Pennsylvania State University

Annual Progress Report: 2010 Nonformula Grant

Reporting Period:

July 1, 2013 – June 30, 2014

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

6/1/2011 – 5/31/2015

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.

Principal Investigator

Patricia Sue Grigson, PhD
Professor
The Pennsylvania State University
500 University Drive
Hershey, PA 17033-0850

Other Participating Researchers

Robert Bonneau, PhD, Loren Evey, PhD, Jidong Fang, PhD, Willard Freeman, PhD, Robert Levenson, PhD, and Kent Vrana, PhD - all employed at Pennsylvania State University

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

Specific Aim 1. Devaluation, Craving, Withdrawal, and Reinstatement. To use short access (SA, 3h) and long access (LA, 8h) to heroin, test whether devaluation of natural rewards and measures of increased craving predict risk for reinstatement and whether depot naltrexone in male and female rats can 'normalize' these neurobehavioral indices of risk.

(1) Model development: Aversive orofacial taste reactivity following intraoral delivery of a heroin-paired saccharin cue (Caesar Imperio and Daniel Appeah). Over the last three years we have demonstrated that rats avoid intake of an otherwise palatable saccharin cue when paired with the opportunity to self-administer heroin and greater avoidance is associated with greater drug seeking, greater drug-taking (escalated intake), a greater willingness to work for drug, and greater drug-induced reinstatement following a period of extended abstinence. What we have been looking for, however, is evidence of dysregulation of affect. We have measured affect in cocaine self-administering rats in two ways: (1) by examining the orofacial responses emitted following intraoral infusion of the drug-paired saccharin cue and (2) by measuring ultrasonic vocalizations (USVs). Here, we sought to determine whether, as occurred with the cocaine-paired cue, rats also would emit aversive taste reactivity behavior (i.e., gapes) following the intraoral delivery of a gustatory cue (saccharin) that has been paired with heroin self-administration. The results showed that, although heroin self-administration was robust, and even escalated over trials, these rats failed to emit sustained aversive taste reactivity following intraoral delivery of the heroin-paired saccharin cue (see Figure 1). There remain at least two key procedural differences between the heroin study and the cocaine study, where gapes to the intraoral delivery of the saccharin cue are robust and sustained. Another experiment is planned to address these procedural differences.

(2) Predicting vulnerability to addiction-like behavior for heroin (Chris Jenney). Last year, a graduate student, Mr. Chris Jenney, and an undergraduate student, Mr. Christian Njatcha, tested whether greater avoidance of a heroin-paired saccharin cue very early in training (i.e., following just three saccharin-heroin pairings) would predict later addiction-like behavior for heroin in the "Piazza" paradigm. This year, Mr. Jenney replicated this study. The final results demonstrate that large suppressors, i.e., those rats that most greatly avoided intake of the saccharin cue when paired with the opportunity to self-administer heroin (see Figure 2), exhibited the greatest 'addiction-like' behavior for heroin (see Figure 3). Addiction-like behavior was determined by the willingness to work for drug when tested on the progressive ratio schedule of reinforcement, persistence during periods of signaled non-availability of drug, and drug-induced reinstatement of heroin-seeking behavior following a period of extinction. These experimental subjects were then sacrificed and their brains dissected. Tissue has gone to the Levenson lab for protein analysis. Tissue from the locus coeruleus in the first replication also was sent to the van Bocksteale laboratory at Jefferson resulting in a joint publication (Jaremko et al., 2014).

(3) Aversive ultrasonic vocalizations following exposure to a drug-paired taste cue when using a 30 minute access period to the taste cue (Danielle Alexander and Raheem McLeod-Dunniehigh). Wheeler et al. (2011) demonstrated that rats emit greater aversive taste reactivity when having to wait 30 min, rather than 0 min for access to cocaine. Here, Raheem gave rats 30 min access to 0.15% saccharin and 5 min later injected the rats with saline, morphine, cocaine, or LiCl. There

was a total of 6 such pairings. The results showed that rats in the morphine, cocaine, and LiCl conditions reduced intake of the saccharin cue, but avoidance was not accompanied by an increase in aversive calls (data not shown). Longer free access to the sweet (i.e., 30 minutes to lick), then, appears to attenuate the aversive state induced by waiting for drug. An increase in aversive calls is evident when only 5 min access to the saccharin cue predicts experimenter delivered drug (manuscript in preparation).

