Vital Signs, and Bowel Function of Cancer Patients* - The purpose of this project is to gather preliminary data on the effectiveness of a specialized music therapy approach to pain management with cancer outpatients who have chronic pain. A cross-over design will be used to examine the effects of music therapy entrainment, a live music intervention based on principles of physics, on reported pain levels, vital signs, pain medication usage, and bowel function in 40 participants. Participants will receive one music therapy entrainment session and one sham treatment consisting of listening to pre-recorded music. In addition to measuring the above mentioned outcomes, three participants will be chosen at random to undergo MRI scans. The purpose of this is to assess brain activity relevant to entrainment music.

### Duration of Project

7/1/2011 – 6/30/2012

### 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**

*Place-Based Interventions for Public Health: A Cross-Disciplinary Approach to the Study of Policing* - The purpose of this study is: 1) to determine if the benefits of focused, foot patrol policing to reduce violent crime translates to a decrease in the incidence of drug-related illnesses (such as overdose) and physical assaults; and 2) to identify community variables that are associated with rates of crime-related illness or injury. Community variables include micro-places (e.g. alcohol outlets, parks) that serve as perpetual 'crime generators' as well as facilities

(e.g. churches, health clinics) that contribute to the promotion of healthy behaviors. These data will provide evidence to support ways in which the police, along with city agencies and services can leverage their different tools and resources in a more targeted and collaborative manner to prevent crime and achieve shared health and safety outcomes.

### **Duration of Project**

1/1/2011 – 6/30/2011

### **Summary of Research Completed**

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

### **Research Project 3: Project Title and Purpose**

*Inflammation and Organ Tissue Damage* - This project seeks to clarify the mechanisms by which alteration in the inflammatory response cause tissue damage and organ failure. Specifically, we will study critical steps of the inflammatory response to understand how: 1) adhesion receptors expressed on endothelial cells and circulating leukocytes affect the immune response and subsequent organ damage, 2) signal transduction processes initiated by such receptors regulate the inflammatory response, and 3) cytokines produced by immune cells affect the overall outcome of the inflammatory response toward resolution or chronic inflammation. The overall goal is to uncover novel pharmacological strategies to prevent or treat the organ complications associated with chronic inflammation (e.g., neurodegenerative disorders, cardiovascular disease, obesity with insulin resistance, and physiological aging). These results will advance our understanding of the mechanism by which systemic inflammation increases organ morbidity and all-cause mortality in the population of the USA.

### **Duration of Project**

9/1/2012 – 6/30/2013

### **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**

*Inhibition of Leptin Receptor Signaling in Cellular Models of Rheumatoid Arthritis and Osteoarthritis* - Osteoarthritis (OA) and rheumatoid arthritis (RA) are debilitating diseases whose progression can be promoted and/or aggravated by obesity. Leptin is a satiety factor regulating appetite and energy expenditure by acting on leptin receptors (ObR) in the

hypothalamus. Leptin represents a critical link among nutritional status, metabolism and immunity. Recent evidence suggests a major role of the leptin / ObR system in the pathogenesis of OA and, perhaps, RA. Consequently, pharmacological downregulation of leptin activity could become a novel treatment for OA and RA. Our laboratories developed highly efficacious, specific and safe leptin-based peptide antagonists of ObR. This collaborative project will explore the applicability of our lead ObR antagonists in reversing characteristic OA and RA processes in vitro.

### **Duration of Project**

7/1/2011 – 12/31/2011

### **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 Infrastructure Project 5: Project Title and Purpose**

*Research Infrastructure Project: Multiphoton Imaging Facility* – This project will fund a laboratory renovation to create a new Multiphoton Imaging Facility. Renovations will be made to existing space in the Department of Biochemistry located on the 6th floor of Kresge Hall. The updated facility will greatly improve capabilities for confocal microscopic imaging.

### **Duration of Project**

7/1/2011 – 6/30/2012

### **Summary of Research Completed**

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

### **Research Project 6: Project Title and Purpose**

*Immunomodulatory Cannabinoids as Potential Therapeutics for Transplant Graft Rejection* – Compounds that bind to Cannabinoid Receptor 2 will be investigated for their potential to increase survival of skin and organ grafts. Compounds of this type should suppress immune reactions but not have psychotropic activity. The efficacy of these compounds in combination with standard anti-rejection therapy will also be investigated.

### **Anticipated Duration of Project**

7/1/2011 – 12/31/2014

## **Project Overview**

Current therapies for organ and skin transplantation have deficiencies, as long-term use results in unwanted side effects including nephrotoxicity, neurotoxicity, diabetes, and liver dysfunction. Synthetic cannabinoids that bind selectively to the cannabinoid receptor 2 (CB2) have been shown to have anti-inflammatory and immunosuppressive activity. Compounds of this class do not have the psychogenic effects of marijuana or its constituents because the CB2 receptor is expressed almost exclusively on cells of the immune system. The psychogenic effects of abused cannabinoids result from binding of ingredients in marijuana to the CB1 receptor, which is expressed on neurons. This anatomical separation of expression of CB2 receptors permits design of compounds that will affect the immune system but not the nervous system. This project seeks to assess the potential of CB2 binding compounds, alone or in combination with standard anti-rejection therapies, to depress graft rejection. There are two Aims. One will use an in vitro assay that is an accepted correlate of graft rejection, the Mixed Lymphocyte Reaction, to assess the immunosuppressive capacity of various compounds, alone and in combination with standard anti-rejection therapies. Aim 2 will test the potency of these compounds alone or in combination with standard anti-rejection therapies in vivo using mice with grafted tissues. The in vitro assays will be used as a screen for compounds and drug combinations. Those that are most effective in vitro will be tried in vivo.

## **Principal Investigator**

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## **Other Participating Researchers**

Rebecca Hartzell, BSc; Joseph J. Meissler, BSc; Senthil Jayarajan, MD – employed by Temple University School of Medicine

## **Expected Research Outcomes and Benefits**

This research has the potential to develop a new class of compounds that bind selectively to Cannabinoid Receptor 2 (CB2), expressed on cells of the immune system, as effective therapies in prolonging skin and organ grafts. Based on preliminary in vitro data, CB2 selective compounds appear to have the ability to decrease reactivity of leukocytes for foreign tissue. CB2 active compounds were shown by us to decrease lymphocyte proliferation in response to foreign tissue and to decrease the secretion of proteins (cytokines) that activate cells of the immune system. These results were obtained using the Mixed Lymphocyte Reaction, an assay that is generally accepted as an in vitro correlate of graft rejection. This project will further evaluate the potential of these compounds for preventing graft rejection by taking the experiments in vivo. Mice receiving grafts will be treated with the CB2 active compounds and graft survival compared with controls and with those of mice receiving standard anti-graft treatment. In

addition, combinations of reduced doses of standard therapies will be tested with addition of CB2 compounds. Combination therapies may reduce toxicity of currently available anti-rejection drugs. The new compounds may be effective by themselves, or may be useful in combination with reduced doses of currently accepted anti-rejection regimens, which may reduce the long-term toxicity of the current standard regimens.

## **Summary of Research Completed**

*Aim 1: Test the effect of cannabinoids that are selective for the cannabinoid type 2 (CB2) receptor, alone, and in combination with standard therapies for graft rejection, using the assay that is an in vitro correlate of graft rejection, the Mixed Lymphocyte Reaction (MLR)*

1.1 Testing of an additional CB2 selective agonist for immunosuppressive activity in the MLR. During this reporting period an additional CB2 selective agonist, GP 1a (Tocris, Minneapolis, MN) was tested for efficacy in the Mixed Lymphocyte Reaction as reviewer comments on the paper in revision (see below) asked for assurance that the results were not due to the activity of only two compounds. GP 1a was found to have robust activity in the assay, and gave immunosuppression equal to or greater than that observed with the previous two CB2 selective compounds tested (O-1966 and JWH-015). Results are presented in Fig. 1.

