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<b>14. ABSTRACT</b> Due to improved battlefield medicine, the majority of Soldiers who are wounded survive and are treated with prescription opioid painkillers. This award had two goals: to test a novel means (Distress Intolerance, DI) 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 used male and female rats that were exposed to an experimenter-administered regimen of escalating-dose morphine (or saline) injections. This design allowed us to be sensitive to sex differences, which exist in human drug abusers, and it allowed us to determine if a model of physician-prescribed opioids alters vulnerability to subsequent abuse. Over the 3-year grant period we completed analysis of pre- and post-morphine DI measures and their correlation with subsequent vulnerability to oxycodone self-administration, and we completed analysis of the impact of prior experimenter-administered morphine on subsequent vulnerability to oxycodone self-administration in male and female rats. We made preliminary progress on the effects of antalarmin on oxycodone self-administration but were hampered by delays in ACURO animal protocol approval and changes in staff that required additional time for training. Regardless, we can use our antalarmin data as pilot data for additional funding opportunities. Our final results therefore include: 1. Multiple linear regression with DI measures and oxycodone self-administration demonstrated that, in males, there is a significant correlation between baseline Tail Flick withdrawal latency and amount of self-administered oxycodone. The data show that the lower the latency to tail withdrawal at baseline (pre-morphine), the more oxycodone is take, and they support our hypothesis that pre-opioid exposure baseline DI measures can predict vulnerability to subsequent opioid misuse. 2. Prior exposure to experimenter-administered morphine and withdrawal results in significantly greater oxycodone intake during the		

acquisition phase in males but not females. These data suggest that there is a sex difference in vulnerability to opioid misuse after being treated with a prescription regimen of opioid painkillers: males are more susceptible to misuse than females. In conclusion, our findings suggest 1) Fast, easily implemented Distress Intolerance measures can be taken before deployment and used to help “flag” whether a Soldier would be at risk for developing an opioid use disorder if they ever required an opioid painkiller prescription, 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)

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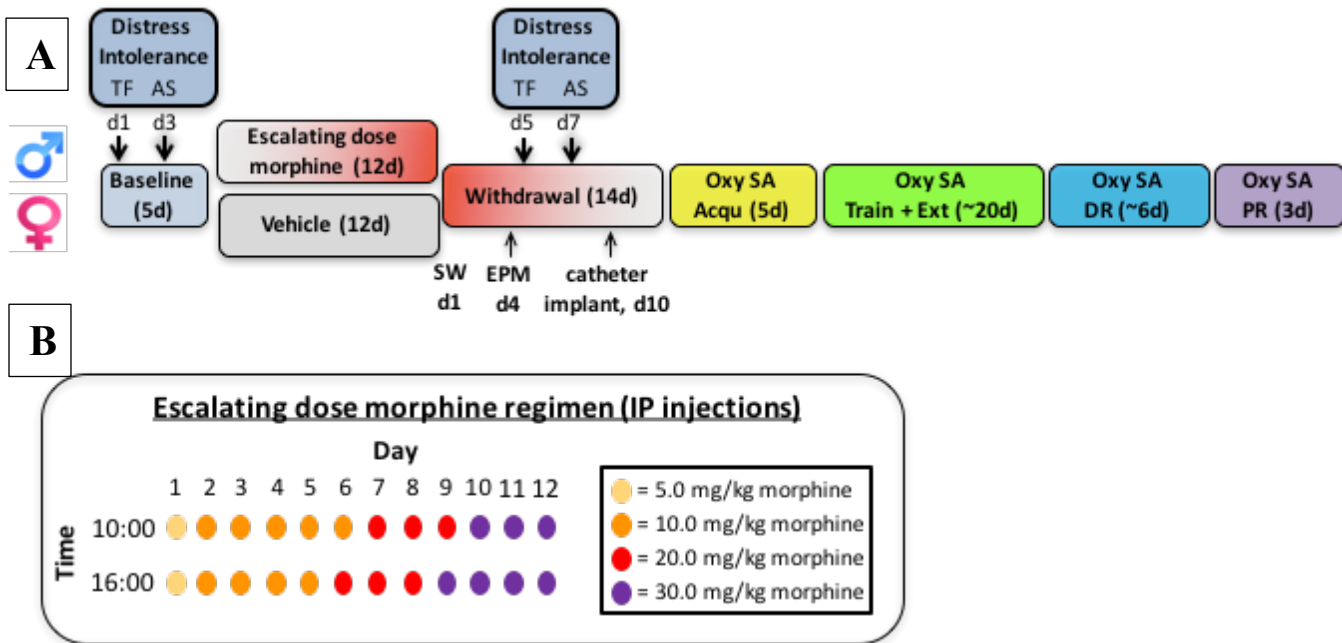
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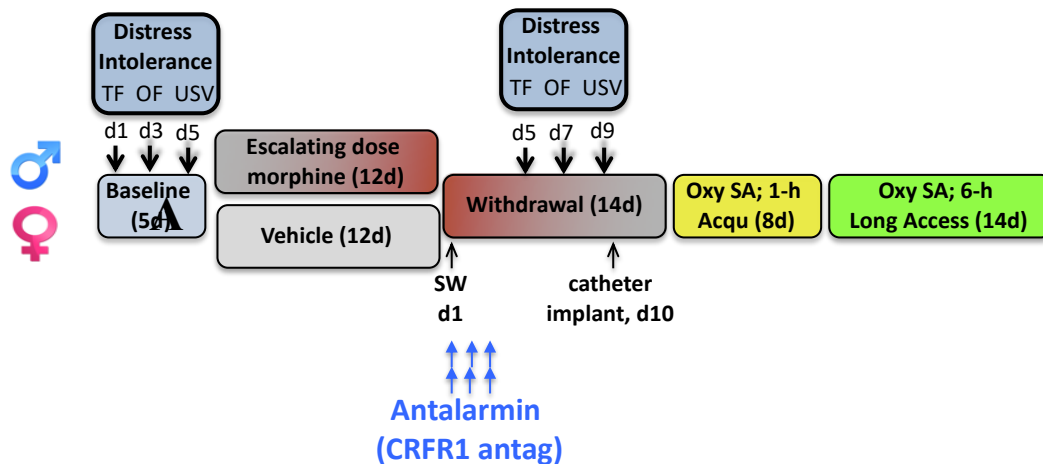
1. **INTRODUCTION:** As a result of improved battlefield medicine, approximately 50% of Soldiers wounded in action are returned to duty. Many are prescribed opioid painkillers as part of their recovery. Unfortunately, increasing numbers of Soldiers misuse prescription opioids and ultimately become addicted. 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; 2) whether prior exposure to chronic morphine administration and withdrawal predisposes males and/or females to increased opioid self-administration; and 3) 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, prior exposure to opioids will increase vulnerability to 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 if prior non-contingent opioid exposure and withdrawal increase the 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 and prior opioid exposure/withdrawal.