Specific Aim 2. Sleep and the HPA axis. To use either short (3h) or long (8h) access to heroin (determined by the outcome of Aim 1), test whether dysregulation in sleep and/or the HPA axis (i.e., corticosterone) predict risk for reinstatement and whether each can be 'normalized' following treatment with depot naltrexone in rats.

(1) Heroin self-administration and sleep architecture (Alissa Coffey, Jidong Fang, Colin Barnstable, and Sue Grigson. Drs. Matt Puhl, Jidong Fang and I published a manuscript showing that chronic sleep deprivation (about a 25% reduction) greatly augmented cocaine self-administration (Puhl et al., 2013). The effect of chronic sleep deprivation on heroin self-administration has not yet been evaluated in full. That said, Alissa Coffey did examine the effect of heroin self-administration, abstinence, and reinstatement on sleep architecture. The results of this first replication showed an initial increase in REM sleep, a decrease in Awake time, and an increase in NREM sleep during acquisition, particularly in high drug-takers. Abstinence was associated with fewer, longer episodes of both Awake and NREM sleep, again, particularly in high drug-takers (data not shown).

Specific Aim 3. Alternative natural rewards. To test whether the effectiveness of depot naltrexone can be augmented by home cage availability of an alternative natural reward.

(1) Preventing the seeking and taking of heroin with an alternative natural reward (i.e., environmental enrichment). As shown previously (2012-2013 progress report), exposure to environmental enrichment in adulthood greatly reduced working for heroin, seeking during extinction testing, and drug-induced reinstatement. The genetic data gleaned from these subjects is now being analyzed.

Specific Aim 4. Analysis of Protein and gene Expression in Heroin Self-Administering Rats. To evaluate the impact of heroin-dependence, withdrawal, and depot naltrexone treatment on candidate brain proteins and genes.

(1) Model development: Individual differences in heroin-induced avoidance of a drug-paired saccharin cue are associated with individual differences in gene expression (Caesar Imperio, Ashley McFalls, Bill Freeman, Kent Vrana, and Sue Grigson). As discussed above, we previously found that rats avoid intake of an otherwise palatable saccharin solution when it predicts the opportunity to self-administer cocaine or heroin and greater avoidance of the saccharin cue is associated with greater seeking and taking of drug. Here, we used RNA-seq to measure gene expression. Figure 4 is a Venn diagram showing the relationship between the pairwise comparisons of the statistically significant changes in gene expression in the three groups: large suppressors, small suppressors, and saline in the hippocampus and the medial prefrontal cortex (mPFC). Results show that some genes are commonly expressed between these

groups, but many are not. We are now looking at the genes that may underlie these large individual differences in drug-taking behavior. For example, as shown in Figure 5, large suppressors (i.e., high escalators), exhibit increased expression of corticotrophin releasing hormone (CRH) in the hippocampus relative to the small suppressors.

Minority Student Training. This year, we trained 2 additional students from Lincoln University. As proposed, along with their research experience, these students also had the opportunity to attend neuroscience seminars and twice weekly scientific and professional development seminars in the Summer Undergraduate Research Internship Program (SURIP). Each student prepared a poster and presented as first author at the end of the summer at the Summer Undergraduate Research Symposium. Exit interviews and follow up evaluations were conducted at the end of the summer.

Presentations: Research findings were presented at a summer undergraduate symposium and a national conference.

Publications:

Ebersole B, Petko J, Levenson R. Bioorthogonal click chemistry to assay mu-opioid receptor palmitoylation using 15-hexadecynoic acid and immunoprecipitation. Anal Biochem 2014; 451:25-27.