### 1.2 Determination of which leukocyte cell subsets in the MLR are producing IL-10

A major question is what is the mechanism(s) of immunosuppression induced by CB2 selective agonists in the MLR. Previously we had found that the immunosuppressive cytokine, IL-10, is markedly elevated in supernatants of MLR cultures treated with O-1966, and that the subclass of T-cells known as T-regulatory (Treg) cells, which are immunosuppressive, are increased in the cultures. Pre-treatment of the MLR cultures with a neutralizing antibody to IL-10, before the addition of O-1966, partially blocked the immunosuppression and also blocked the increase in Treg cells. We are currently extending this observation by carrying out experiments to determine which cells are making the IL-10. Flow cytometry was used to tag various leukocyte cell subsets using extracellular markers, along with intracellular staining to detect IL-10 and Foxp3 (a marker for Treg cells). MLR cultures were set up in the normal manner, and treated with O-1966, JWH-015 or GP 1a to induce immunosuppression. Control cultures were treated with vehicle. A sufficient number of wells were used to obtain approximately  $8-9 \times 10^6$  cells for staining. After three days incubation, the wells were treated with GolgiStop<sup>®</sup> (BD Biosciences), a protein transport inhibitor containing monensin, for at least 4 hours at 37° C. The cultures were then harvested, spun down and washed 2X with Staining Buffer (PBS + 1% BSA), then stained for outer membrane markers. The antibodies used are markers for T-cells (anti CD3 $\epsilon$  eFluor 450, eBioscience, San Diego, CA), macrophages, (anti-CD11b BV605, BioLegend, San Diego, CA), and B-cells (anti-CD45R APC eFluor 780, Biolegend). Cells were stained with a cocktail of all surface markers for 30 min at 4° C. Cells to be used for intracellular staining were then spun down and washed 2X, and BD Cytotfix/Cytoperm<sup>®</sup> fixation and permeabilization solution (BD Biosciences) was added for 20 min at 4° C. The fixed cells were then washed 2X in BD Perm/Wash buffer. The cells were then stained with a cocktail of anti-IL-10 APC and anti-Foxp3 PE (BD Biosciences) in Perm/Wash buffer, for 30 min at 4° C in the dark. Cells were then washed 2X in Perm/Wash buffer, resuspended in Staining Buffer at 400  $\mu$ l, and analyzed using

the LSR II instrument in the Flow Cytometry Core Facility of Temple University School of Medicine. Dr. Xiaoxuan Fan, Director of the Flow Facility, provided assistance in selection of the fluorochromes, setting of the gates, and in analysis of the data.

Results from three experiments have been inconclusive in detecting the intracellular staining of either IL-10 or Foxp3. The reasons for the low fluorescent signals for these two intracellular molecules may be due to 1) bleed-over of the reagents for the extracellular markers, which are quite robust and abundant compared to the intracellular fluorescent signals, 2) failure of the monensin to fully block the release of IL-10, or 3) non-optimal permeabilization conditions. We are currently trouble shooting this assay by reducing the number of extracellular markers in order to reduce the amount of spillover that may be obscuring the intracellular stains, and also by changing the buffers used for the IL-10 stain.

### 1.3 Investigation into another mechanism by which a CB2 agonist might inhibit T-cells.

In the MLR, T-cells are the main leukocyte subset that responds to the foreign antigens. It is well established that when the T-cell receptor (TCR) is triggered by contact with antigen, a leukocyte-specific protein tyrosine kinase (LcK) is recruited, autophosphorylated, which then induces subsequent downstream TCR signaling. It has been shown that Lck deficient mice accept allografts indefinitely. We formulated the hypothesis that CB2 agonists inhibit LcK. To test this possibility, experiments were carried out to see if treatment of mouse splenic T-cells with O-1966 inhibited LcK phosphorylation. Antibodies to pLcK were used in Western blots. CD4 T-cells were purified from mouse spleens using magnetic beads (Miltenyi). These cells were then stimulated with anti-CD3 and anti-CD28, with or without O-1966 at various concentrations similar to ones used in the MLR. Western blots were performed, and cells were also examined by confocal microscopy using an antibody to LcK to detect inhibition of pLcK resulting from treatment with the CB<sub>2</sub> compound. The results were inconclusive.

1.4 Efficacy of combinations of a CB2 selective agonist and FK506, a standard anti-rejection medication. One objective of this research is to determine the utility of a CB2 agonist used in combination with one of the standard anti-rejection medications, with the goal of reducing the dose of the standard therapy. The rationale for this objective is that FK506, one of the standard anti-rejection medications, when used chronically, leads to islet cell destruction and diabetes, neuronal dysfunction, and renal toxicity. The possibility of using FK506 with another compound that has anti-rejection properties, to reduce the FK506 dose, would be a promising strategy for managing rejection. These experiments were designed with the assistance of Ronald Tallarida, Ph.D., an expert in drug combinations. The strategy that was pursued was to combine FK506 and O-1966 at their individual ED<sub>50</sub> values. To effect this method, 5 separate experiments were carried out titrating the immunosuppressive capacity of FK506 and O-1966, each used alone, in order to determine the ED<sub>50</sub> of each compound (Fig. 2). When the two compounds were combined at a 1:1 ED<sub>50</sub>, the interaction was additive. Two other ratios of these two compounds were also tried, 2:1 and 1:2, O-1966: FK506, respectively. All three of these combinations showed significant additive interactions (Figs. 3), as the dose response curves of the combinations were shifted to the left compared to FK506 alone.

*Aim 2: To test the effect of cannabinoids that are selective for the CB2 receptor, alone or in combination, with standard therapies for graft rejection, on the course of graft rejection, using*

*mouse models of skin grafts and heart transplants.*

No further work was done on Aim 2 in the 2013-2014 year.

### Publications

Robinson RH, Meissler JJ, Fan, X, Yu, D, Adler MW, Eisenstein TK An immunosuppressive CB2-selective cannabinoid suppresses T-cell activities, and increases Tregs and IL-10. Submitted and in revision.

### Presentations

Robinson RH, Meissler JJ, Jayarajan, S, Adler MW, Eisenstein TK. CB2-selective cannabinoids as potential therapeutics to prevent graft rejection. Poster presented at Temple University School of Medicine, Translational Symposium, Sept. 2013.

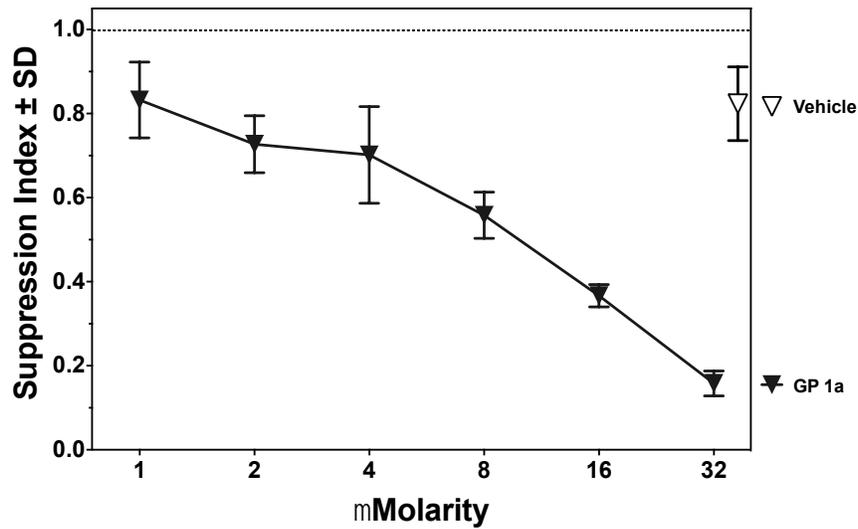
Robinson, RH. CB2-selective compounds as immunosuppressants. Mid-Atlantic Pharmacology Meeting, 2013. Philadelphia, PA Invited Trainee talk.

