Establish & Characterize An Oral Opioid Self-Administration Model

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FINAL REPORT

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Trends & Trajectories of Prescription Opioids in the Military Health System

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1.0 SUMMARY

Non-medical opioid use has reached epidemic proportion in the United States. Due to the nature of the work, many service members may be vulnerable to developing opioid use problems. Animal models have already provided valuable information about the reinforcing effects of opioids; however improving animal models to better reflect aspects present in human use and could help us better understand, prevent, and treat this problem. Thus, we developed an animal model of opioid use that has better face validity (oral rather than intravenous opioid delivery, as is found in many cases of non-medical prescription opioid abuse) and incorporates alternative reinforcement, which is ever present and likely important in mitigating opioid abuse in humans, but which is rarely incorporated in to animal models. The project had four Aims: **1)** Examine the impact of extinction on oral opioid self-administration and b) examine the impact of opioid-associated cues on reinstatement of extinguished responding for oral opioid access. Rats were trained to work for opioid solution access as described above, then responding was extinguished (no stimuli were present and responses no longer result in opioid access) and responding was assessed. Once responding was extinguished (<20& of pre-extinction levels), we presented a stimulus that had signaled opioid availability (the dipper that had contained the opioid solution after every 5 lever responses), and reinstatement (the number of responses over 30-min in the presence of the stimulus) was measured. **2a)** Examine the impact of providing concurrently available food on oral opioid self-administration, and **2b)** examine the impact of changing the contingency for food on those effects. Rats were trained to respond on a lever for opioid solution as described above. For these studies, a second lever was present in the chamber. Initially, this lever was inactive. Once opioid responding stabilized, the light above the inactive lever was illuminated and responses on this lever produced a single 45mg food pellet. Over the next few sessions, the response requirement for food was increased to 5. The lever that produced opioid access was inactive and the light was turned off during these sessions. Subsequently, access to opioid solution or food alternated over subsequent daily sessions, with the light illuminated above the active lever. Finally, lights above both levers were illuminated and both levers were active during each daily session. The response requirement for food was varied (5,25,50,150), with each response requirement in effect for at least 10 sessions and the effect on opioid use was assessed. In addition, varying periods (1, 2, or 4 consecutive sessions) of unsignaled food extinction were imposed and the effect on opioid responding was assessed during and after each extinction period. **3)** Characterize responding for various concentrations of opioid solution, including vehicle alone. During two hour daily sessions, a light above a lever was illuminated and responding on this lever provided water access. Rats were fed their daily ration in the operant chamber at the end of the first hour. Once rats reliably responded on the lever for water access, feeding did not occur until rats returned to their home cage after the two-hour session. Once this responding stabilized, varying concentrations of etonitazene (0.625, 1.25, 2.5, and 5 µg/ml) were introduced and responding maintained by each concentration was assessed. **4a)** Examine the impact on self-administration of supplemental opioid administration and **4b)** withdrawal of supplemental opioid. One group of rats were trained to respond on a lever for opioid solution as described above for Aim 2 (concurrent access to etonitazene and food during daily sessions) and another group as described above for Aim 3 (responding for etonitazene alone). Once responding stabilized, supplemental morphine HCl (32 mg/kg, i.p.) was administered (2 or 6 hours) prior to daily self-administration sessions. Responding on the day of and following supplemental administration was assessed in each group. This research resulted in a number of key findings. We were able to train rats to respond for an oral opioid solution (etonitazene) which is similar to new, high-potency opioids responsible for increasing numbers of overdoses, such as fentanyl. Consumption of this opioid produced observable signs of opioid intoxication, including tail rigidity, stereotypies, and flat body posture. As expected, responding declined when access to the solution was removed (extinction) demonstrating that rats were responding for solution access. Importantly, extinction was faster in rats responding under a fixed-ratio schedule compared to rats responding under a random-interval schedule. This slower decline in responding in extinction under the random-interval schedule is characteristic of habitual responding. This indicates random-interval schedules function similarly for oral opioid self-administration as for intravenous administration of other abused substances, and further, suggests this is a useful model of habitual oral opioid use. Responding for the opioid solution was reinstated by presenting the empty solution dipper contingent upon lever responses. Providing alternative reinforcement on alternating days did not affect the amount of opioid consumed when it was available, however, providing alternative reinforcement concurrent with opioid availability reduced opioid use.