Jaremko KM, Thompson Jr. NL, Reyes BAS, Jin J, Ebersole B, Jenney CB, Grigson PS, Levenson R, Berrettini WH, Van Bockstaele EJ. Morphine-induced trafficking of a mu-opioid receptor interacting protein in rat locus coeruleus neurons. Prog Neuropsychopharmacol Biol Psychiatry 2014; 50:53-65.

Manuscripts: Two manuscripts were submitted and one manuscript is being prepared.

Grants Submitted:

Sponsor: National Institutes of Health R01 DA024271
Title: The Effects of Chronic Sleep Deprivation on Addiction and Relapse
Period: 08/01/14 -08/31/2019
Role: Grigson (PI); Fang (Co-I); Barnstable (Co-I)

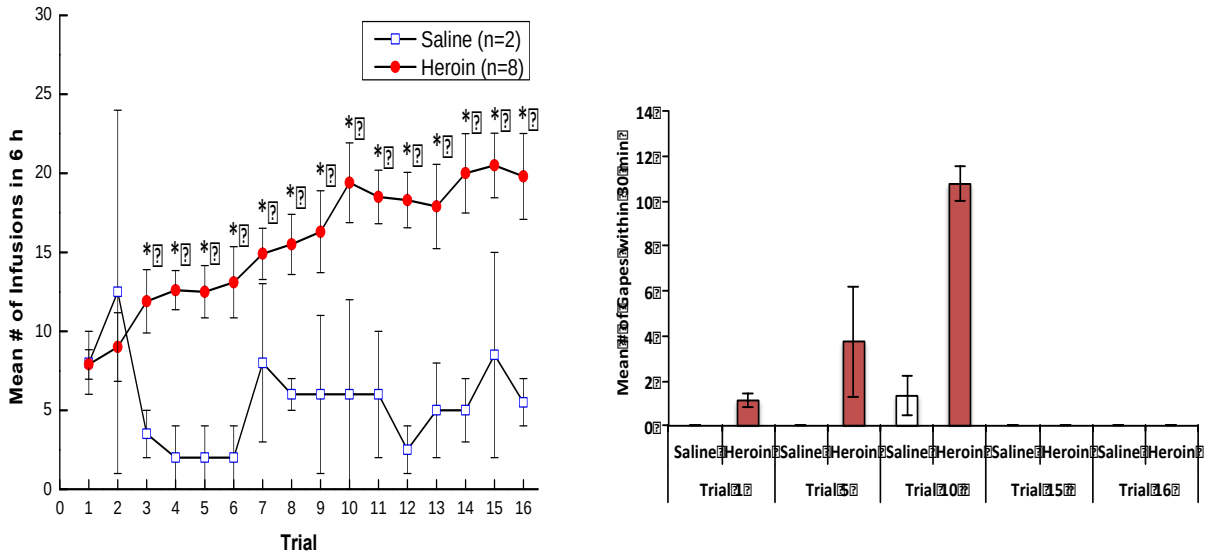

Figure 1: Left panel. Heroin Infusions/6h. Rats in the saccharin-heroin condition self-administered increasing doses of heroin per daily 6 h session (i.e., exhibited escalation), ps < .05. Self-administration of saline, on the other hand, was not surprisingly flat, ps > .05. **Right panel. Taste Reactivity Behavior.** Unlike previous studies using cocaine, it appears that aversive taste reactivity behavior following delivery of the heroin-paired saccharin cue is not sustained across trials. Thus, gaping behavior to the saccharin cue in anticipation of heroin self-administration reached its peak at trial 10, but was abolished by trials 15 and 16.