Eisenstein, TK: Symposium presentation on “CB2 Agonists as Immunosuppressants” in a symposium entitled, “CB2 Functions in the Brain and the Periphery” at the meeting of the College on Problems of Drug Dependence, June 2014.

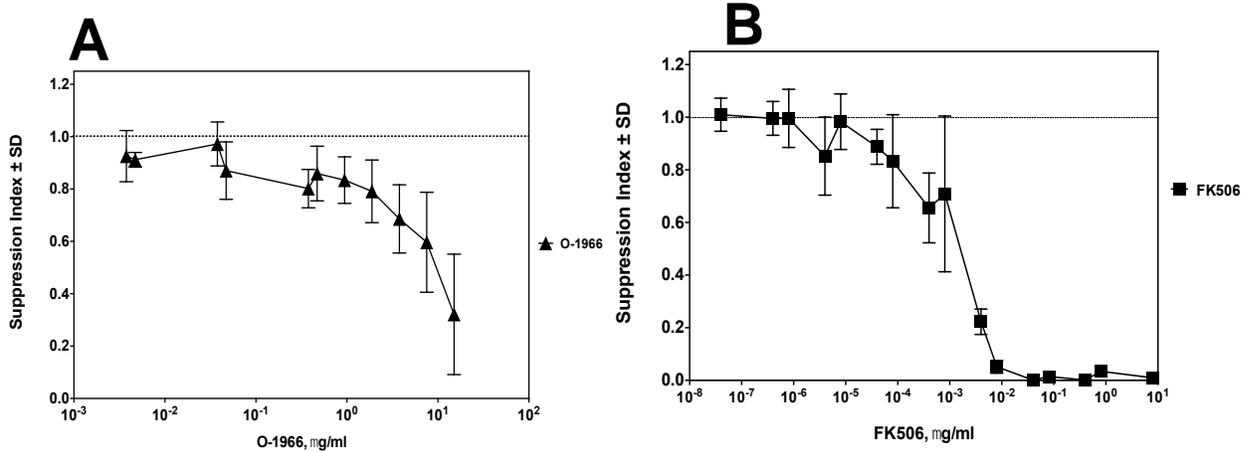
### Patent

The preliminary patent application, “Cannabinoid Receptor Treatments” emanating from this work was originally filed August 17, 2012, and was finalized on 3/6/14. The application No is PCT/US12/51330. The abstract of the patent is:

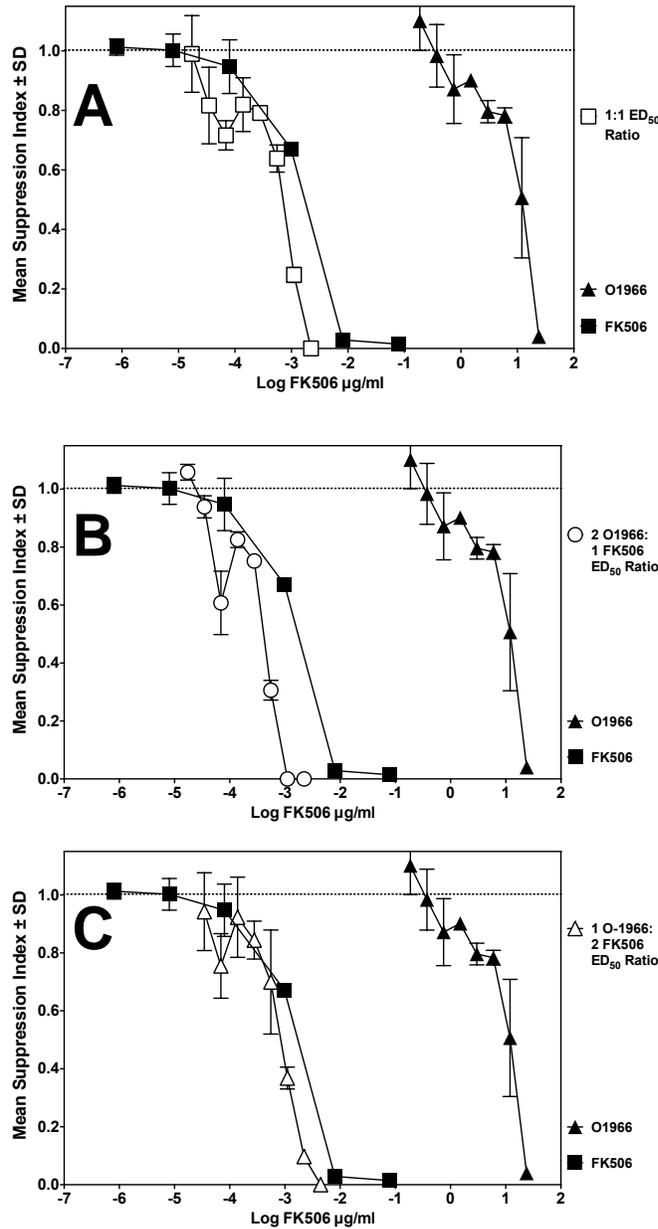
“The present invention includes methods and compositions for treating a transplant recipient by administration of a CB2 receptor agonist either alone or in combination with one or more active pharmaceutical ingredients to block the rejection of foreign tissue and prolong grafted organs, tissues and cells.”



**Figure 1.** Immunosuppressive activity of a CB2 receptor-specific agonist, GP 1a, in the Mixed Lymphocyte Reaction (MLR) assay. Data are expressed as Suppression Index  $\pm$  SD for each concentration (average of quadruplicate cultures), which is the experimental counts per minute (CPM) divided by the CPM of untreated control cultures. Vehicle for GP 1a was absolute ethanol, used at the same concentration as is present in wells receiving 32  $\mu$ M of the cannabinoid. Data are a pool of two experiments.



**Figure 2.** Titration of immunosuppressive effect of O-1966 (Panel A) and FK506 (Panel B) in the MLR assay. Data are expressed as mean Suppression Index  $\pm$  SD for each concentration. Data for both panels are pooled results of 5 experiments with each point done in quadruplicate in each experiment.



**Figure 3.** Titration of immunosuppressive effect of combinations of O-1966 and FK506 in the MLR assay. Combinations were based on ratios of the ED<sub>50</sub> of O-1966 and FK506, respectively, diluted over a range of concentrations. Combinations were ratios of O-1966:FK506. Panel A, 1:1; Panel B, 2:1; and Panel C, 1:2. Data are expressed as Suppression Index ± SD for each concentration. Data are from a single experiment, but each point represents an average of responses in quadruplicate wells.

**Research Project 7: Project Title and Purpose**

*Differentiation Therapy for Leukemia* – The purpose of the project is to evaluate the therapeutic efficacy of the peptide, angiocidin, for the treatment of leukemia. We propose to determine if angiocidin can block the engraftment of human leukemia cells in a mouse model of leukemia. We will evaluate the effect of angiocidin on the engraftment of at least three patient leukemia cells. These will be patients with acute myeloid leukemia.