This result indicates that ensuring patients have other, competing activities available to them while undergoing opioid pharmacotherapy might help to reduce the development of non-medical opioid use. The amount of opioid use varied inversely with the availability of the alternative reinforcer (decreased alternative availability resulted in increased opioid use). Higher concentrations of etonitazene (up to 5 µg/ml) maintained greater amounts of responding. Supplemental opioid exposure slightly decreased responding for etonitazene when administered 2 hours, but not 6 hours, before etonitazene access. Withdrawing supplemental morphine did not have any apparent effect on responding for etonitazene or food (responding returned to pre-supplement levels). Unexpected extinction of alternative reinforcement resulted in increased opioid use, but this use did not exceed levels observed before food was made available in the operant chamber. This indicates that "frustration" does not result in excessive substance use due to dysphoria or stress, but rather substance use follows from allocation of behavior within the time available to the individual, again underscoring the importance of ensuring patients have alternative activities available to them while convalescing. Finally, mice might also be trained to self-administer etonitazene, which could help identify genetic factors that produce vulnerability to opioid abuse. However, mice were insensitive to extinction, suggesting habitual responding might develop faster in mice than in rats. This work provides a potentially useful model to examine factors involved in the development of habitual opioid use, and how providing alternative activities in the context where opioid use is problematic might help reduce that use. Further, this work demonstrates that the concept of 'frustration stress' leading to relapse of opioid use is likely better understood as the unexpected loss of alternative reinforcement and thus the alternative behavior associated with it, which may be replaced by substance use in drug-experienced individuals. This research could help the military arrange activities that can minimize the occurrence of non-medical opioid use by competing with it. Providing an enriched environment with highly available and rewarding alternative activities is likely to help mitigate non-medical opioid use among the armed forces.

2.0 INTRODUCTION

Non-medical abuse of prescription opioids: Non-medical use of prescription opioids is the most rapidly escalating substance abuse problem the United States currently faces. Over the past decade, non-medical use of prescription drugs (including opioids) has remained more prevalent than use of any other illicit substance except marijuana[1]. Further, overdose deaths due to prescription opioid misuse have also increased[2]. Prescription opioids are most often taken orally, in contrast with heroin which is most often injected[3,4]. Trends among military personnel are similar[5], resulting in a growing population of military personnel struggling with oral opioid addiction. There is growing recognition that effective therapies for substance use disorders are likely to require a long-term course of treatment[6]. An animal oral opioid self-administration procedure would provide a long-lasting preparation that parallels the most common route of human use and could facilitate identification of effective therapies, particularly those that require long-term treatment.

Effective therapies for oral opioid use disorder are likely to require chronic treatment. Most available treatments for substance use disorder are evaluated over relatively short periods and then assessed for effectiveness 6-12 months after the study (and the treatment) are terminated. Similarly, preclinical studies often examine acute treatment effects[7]. Recently, this strategy has been questioned, as substance use disorder is more similar to chronic illnesses such as diabetes and hypertension that require long-term management[6], and acute effects may not persist during repeated treatment[8]. Thus, in order to identify therapies that remain effective with such long-term management, we need animal models that allow relatively lengthy courses of treatment.

Oral opioid self-administration provides a long-lasting preparation that parallels the most common route of human use. Oral opioid self-administration procedures were pioneered several decades ago[e.g. 9], yet most preclinical studies of opioid use intravenous injection of the drug. These procedures require implantation of intravenous catheters which have only a limited period of viability (often measured in weeks or months). In contrast, oral self-administration requires no surgery and can continue throughout the lifespan of the subject. In fact, we have used a similar procedure to study ethanol self-administration in rats for up to two years[10]. This longer viability allows us to examine effects of long-term therapies and the consequences of termination of the therapy in ways that are complicated by intravenous preparations [8,11].

An animal model of oral opioid use could help identify more effective treatments. Recently, we used a similar procedure to examine potential therapies for excessive drinking. We used this model to examine potential pharmacotherapies for drinking, and found that fluvoxamine, a selective serotonin reuptake inhibitor, did not produce robust, long-lived selective decreases in responding for ethanol versus food [7,8,12], consistent with recent clinical results[13]. We found similar results for the nicotinic agonist varenicline[14,15]. These results indicate that further development of varenicline as a treatment for drinking should proceed with caution, despite some early indications of clinical effectiveness[16].

We have also used a similar procedure to model behavioral therapies for drinking. We found that providing alternative reinforcement can reduce ethanol self-administration, and that providing longer periods of alternative reinforcement can increase the persistence of the alternative behavior when rats were re-exposed to alcohol cues[11,17,18]. This procedure models behavioral therapies that have been shown to be effective in studies of human substance users[19]. Increased persistence of alternative behavior, even when re-exposed to cues that had set the occasion for drinking is a desirable pattern of behavior for those in recovery and suggests that this therapy could reduce subsequent cue-induced relapse.

