Pennsylvania State University

Annual Progress Report: 2010 Nonformula Grant

Reporting Period:

July 1, 2011 – June 30, 2012

Nonformula Grant Overview

The Pennsylvania State University received $2,191,427 in nonformula funds for the grant award period June 1, 2011 through May 31, 2015. Accomplishments for the reporting period are described below.

Research Project: Project Title and Purpose

A Multidisciplinary Research Paradigm for Assessing and Guiding Addiction Treatment - The overall goal of our program is to develop a preclinical basic science model to study the dysregulated state (variously called allostasis or protracted abstinence) that predicts relapse in an opiate addicted subject and to test whether this state can be reversed following treatment with depot naltrexone. Depot naltrexone is a newly formulated drug that was recently approved for the treatment of opiate addiction in humans. It is our hope that these data will identify a complex of measures that indicate susceptibility to relapse and to treatment and, thereby inform the strategy employed for the diagnosis and treatment of addiction in humans.

Anticipated Duration of Project


Project Overview

There are two broad research objectives: 1) To establish a rodent model of multisystem dysregulation that persists following opiate withdrawal and is believed to contribute to risk of relapse. While neuroscientists have described abnormalities in hypothalamic pituitary adrenocortical (HPA) axis function, stress response, response to natural rewards and drug-related cues, as well as epigenetic changes associated with this state, they have not been studied in concert, and there are insufficient data on the duration or reversibility of these abnormalities over time or with medication. 2) To provide meaningful research experiences to minority undergraduate students through our summer research internship program, as well as the mentorship needed to help prepare these students for graduate training in biomedical research and/or medical school.

In the animal model studies, the specific aims are: 1) To track heroin-induced dysregulation of behavioral, physiological, neural, and genetic measures; 2) To determine whether and when depot naltrexone will reverse specific elements of systemic dysregulation; 3) To test whether
depot naltrexone-associated normalization of these parameters will persist following a return to a drug-free state (i.e., following discontinuation of depot naltrexone treatment); and 4) To determine whether normalization of these parameters will shift the balance from drug-related to alternative natural rewards.

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Expected Outcomes and Benefits

This project has a number of expected outcomes. (1) The proposed studies will allow for the development of a sophisticated animal model that will use multiple measures to establish a profile of vulnerability vs. resilience in the face of addiction and, ultimately, in response to treatment. Evidence suggests that these indices translate nicely to the human condition and, as such, these preclinical data will serve to inform the diagnosis and treatment of addiction in the human population. (2) From a practical standpoint, depot naltrexone has a great deal of potential, but it also is very expensive (about $1,100/monthly injection). The present set of studies will be the first to examine the consequences of discontinued treatment. What happens when one comes off of the drug after a series of monthly treatments? (3) While depot naltrexone can be a very effective treatment, the literature suggests that about half of the subjects drop out of the treatment program. Might it be possible to increase compliance with the addition of alternative rewards? The present study will be the first to examine, using the animal model, whether the availability of an alternative reward will serve to increase the effectiveness of the drug. (4) While there is a great deal of overlap between the addictive state in humans and animals, some assessments simply cannot be made in the human population. The proposed studies in rodents, then, will enable us to explore variables (e.g., protein and gene expression in brain regions) that cannot be studied in patients and which may suggest new directions for medications development. (5) Our educational partnership with Lincoln University is designed to provide hands-on research experience and ongoing mentorship to young people to help to prepare them for graduate school or medical school. (6) Finally, our external advisory committee of distinguished basic and clinical scientists and Pennsylvania-based health policy makers will help to ensure the relevance of the research to the citizens of our state and proximal area.
Summary of Research Completed

During this reporting year we have completed the following:

**Order and set up equipment.** Our first requirement was to order and build 12 chambers to measure saccharin intake and heroin self-administration and 2 additional chambers for the measurement of aversive taste reactivity behavior (i.e., gapes). Although we were slowed a bit by a time-consuming vendor error, we were up and running by February, 2012.

**Experiment 1.** Twenty-two male Sprague-Dawley rats were anesthetized and implanted with an intrajugular catheter and intraoral cannulae. Once recovered, they were all given 5 min access to saccharin and then 3 hours to intravenously (iv) self-administer either saline (n=4) or 0.06 mg/kg heroin (n=18). There was one such pairing a day, 5 days per week for 20 trials. Twenty-four hours later, all rats were given one 10 min taste reactivity test where the saccharin cue was infused into the oral cavity once/min and orofacial responses were videotaped. The animals were then anesthetized and implanted with a methadone-filled mini-pump to allow for 5 days of detoxification. After 14 additional days of abstinence (see diagram below), heroin experienced rats were implanted with either a vehicle pellet or an extended release naloxone pellet. The pellet remained in place for 60 days, with probe tests occurring at 30 day intervals. Thereafter, the pellet was removed and a final probe test was conducted following 30 additional days abstinence.