## **Duration of Project**

1/1/2011 – 12/31/2012

## **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 8: Project Title and Purpose**

*Social Influences on Alcohol Consumption in Adolescent Versus Adult Mice* – The presence of peers is associated with increased risk taking during adolescence, including alcohol and drug use, but the underlying neural mechanisms for this “peer effect” are not understood. Importantly, a similar effect is not seen among adults. Previous research by one of the present investigators indicates that the effect may operate via the impact of peer presence on the adolescent brain’s reward processing system, such that the presence of peers increases the salience of other rewards and thereby biases individuals toward the potential benefits of a risky choice. This project will examine the feasibility of modeling this process in rodents, and will examine the differential impact of “peer” presence on alcohol consumption among juvenile and adult mice.

## **Anticipated Duration of Project**

7/1/2011 – 12/31/2014

## **Project Overview**

The presence of peers increases risky behavior among adolescents, but not adults. This “peer effect” is likely due to the impact of the presence of peers on reward processing and is mediated neurally by hyperactivation of brain regions known to be associated with reward sensitivity. Understanding the processes through which peers influence adolescent risk-taking has important implications for public health, since a preponderance of adolescent recklessness, including binge drinking, occurs in the presence of their friends. To date, experimental documentation of the peer effect has relied on simulated outcome variables that mimic real-world risky behavior. It is important to show that the presence of peers affects actual risky behavior, such as drinking alcohol, but legal and ethical issues preclude the conduct of experiments in which alcohol consumption is compared among teenagers when they are alone versus with their peers. However, this is a topic to which animal models can be readily applied, and lessons learned from such work can inform our understanding of human behavior and development.

All mammals experience a stage of development comparable to the biological stage in humans that is labeled adolescence. Importantly, the behavior of adolescent and adult rodents is quite different, as are their responses to alcohol. For example, social interaction among adolescent, but not adult, rats is facilitated in familiar environments by low doses of alcohol; whereas inhibition of social interaction is seen at moderate doses of alcohol in adults and only at high

doses in adolescents. Adolescent and adult rodents also differ with respect to voluntary consumption of alcohol, with adolescent rats and mice consuming more alcohol than adult rats and mice. A key unanswered question is whether there is an interaction between social setting and alcohol consumption among rodents. The aim of the proposed research is to test the hypothesis that adolescent mice will consume more alcohol when the alcohol is available in a social setting than they will consume when they are alone. Being able to evaluate social behavior and alcohol consumption simultaneously in rodents will provide valuable novel information that will aid in understanding the factors involved in alcohol consumption in adolescent humans.

### **Principal Investigator**

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### **Other Participating Researchers**

Jason Chein, PhD., Thomas J. Gould, PhD; Sheree F. Logue, PhD – employed by Temple University

### **Expected Research Outcomes and Benefits**

The chief contributors to morbidity and mortality among adolescents are self-inflicted, and one of the most common threats to adolescent health and well-being is alcohol use. Adolescents typically engage in risky behavior (including drinking) when they are with their peers. Previous experimental research has demonstrated that effect of peers on adolescent risk taking is mediated through the impact of peer presence on the brain's reward processing system, but this "peer effect" only has been demonstrated experimentally using laboratory tasks that merely simulate real-world risk taking. It is important to know whether a comparable peer effect is seen when the outcome in question is a genuine, health-compromising risk behavior, such as drinking. Legal and ethical issues, however, preclude the possibility of studying peer effects on adolescent alcohol use experimentally. We believe that the peer effect on adolescent alcohol consumption can be profitably studied using animal models, which permit a more detailed examination of the underlying neural processes. In order to fully study this phenomenon, we must first demonstrate that we can create it experimentally in a controlled laboratory context. The expected outcome of this project therefore is the demonstration of the feasibility of studying peer influences on adolescent alcohol consumption using mice. The long-term benefit of this program of research is a fuller understanding of factors that influence alcohol use during adolescence and, ultimately, more effective preventive interventions to reduce alcohol use and abuse.

## Summary of Research Completed

The scientific experiment was completed, and results reported, during a previous reporting period (July 1, 2012- June 30, 2013).

During the current reporting period, Dr. Steinberg disseminated the findings of this study at academic meetings and colloquia via two separate presentations as follow:

### Talk #1 - Abstract

Adolescence is a period of heightened engagement in risky and reckless behavior, including unprotected sex, substance use, reckless driving, and criminal activity. In this keynote, I present the results of a program of research on the underpinnings of risk-taking adolescence that is informed by recent advances in developmental neuroscience. According to the Dual Systems Model of adolescent brain development, reward-seeking and impulsivity develop along different timetables and have different neural underpinnings, and the difference in their timetables helps account for heightened risk-taking during adolescence. Consistent with predictions, in a sample of nearly 1,000 Americans between the ages of 10 and 30, we find that there is a substantial increase in reward-seeking during early adolescence, with sensitivity to rewards and preference for immediate rewards especially pronounced. In contrast, age differences in impulsivity follow a linear pattern, with impulsivity declining steadily from age 10 on. Heightened vulnerability to risk-taking in middle adolescence may be due to the combination of relatively higher inclinations to seek rewards and still maturing capacities for self-control. Preliminary findings from an ongoing replication of this study of 5,000 individuals in 10 diverse nations support the conclusions drawn from the study of American individuals. I also present findings showing that adolescents' sensitivity to rewards is heightened by the presence of peers, as well as evidence that this effect is mediated by hyper-activation of the brain's reward circuitry. I conclude by discussing the implications of this work for policy and practice.

### Talk #1 Presentations

Society for Research in Psychopathology, Oakland, September, 2013.

Sulzberger Distinguished Lecture, Center for Child and Family Policy, Duke University, September, 2013.

Boyd McCandless Lecture in Developmental Psychology, Department of Psychology, Emory University, October, 2013.

2013 Youth Services Conference, Wisconsin Department of Children and Families, Wisconsin Dells, WI, October, 2013.

University of Virginia Center to Promote Effective Youth Development, October, 2013.

Talks to Teachers, University of Pennsylvania, November, 2013.

Camden County Council on Alcoholism and Drug Abuse, Camden, December, 2013.

Birch Lecture, International Neuropsychological Society, Seattle, February, 2014.

Advocates for Children of New Jersey, East Windsor, New Jersey, March, 2014.

*Grimes Lecture, Department of Psychology, LaSalle University, March, 2014.*

Institute of Medicine, Committee on the Health Implications of Raising the Minimum Age for Purchasing Tobacco Products, April, 2014.

Conference on the Third Decade of Life, Volkswagen Foundation, Hannover, Germany, June, 2014.

Workshop on Development of Recommendations for the Assessment of Adolescents' Competence in Clinical Care. World Health Organization, Brocher Foundation, Geneva, June, 2014.

Conference on Preventing and Responding to Prescription Drug Abuse on Campus, Temple University, June, 2014.

#### Talk #2 - Abstract

In the past decade, the United States Supreme Court has issued landmark opinions in three cases that involved the criminal culpability of juveniles. In 2005, the Court abolished the juvenile death penalty. In 2010, the Court banned life without parole for juveniles convicted of crimes other than homicide. And in 2012, the Court prohibited states from mandating life without parole for any crimes committed by minors. In all three cases, the Court drew on scientific studies of the adolescent brain in concluding that adolescents, by virtue of their inherent psychological and neurobiological immaturity, are not as responsible for their behavior as adults. Drawing on findings from a 15-year program of work on adolescent decision making and risk taking, this presentation will discuss the Court's rationale in these cases and the role that scientific evidence about adolescent brain development played in its decisions. I conclude that in discussions of adolescents' treatment under criminal law, juveniles' greater amenability to rehabilitation is more important than their diminished culpability. Moreover, I argue that neuroscientific evidence should supplement, rather than supplant, findings from behavioral science.

#### Talk #2 Presentations

Models for Change National Court Leadership Symposium, Washington, October, 2013.