**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:* We completed Objective 1 and are in the process of writing 2 manuscripts on the results. We made promising headway on Objective 2, but were not able to complete it as described. We will seek additional funding to finish Objective 2, as it is important to see if blocking CRF actions during opioid withdrawal has a sex-dependent therapeutic effect on opioid abuse and OUD. 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.

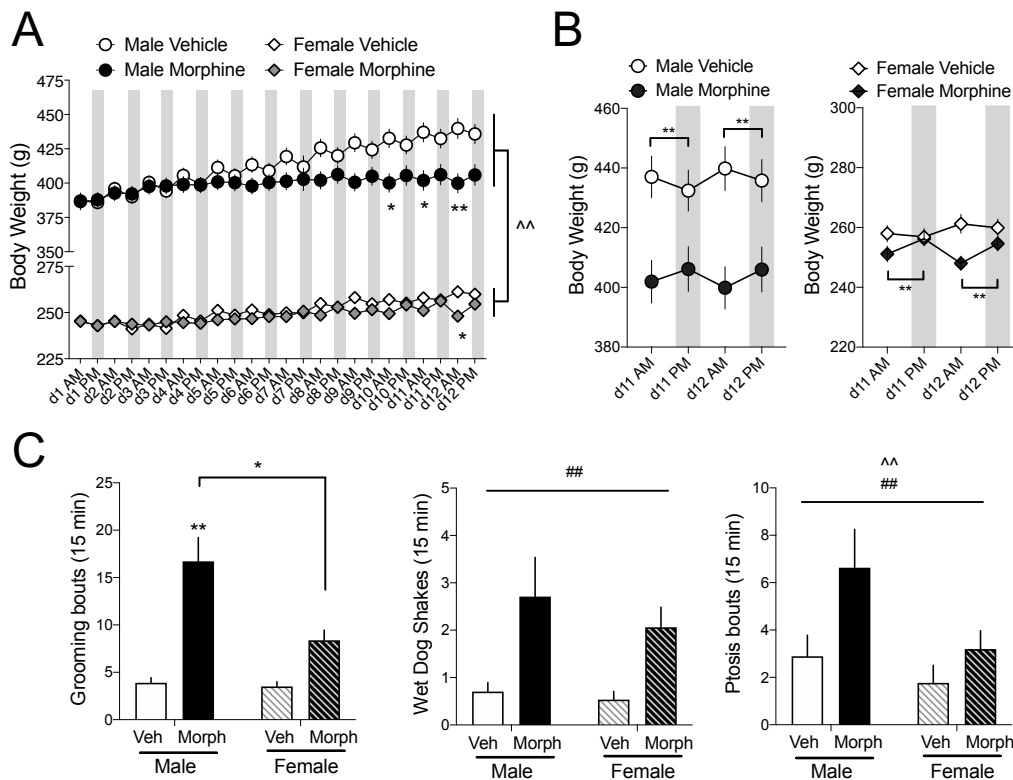


**Figure 2. Experimental schematic for Objective 2.** We made the following changes to the experimental design shown in Figure 1: Acoustic Startle (AS) and Elevated Plus Maze (EPM) behaviors were eliminated because they were not modified by morphine withdrawal as expected. AS and EPM were replaced by 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

