AWARD NUMBER: W81XWH-17-1-0004

TITLE: Sex differences in the ability to predict and treat opiate abuse

PRINCIPAL INVESTIGATOR: Elena Chartoff

CONTRACTING ORGANIZATION: The McLean Hospital Corporation
Belmont, MA 02478

REPORT DATE: November 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
November 2018	Annual	1 Nov 2017 - 31 Oct 2018

4. TITLE AND SUBTITLE

Sex differences in the ability to predict and treat opiate abuse

5a. CONTRACT NUMBER

5b. GRANT NUMBER

W81XWH-17-1-0004

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Elena Chartoff

E-Mail: echartoff@mclean.harvard.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

The McLean Hospital
Corporation
115 Mill Street
Belmont, MA 02478-1041

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Due to improved battlefield medicine, the majority of Soldiers who are wounded survive and treated with prescription opioid painkillers. This award has two goals: to test a novel means (Distress Intolerance) of predicting risk for prescription opioid abuse, and to test a pharmacological compound (CRF receptor antagonist) for prevention of prescription opioid abuse and addiction. We use male and female rats that were exposed to an experimenter-administered regimen of escalating-dose morphine (or saline) injections. This design allows us to be sensitive to sex differences, which exist in human drug abusers, and it allows us to determine if a model of physician-prescribed opioids alters vulnerability to subsequent abuse. In this second year of funding we have completed the first goal and have begun testing the changes to the self-administration paradigm we obtained IACUC and ACURO approval for. Specifically, for Aim 2, we are now going to test the effects of the CRF receptor antatonist antalarmin on escalation of oxycodone intake in a long-access self-administration paradigm. To date, we found that certain measures of Distress Intolerance (e.g., warm water tail flick latency and acoustic startle response) in drug-naïve rats were significantly correlated with the amount of oxycodone rats self-administered during the acquisition phase. After completion of Aim 1, we found that prior morphine experience in males, but not females, increased oxycodone self-administration. In conclusion, our findings suggest 1) Distress Intolerance measures can be taken before deployment and used to help decide whether a Soldier would be at risk for developing an opioid use disorder, and 2) prior exposure to, and withdrawal from, a regimen of opioids (administered in a manner akin to a prescription in people) increases vulnerability of males, but not females, to increased addiction-like behavior.

15. SUBJECT TERMS

Distress Intolerance (DI); Self-administration (SA); Tail Flick assay (TF); Acoustic startle (AS)

16. SECURITY CLASSIFICATION OF:	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
			USAMRMC

a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	14	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Table of Contents

Page

1. Introduction…….4

2. Keywords…….4

3. Accomplishments……..……...4

4. Impact……...……11

5. Changes/Problems...….……12

6. Products…….…….…..…….13

7. Participants & Other Collaborating Organizations……13

8. Special Reporting Requirements……14

9. Appendices……NA

1. **INTRODUCTION:** As a result of improved battlefield medicine, approximately 50% of Soldiers wounded in action are returned to duty. Many are prescribed opioid painkillers as part of their recovery. Unfortunately, increasing numbers of Soldiers misuse prescription opioids and ultimately become addicted. Considering that women represent an increasing percentage of active duty military personnel, it is imperative that efforts to address the burgeoning prescription painkiller abuse problem account for sex differences in addictive behavior and treatment response. Substance abuse is associated with heightened levels of Distress Intolerance—defined as the perceived inability to tolerate negative physical (e.g. pain) and emotional states. This raises the possibility that baseline levels of distress intolerance can be used to *predict* the likelihood of prescription opioid abuse. Chronic opioid administration leads to dependence, characterized by a withdrawal syndrome comprising intense, short-lived physical symptoms and protracted stress-like psychological symptoms. Indeed, many key signs of drug withdrawal are mediated by the stress hormone corticotropin releasing factor (CRF). This raises the possibility that CRF blockers can be used to *prevent* drug withdrawal-induced negative affective states and the likelihood of abuse. Interestingly, females are more sensitive to CRF and are more likely than males to use opioids to cope with negative emotional states. The proposed studies use a well-established rodent model of opioid dependence and withdrawal in male and female rats to determine 1) whether Distress Intolerance can be used as a predictor of opioid withdrawal-induced negative affective states and likelihood to engage in oxycodone self-administration and 2) whether the CRF receptor type 1 (CRFR1) antagonist antalarmin administered during opioid withdrawal blocks withdrawal-induced negative affective states and likelihood to self-administer oxycodone. We hypothesize that Distress Intolerance will positively correlate with withdrawal-induced anxiety and measures of addictive-like behavior, and antalarmin will attenuate these measures, more strongly in female compared to male rats.

2. **KEYWORDS:** Distress Intolerance, Oxycodone, Morphine, Substance Use Disorder, Addiction, Anxiety, Sex Differences, Intravenous Self-Administration (IVSA), Acoustic Startle, Pain

3. **ACCOMPLISHMENTS:**

- **What were the major goals of the project?**

  Objective 1. The proposed studies will determine if baseline Distress Intolerance (DI) can be used as a predictor of morphine withdrawal-induced negative affective states and likelihood to engage in oxycodone intravenous self-administration (IVSA). These studies will be done in male and female rats in parallel to determine if biological sex impacts the predictive ability of DI.

  Objective 2. The proposed studies will determine if the CRF receptor antagonist antalarmin administered during morphine withdrawal blocks withdrawal-induced negative affective states and likelihood to engage in oxycodone IVSA. These studies will be done in male and female rats in parallel to determine if biological sex impacts the ability of CRF antagonism to block abuse-related behaviors.

- **What was accomplished under these goals?**

  1. *Major Activities* in Year 2 of this grant focused on completing Objective 1, modifying the experimental design for Objective 2 based on our findings from Objective 1, and initiating Objective 2 studies. For Objective 1, the goal was to determine if baseline distress intolerance (DI) can be used as a predictor of morphine withdrawal-induced negative affective states and likelihood to engage in oxycodone intravenous self-administration (IVSA). The experimental design for these studies is depicted below in **Figure 1**. We made several changes to the experimental design for Objective 2 (approved by ACURO), and these are reflected in **Figure 2**.

