Pennsylvania State University

Annual Progress Report: 2010 Nonformula Grant

Reporting Period:

July 1, 2012 – June 30, 2013

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.

Principal Investigator

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Other Participating Researchers

Robert Bonneau, PhD, Loren Evey, PhD, Jidong Fang, PhD, Willard Freeman, PhD, Robert
Levenson, 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: Significant avoidance of the saccharin cue when paired with 6 h access to heroin self-administration. We and the reviewers were concerned with our basic behavioral paradigm. Specifically, rats were not exhibiting strong avoidance of the heroin-paired saccharin cue when using a 3 h access period to heroin, further, aversive taste reactivity behavior, the primary measure of dysregulation (an indicator of allostasis/addiction), also was weak and not sustained across repeated tests. In an effort to improve the model, Mr. Imperio switched to the extended access model, where access to the saccharin cue was followed by 6 h, rather than only 3 h, of heroin self-administration. The results, shown in Figure 1, revealed that, as is typical, some of the out bred male Sprague-Dawley rats greatly avoided intake of the heroin-paired saccharin cue (referred to as the high suppressors), while others (referred to as the low suppressors), did not (see Figure 1, panel A). Those rats that most greatly avoided the saccharin cue also took the most infusions of heroin per 6 h session (see Figure 1, panel B). Importantly, when looking at drug intake only across the 1st h, the high suppressors, but not the low suppressors, escalated their intake of drug across trials (see Figure 1, panel C). Finally, the high suppressors, but not the low suppressors, exhibited a strong, and significant, willingness to work for heroin when tested on a progressive ratio (PR) schedule where more work (i.e., more licks on the empty spout operant) was required for each successive infusion (Figure 1, panel D). This model, then, is expected to support greater aversive taste reactivity (i.e., more gapes) following intraoral infusion of the drug-paired cue and this aversive taste reactivity behavior is expected to be sustained across repeated tests. The brains of the present subjects are being examined in the Freeman lab to link these differences in behavior to gene expression and epigenetic differences. The epigenetic studies will take advantage of a new next generation sequencing technique developed in the Freeman lab – Bisulphite Amplicon Sequencing (BSAS) (Masser et al., Submitted) to examine cytosine methylation (5-mC) in promoter regions of genes with differential gene expression. A follow up study will be conducted to test whether this new model will support the aversive taste reactivity as predicted. Once this phase of development is complete, depot naltrexone can be tested.

(2) Model development: Predicting vulnerability to addiction-like behavior for heroin. The data from our laboratory and others now show that about 15% of the out bred male Sprague-Dawley population will exhibit high addiction-like behavior for not only cocaine (Deroche-Gamonet et al., 2004; Puhl et al., 2011), but also for heroin (Tacelosky et al. in preparation). Clearly it would be highly advantageous to predict who will be whom (i.e., who is drug-prone and who is resilient) prior to drug exposure, or at the very least, early in training. We have found that greater avoidance of a cocaine-paired saccharin cue is associated with greater cocaine seeking and taking behavior (Grigson & Twining, 2002). Here 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. The results,
shown in Figure 2, suggest that large suppressors, those rats that most greatly avoided intake of the saccharin cue when paired early on with the opportunity to self-administer heroin, exhibited the greatest willingness to work for drug when tested on the progressive ratio schedule of reinforcement and the greatest drug-induced reinstatement of heroin-seeking behavior following a period of extinction. These experimental subjects were sacrificed and their brains dissected. The left hemisphere was sent to our colleagues at the University of Pennsylvania to measure synaptopodin, as an index of dendritic spine density. Nuclei from the right hemisphere have gone to the Levenson lab for protein analysis. This behavioral study will be replicated, proteins measured, and then a manuscript prepared for publication.