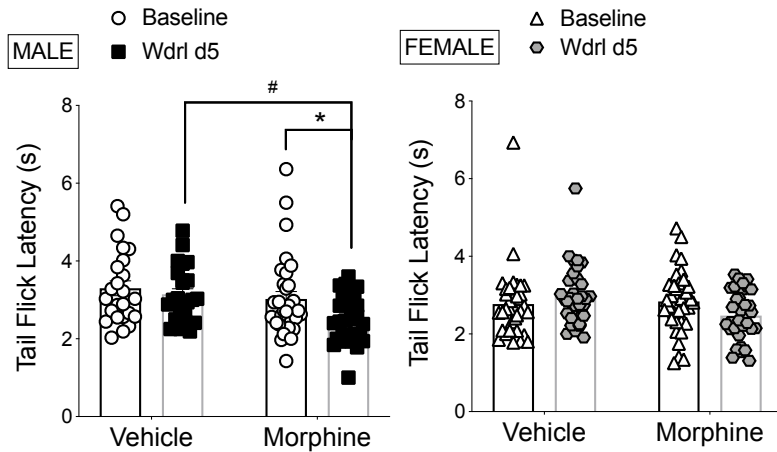
**Somatic withdrawal and body weight during escalating dose morphine:** To make sure rats were becoming morphine-dependent, we recorded body weights throughout the 12-day escalating morphine injection regimen (**Fig. 3A, B**) and recorded and scored somatic withdrawal (SW) behaviors (**Fig. 3C**) 16 hr after the last morphine injection. Morphine-treated male, but not female, rats failed to gain weight normally (**A, B**). Chronic morphine treatment also changed the diurnal pattern of food intake: While saline-treated rats show weight loss throughout the day (they eat at night), the opposite became true in morphine-treated rats. In both males and females, rats showed weight gain during the day (**Fig. 3B**), presumably due to the effects of the twice-daily morphine injections. As expected, day 1 of morphine withdrawal was associated with increased somatic withdrawal signs (**Fig. 3C**), which is indicative of morphine dependence. These data support the premise that the rats are morphine-dependent.



**Figure 3. Effects of morphine and withdrawal on body weight and somatic withdrawal.** 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 changes in body weight over the 12-d regimen. A closer look at body weights on morphine days 11 and 12 (B) show that the diurnal pattern of weight gain switches in morphine-treated rats. On day 1 of morphine withdrawal (i.e., 16 hr after the last morphine or vehicle injection), rats were videotaped for 15 min and somatic withdrawal behaviors were scored (C). Both males and females showed increased somatic withdrawal behaviors, including Grooming bouts, Wet Dog Shakes, and Ptosis. Males tended to show more withdrawal signs than females. 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,

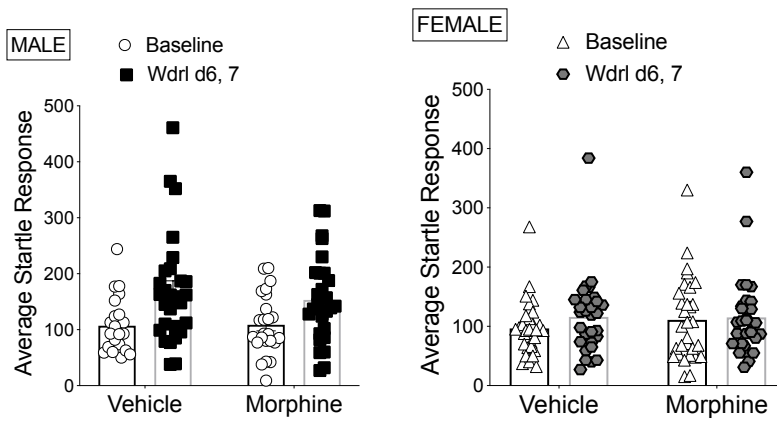
we used the TF as a proxy for measures of Discomfort Intolerance in humans, one domain of DI that measures pain sensitivity. Briefly, rats' tails are submerged 2-3cm in a 52°C water bath and the latency (sec) to flick the tail out of the water is measured. A cutoff time of 15 sec is used to prevent tissue 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. Males (left panel), but not females (right panel) showed withdrawal-induced hyperalgesia, as the tail flick latency was reduced in morphine-withdrawn rats compared to baseline and compared to vehicle-withdrawn rats. N=24/group. \*p<0.05.

**Acoustic Startle (AS):** The acoustic startle (AS) response is a simple reflex observed in many animal species including