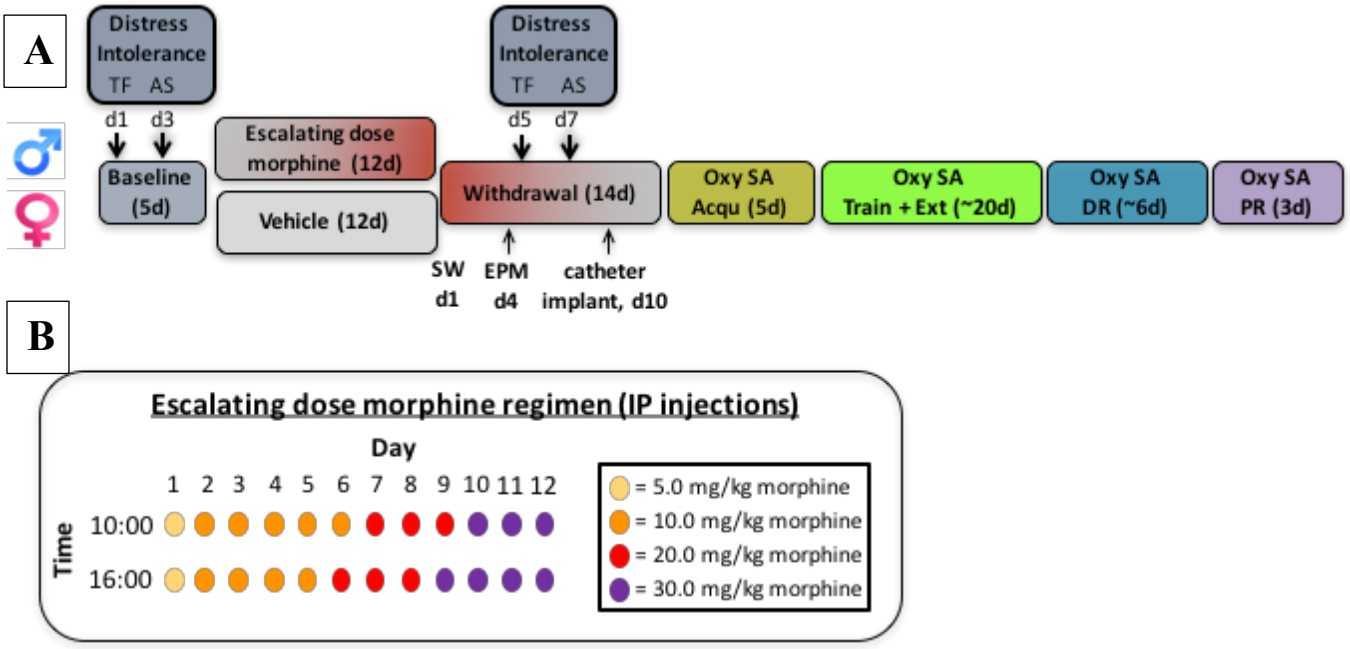

Figure 1. Experimental schematic for Objective 1. Male and female rats were housed separately from each other and 3/cage. (**A**) During the baseline Distress Intolerance (DI) measures, the warm water Tail Flick (TF) assay and the acoustic startle (AS) assay were done on each rat. This was followed by 12 days of saline or escalating dose morphine injections (**B**). During the withdrawal period (**A**), Somatic Withdrawal (SW) and Elevated Plus Maze (EPM) behaviors were measured and DI measures (TF and AS) were taken again. On day 10 of withdrawal, all rats were implanted with chronic indwelling jugular vein catheters and on day 14 of withdrawal, oxycodone IVSA (Oxy SA) was initiated. The first 5 days were used to assess acquisition (Acqu) of Oxy SA. The following ~20 days were used as a training period in which all rats were stabilized on the IVSA behavior for approximately 10 days. This was followed by 4 days in which lever pressing was extinguished by replacing Oxy with saline and then 6 days in which saline or Oxy was given on alternating days. The purpose of this extinction and alternating drug regimen was to ensure that the rats would press more for Oxy than they would for saline. During the following 6 days, a dose response (DR) curve was generated by presenting rats with a range of 6 Oxy doses (0.0 – 0.30 mg/kg) in random order (1 per day). Finally, rats were tested on a Progressive Ratio (PR) schedule of reinforcement, in which each infusion of Oxy required successively more presses on the active lever. Each rat was tested on the PR schedule two times, separated by one day off.