P. Browning Hoffman Memorial Lecture in Law and Psychiatry, University of Virginia School of Law, October, 2013.

Center for Psychology and Law, University of California, Irvine, March, 2014.

*Society for Research on Adolescence, Austin, March, 2014.*

*National Center for Mental Health and Juvenile Justice, Washington, May, 2014.*

### **Research Project 9: Project Title and Purpose**

*The Role of G Protein-Coupled Receptor Associated Sorting Protein 1 (GASP-1) in Breast Cancer Progression* – The purpose of the project will be to evaluate the prognostic significance of GASP-1 in breast cancer detection and diagnosis.

#### **Duration of Project**

1/1/2011 – 12/31/2012

#### **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**

*p38SJ, a Novel DING Protein from St. John's Wort Inhibits Tumor Cell Growth via Induction of Cell Cycle Arrest* – p38SJ is a novel DING phosphatase isolated from St. John's Wort. Our preliminary data demonstrate that p38SJ inhibits proliferation of human T98G glioblastoma cells, as increased toxicity was observed upon treatment of cells with p38SJ. Furthermore, pre-treatment of rapidly proliferating U87MG cells with p38SJ leads to cell growth delay and induces cell cycle arrest in G0/G1 phase. Our observations provide evidence that p38SJ alters signaling pathways that impact cell growth and proliferation. The purpose of this project is to demonstrate that treatment of primary glioblastoma tumors from patients with p38SJ will result in the suppression of tumor growth. Thus p38SJ can be developed and used as an anti-cancer agent.

### **Duration of Project**

1/1/2011 – 6/30/2012

### **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 11: Project Title and Purpose**

*Body Sensor Networks and Their Applications in Maternal Fetal Monitoring* – Assessment of fetal health during pregnancy constitutes a very important task of modern obstetrics. It is applied in high risk patients in the third trimester and in almost all patients during labor and delivery. Currently, the monitoring devices needed for fetal heart rate (FHR) and uterine contractions are hardwired to a large monitor (about 15 lbs), and require the patient to remain relatively immobile in order for the monitor to function optimally and continuously. Many women feel more comfortable if they are able to move during labor, and therefore feel constrained by the monitor. The project seeks to design a body sensor network (BSN), a network consisting of one or more on-body sensing units coupled with a smart local processing unit, to allow normal mobility during the monitored period. The BSN system has to be both energy-efficient and secure.

### **Anticipated Duration of Project**

7/18/2011 – 12/31/2014

### **Project Overview**

A body sensor network (BSN) consists of one or more on-body sensing units coupled with a smart local processing unit. The on-body sensors are placed on a person's body to collect physiological information, such as blood pressure, body temperature, heart rate, oxygen saturation, and so on. These data are transmitted periodically using a short-range wireless

channel, to the smart local processing unit, such as a microprocessor unit or a mobile device, like smartphones, for temporary storage and impending long range data transmission. When appropriate or necessary, the processing unit will relay the collected data to remote centralized servers. Medical professionals can use the aggregated data for remote diagnosis and treatment. The objective of this proposed project is to build a BSN platform using state-of-the-art wearable wireless technology for the purpose of remotely monitoring the health and well-being of a person in care. Our project will consist of both a working BSN prototype, as well as a comprehensive suite of communication and security protocols to ensure reliable delivery and safe transmission of the medical data according to HIPPA regulations.

The specific research aims are (1) Design a working BSN prototype with wearable on-body sensors which provide unobtrusive support for daily life based on context and the situation of a patient or an assisted elder. (2) Design algorithms to schedule the sleep/wake cycle of the sensors together with data transmission so as to reduce power consumption while being responsive to the application demands. (3) Design security framework to safeguard the data collected at various states of a BSN application cycle.

### **Principal Investigator**

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### **Other Participating Researchers**

Chiu C. Tan, PhD; Li Bai, PhD; Dimitrios S. Mastrogiannis, MD, PhD, MBA – employed by Temple University

### **Expected Research Outcomes and Benefits**

We expect to develop one or more integrated on-body sensor units that combine various sensing capabilities into a small hardware package. Custom designed software will be developed on commercial smartphones, such as the iPhone, Windows Mobile phones, or Android phones, to allow the on-body units to interface with the smartphone. Our prototype BSN system is intended to have an extensible design to accommodate future developments, such as new wireless frequencies.

We also expect to develop an algorithmic framework to better understand the tradeoffs between the responsiveness and power consumption. This framework will be used to design scheduling policies to regulate the different wireless communications and duty cycles of the on-body units and the local processing unit. A BSN simulator will be developed to test the performance of various algorithms.

A comprehensive study of the security requirements for BSNs to manage medical data in accordance with the necessary regulations will be completed in anticipation of clinical experiments on human volunteers. We also expect to adapt existing security protocols and design new ones, as needed, to satisfy any additional requirements. Using our prototype design, we expect to conduct isolated experiments designed to collect system microbenchmarks, such as power consumption and data fidelity, to improve our system design.

This project has the potential for significant benefits to the research community. We intend to make freely available the hardware schematics and software packages to help other researchers develop their own BSN prototypes. A depository database on collected trace data (energy consumption, physiological data, and so on) will also be made available to researchers. Appropriate steps on concealing any personal identifiable information (PII) will be undertaken to protect the privacy of the experimental volunteers.

### **Summary of Research Completed**

In the third year of the project, we have used the prototype built in the second year to determine the safety and accuracy requirements necessary for the hardware to be deployed in potential future field trials. Details are given below.

Once the system is deployed in the field, we expect to use commercial cellular service to transmit the collected data from the patient's home to the hospital. There are marked differences between deploying a monitoring system in the patient's home versus the hospital. In a hospital, there are trained personnel on site to interpret the data in a timely fashion, and respond to any emergency conditions, should any arise. However, in a home based monitoring system, the patient is unable to make the same kind of diagnosis. The collected data may not be read by a clinician immediately, thus making it possible that certain emergency conditions are not detected in time.

Interpreting ECG data requires substantial clinical training and experience, and it is not feasible to build a system that is able to replicate human physician interpretation. Instead, we focused on trying to determine critical states, where the home monitoring device will instruct the user to immediately visit the hospital. The computation of these critical states will need to be performed on the local monitoring hardware, since commercial cellular service can be unreliable at times. Since a local computation will only rely on the monitoring system itself, and the patient can still travel to the hospital even if the cellular network service is unavailable.

Based on our discussions with an OB/GYN, we have established a partial critical state model shown in Figure 1. The model maps out certain conditions may indicate potential danger, and that action should be taken, rather than waiting for the data to be transmitted to, and analyzed in, a hospital. This is denoted as "warning" state in Figure 1. As the figure indicates, the decisions for a warning can depend on multiple factors, one of which is time. For example, after a 10 minute monitoring window, depending on the beats per minute and weeks to delivery, we may need to have additional monitoring times before deciding whether to enter the warning state. In addition, some of the decisions to enter the warning state will require more than one sensor. For example, to detect deceleration requires input from both the heart beat monitor and the

tocodynamometer (used to measure contractions).

We have started to use unified modeling language (UML) and petri nets as a way of modeling critical states. This is the preliminary step necessary for the eventual code implementation into the prototype. UML is easy to learn and use among three teams from three disciplines: medical, science, and engineering. It will be used to describe high-level design. This approach facilitates the early discovery of design problems. Two sets of design validation will be used. The first is the completeness check. This process validates that all possible test results are represented as transition conditions and all states are reachable (i.e. live states). The second is the minimization check. This process detects, and then, removes all redundant states. This is done by finding a set of maximal compatible states (equivalent state groups) that behave consistently for all inputs. By consistent behavior, we mean that all states in the same equivalent class group will transit to the same next state group for a given input. We then use formal timed Petri Nets to capture specific diagnosis that is sensitive to timing information. A Petri net is one of the mathematical modeling languages for the description of distributed systems.