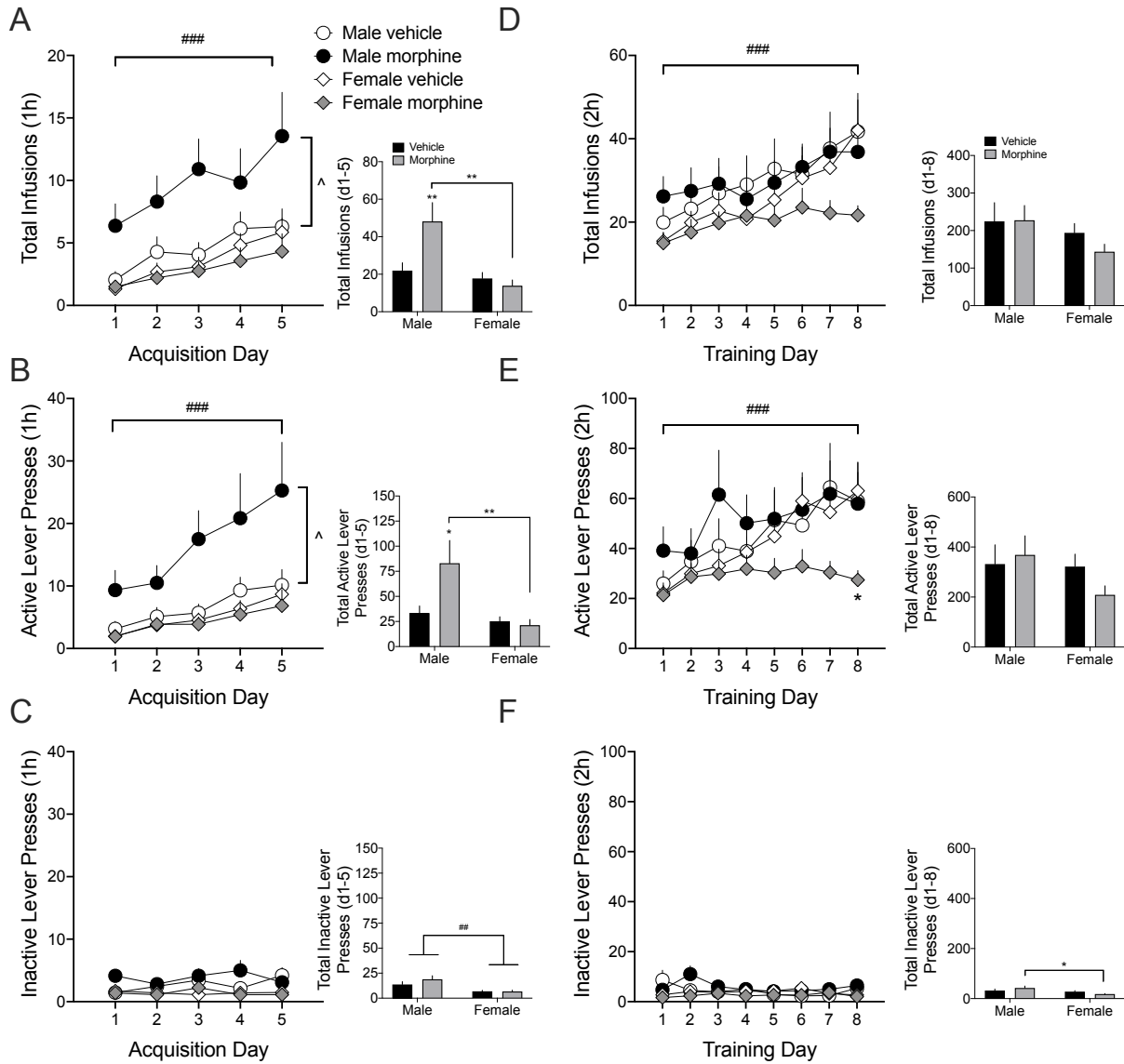
rodents and humans. In humans, it is thought that increased baseline startle sensitivity is present in people with high negative affect, which can be associated with anxiety, depression, and PTSD. In the laboratory, acoustic startle is typically elicited by short white noise bursts. We find that there 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) was still 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 (left panel), the increase in AS amplitude on Wdrl d7 is due to the increase in weight observed over time in males, but not females (right panel). Morphine withdrawal has no effect on AS responses in either males or females.

**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

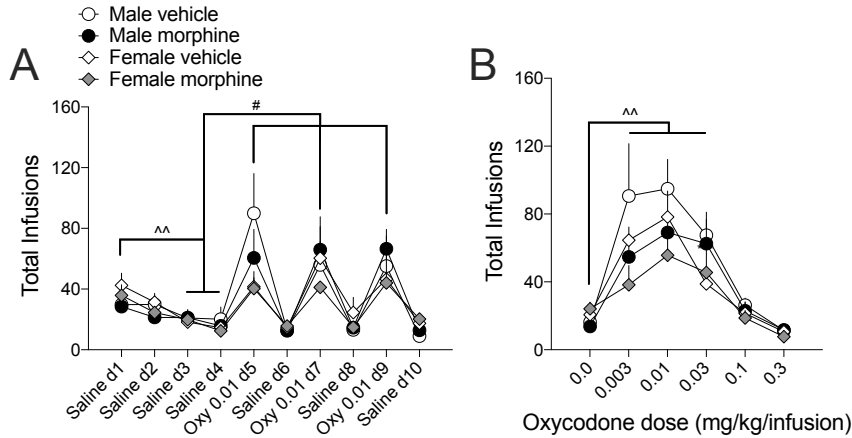
signal availability of the next unit dose. We hypothesized that rats in morphine withdrawal would acquire IVSA behavior faster and take more oxycodone infusions across a range of drug doses (**Fig. 6**).



**Figure 6.** After 14 days of morphine withdrawal, rats self-administered oxycodone on a Fixed Ratio 1 (FR1) schedule of reinforcement. Left panels are per-day graphs of oxycodone infusions (**A**), active lever presses (**B**), and inactive lever presses (**C**) during the 5-d, 1 h/d acquisition period. Right panels are per-day graphs of oxycodone infusions (**D**), active lever presses (**E**), and inactive lever presses (**F**) during the 8-d, 2 h/d training period. For both left and right panels, insets represent the sum of infusions/presses over the acquisition or training time course. Morphine-withdrawn males, but not females or controls, self-administered more oxycodone during acquisition. In contrast, morphine-withdrawn females tended to self-administer less oxycodone during the last half of the training regimen (**D**, **E**). N=22-24 rats/group.