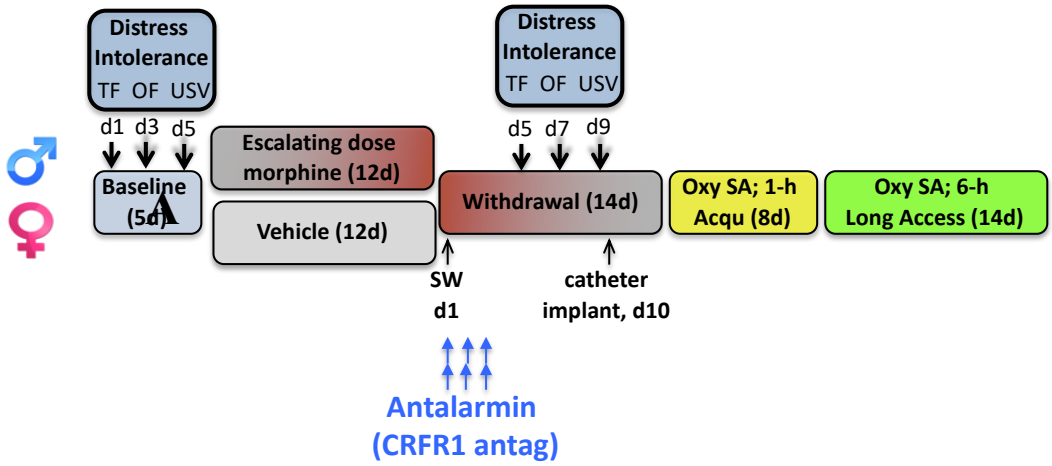

Figure 2. Experimental schematic for Objective 2. We made the following changes to the experimental design shown in Figure 1: Acoustic Startle (AS) and Elevated Plus Maze (EPM) behaviors were eliminated because they were not modified by morphine withdrawal as expected. AS and EPM were replaced by the Open Field (OF) test and Ultrasonic Vocalization (USV) measurements, which have been shown to reflect negative affective states including anxiety and dysphoria. The oxycodone self-administration (SA) paradigm was changed such that the number of days of 1-h, FR1 training was reduced, and the dose response (DR) and progressive ratio (PR) components were eliminated and replaced by 14-days of Long-Access (LgA, 6-h/d) SA. These changes to SA were made because the design in Objective 1 was very lengthy and led to loss of a number of subjects due to catheter failure over time. Furthermore, the LgA paradigm is considered a highly translatable model of the transition from drug abuse to addiction, as many animals will begin to escalate their intake during LgA periods, a phenomenon seen regularly with people.

Objective 1

Somatic withdrawal and body weight during escalating dose morphine: To make sure rats were responding to the morphine and becoming morphine-dependent, we recorded body weights throughout the 12-day escalating morphine injection regimen (**A**) and recorded and scored somatic withdrawal (SW) behaviors (**B**) 16 hr after the last morphine injection (**Figure 3**). Morphine-treated male, but not female, rats failed to gain weight normally (**A**). As expected, day 1 of morphine withdrawal was associated with increased somatic withdrawal signs (**B**), which is indicative of morphine dependence. These data support the premise that the rats are responding to morphine and are morphine-dependent.

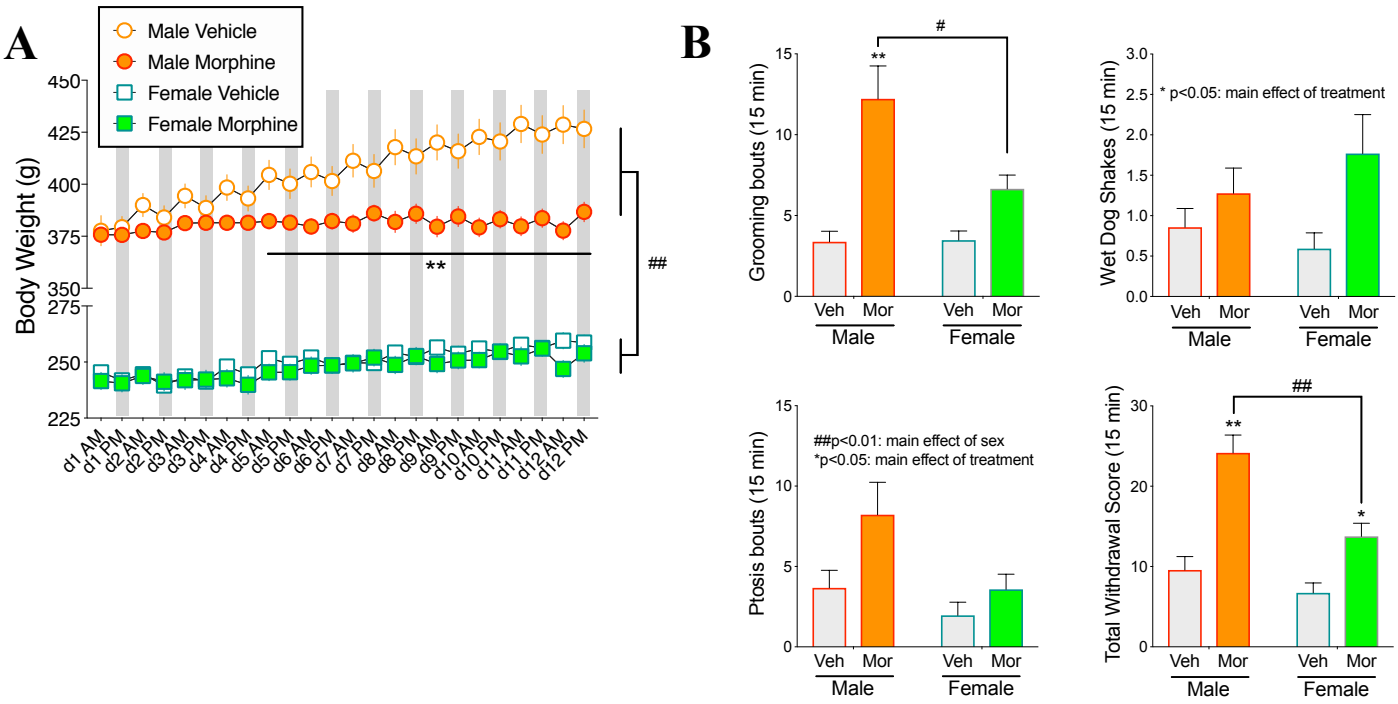

Figure 3. **Effects of morphine and acute withdrawal on body weight and somatic withdrawal signs**. Body weights throughout the 12-day morphine regimen are plotted in (**A**). Vehicle-treated male rats continue to gain weight throughout, whereas morphine-treated males did not gain weight. Neither treatment group of females showed any change in body weight over the 12-d regimen. On day 1 of morphine withdrawal (i.e., 16 h after the last morphine or vehicle injection), rats were videotaped for 15 min and somatic withdrawal behaviors were scored (**B**). Both males and females showed increased somatic withdrawal behaviors, with males expressing higher levels of Grooming, Ptosis, and the Total Withdrawal Score (weighted average of the 3 specific behaviors Grooming, Wet Dog Shakes, and Ptosis. N=24 rats/group.