(3) Evidence that the opiate-paired taste cue elicits not only avoidance, but withdrawal as well. A reviewer of this project’s early progress stated: “The investigators are encouraged to be more hypothesis-driven in their model development work. If the hypothesis is that the saccharin aversion is a reflection of the opponent-processing conditioning, it would be useful to have ancillary measures that reflect some of the contributing variables, particularly withdrawal severity.” To this end, we recently conducted a study with Ms. Danielle Alexander, a senior research technician, and Mr. Raheem Mcleod-Dunniehigh, an undergraduate student from Lincoln University, to test whether ingestion of a morphine-paired saccharin cue would lead to a reduction in body weight when measured 2 h later. Body weight loss is a very reliable indicator of withdrawal and, in fact, we know that rats do lose weight at 2 h when intake of the morphine- or cocaine-paired saccharin cue is followed by an injection of a low dose of naloxone (Nyland & Grigson, 2013). In Raheem’s study, however, some small and large suppressors were injected with saline, rather than naloxone. The results showed that not only the naloxone-challenged rats, but also the saline controls, reduced body weight 2 h following ingestion of the morphine-paired taste cue (Figure 3). The taste cue alone, then, supports the onset of conditioned withdrawal in anticipation of drug availability. This study will be replicated and a manuscript prepared for publication.

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.

Dr. 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). Our undergraduate student from Lincoln University, Ms. Carolina Biran, conducted an experiment to test whether chronic sleep deprivation also would facilitate heroin self-administration behavior (see Biran et al., below). This study involved a very small sample size and requires replication. Studies of HPA axis function and assessment of sleep patterns await full model development.

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.

Preventing the seeking and taking of heroin with an alternative natural reward (i.e., environmental enrichment). A goal of our original Specific Aim 3 is to test whether the availability of an alternative natural reward would increase the effectiveness of depot naltrexone
in the treatment of addiction. Of course this requires not only that we can test depot naltrexone (a part of the model still in development), but also that natural rewards are, themselves, effective in reducing responding for an opiate. We have shown that exposure to an enriched environment will greatly reduce responding for cocaine. The present experiment tested whether, likewise, exposure to an enriched environment also would be effective in reducing responding for an opiate – heroin. The results showed that rats housed in enriched (i.e., housed 2-3 subjects/enlarged cage, novel objects presented daily, and continuous access to a 12” exercise wheel) rather than standard (singly caged) conditions throughout all of training and testing took a similar number of infusions/3h of 0.06 mg/inf heroin across the 12 day acquisition phase of the study (data not shown). When challenged, however, rats housed in the enriched environment were less willing to work for heroin on the PR schedule of reinforcement than were standard housed rats (see Figure 4, panel A); they exhibited less seeking during the first h of the 6 h reinstatement test (Figure 4, panel B); and they exhibited less heroin-induced reinstatement (Figure 4, panel C). Enrichment, then, greatly reduces responding not only for cocaine, but also for heroin. Follow up studies on brain in the Freeman lab showed that enrichment tended to reduce expression of EGR2, fos, and Arc (a cytoskeletal associated protein) in the PFC, particularly compared to the standard housed high drug-takers (Figure 5). This portion of the study is awaiting replication. As is, these results demonstrate that enriched housing conditions can change both behavior and brain, even when experienced in adulthood. These data will be presented by Mr. Imperio at this year’s meeting of the Society for Neuroscience and will be prepared for publication soon thereafter.

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

Protein expression in heroin self-administering rats. Rats were given the opportunity to self-administer heroin (0.06 mg/inf 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 measure 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 timeout 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 ranked in the top third. The expression of a number of brain proteins was then assessed in the hippocampus, nucleus accumbens (NAc), and prefrontal cortex (PFC). Here we found that D2 receptor expression in the PFC, NAc, and hippocampus is not related to the amount of drug taken, per se (all rats took about the same amount of heroin), but to the strength of the addiction-like behavior exhibited for the drug (see Table 1). Thus, lower expression of D2 receptors in the hippocampus, NAc and PFC was significantly correlated with a greater “addiction-like” behavior score. Expression of the D2 receptor in the hippocampus was correlated with all three components: signaled non-availability, progressive ratio, and timeout period responses, while the expression of this receptor in the NAc and PFC was correlated with only one component, signaled non-availability responding and progressive ratio responding,
respectively. Interestingly, high addiction-like behavior also was correlated with low WLS expression in the PFC and low WLS was correlated with low expression of the D2 receptor. These data provide a novel link between D2 receptor function and that of this mu opioid receptor interacting protein, WLS (Tacelosky et al. In preparation).