The proposed procedure provides the opportunity to examine the potential benefit of combining pharmacotherapy with behavioral therapy. Presumably, agents that reduce the motivation to use opioids in combination with a therapy that reduces the power of cues to trigger relapse could be more effective than either alone[20]. While this strategy has been suggested, few data are available to evaluate its validity. Further, animal models of such combination therapy have not been reported. Such an animal model could help identify critical aspects of this approach and result in more efficient and effective treatment. This project begins to develop an animal model of oral opioid abuse which could be used to study pharmacological, behavioral or combined interventions.

While we have experience training rats to self-administer oral oxycodone and ethanol solutions, we may encounter unanticipated difficulties. To address this possibility and extend our study, we also piloted oral opioid self-administration in mice using a modified operant conditioned taste preference procedure. Demonstrating we

are able to detect a preference for the opioid-containing solution would have moved toward establishment of a mouse model of oral opioid use; unfortunately, we were unable to demonstrate a preference for the opioid solution. There is some precedent for these types of procedures, including a study in which responding for intravenous oxycodone was compared to responding for food in separate groups of mice[21], an oral opioid self-administration procedure[22], and conditioned taste preference using the proposed flavored solutions[23]. This procedure would allow within-subject comparison of responding maintained by opioid versus a similar alternative reinforcer (both are isocaloric flavored solutions), which could facilitate future studies using established mouse models, including genetically modified mice that have human mu-opioid receptors thought to confer vulnerability to substance use[e.g. 24]. We can also examine strain differences and potentially relate differences to genetic mutations that might be homologous to human polymorphisms that increase vulnerability to opioid use. Finally, we can use this model to identify treatments that reduce responding for the opioid, but not the alternative solution. Such treatments would be expected to have clinical utility.

Oral opioid addiction is a health problem in the military[5]. Establishing a long-lived preclinical opioid self-administration procedure facilitates identification of effective strategies for long-term management of this growing problem. The military has unique characteristics that make it particularly amenable to implementation of behavioral therapies such as contingency management. Contingencies are extremely effective when a patient's continued employment and advancement is incorporated in such strategies. For example, when physicians found to have substance use problems are subjected to monitoring and probation as a contingency to maintain their medical license and prescription authority, recovery rates can exceed 80%, far greater than rates among the general population[25]. The addition of a pharmacotherapy could further enhance the effectiveness of such behavioral therapies. This preclinical procedure could be used to optimize treatment strategies that combine pharmacological and behavioral aspects.

3.0 METHODS, ASSUMPTIONS, AND PROCEDURES

Subjects

Rats: Male Lewis rats (Envigo, Inc., Indianapolis, IN, n=48) arrived at 6 weeks of age weighing approximately 275g. Rats were individually housed and allowed to habituate to vivarium routines for at least 2 weeks. During this time, rats had ad libitum access to food and water in their cages. Once rats weighed 300g, food was restricted to 12-15g/day to maintain rats' weights at approximately 330g (median: 329g; range: 302-364g) for the rest of the study. Water remained available in the home cage at all times, except as noted in the Postprandial Drinking Procedure. All studies were approved by the Institutional Animal Care and Use Committee as well as by the United States Air Force AFMSA/SGE-C Animal Use Program, and were conducted in accordance with the Guide for Care and Use of Laboratory Animals[26]. Animals were housed under a 14/10 hour light/dark cycle and tests were conducted during the light cycle.

Mice: Male C57/BL6 mice (Jackson Labs, Bar Harbor, ME, n=8) arrived at 6 weeks of age. Mice were singly housed, and water was freely available except during experimental sessions. After spending at least one week habituating to vivarium routines, food was limited to 2.5 g rodent chow provided each day following experimental sessions.

Apparatus

Training and testing occurred in standard rodent operant chambers from a commercial vendor (Med-Associates, Georgia, VT). Chambers were equipped with a liquid dipper that delivered 0.1 ml of a solution into an accessible location in the center of one chamber wall. A food dispenser was also present which delivered 45mg rodent chow flavored pellets (BioServ, Flemington, NJ) to the same receptacle. Two response levers were present on either side of the receptacle and a stimulus light was located above each lever. A house light was present at the top of the opposite wall. Chambers were enclosed in ventilated, sound and light-attenuating enclosures.