Warm Water Tail Flick (TF) Assay: The TF assay is a measure of the spinally mediated nociceptive response in which an animal (rat) flicks its tail away from a heat source. This reflexive behavior is non-invasive and can be repeatedly measured. Opioids and other analgesics increase tail flick withdrawal latency, making this a commonly used and reliable assay for testing the potency of compounds with putative analgesic efficacy. Here, we used the TF as a proxy for measures of Discomfort Intolerance in humans, one domain of DI that measures pain sensitivity. Briefly, rats' tails are submerged 2-3cm in a 52˚C water bath and the latency (sec) to flick the tail out of the water is measured. A cutoff time of 15 sec is used to prevent tissue damage. We find that on morphine withdrawal day 5, morphine-treated males, but not morphine-treated females, show a decrease in the latency to withdraw their tails from the warm water bath (**Figure 4**). This is indicative of withdrawal-induced hyperalgesia, a common finding in opioid-withdrawn people. Our results suggest this effect is sex-dependent. There is no effect of vehicle treatment over time on TF latency in either males or females.

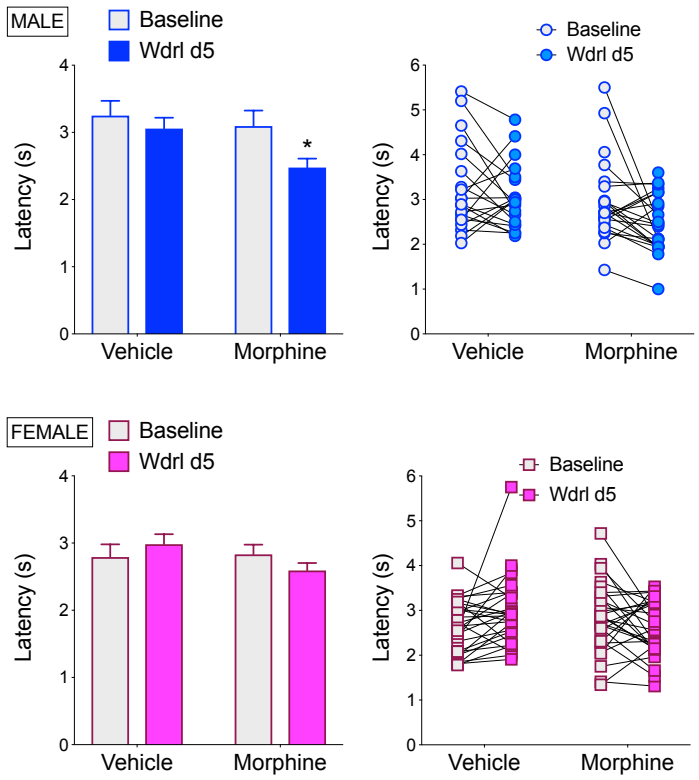

Figure 4. Prior to the start of morphine (or saline Vehicle) treatment ("baseline"), and after 5 days of withdrawal from chronic, escalating dose morphine (or vehicle) ("Wdrl d5"), rats were tested in the Tail Flick assay. In the left panels, averaged data are plotted. In the right panels, data are shown for individual rats. In morphine-, but not vehicle-treated, males, there is a significant reduction in TF latency on wdrl d5 (blue, upper panels). There is no change in TF latency in females under any condition (pink, lower panels). N=24/group. *p<0.05.

Acoustic Startle (AS): The acoustic startle (AS) response is a simple reflex observed in many animal species including rodents and humans. In humans, it is thought that increased baseline startle sensitivity is present in people with high negative affect, which can be associated with anxiety, depression, and PTSD. In the laboratory, acoustic startle is typically elicited by short white noise bursts. We find that there is no effect of chronic morphine on startle amplitude compared to vehicle-treated rats on withdrawal day 7 (**Figure 5**). This is not consistent with our hypothesis that morphine withdrawal would be associated with an increase in startle reactivity, which is indicative of withdrawal-induced anxiety, a negative affective state that may be linked to increased prescription opioid abuse. However, individual acoustic startle reactivity (either before or after morphine) can still be used in correlational analyses to determine if startle can predict vulnerability to oxycodone abuse.

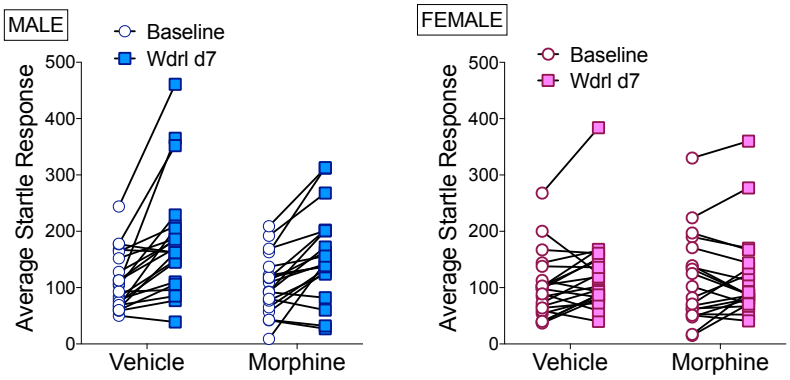

Figure 5. Prior to the start of morphine (or saline Vehicle) treatment ("Baseline"), and after 7 days of withdrawal from chronic, escalating dose morphine (or vehicle) ("Wdrl d7"), rats were tested in the AS assay in which rats were exposed to 3 decibels of noise bursts presented in random order in sound attenuated chambers. Data are startle amplitudes collapsed across the 3 decibels and are shown for individual rats. In males, the increase in AS amplitude on Wdrl d7 is due to the increase in weight observed over time in males, but not females. Morphine withdrawal has no effect on AS responses in either males or females.

Elevated Plus Maze (EPM): The EPM is a well-validated animal model of anxiety-like behavior in which rats are placed in the center of an elevated maze containing two closed and two open arms and videotaped from above for 5 min. Previous work has shown that manipulations that decrease anxiety (e.g., clinically effective anxiolytics) increase the number of open arm entries and the amount of time rats spend in the open arms, whereas anxiogenic treatments decrease these measures. We hypothesized that rats undergoing morphine

withdrawal would spend less time in the open arms compared to control rats, which would be consistent with withdrawal-induced anxiety. Paradoxically, we found that both male and female morphine-withdrawn rats tended to spend more time in the Open Arms compared to vehicle control rats on morphine withdrawal day 4 (data not show). As such, we decided to stop testing rats in the EPM for Objective 2.