**Minority Student Training.** This year, we trained 3 additional students, two from Lincoln University and a third, Mr. Christian Njatcha, from the Harrisburg University of Science and Technology. Mr. Njatcha has accepted a graduate position at the University of Minnesota for the coming fall. 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). Each student prepared a poster and presented as first author at the end of the summer at the Summer Undergraduate Research Symposium. Given her strong interest in medicine, arrangements have been made for Ms. Biran to shadow a number of physicians at the Penn State College of Medicine in Hershey. Exit interviews and follow up evaluations were conducted at the end of the summer.

**Presentations Associated with the Grant**


**Publications Associated with the Grant**


Grants Active and Submitted

1. **Active.** Mr. Caesar Imperio, 4th year graduate student, was awarded a predoctoral NRSA from NIDA (F31 DA036322) entitled “Substance Abuse: Individual differences in behavior and epigenetics”. Drs. Grigson and Freeman are co-Sponsors allowing for combined behavioral and genetic analysis of the protective effects of environmental enrichment. This grant will support Mr. Imperio from August 1, 2013 – July 31, 2016.

2. **Submitted.** DA R01 DA036591-01 entitled “Wntless in Opioid Addiction” was submitted with Dr. Wade Barrettini at the University of Pennsylvania as the PI on the project. Drs. Levenson and Grigson as co-Investigators. This grant was unscored. A new grant will be submitted by this team in October.

3. **Submitted.** R21 DA 035608 entitled “Heroin Addiction: Prediction Vulnerability and Identification of a Novel Treatment”, is a collaborative project between Drs. Levenson and Grigson (Dr. Levenson is the PI). It is a CEBRA (Cutting Edge Basic Research Award) grant that is designed for high risk/high gain projects. This collaborative project also has been shaped by this CURE project as it seeks to identify a novel peptide that will act to prevent the mu/WLS interaction and, thereby, the development of opiate addiction. The grant received a score of 43 and will be resubmitted August, 2013.
Figure 1. Drug-induced suppression of saccharin intake following pairings with 6 h access to heroin. (A) Separation of Intake of the saccharin cue between Heroin High & Low Suppressors. (B) Heroin High Suppressors infused more drug compared to Low Suppressors. (C) Heroin High Suppressors infused more drug within the first hour compared to Heroin Low Suppressors. (D) High Suppressors displayed higher motivation to work for drug.

Figure 2. Average number of heroin infusions during progressive ratio (PR) and drug-induced reinstatement testing for rats in the saccharin-saline condition (Saline Controls), and Small and Large Suppressors in the saccharin-heroin condition. Large suppressors were more willing to work for drug on the PR schedule and they exhibited greater reinstatement of heroin-seeking.
Figure 3. Average Weight Change 2 hrs after Naloxone Challenge. Rats in both the Morphine High and the LiCl groups significantly lost body weight at 2 h compared to rats in the Saline and Morphine Low condition. Interestingly, body weight loss occurred at the 2 h time point in these two treatment groups, whether injected with saline or naloxone. Thus, exposure to the drug-paired taste cue, alone, can elicit evidence of withdrawal (i.e., body weight loss).

Figure 4 (A) Enriched animals showed a decrease in motivation to work for drug on the progressive ratio schedule. (B) Enriched animals displayed a significant decrease in extinction behaviors versus Standard animals during the first h of extinction testing (Singly Housed-no form of enrichment). (C) After a drug prime, enriched animals showed a significant reduction in attempted infusions (i.e., seeking) compared to standard animals. *p<0.05.
Figure 5. The effect of enrichment on brain. Rats housed in the enriched environment exhibited a decrease in EGR2, Arc, and an increase in BDNF in the medial prefrontal cortex (mPFC) compared to standard housed rats. Other brain regions also showed interesting differences in protein expression include the hippocampus, the VTA and the nucleus accumbens. This study is currently being replicated.

Table 1. Reflects significant (+) correlations between expression of the D2 receptor, spinophysin, and Wntless (WLS/GPR177) and the number of “Addiction-like” criteria met, signaled non-availability responses, progressive ratio breakpoint infusions, and timeout period responses in the hippocampus, nucleus accumbens, and prefrontal cortex of rats with a history of heroin self-administration in the “Piazza” paradigm.