**Acquisition:**
- 5 min saccharin – 3 h saline self-administration
- 5 min saccharin – 3 h heroin self-administration

**Probe Test:**
- Day 1: 10 min Taste reactivity test, blood extraction for corticosterone, 5 min Open Field test
- Day 2: Extinction/drug-induced reinstatement test
**Results.** In accordance with expectations, rats consumed less of the otherwise palatable saccharin cue when paired with the opportunity to self-administer heroin relative to the saccharin-saline paired controls (see Figure 1, panel A). The rats with a history of heroin self-administration also exhibited a shorter latency to take heroin (Figure 1, panel B) and, greater avoidance of the taste cue predicted a shorter latency to take drug (see Figure 1, panel C). Finally, we have begun to examine the taste reactivity behavior and have determined that rats exhibit frank aversive orofacial responses (i.e., gapes) following the intraoral infusion of the heroin paired saccharin cue (see Figure 1, panel D). Additional testing will be conducted to determine whether treatment with depot naltrexone will serve to reverse this aversive taste reactivity behavior and the seeking of heroin. This work is ongoing and will require a replication.

**Experiment 2.** Experiment 2 was conducted somewhat differently, but provided a great deal of insight into underlying neural mechanisms. In this study, rats were not given a taste cue. They were, instead, given the opportunity to self-administer heroin (0.06 mg/kg) over three 40 min access periods, separated by two 15 min periods of signaled non-availability. There were 5 such sessions a week for about 5 weeks. In an effort to assess “addiction-like” behavior, progressive ratio trials were conducted once/week to assess the willingness to work for drug. Persistent responding was indexed by the number of infusion attempts emitted during periods of signaled non-availability. Finally, evidence for disinhibition was determined by measuring the number of responses emitted during mandatory time out periods that followed each infusion of drug. Rats were then ranked on each of these measures and given a score of 1 for each measure on which they fell in the top third. Each subject’s addiction score, then, could range from 0 to 3, depending upon the number of criteria on which the subject was positive (i.e., ranked in the top third). As shown in Figure 2, rats scoring positive on all three measures, exhibited the greatest amount of responding during signaled non-availability (2A), time out (2B), and progressive ratio (2C). At 25 days of exposure, the composite ‘Addiction Score’ was found to increase systematically as a function of the number of positive criterion (2D). Finally, while all rats tended to take about the same amount of heroin during daily fixed ratio testing (2F), rats exhibited different degrees of ‘addiction-like’ behavior when challenged (2E). Fourteen percent of these out bred male Sprague-Dawley rats exhibited high ‘addiction-like’ behavior for heroin. This is in keeping with that found for cocaine in rats and man.

At the end of experimental testing, the rats were sacrificed and a number of brain regions were extracted to assess proteins related to the function of the mu and the dopamine receptor (e.g., spinophilin, wntless, VAPA, and D2 receptor). Importantly, the results showed that the expression of such proteins relates not to the amount of drug taken, per se, but to the drive with which the subject seeks drug. For example, Figure 3 below shows that low D2 receptor expression in the prefrontal cortex is associated with a high Addiction Score and high break point responding, while low D2 receptor expression in the hippocampus and the nucleus accumbens is associated with high drug-seeking. These are fascinating new data that serve to inform subsequent work.

**Experiment 3.** In a final study conducted like that described in Experiment 1, we sacrificed the rats immediately following acquisition and the first probe trial. Tissue samples were taken from the ventral tegmental area, nucleus accumbens, striatum, hippocampus, amygdala, and prefrontal cortex. This analysis will allow for an assessment of protein and gene expression as a function of
individual differences in responding to drug and drug-related cues prior to detoxification and prior to treatment with depot naltrexone. As such, these will serve as important baseline data.

**Minority Student Training.** Thus far, we have trained 4 students from Lincoln University in the laboratory, 2 in the summer of 2011 and 2 in the summer of 2012. As proposed, along with their research experience, these students also have attended neuroscience seminars and weekly seminars in the Summer Undergraduate Research Internship Program (SURIP). Finally, they prepared a poster, which they presented as first author at the end of the summer.

**Publications/Presentations Associated with Grant**

Harrell, S.A., Alexander, D., Nyland, J., Grigson, P.S. Bilateral lesions of the thalamic orosensory area (TOAx) disrupt cocaine-induced intake suppression when using short, but not long, CS access periods in rats. Summer Undergraduate Research Symposium, August, 2011


de Winton Cummings, P., Imperio, C.G., & Grigson, P.S. Rats emit aversive orofacial responses to a heroin-paired taste cue and these learned responses dissipate with abstinence in rats. Summer Undergraduate Research Symposium, August, 2012.


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**Figure 1. Experiment 1 Results**
Figure 2. Experiment 2 Results

Figure 3.