From the hardware perspective, we determined that detection of critical states is dependent on the scheduling of multiple sensors. Our prototype originally used Bluetooth Low Energy (BLE) as a starting point to allow integration of multiple sensors. BLE makes it possible to have application control over some of the parameters associated with the low energy operation of the protocol through the Host Controller Interface (HCI). With BLE, now branded as Bluetooth Smart, enabled devices require Smart ready base stations for communications. Though multiple available Bluetooth hardware supports Smart, the software is not available. For example Android currently does not support Smart even if the hardware is Smart ready. Depending on the network size and proximity of nodes, the bit error rates can significantly affect latency. The default scheduling of multiple sensors via BLE is not suitable for our purpose. This is because when a single one vital sign becomes more important, a fixed scheduling can miss a critical measurement, or diminish collected data necessary for analysis to draw important medical decisions. The HCI of BLE allows us to tune the network for maximum performance in throughput, energy, and so on by providing control to the application level. We intend to use constrained optimization techniques to tune the parameters associated with network scheduling, and access control, in order to improve functionality with respect to general body sensor networks' throughput, latency, and power performance. Doing so dynamically based on initial sensor-sensor level signal processing can improve both performance and accuracy of important fetal heart rate data. For example, in situations where levels are close to baseline, the network configures itself for maximum efficiency, or if sensors identify signs of tachycardia or bradycardia, efficiency is not a priority and throughput and latency performance is maximized.

The requirement of detecting potential dangerous patient conditions locally on the patient's device led to the exploration of potentially more powerful hardware platform than was originally designed. Our original designed used a PIC24 microcontroller with IOIO firmware. We have established design requirements for the updated interface device in order to meet the new standards of operation. The interface device must be able to coordinate sensor operations in terms of scheduling and sleep and awake cycles at the application layer. In this way, we can improve energy efficiency while maintaining a balanced energy consumption of the network. This interface device must support multiple communication protocols for interoperability, handle

synchronous and asynchronous sensing events, and support ADC and DAC capability, while providing the user with simple feedback via a push button or graphic interface.

One option for future expansion is Freescale, where the i.MX25 Applications Processor will be a good platform for next-stage prototype development. This provides a power-efficient implementation of the ARM926EJ-S core, with speeds of up to 400 MHz, support for up to 133 MHz DDR2 memory, integrated 10/100 Ethernet MAC, and two on-chip USB PHYs. Its common application is automotive, providing a user with control of the infotainment system through basic speech recognition or touch screen, smart toll and metering applications, and secure data black box applications. Given the stringent interoperability requirements of the automotive application, this application processor can function with high efficiency in the medical environment.

As of June 30, 2014, we have expanded on one of the earlier conference papers that was accepted into IEEE International Conference on E-Health Networking, Application and Services (HealthCom), into a journal submission to the journal *International Journal of E-Health and Medical Communications*. The journal version contained new material that was not found in the conference version.

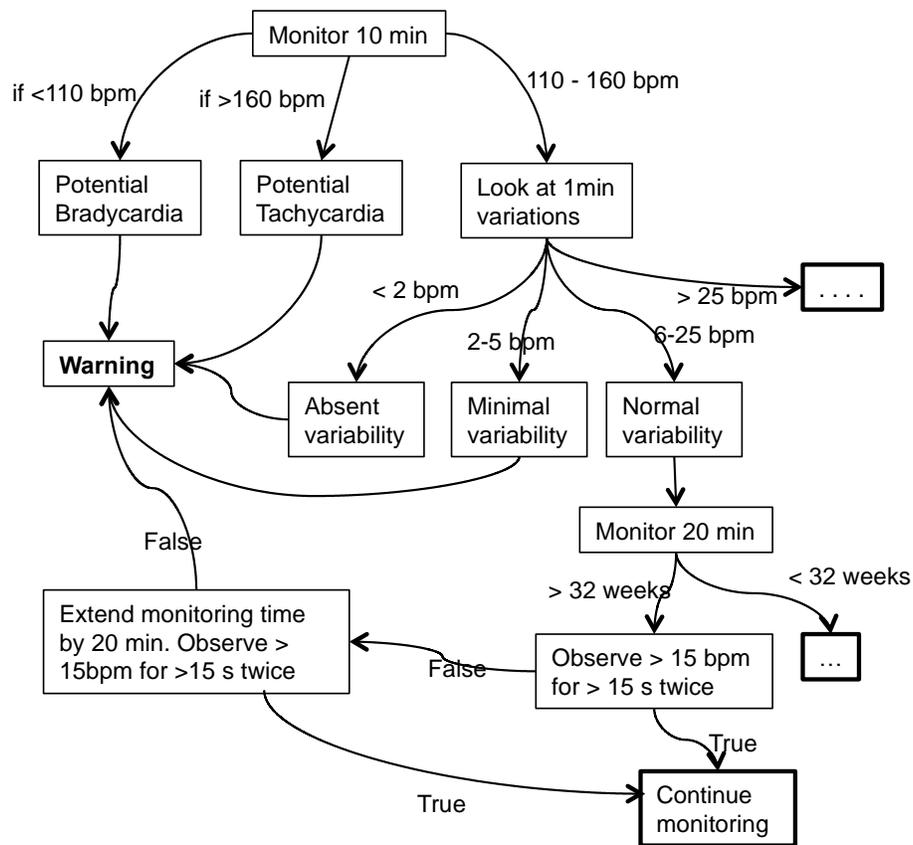


Figure 1. Partial state diagram for detecting critical state, denoted as “Warning”

## **Research Project 12: Project Title and Purpose**

*Clinical Management of Anxiety and Access to Health Care* – The purpose of this project is to develop and evaluate a computer-administered dental anxiety management program that can easily be implemented in dental and non-dental healthcare settings. Anxiety is a major reason for avoidance of dental health care, especially among low-income individuals. The proposed dental anxiety management program will have the following features: 1) A dental anxiety screening questionnaire for patients; 2) a computer-administered algorithm to classify patients according to their level of dental anxiety, 3) a menu of interventions for managing dental anxiety endorsed by each patient and delivered using a tablet PC and 4) an evaluation component (pre-post intervention) The program will also be designed so that it can be administered online and accessed from a prospective patient's home.

### **Anticipated Duration of Project**

7/1/2012 – 10/31/2014

### **Project Overview**

Dental and health anxiety are common and potentially distressing problems, for both patients and healthcare providers. Anxiety has been identified as a barrier to regular dental visits and as an important target for enhancement of oral health-related quality of life. The aims of this study are 1) to screen for dental anxiety among patients using a brief screening questionnaire; 2) to evaluate a computer algorithm to determine patients' level of dental anxiety; 3) to deliver a menu of interventions for managing dental anxiety endorsed by each patient and delivered using a tablet PC or via an online website, and 4) to evaluate the program in a small randomized controlled trial in comparison to a delayed treatment control. The proposed intervention would involve screening for dental anxiety in the reception area of the Temple University Kornberg School of Dentistry (TUKSoD) dental clinic while the patient is waiting for his/her appointment and employing a specifically adapted version of the motivational enhancement system (MES) program which will use the concepts of cognitive-behavioral therapy (CBT) to assist the participant in preparing a personal plan for managing his or her dental anxiety. The same program would be available for online access. The respondent will have a range of CBT choices presented to him/her as part of the intervention. Each option will be presented using audiovisual clips of the dental experiences that he or she may encounter. Using the graduated exposure approach typical of CBT, respondents will select the sequence of dental encounters they wish to experience when they meet the dentist. When participants visit the dentist, information on their anxiety score and graduated exposure plan will be made available to the dental provider. At subsequent dental encounters, the dental anxiety module would be reviewed with the participant. A CBT anxiety management component will also be delivered via video as part of the intervention package to supplement the graduated exposure plan. All of the intervention components will be primarily delivered via a video interface but all will be supported by personnel (dental students, residents, faculty) providing treatment to their patients. Caregivers participating in the project will be trained in cognitive-behavioral methods so that they can answer questions and model procedures when appropriate. The range of potential CBT interventions will be presented to the patient in the waiting area via a tablet PC or via the internet

if anxious patients wish to prepare for the visit at home prior to their appointment. Primary outcomes such as anxiety levels, attendance, and satisfaction will be assessed periodically during the study. Secondary outcomes such as oral health related quality of life will also be evaluated.