We found that morphine withdrawn male, but not female, rats self-administered more oxycodone and pressed the active, drug-paired lever, more than vehicle-treated male rats during the initial 5 days of oxycodone IVSA acquisition (**Fig. 6A, B**) and as overall sums (**Fig. 6A, B insets**). These findings suggest that prior opioid exposure and withdrawal make males more vulnerable to taking oxycodone, which could have important, sex-dependent implications for pain treatment and OUD. After the initial 5 days of acquisition, rats continued to train on oxycodone IVSA, but in 2-h/d sessions. Except for females that had been previously morphine-exposed, the other 3 treatment groups showed a gradual escalation in oxycodone intake and active lever presses

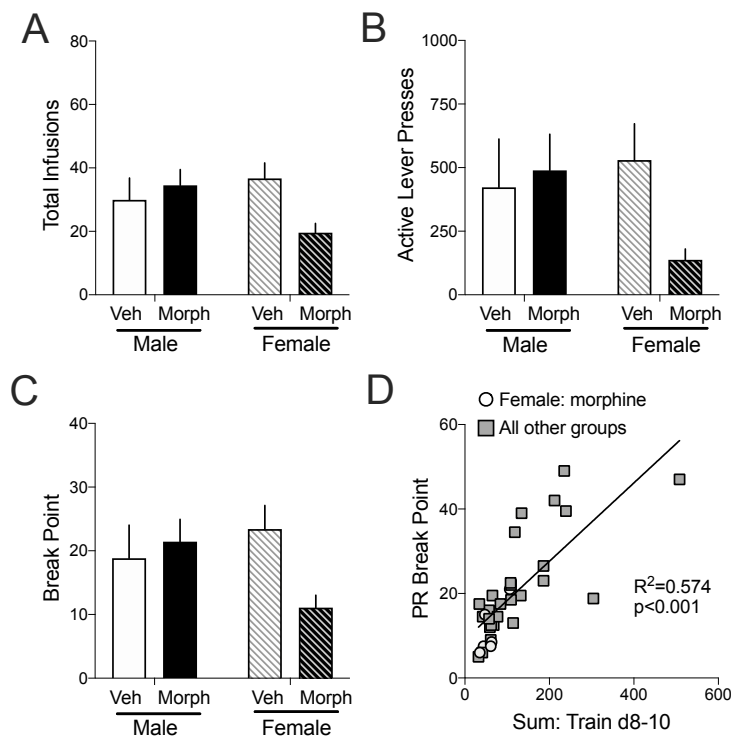
(Fig. 6D, E) over the 10 days of training. These findings suggest one of two things: 1. Morphine-exposed females are protected from the reinforcing effects of oxycodone or 2. Morphine-exposed females are sensitized to the rewarding effects of oxycodone and thus self-administer less drug to obtain the same reinforcing effect. This type of finding is critical to understand for appropriate treatments to be developed.



**Figure 7.** Preparation for generating an oxycodone dose-effect curve involved extinguishing responding with saline followed by alternating days of saline and oxycodone (0.1 mg/kg) to demonstrate that all groups could distinguish between saline and drug (A). Oxycodone dose-dependently caused the typical inverted U dose effect function in which all groups of rats self-administered more infusions as the dose increased to 0.01 mg./kg and then self-administered progressively fewer infusions as the dose continued to increase to 0.3 mg/kg (B). There were no significant group differences. N=10-16/group.