Oxycodone

Oxycodone HCl was obtained from a commercial supplier (Sigma, Inc, St. Louis, MO) and dissolved in drinking water provided by vivarium staff at a concentration of 1 mg/ml to produce a stock solution. This stock solution was then diluted to the working concentrations (described below) in drinking water. Working solutions were made fresh every 2-5 days, as needed.

Postprandial drinking

After the two-week habituation period, rats had water removed from their cage two hours before daily sessions. Rats were trained to respond on a lever when the light above it was illuminated for 10-sec access to 0.1 ml of water during two hour sessions. One hour into the session, rats were fed their daily food ration in the operant chamber. Initially a single response produced 10-sec dipper access, turned off the stimulus light above the lever, and turned on the house light. After 3-4 sessions responding for water, oxycodone (0.001 mg/ml) was added to the solution. This concentration was rapidly increased [0.003, 0.01, 0.1 mg/ml for 1-2 sessions each] to 0.3 mg/ml then maintained for 36 ± 6 sessions, then increased again to 0.56 for 6 ± 4 sessions, and finally to 1 mg/ml. Over the next several sessions, the response requirement was increased from fixed-ratio 1 to 5 (FR1 to FR5), at which point rats were no longer fed food rations in the operant chamber, but instead in their home cage, after the daily session.

Etonitazene

Etonitazene HCl was obtained from a commercial supplier (Sigma, Inc, St. Louis, MO). Etonitazene was dissolved in drinking water at a concentration of 1000 µg/ml to produce a stock solution. This stock solution was then diluted to the working concentrations (described below) in drinking water. Working solutions were made fresh every 2-5 days, as needed.

Postprandial drinking

After the two-week habituation period, rats had water removed from their cage two hours before daily sessions. Rats were trained to respond on a lever when the light above it was illuminated for 10-sec access to 0.1 ml of water during two hour sessions. One hour into the session, rats were fed their daily food ration in the operant chamber. Initially a single response produced 10-sec dipper access, turned off the stimulus light above the lever, and turned on the house light. Over the next few sessions, the response requirement was increased from fixed-ratio 1 to 5 (FR1 to FR5). After 3-4 sessions responding for water, etonitazene (0.000625 mg/ml) was added to

the solution. This concentration was then increased [0.00125 - 0.025 mg/ml for 5-10 sessions each] to 0.005 mg/ml, for 5-10 sessions at which point daily feeding took place after the session in each rat's home cage. This condition was then maintained for the next several months. It was decreased to 0.003 mg/kg for 5-7 sessions, then increased again to 0.005 mg/ml, at which point rats were no longer fed food rations in the operant chamber, but instead in their home cage, after the daily session.

Sucrose Fading Procedure

After the two-week habituation period, rats were trained to respond on a lever when the light above it was illuminated for 10-sec access to 0.1 ml of a sucrose solution (8% w/v) during a two-hour session. Sucrose was purchased from a local grocery store (H-E-B, San Antonio, TX) and dissolved in drinking water provided to the colony by vivarium staff. Initially a single response produced 10-sec dipper access, turned off the stimulus light above the lever, and turned on the house light. During the initial three sessions, the number of deliveries earned increased from 87 ± 35 to 204 ± 17 (mean ± S.E.M.). For the next session, oxycodone (0.001 mg/ml) or etonitazene (0.625 µg/ml) was added to the sucrose solution. This concentration was maintained for the next 8 sessions. For rats responding for oxycodone, the oxycodone concentration was then rapidly increased [0.003, 0.01, 0.1 mg/ml for 1-3 sessions each] to 0.3 mg/ml then maintained for 10 sessions, then increased again to 1 mg/ml. For those responding for etonitazene, the concentration was increased to 1.25 µg/ml for the next five sessions and then to 2.5 µg/ml for the next six sessions, and then to the final concentration of 5.0 µg/ml. Over the next several sessions, the response requirement was increased from fixed-ratio 1 to 5 (FR1 to FR5), then sucrose was gradually removed from the solution, until rats were responding for deliveries of 0.1 ml of 1 mg/ml oxycodone or 5.0 µg/ml etonitazene in drinking water. Once responding was maintained by the opioid in water alone, rats continued training until responding stabilized: amount earned over four consecutive sessions varied by less than 30% of the mean for each subject. Sessions occurred on weekdays.