Oxycodone intravenous self-administration (IVSA): After 14 days of abstinence from the escalating chronic morphine regimen, IVSA was initiated. Rats were trained to respond under a fixed ratio (FR) 1 schedule in which each press of an active lever produced an infusion of oxycodone (0.06 mg/kg, i.v.). Sessions began with extension of two levers (active and inactive) and illumination of a house light to signal availability of drug. Each drug infusion was signaled by offset of the house light and onset of cue lights over the active lever during the infusion and a subsequent 6-s timeout, during which responses are recorded but have no programmed consequence. Following the timeout, the cue lights were extinguished and the house light was illuminated to signal availability of the next unit dose. We hypothesized that rats in morphine withdrawal would acquire IVSA behavior faster and take more oxycodone infusions across a range of drug doses.

After completion of Objective 1, we found that morphine withdrawn male, but not female, rats self-administered more oxycodone than vehicle-treated male rats (First panels of Figure **6A, B**). This finding suggests that prior opioid exposure and withdrawal makes males more vulnerable to taking oxycodone, which could have important, sex-depedent implications for pain treatment. After the initial 5 days of acquisition, rats continued to train on oxycodone IVSA, but in 2-h/d sessions. In both males and females (Middle panels, Figure 6A, B), oxycodone intake gradually increaded over the 10 days of training. There were no sex or morphine-exposure differences noted. Prior to conducting the dose response experiment (Third panel, **Figure 6A, B**), rats went through an extinction phase followed by alternating days of saline or oxycodone (not shown). For the dose response experiment, rats were exposed to one of 6 oxycodone doses presented in random order each day (one dose per day). Interestingly, morphine-exposed male and female rats showed a decrease in the amount of

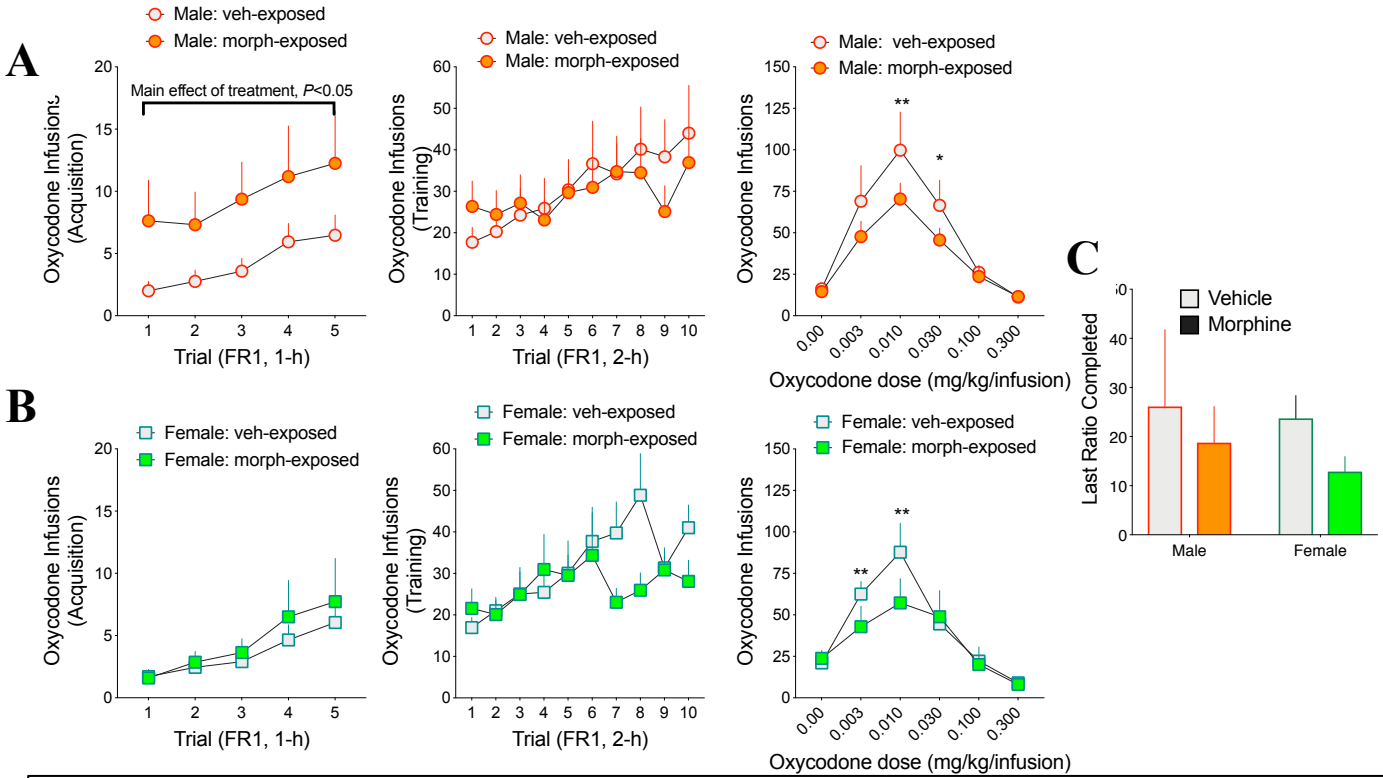

Figure 6. After 14 days of morphine withdrawal, rats self-administered oxycodone on a Fixed Ratio 1 (FR1) schedule of reinforcement. Row (**A**) refers to males and row (**B**) refers to females. Left panels are oxycodone infusions during the 5 days of FR1 1-h/d acquisition; Middle panels are oxycodone infusions during the 10 days of FR2 2-h/d training; Right panels are dose response data. Morphine-exposed males, but not females, self-administered more oxycodone during acquisition (Left panels). In contrast, morphine-exposed males and females self-administered less oxycodone during the dose response experiment and the progressive ratio experiment (**C**). N=22-24 rats/group, except for progressive ratio, which had 5-6 rats/group.

oxycodone self-administered at the doses that elicited peak responding (0.003, 0.01, and 0.03 mg/kg/inf) (Third panel, **Figure 6A, B**). This type of finding suggests that – after substantial oxycodone exposure – morphine-exposed male and female rats had decreased vulnerability to the reinforcing effects of oxycodone. This was substantiated by the progressive ration experiment (**Figure 6C**), in which both morphine-exposed male and female rats showed a nonsignificant trend for a decreased motivation to work for oxycodone, as the effort required for each successive drug infusion increases. Although at first these data appear contrary to our hypothesis and in contrast to the finding observed in **Figure 6A** (males), it is likely that the lengthy exposure to oxycodone necessary to get to the point of testing dose response and progressive ratio behavior interacted with the prior morphine exposure to increase tolerance or produce some other neurobiological change that rendered the animals less sensitive to opioid reinforcement. Although more studies are clearly required to understand this, it may be that carefully monitored prescription opioid administration (by a doctor) can decrease an individual's sensitivity to the reinforcing effects of the drug, thus reducing the likelihood of pursuing abuse.