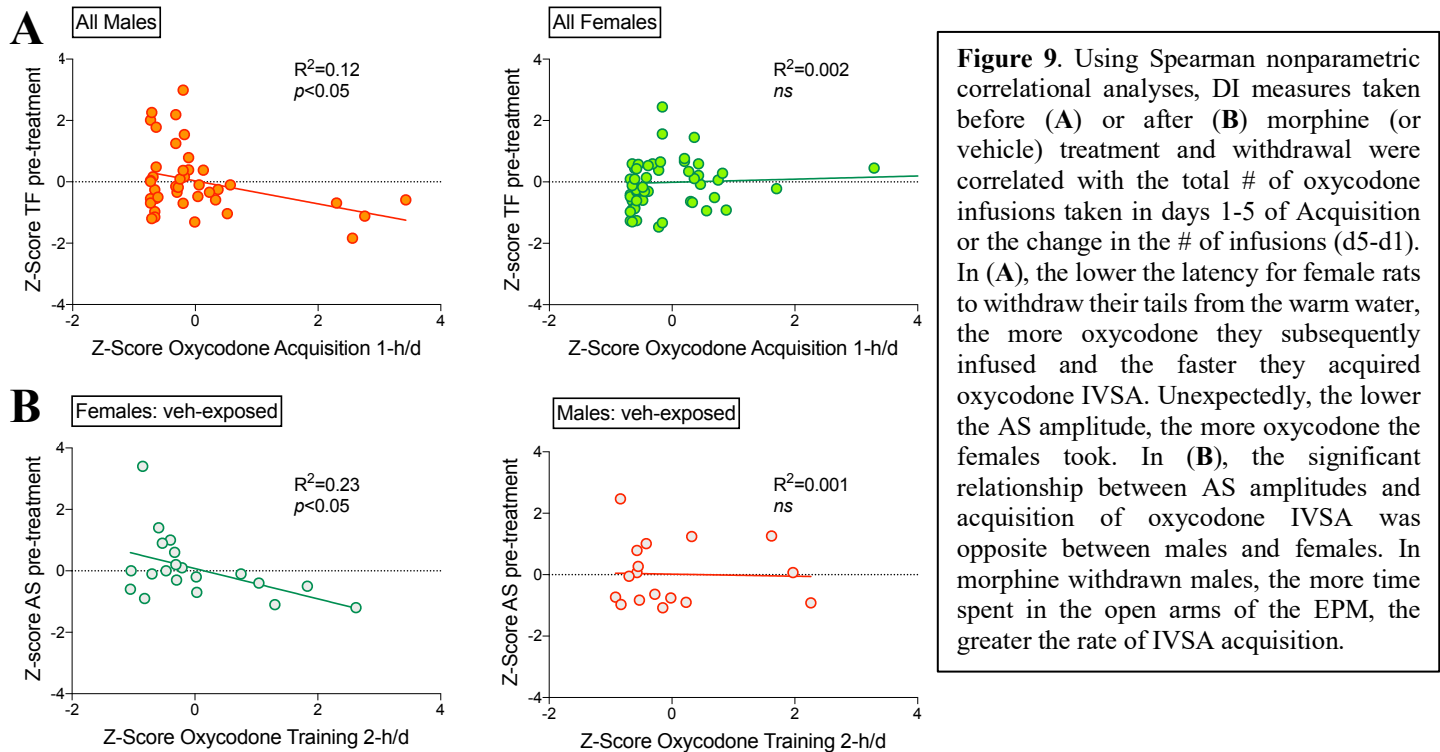
Prior to conducting the dose response experiment (Fig. 7), rats went through an extinction phase followed by alternating days of saline or oxycodone (Fig. 7A) to establish that rats could distinguish between saline and oxycodone. For the dose response experiment, rats were exposed to one of 6 oxycodone doses presented in random order each day (one dose per day). Although there was no significant Treatment X Dose interaction, there was a trend for morphine-exposed females to self-administer the least oxycodone at the peak doses (0.003, 0.01, and 0.3 mg/kg; Fig. 7B). This type of finding suggests that – after substantial oxycodone exposure – morphine-exposed female rats had decreased vulnerability to the reinforcing effects of oxycodone. This was substantiated by the progressive ratio experiment (Fig. 8), in which morphine-exposed 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 these data appear contrary to our hypothesis that morphine-

withdrawn females would be more motivated to take oxycodone, 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 a female’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



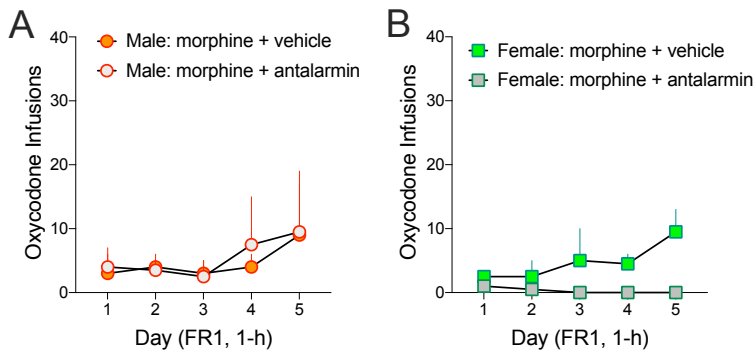
**Figure 9.** Using Spearman nonparametric correlational analyses, DI measures taken before (A) or after (B) morphine (or vehicle) treatment and withdrawal were correlated with the total # of oxycodone infusions taken in days 1-5 of Acquisition or the change in the # of infusions (d5-d1). In (A), the 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.

oxycodone self-administration measures in a sex-dependent manner (Fig. 9). 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 (Fig. 9A), whereas in females, baseline AS amplitudes can predict which vehicle-exposed rats will self-administer the most oxycodone throughout the 10 days of 2-h/d training (Fig. 9B). 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

The proposed studies were designed to determine if the CRF receptor antagonist antalarmin administered during morphine withdrawal blocks withdrawal-induced negative affective states and likelihood to engage in oxycodone IVSA in male and female rats. We were able to complete preliminary studies in this objective, with the most relevant highlight reported here. As shown in Experimental Schematic (Fig. 2), morphine-dependent and withdrawn rats were treated multiple times with the short-acting CRF receptor antagonist, antalarmin prior to acquisition of oxycodone IVSA. Our hypothesis was that blockade of CRF actions would reduce opioid withdrawal signs and hence reduce oxycodone self-administration. We predicted this would have a greater effect in females, as women report greater negative affect during opioid withdrawal than men. Figure 10 shows preliminary data from the first 5 days of IVSA acquisition. As predicted, antalarmin blocks oxycodone IVSA in

females, but has no effect in males. Objective 2 sought to continue this work to determine if antalarmin also interfered with subsequent escalation of oxycodone intake, a phenomenon that occurs when animals are given extended access to drug. We also sought to determine if antalarmin would reduce somatic withdrawal signs as well as withdrawal-induced hyperalgesia in the TF test. These studies still need to be done, and we plan to submit grant applications to finish this promising and important work.



**Figure 10.** The CRF receptor antagonist, antalarmin (20 mg/kg) or vehicle, was injected on 3 separate days during the 14-d morphine withdrawal period. Rats were then tested in oxycodone IVSA. (A) In males, pretreatment with antalarmin did not change the level of oxycodone intake during the initial 5-d acquisition period. However, in females (B), antalarmin pretreatment blocked oxycodone IVSA. These preliminary studies have an N of 4-5/group.