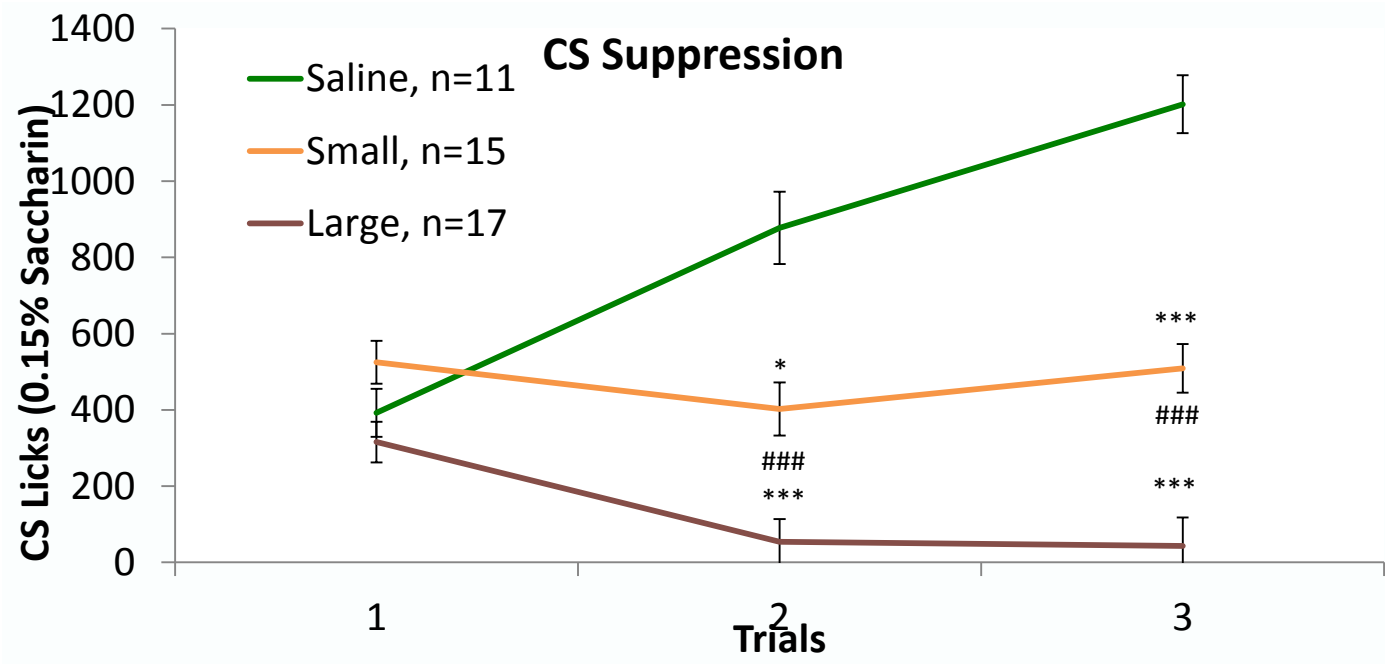

Figure 2. Phase I. Saccharin-heroin pairings. Average number of licks of 0.15% saccharin/5 min for rats across three saccharin-saline or saccharin-6 h heroin pairings. Rats in the saccharin-heroin condition rapidly split into two groups, small suppressors (Small) or large suppressors (Large). Large suppressors exhibited significantly greater avoidance of the saccharin-paired cue following a single saccharin-heroin pairing.