Using Distress Intolerance to predict vulnerability to prescription opioid abuse: Using regression analyses of Z-score transformed data from baseline TF latencies and AS amplitudes (DI measures) and amount of oxycodone infused during either acquisition or 2-h training components to determine if DI measures could significantly predict drug-taking behavior, we found that both DI measures significantly correlated with oxycodone self-administration measures in a sex-dependent manner (**Figure 7A**). These findings support our Objective #1 and suggest that, in males, baseline TF latencies can predict which rats will self-administer the most oxycodone during the acquisition phase **(Figure 7A**), whereas in females, baseline AS amplitudes can predict which rats will self-administer the most oxycodone throughout the 10 days of 2-h/d training (**Figure 7B**) females, baseline DI measures can be used to predict whether an animal is more or less vulnerable to the abuse-

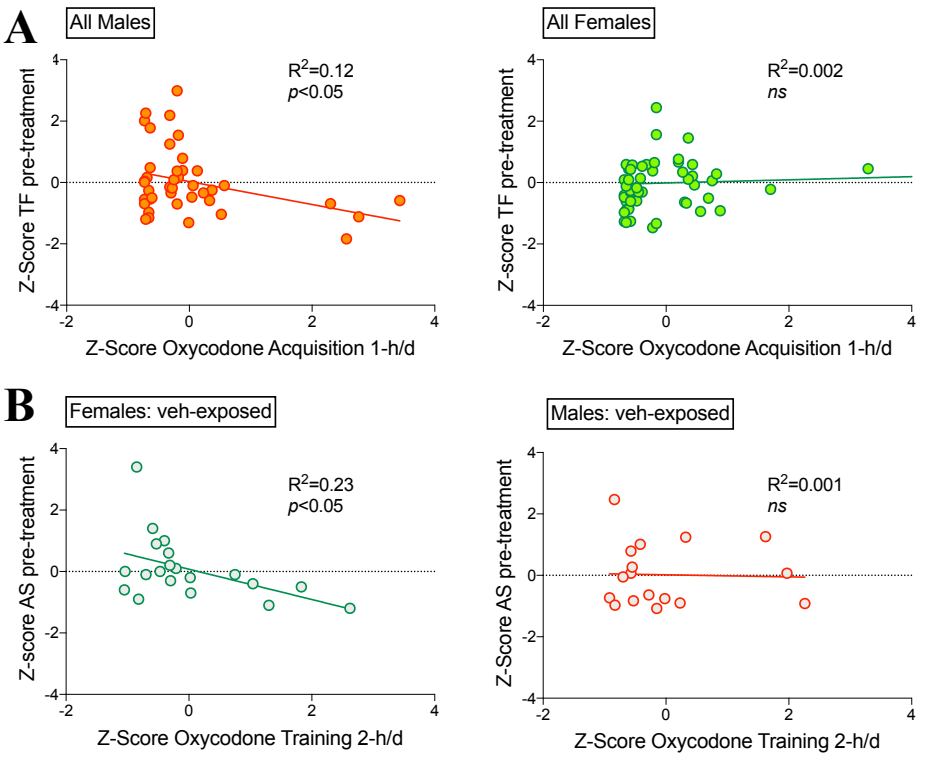

Figure 7. Using Spearman nonparametric correlational analyses, DI measures taken before (**A**) or after (**B**) morphine (or vehicle) treatment and withdrawal were correlated with the total # of oxycodone infusions taken in days 1-5 of Acquisition or the change in the # of infusions (d5-d1). In (**A**), the lower the latency for female rats to withdraw their tails from the warm water, the more oxycodone they subsequently infused and the faster they acquired oxycodone IVSA. Unexpectedly, the lower the AS amplitude, the more oxycodone the females took. In (**B**), the significant relationship between AS amplitudes and acquisition of oxycodone IVSA was opposite between males and females. In morphine withdrawn males, the more time spent in the open arms of the EPM, the greater the rate of IVSA acquisition.

related effects of oxycodone. These findings are consistent with human studies in which higher DI in people currently taking prescription opioids for pain is associated with higher rates of opioid abuse. However, our findings are important and novel because they demonstrate for the first time that DI measures in drug-naïve animals can also predict vulnerability to abuse.

Objective 2

Open Field Test during morphine withdrawal. We used the open field as a measure of putative anxiety-like behavior. Rodents typically avoid the center of an open field, and the more time spent exploring this ethologically risky center space is considered a marker of anxiolytic behavior. Given that opioid withdrawal increases anxiety-like behavior, we hypothesized that morphine-exposed and withdrawn rats would spend less time in the center zone of an OF. Indeed, **Figure 8** shows that this is what was observed in both male and female rats, with a more robust effect in males. This finding is important because it provides evidence that on day 7 of opioid withdrawal, there are detectable behavioral measures of negative affective state.