Extinction and Reinstatement

Rats were placed in the operant chamber and presented the stimulus light associated with opioid solution availability, however, responses on the previously active lever produced no programmed consequence, effectively eliminating opioid access. This condition was maintained until each rat completed less than 20% of the responses completed when the opioid was available. Subsequently, responses on the previously active lever resulted in presentation of a dry (empty) dipper and responses during the session were recorded.

Random-interval responding

Rats were trained to self-administer etonitazene as described above for the Sucrose fading procedure. Initially, 3-s presentations of a 0.1 ml dipper filled with 5.0 µg/ml etonitazene occurred under a Random Time (RT) 10-s schedule. This continued until rats reliably retrieved ethanol deliveries. Subsequently,
ethanol was delivered for each lever press, then under a Random Interval (RI) 20-s schedule and finally under a RI 30-s schedule. Thus, the first response after an interval (which varies across the session, but averages 30-s) results in opioid solution delivery. A second group was trained to self-administer etonitazene as described above for the Sucrose fading procedure, and remained on the fixed-ratio schedule to serve as a control for the habitual responding expected to be engendered by the random-interval schedule.

Alternating sessions of etonitazene and food reinforcement

Subsequently, rats were placed in the operant chamber and the light above the other lever was illuminated. Responses on this lever resulted in delivery of a food pellet (45mg “rodent chow” flavor, Bioserv, CAT#F0165), turned off the stimulus light, and turned on the house light. During the next three sessions (Sessions 103-106), the response requirement for food was increased to FR5. Sessions then alternated between food and etonitazene for the next 12 sessions (Sessions 107-118), with each session lasting two hours.

Concurrent access

After these alternating sessions, food and etonitazene were made concurrently available during 2-hour sessions (Sessions 119-124). Lights above both levers were illuminated and responses were reinforced under independent FR5 schedules. Completion of a FR resulted delivery of food or etonitazene, as appropriate, and the light above both levers turning off and the house light illuminating for 30-sec.

Effect of unsignaled extinction of food

Subsequently, rats were exposed to unsignaled extinction of responding for food for 1, 2, or 4 sessions (Sessions 125, 129-130, 136-139). During these sessions, stimuli remained the same as during concurrent access sessions, but responses on the food lever had no programmed consequence.

Supplemental opioid exposure Rats responding under a fixed-ratio (FR5) schedule and rats responding under the concurrent access procedure were administered morphine sulfate (10 mg/kg) either 2 or 6 hours prior to five consecutive daily sessions. On the preceding week, rats received saline injections 2 or 6 hours before five consecutive sessions; these sessions served as the paired control for the appropriate supplemental condition for each rat. Between the two determinations, rats were allowed to recover for at least one week, and testing only resumed when responding over five consecutive sessions did not differ from the baseline established before subjects were exposed to supplemental morphine.

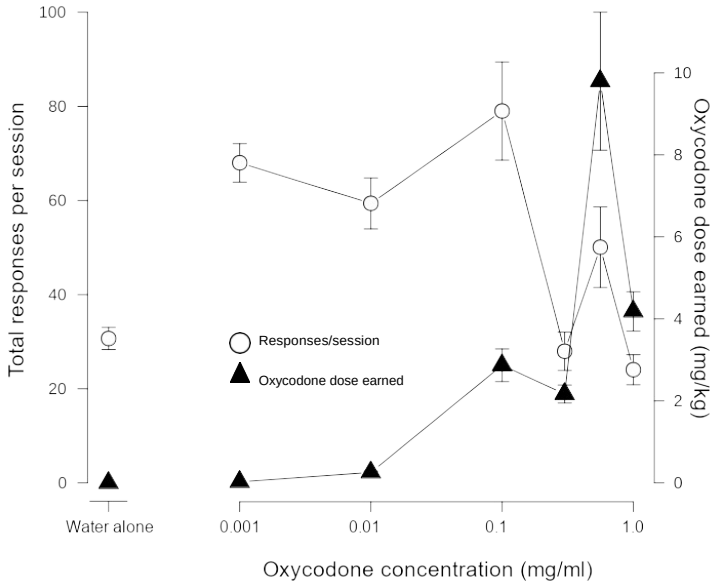
Mouse pilot studies

Mice were trained to respond by breaking a photobeam in an aperture to receive delivery of 0.01 ml etonitazene (5 μg/ml) in the same aperture. The procedure followed that described for the sucrose fading procedure in rats, except for the operant used (beam-break rather than lever) and the volume delivered. Subsequently, the responding was put into extinction, where responses no longer delivered opioid solution or had any other programmed consequence. This condition was maintained for over 50 sessions, yet responding never consistently declined.