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

## 2. Specific Objectives

In Year 1 of this project, our specific objectives outlined in the Statement of Work were as follows:

- Submit and obtain approval for animal studies from McLean IACUC and ACURO.
- Set up and calibrate acoustic startle equipment.
- Obtain controlled substances (morphine, oxycodone) from NIDA Drug Supply.
- Hire and train a research assistant to replace the one working in the lab at the time of this award notice.
- Initiate experiments in Aim 1.
- Present findings at a scientific conference.
- Prepare an annual Progress Report.

We accomplished each of the stated objectives, and we were able to fully test 3 cohorts of rats (N=24 rats each) in this first year. We also presented our data at the annual Society for Neuroscience meeting (2017)

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

- Objective 1 studies will be completed.
- Objective 2 studies will be initiated.
- Data will be disseminated at a scientific conference
- The PI will prepare a yearly progress report

We completed  $\frac{3}{4}$  of Objective 1 and did not start Objective 2 in Year 2. We accomplished the remainder of the stated objectives.

In Year 3 of this project, our specific objectives outlined in the Statement of Work were as follows:

- Aim 2 studies will be completed before the fourth quarter of Year 3.
- The PI will begin preparing manuscripts for publication beginning in the second half of Year 3.
- Data will be disseminated at a scientific conference.
- The PI will prepare a final report describing sex differences in the ability of distress intolerance to predict the likelihood of opiate abuse and CRF antagonism to block opiate abuse in rats.

We completed Objective 1 and initiated (2 cohorts of rats) Objective 2. We accomplished the remainder of the stated objectives.

### 3. *Significant Results*

The most important, impactful findings thus far are illustrated in **Figures 6 and 9** 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 (**Fig. 6**). 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 (**Fig. 9**).

We also had negative findings that did not support our hypotheses. For example, 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, we found no change in AS amplitude (**Fig. 5**), and the EPM failed to capture any anxiogenic effects and was overall inconclusive (data not shown).

### 4. *Other achievements*

- **What opportunities for training and professional development has the project provided?**  
We presented this work at the 2017 and 2018 Society for Neuroscience meetings, which served as professional development opportunities for Drs. Chartoff and Mavrikaki, as well as Tania Lintz (Research Assistant). Due in part to this experience, Ms. Lintz was accepted to a number of graduate programs in neuroscience for the 2020-2021 academic year and is in the process of choosing a school. In addition, Dr. Mavrikaki was promoted from postdoctoral fellow to Instructor at Harvard Medical School, which is a junior faculty position.
- **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, based on this DoD-funded work.
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - This is the final report. We plan to submit two manuscripts of the data and apply for additional funding in the near future.

### 5. **IMPACT:**

- **What was the impact on the development of the principal discipline(s) of the project?**
  - Our findings have the ability to significantly impact 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. In addition, our findings show that prior prescription opioid

exposure (modeled in our studies with noncontingent morphine injections) increases vulnerability to addictive-like behavior in males but not females. Together with DI measures, our findings could equip physicians and the military with predictive tools to curb opioid abuse and OUD.

- 
- **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. Our findings impact our understanding of sex differences in behavior in that there are clear sex differences in opioid-related withdrawal and self-administration behaviors. This will help inform the medical community that men and women cannot be treated for OUD the same.

- 
- **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**
- No changes for Year 3 for this final report.
- 
- **Actual or anticipated problems or delays and actions or plans to resolve them**
- Our institutional IACUC protocol required renewal. It was approved in August, 2019. We submitted our approved IACUC documentation and protocol to ACURO but did not hear back before the grant ended. As such, we were not able to get as far as we intended with Objective 2.
- 
- **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 issues with delayed ACURO approval of our renewed IACUC protocol.
- 
- **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, Constantino N, 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, Esayias 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: 420.13.

- **Journal publications.**

*In preparation:*

Mavrikaki M, Lintz TL, Constantino N, Page S, Chartoff EH. Prior exposure to a chronic morphine regimen differentially modulates oxycodone self-administration in male and female rats.

Lintz TL, Mavrikaki M, Ravichandran CT, Constantino N, Page S, Chartoff EH. Baseline measures of stress sensitivity in rats can predict future oxycodone self-administration behavior.

- **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:	<i>Elena Chartoff</i>
Project Role:	<i>PI</i>
Nearest person month worked:	<i>3.6</i>
Contribution to Project:	<i>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.</i>
Funding Support:	

- |       |                        |
|-------|------------------------|
| Name: | <i>Maria Mavrikaki</i> |
|-------|------------------------|

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

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

1P50MH115874-01A1 (PI: Carlezon; co-I: Chartoff) 04/01/2019-03/31/2024

NIH/NIMH

*Silvio O. Conte Center for Stress Peptide Advanced Research, Education, & Dissemination (SPARED) at McLean Hospital*

NIH/NIMH \$196,468 3.0 cal mo

*Admin Core SPARED Center*

The Administrative Core of the SPARED Center will support and complement the comprehensive qualities of the science and will also support a broad range of training and educational activities, including expansion of already piloted outreach programs as well as new approaches to engage both scientists and lay-persons.