### **Principal Investigator**

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### **Other Participating Researchers**

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Joseph Himle, PhD – employed by the University of Michigan, Ann Arbor, MI  
Steve Ondersma, PhD – employed by Wayne State University, Detroit, MI

### **Expected Research Outcomes and Benefits**

#### Expected Outcomes:

- Patients will find the program easy to use and practical.
- Caregivers (including dental students) will be able to assist patients with the program without adding significant time to the dental visit.
- Caregivers (including dental students) and patients will experience high levels of satisfaction with the program.
- Patients exposed to the program will experience reduced anxiety levels during, and after post-intervention dental visits compared to those not receiving the intervention package.
- Patients exposed to the program will attend more dental appointments and will improve their oral health outcomes compared to those receiving the intervention package.

#### Benefits:

- The intervention package could be commercialized and patented.
- The dental anxiety management training, if successful, will become a best practice model to be replicated in other health care settings in the country.
- The information from the program will be used to predict dental attendance and referral patterns at the school's clinics, improving efficiency of clinic operations.
- The proposed study will provide an opportunity for fostering interdisciplinary research between Psychology and Dentistry and will also allow one doctoral candidate in Psychology to develop expertise in dental anxiety and dental health.
- The data generated from this pilot project, if successful, will provide the basis for the design of a proposal for a randomized controlled trial to be submitted to NIH to test the implementation of the program on a larger scale, consistent with the goals of the NIDCR Behavioral or Social Intervention Planning and Pilot Data Grant (R34) program which

supports the planning, design, documentation, and pilot data collection for investigator-initiated studies of behavioral or social interventions relevant to oral, dental or craniofacial health, and is intended to provide support for the development of a comprehensive clinical trial protocol (i.e., behavioral or social intervention study protocol) or R01 funding.

## **Summary of Research Completed**

Activities conducted during the last year of the study involved: 1-Pilot study: An open pilot trial examining the feasibility and acceptability of the intervention, as well as client satisfaction was conducted. Six patients of a community dental clinic who were identified as having high dental anxiety participated. Three of the five participants available for follow-up assessment demonstrated clinically significant reductions in their dental anxiety, with MDAS scores dropping from the high/moderate range to the low range. 2- Conduct clinical trial: 120 subjects have been enrolled and randomized to one of the 2 arms of the trial (Control N=63, Immediate Treatment N=57). Among those who have completed the baseline and follow up assessments, MDAS scores decreased substantially from baseline ( $18.7 \pm 3.7$ ) to 1 month follow up ( $14.1 \pm 5.4$ ) for the immediate treatment group, and significant differences were observed when compared to the control ( $p=0.017$ ). No significant between group differences were found for change in fear of dental procedures as assessed by the ADIS or in oral health-related quality of life; however, means were in the expected direction, with the immediate treatment group showing greater decreases in ADIS – fear and oral health-related quality of life at follow up. The Client Satisfaction Questionnaire (administered only to those in immediate treatment about their satisfaction with the computerized dental anxiety management program) was high. 3- Development web based version of intervention: The originally developed MES intervention was migrated to a web based version. The technical configuration tasks (server configuration and security setup) and production of technical documentation for reference have been developed. Programming, user-facing design and development and recording the results on the back-end and allowing for a spreadsheet export of the data have also been completed. In summary, the immediate treatment group reported significantly lower dental anxiety at 1 month follow up. Patients were highly satisfied with the computerized dental anxiety management program. Improvement in oral health related quality of life scores and reduction of fear of dental procedures seem to be higher for the test group. Further analyses are needed.

### *Pilot Study Results*

An open pilot trial examining the feasibility and acceptability of the intervention, as well as client satisfaction was conducted. Six patients of a community dental clinic (5 females,  $M_{\text{age}} = 49.50$ ,  $SD = 10.48$ ) who were identified as having high dental anxiety on the Modified Dental Anxiety Scale participated in the pilot trial. All completed the intervention during the hour before a scheduled dental appointment, and 5/6 patients completed a one-month follow-up assessment. To assess changes in dental anxiety, participants were administered the MDAS just before participating in the intervention and at one-month follow-up. Participants also completed the Client Satisfaction Questionnaire (CSQ-8) immediately upon completing the intervention. All six of the pilot participants were able to complete the intervention with minimal assistance. Three of the five participants available for follow-up assessment demonstrated clinically significant reductions in their dental anxiety, with MDAS scores dropping from the high/moderate range to the low range. These participants reported finding the intervention

“informative” and “very helpful.” Two of the pilot participants demonstrated unchanged dental anxiety at follow-up. One of these participants reported finding the intervention “very helpful” even though some aspects of the exposure videos of dental procedures were “hard to watch.” The other participant appeared annoyed/anxious at times during the intervention, but reported still finding it helpful overall. Results from the CSQ-8 suggest that all six participants were very satisfied with the intervention (average CSQ-8 item score = 3.60; possible range = 1, *quite dissatisfied*, to 4, *very satisfied*). Findings from the pilot trial support the feasibility and acceptability of this intervention.

### *Conduct Clinical Trial*

120 patients were enrolled and randomized to a Control (N=63) and Immediate Treatment (N=57) group (Consort Flow Chart). The mean age was 44 years old. The majority of the participants were black followed by whites and reported a Non-Hispanic/Latino ethnicity. No significant differences in any of the demographic factors were found at baseline between test and control groups. No significant differences were detected in various clinical characteristics at baseline between test and control groups. The mean screener and pre-treatment MDAS ranged from 18.81 to 19.97, while the pre-treatment ADIS fear ranged between 5.02 and 5.40. The mean score for the oral health impact profile ranged from 23.64 to 25.19. The same number of subjects met criteria for dental phobia on the ADIS assessment in both groups.

Differences between RCT conditions in primary and secondary outcomes at baseline and follow-up.

### *Modified Dental Anxiety Scale*

A significant reduction in the pre-treatment MDAS to follow up-MDAS was observed among those assigned to the immediate treatment group ( $p=.017$ ). However, no differences were observed when the screener MDAS was compared to the follow up MDAS ( $p=.109$ ). The trend was in the expected direction.

### *ADIS fear*

A non-significant reduction in the pre-treatment ADIS fear was observed when compared to the follow up-ADIS fear among those assigned to the immediate treatment group ( $p=.251$ ). However, means were in the expected direction.

### *ADIS avoidance*

A non-significant reduction in the pre-treatment ADIS avoidance was observed when compared to the follow up-ADIS avoidance among those assigned to the immediate treatment group ( $p=.896$ ).

### *Oral Health Impact Profile OHIP*

A non-significant reduction in baseline OHIP to follow up-OHIP was observed among those assigned to the immediate treatment group ( $p=.829$ ).