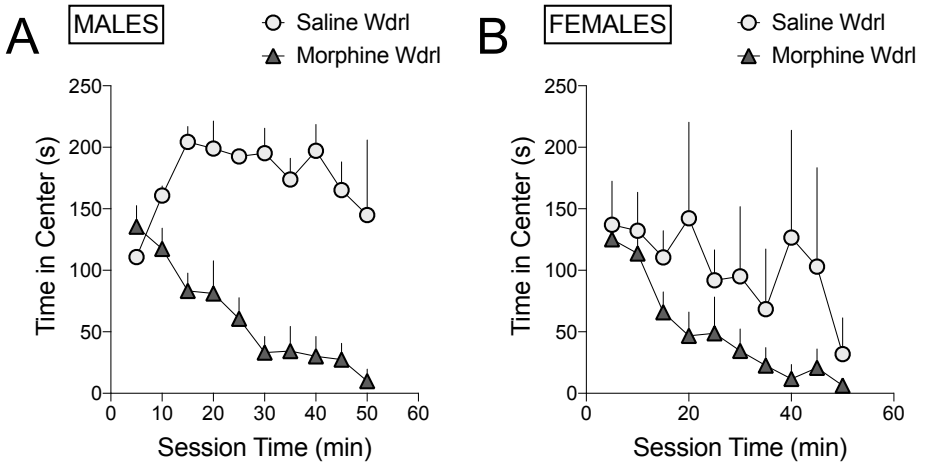

Figure 8. On morphine (or saline) withdrawal day 7, rats were placed in the OF arena and the time spent in the center zone was recorded. Saline-exposed rats spent more time in the Center zone compared to morphine-exposed rats, with the difference between groups more robust in males (**A**) compared to females (**B**). N=6/group.

Ultrasonic Vocalizations during morphine withdrawal. There is a dramatic sex difference in the number of 50 Hz calls after morphine (or vehicle) treatment and withdrawal (**Figure 9**). Overall, males make far fewer calls than females, and males slightly increase, whereas females robustly decrease, the number of calls made during morphine withdrawal. This work is still early in development, as it was just approved for Objective 2. But it suggests strong sex differences in baseline USVs as well as the effect of morphine exposure and withdrawal on USVs.

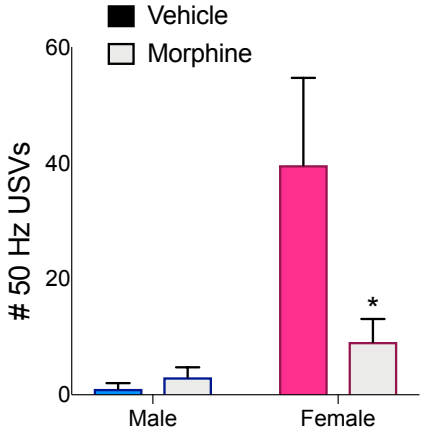

Figure 9. On morphine (or saline) withdrawal day 9, USVs were recorded for 5 minutes from each rat. The majority of calls occurred at 50 Hz, although a few calls were detected at 22 Hz. Here we present the total # of calls at 50 Hz for each sex and treatment. N=6/group.

Oxycodone self-administration using a Long-Access (LgA, 6-h/d) protocol. The final change made to the experimental design for Objective 2 was to use a Short-Access followed by a LgA self-administration paradigm in order to 1) shorten the length of the experiment, thus allowing more catheters to remain patent, and 2) test the effects of morphine exposure and withdrawal on escalation of oxycodone intake under LgA conditions. This is thought to closely mimic that transition of opioid abuse to compulsive opioid use disorder. In our preliminary studies (one cohort of 6 rats/group), we find that morphine-exposed male rats self-administer more oxycodone than vehicle-exposed rats (Figure 10), a finding that is consistent with **Figure 6A**. Now that we know this protocol works, we will proceed with the crux of Objective 2, which is to test the effects of the CRFR1 antagonist on morphine withdrawal signs and oxycodone self-administration.

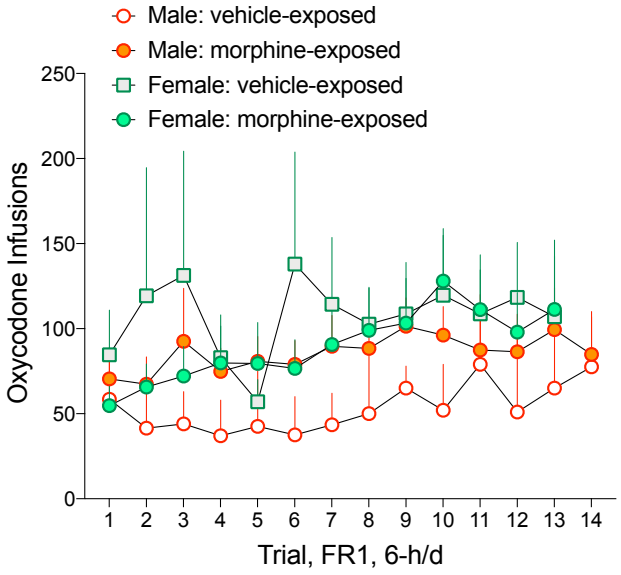

Figure 10. 14 days after cessation of chronic, escalating morphine treatments, rats were implanted with jugular catheters and allowed to train for 1-h/d for 8 days to learn oxycodone self-administration. After this, rats were switched to the LgA paradigm, in which they were allowed to self-administer drug for 6-h/d for 14 days. In this pilot study, we observe that morphine-exposed males take more oxycodone than vehicle-exposed controls. N=6/group.

Stated Goals Not Met: As seen below under Specific Objectives, we met all of our stated goals (as outlined for Year 2 in the Statement of Work [SOW]).

2. *Specific Objectives*

In Year 2 of this project, our specific objectives outlined in the Statement of Work were as follows:

a) Objective 1 studies will be completed.
b) Objective 2 studies will be initiated.
c) Data will be disseminated at a scientific conference (e.g. Society for Neuroscience, College on Problems of Drug Dependence).
d) The PI will prepare a yearly progress report

As discussed in #'s 1 and 3, we accomplished each of the stated objectives.

3. *Significant Results*

The most important, impactful findings thus far are illustrated in **Figures 6A and 7** and described above. Briefly, we found that prior morphine exposure significantly increased the vulnerability of male, but not female, rats to self-administer more oxycodone during early, acquisition stages. We also found that our rat analogs of Distress Intolerance (DI), which include a measure of pain tolerance (TF) and startle reactivity (AS) can reliably predict vulnerability to oxycodone self-administration in a sex- and DI-specific manner.