(NEW)

1R21DA048399-01 (PI: Chartoff) 09/30/2019-08/31/2021 2.4 cal mo

NIH/NIDA \$185,817

*Prenatal oxycodone exposure: developmental effects on microglia and addiction-like behavior in rats*

These studies test the hypothesis that prenatal exposure to oxycodone causes both acute and long-lasting disruptions in microglia function and microglia-mediated pruning of dopamine D1 receptors in the nucleus accumbens in rats.

(NEW)

1R01DA045632-01A1 (PI: McHugh; co-I: Chartoff) 9/30/19-7/31/23 0.6 cal mo

NIH/NIDA \$234,420

*Affective and Inflammatory Reactivity to Pain in Opioid Use Disorder*

The goal of this project is to quantify the association between pain-induced negative affect and peripheral inflammation and opioid use disorder outcomes.

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

Nothing to Report.

8. **SPECIAL REPORTING REQUIREMENTS**

- **COLLABORATIVE AWARDS:** Not applicable

- 

- **QUAD CHARTS:** Attached.

- 

9. **APPENDICES:**

# Sex differences in the ability to predict and treat opiate abuse

Log #: BA150026

Award #: W81XWH-17-1-0004



PI: Elena Chartoff

Org: McLean Hospital

Award Amount: \$732,587

## Study/Product Aim(s)

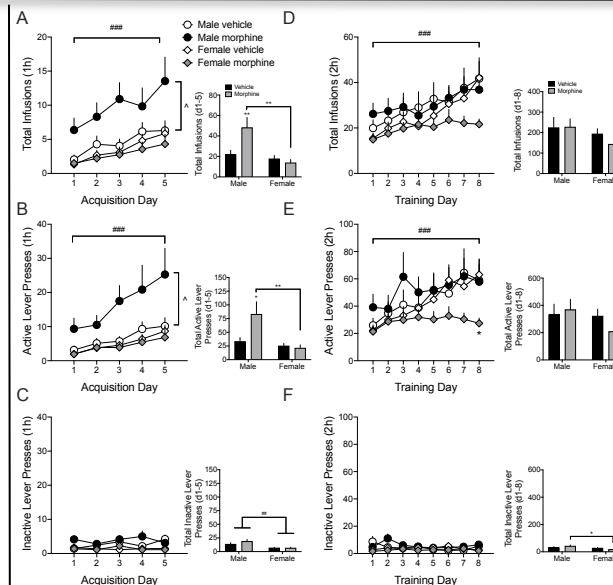
Prescription opioid addiction is an increasing problem in male and female Soldiers.

**Aim 1.** Identify behavioral measures of Distress Intolerance (DI) to predict vulnerability to opioid painkiller abuse.

**Aim 2.** Determine if pharmacological blockade of the stress peptide corticotropin releasing factor (CRF) reduces withdrawal signs and prevents opioid abuse.

## Approach

- Behavioral measures of Distress Intolerance taken prior to chronic morphine treatment and during protracted morphine withdrawal in male and female Sprague Dawley rats.
- Rats will be treated with the selective CRF receptor antagonist Antalarmin during morphine withdrawal and tested for addiction-like behavior in an oxycodone self-administration paradigm.



After 14 d of morphine withdrawal, rats self-administered oxycodone. Left panels represent oxycodone infusions (A), active lever presses (B), and inactive lever presses (C) during the 5d, 1 h/d acquisition period. Right panels represent oxycodone infusions (D), active lever presses (E), and inactive lever presses (F) during the 8d, 2 h/d training period. Insets represent the sum of infusions/presses. Morphine-withdrawn males self-administered the most oxycodone during acquisition. Morphine-withdrawn females tended to self-administer less oxycodone during the last half of the training regimen (D, E). N=22-24 rats/group.

## Timeline and Cost

Activities	CY	17	18	19
Milestone: ACURO approval, personnel training, equipment set up		█		
Milestone: Behavioral analyses (DI), morphine treatment, oxycodone self-administration in 1 cohort		█		
Milestone: Complete behavioral analyses (DI), morphine treatment, oxycodone self-administration.			█	
Milestone: Complete behavioral analyses (DI), morphine treatment, Antalarmin treatment, oxycodone self-administration.			█	█
Milestone: Data analysis, interpretation, and preparation of manuscript(s) for publication. Scientific conferences.			█	█
<b>Estimated Budget (\$K)</b>		\$209,403	\$260,000	\$263,184

Updated: (03/05/2020)

## Goals/Milestones

**CY17 Goal** – Administrative setup

- ☑ ACURO approval, personnel training, equipment setup
- ☑ Initiate behavioral experiments

**CY18 Goals** – Experimental Aims

- ✓ Complete 100% of behavioral studies in Aim 1
- ✓ Analyze, interpret, and write up data for publication
- ✓ Present findings at scientific conference(s)

**CY19 Goals** – Experimental Aims

- ☐ Complete 100% of behavioral studies in Aim 2
- ✓ Analyze, interpret, and write up data for publication
- ✓ Present findings at scientific conference(s)

## Comments/Challenges/Issues/Concerns

- None.

## Budget Expenditure to Date

Projected Quarterly Expenditure: \$64,913

Actual Quarterly Expenditure: \$79,603 (as of 10/31/19).

Cumulative Expenditure: \$263,184 (as of 10/31/19).