### *Met Diagnostic Criteria for Phobia*

Trend was in the expected direction. Fewer people in the immediate treatment group met diagnostic criteria for dental phobia at follow up as compared to the control group ( $p=0.96$ ). This

same trend existed at pre-treatment. 47% (9/19) of those in the immediate treatment group met criteria for dental phobia at follow-up compared to 72% (18/25) of those in the control group.

Activities completed under the grant: Publications and Conference presentations

*Journal Articles Under Review and Posters*

- Potter C, Kinner D, Tellez M, Ismail AI, Heimberg R. Panic Symptoms in Dental Phobia: Implications for Treatment. Under review. Submitted March 2014.
- Tellez M, Kinner D, Heimberg R, Lim S, Ismail AI. Prevalence and Correlates of Dental Anxiety in Patients Seeking Dental Care. Under review. Submitted March 2014.
- Potter, C. M., Jensen, D., Kinner, D. G., Tellez, M., Ismail, A. I., & Heimberg, R. G. Feasibility and acceptability of a computerized cognitive behavioral therapy intervention for dental anxiety: An open pilot trial. Poster accepted for presentation at the 48<sup>th</sup> annual Association for Behavioral and Cognitive Therapies convention. November 2014 Philadelphia, PA.
- Schulman S, Potter CM, Jensen D, Tellez M, Ismail AI, Heimberg RG. Comparison of Standard and Alternative Methods of Scoring the Modified Dental Anxiety Scale as a Screener for Dental Phobia. Poster accepted for presentation at the 48<sup>th</sup> annual Association for Behavioral and Cognitive Therapies convention. November 2014 Philadelphia, PA.
- Kinner DG, Tellez M, Heimberg RG, Ismail AI. The Moderating Role of Mindfulness on the Relationship between Dental Anxiety and Oral Health-Related Quality of Life among Adults Seeking Dental Care. Poster accepted for presentation at the 48<sup>th</sup> annual Association for Behavioral and Cognitive Therapies convention. November 2014 Philadelphia, PA.

Additional funding secured related to grant

Carrie Potter, who is a fourth year student in the Temple University doctoral program in clinical psychology and served as study coordinator for the second year of this grant, received an NIDCR funded National Research Service Award to fund her doctoral dissertation. Her dissertation study builds off of the current grant to examine the role of two potential risk factors for dental anxiety, anxiety sensitivity and pain expectancy. Drs. Marisol Tellez and Richard Heimberg are serving as the co-sponsors for her dissertation. In addition, an R34 Grant titled “Dental Anxiety Treatment among Patients in a university Clinic Setting” was submitted to NIDCR and receive an impact score of 34. This is an application for a Clinical Trial or Biomarker Clinical Evaluation Study Planning Grant. It is intended to support activities in preparation for a later Clinical Trial Application (U01). The subsequent clinical trial will evaluate the efficacy of a brief internet-based cognitive-behavioral intervention for the treatment of impairing dental anxiety among those seeking dental care at a university clinic. The planned U01 clinical trial will evaluate the efficacy of the internet-based version of this intervention assisted by clinical psychology personnel and compare it to a control condition in which the treating dentist is simply informed of the patient's score on a dental anxiety scale. More importantly, it will also evaluate the efficacy of the intervention when assisted by dental hygienists. Final funding decisions will be known early fall 2014.

## **Research Project 13: Project Title and Purpose**

*Relationship between Neuropsychological Function and Monocyte Dysregulation in HIV Infection* – The purpose of this project is to investigate the expansion of a circulating monocyte subset that may be important to development of neurocognitive impairment in HIV infection and how it relates to neurocognitive function in a small cohort of HIV infected subjects.

### **Anticipated Duration of Project**

3/20/2012 – 10/31/2014

### **Project Overview**

The studies proposed here will expand on an R01-funded longitudinal study of HIV infected subjects to include neuropsychological testing. The overall objective of this project is to test the hypothesis that improved neurocognitive function in anti-retroviral therapy (ART) treated HIV infected individuals is due, at least in part, to the ability of ART to restore monocyte/macrophage homeostasis. To test this hypothesis, 20 ART-naïve patients with detectable plasma viremia will be recruited into these studies and followed for 6 months. Whole blood will be obtained from volunteers at the time of enrollment (pre-ART) and at weeks 2 and 4 post-ART initiation and 2, 3, 4 and 6 months thereafter. For each draw, peripheral blood monocytes from HIV-1 infected donors will be assessed by flow cytometry for alterations in immune homeostasis using the following antibodies: CD14, CD16, CD163, CD11b (activation epitope), CCR5, CD115, M-CSF, IL-10 and IL-12. Patients will also be assessed for neurocognitive function prior to the start of ART and at 3 and 6 months post-ART. Participants will be assessed across multiple cognitive domains, including verbal learning and memory, motor speed and dexterity, auditory attention/working memory, speed of information of processing and executive function. In addition, several behavioral self-report measures will be included to assess psychological functioning and independence of daily living activities. Collected data will be analyzed for correlations between neurocognitive performance and restored or altered monocyte/macrophage homeostasis.

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### **Other Participating Researchers**

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## **Expected Research Outcomes and Benefits**

These studies are designed to answer key questions in the area of neurocognitive impairment in HIV infection:

1. Does ART restore monocyte/macrophage homeostasis comparable to that seen in seronegative individuals?
2. Does restored monocyte/macrophage homeostasis relate to improved neurocognitive performance in ART-treated HIV infected subjects?

We anticipate that this longitudinal study will provide information regarding the phenotype and potential function of the expanded CD163<sup>+</sup>/CD16<sup>+</sup> monocyte subset in HIV-1 infection, as well as the kinetics of suppression of this expanded monocyte subset (restored monocyte homeostasis) and how it relates to neurocognitive function in HIV infection. We further anticipate that the information obtained from these studies may reveal valuable biomarkers and/or additional insights into the pathogenesis of neurocognitive dysfunction in the setting of HIV infection.

## **Summary of Research Completed**

To date we have recruited 13 HIV infected, anti-retroviral therapy (ART)-naïve subjects into this longitudinal study. 20ml of blood was collected in K<sub>3</sub>EDTA containing Vacutainer collection tubes by a qualified phlebotomist at the time of donor enrollment (pre-ART) and at weeks 2 and 4 and months 2, 3, 4 and 6 post-ART initiation from 9 of the 13 subjects. The remaining participants have completed up to, and including 4 months post-ART initiation. An additional 20ml is collected at each study time point for CD4<sup>+</sup> and CD8<sup>+</sup> T cell counts, complete blood count (CBC) and HIV-1 viral load. At the time of each lab draw, PBMC was isolated and used for flow cytometric studies for each subject, described in a previous report. Neuropsychological testing was also performed at the time of the pre-ART draw and the 3 and 6 month post-ART draws, as described previously.

At the time of this report, 9 of the 13 recruited subjects have completed the 6-month study. Two individuals completed the pre-ART and 3 month post-ART testing before being lost to follow-up. The remaining 2 participants have only to complete their 6-month blood collection and neuropsychological evaluation, which will be done by the end of August 2014. We are no longer actively recruiting additional ART-naïve subjects, but do have a final subject recruited this month, who will be included in our final report if he is able to participate. As such, we will have 13-14 subjects at the time the study closes who have completed the study up to 3 months post-ART and 9-12 who have completed the study in its entirety (to 6 months post-ART).

We are currently in the process of analyzing our flow cytometric and neuropsychological testing analyses for the subjects who have completed the studies and/or their participation in the studies. We have found that, due to the longitudinal nature of this work, it is best to analyze the flow cytometric data findings after all collections are made from each subject to maintain consistency in gating among collections for the individual subject, as well as across all subjects. After we've made the final blood collection we will complete our flow analyses and identify potential relationships between our monocyte subsets of interest and neurocognitive function.