We also had negative findings, which are illustrated in Figure 5 or mentioned in the section on the EPM. Specifically, we hypothesized that, during morphine withdrawal, we would observe an increase in AS amplitude (anxiogenic effect), and a decrease in open arm time in the EPM (anxiogenic effect). However, our data do not support this hypothesis. There are many potential explanations for these negative findings, but we thought the most productive approach would be to test additional measures of withdrawal. As such, we proposed and were approved to eliminate AS and EPM and instead measure ultrasonic vocalizations (USVs) and locomotor activity in an open field assay for Objective 2.

4. Other achievements

- Nothing to report
- **What opportunities for training and professional development has the project provided?**
  We presented this work at the 2018 Society for Neuroscience meeting in San Diego, CA which served as a professional development opportunity for both Drs. Chartoff and Tania Lintz (Research Assistant).

- **How were the results disseminated to communities of interest?**
    - We presented this work at the 2017 & 2018 Society for Neuroscience meetings.
    - Dr. Chartoff was invited to participate in an SfN Press Conference on Opioids at the 2018 SfN meeting in San Diego.

- **What do you plan to do during the next reporting period to accomplish the goals?**
- During the next reporting period we will complete our studies for Objective #2, which involve testing the effects of antalarmin.

5. **IMPACT:**
- **What was the impact on the development of the principal discipline(s) of the project?**
- Our findings will likely have a significant impact on prescription opioid abuse in the military and in the general public because they demonstrate that relatively simple behavioral measures of Distress Intolerance, which are already used clinically, can be assessed prior to any drug treatment and can then be used to predict an individual's risk for subsequent opioid abuse. The use of baseline DI measures as a predictive tool will be most easily translated into humans by the military, since all Soldiers undergo extensive medical exams prior to deployment. DI measures can easily be incorporated into these exams. In the event a Soldier sustains an injury while on duty and needs a prescription opioid painkiller, the baseline DI measures can inform physicians about the relative risk of future opioid abuse. If the baseline DI measures suggest a Soldier is in a high risk category, steps can be taken to mitigate this risk, thus reducing the overall rates of opioid abuse and addiction in the military.
-
- **What was the impact on other disciplines?**
- As mentioned above, our findings will likely have a significant impact on the opioid epidemic that affects civilians as well, although translation of these DI measures to the general public will likely be more difficult.
-
- **What was the impact on technology transfer?**
- Nothing to report.
-
- **What was the impact on society beyond science and technology?**
  Nothing to report.

6. **CHANGES/PROBLEMS:**
- **Changes in approach and reasons for change**
- As described above, our results from Objective 1 strongly indicated that neither the AS nor the EPM were providing useful data on morphine withdrawal. In addition, the lengthy design of the IVSA experiments resulted in approximately 33% of rats losing catheter patency as they neared the end of the study (i.e. as they neared the progressive ratio portion). As such, we applied for and obtained ACURO approval (approval granted 9/26/18) to alter our experimental design as described above.
-
- **Actual or anticipated problems or delays and actions or plans to resolve them**
- Nothing to report.
-
- **Changes that had a significant impact on expenditures**
- Nothing to report.
-
- **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

- Nothing to report.
-
- **Significant changes in use or care of human subjects**
- Not applicable
-
- **Significant changes in use or care of vertebrate animals.**
- See above about ACURO-approved changes to experimental design. Note that this didn't significantly change our use or care of the rats.
-
- **Significant changes in use of biohazards and/or select agents**
- Not applicable
-

7. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  M. Mavrikaki, S. Page, E. H. Chartoff. 2017. Effects of morphine abstinence on oxycodone self-administration in male and female rats. Society for Neuroscience poster:793.09.
- Lintz T, Mavrikaki M, Esayeas B, Page S, Chartoff EH. 2018. Effects of morphine withdrawal on oxycodone self-administration in male and female rats. Society for Neuroscience conference, San Diego. *Poster*.
- **Journal publications.**
  Nothing to Report.
-
- **Books or other non-periodical, one-time publications.**
  Nothing to Report.
-
- **Other publications, conference papers, and presentations.**
  Nothing to Report.
-

Website(s) or other Internet site(s)
Nothing to Report.

-

Technologies or techniques
Nothing to Report.

Inventions, patent applications, and/or licenses
Nothing to Report.

-

Other Products
Nothing to Report.

-
-
- **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
- **What individuals have worked on the project?**

Name:	*Elena Chartoff*
Project Role:	*PI*

Nearest person month worked:	*3.6*
Contribution to Project:	*Dr. Chartoff is the PI. She designed the experiments, contributes to conducting the experiments, oversees research staff, analyzes and interprets data and is responsible for dissemination of findings.*
Funding Support:	\

-

Name:	*Maria Mavrikaki*
Project Role:	*Postdoctoral fellow*
Nearest person month worked:	*10.8*
Contribution to Project:	*Dr. Mavrikaki is primarily responsible for conducting the experiments. She provides daily supervision of the research assistant, and helps analyze and interpret data.*
Funding Support:	\

Name:	*Tania Lintz*
Project Role:	*Research Assistant*
Nearest person month worked:	*3.0 (from 11/1/17 – 8/31/17); 75% (from 9/1/17 – present)*
Contribution to Project:	*Tania assists Dr. Mavrikaki with the behavioral studies, including DI measures, morphine injections, and oxycodone IVSA.*
Funding Support:	

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**
-

PI: Elena Chartoff
R01 DA045000-01 (Chartoff) 9/01/2017 – 8/31/2022 4.20 calendar
NIH/NIDA $272,229
Neurobiological mechanisms of prescription opioid withdrawal
The major goal of the proposed studies is to identify mechanisms by which glutamatergic signaling mediates withdrawal from the prescription opioid oxycodone in male and female rats such that novel, drug-able targets can be identified for treatment of the opioid withdrawal syndrome.

-
- **What other organizations were involved as partners?**

Nothing to Report.

8. **SPECIAL REPORTING REQUIREMENTS**

- **COLLABORATIVE AWARDS:** Not applicable
-
- **QUAD CHARTS:** Attached.

•

9. **APPENDICES:n/a**