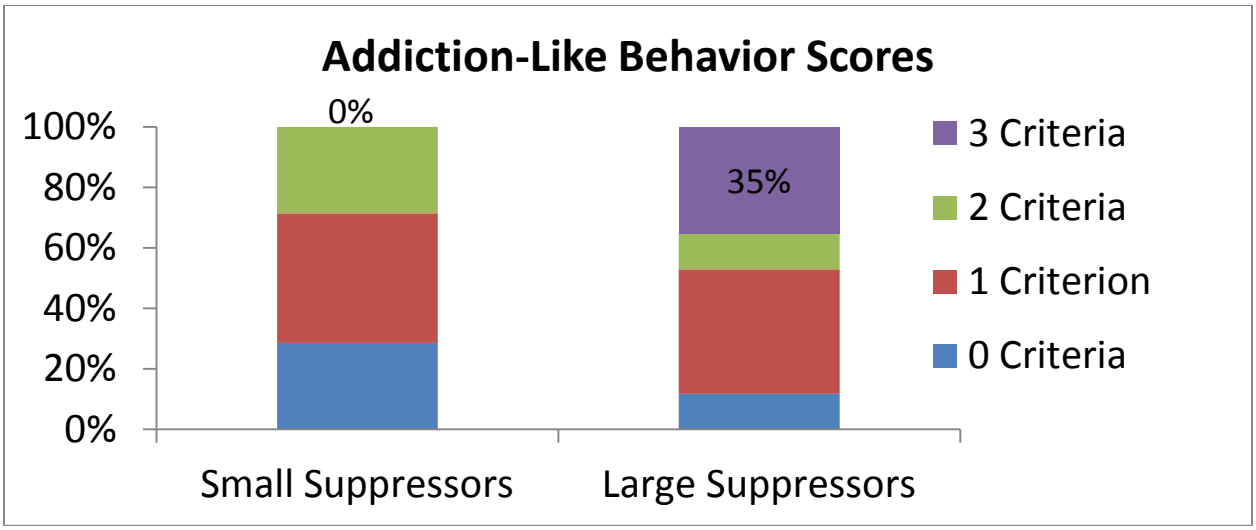

Figure 3. Phase II. 'Addiction-Like' Behavior score. 35% of the Large Suppressors exhibited a high 'addiction-like' behavior score of 3 compared to 0% of the subjects identified as Small Suppressors in Phase I testing.

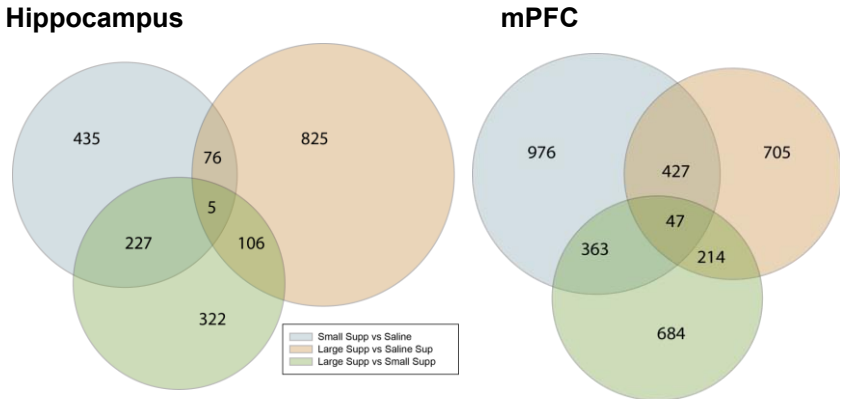

Fig. 4 RNA-seq assessment of the Hippocampus and mPFC in extended access heroin animals. Each Venn diagram shows the relationship between the pairwise comparisons of the statistically significant changes in gene expression of the three extended access groups: large suppressors, small suppressors, and saline. *Left panel* shows the analysis for the hippocampus and the *right panel* shows the relationship for the medial prefrontal cortex (mPFC). RNA-seq analysis demonstrated a number of different mRNA changes across the three groups, with slight overlap between. Closer inspection revealed regional differences in the number of changes in gene expression between the hippocampus and mPFC (n=3/group).

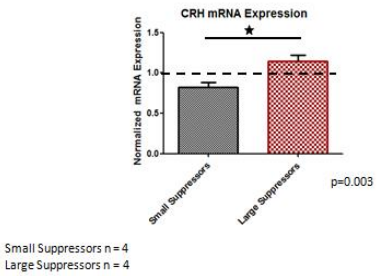

Figure 5**. Corticotrophin Releasing Hormon (CRH) mRNA Expression.** The expression of CRH mRNA in the Hippocampus was significantly greater in large suppressors (i.e., rats that most greatly avoided intake of the saccharin cue and escalated heroin self-administration).