4.0 RESULTS AND DISCUSSION

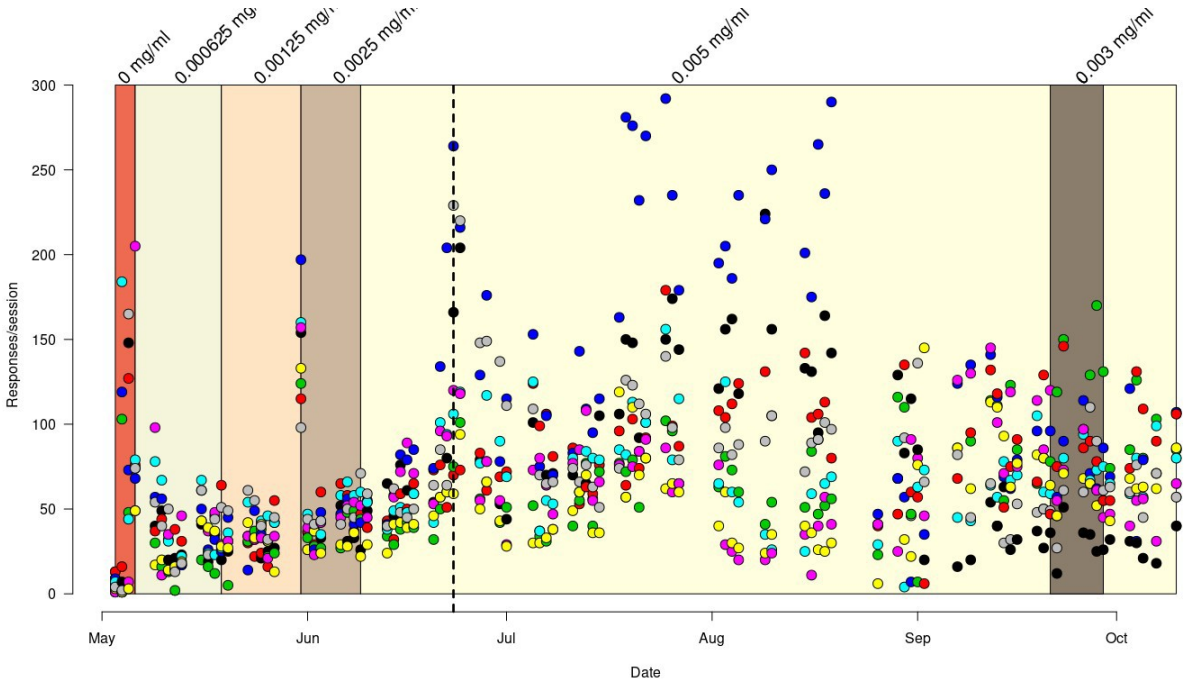
Oxycodone self-administration

Responding was maintained by increasing oxycodone concentrations (points represent mean ± S.E.M. for n=8 rats). The amount of responding and dose consumed increased up to 0.1 mg/ml. Dose earned continued to increase up to 0.3 mg/ml. Both dose earned and responses per session decreased at 1 mg/ml. Oxycodone failed to maintain responding in a second group of n=8 rats.



Etonitazene self-administration

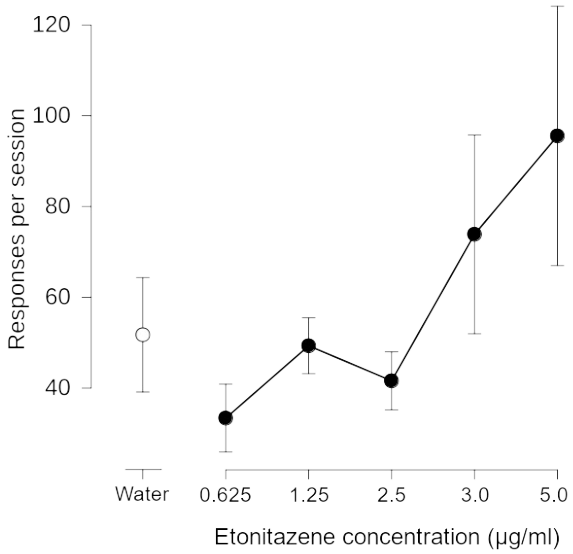
Postprandial drinking procedure: Rats were trained to respond for another opioid solution (etonitazene) which is easier to train, and is more similar to new, high-potency opioids such as fentanyl which are resulting in increasing numbers of overdoses. Total responses during two hours of access each day are shown; each color represents a different subject. Initially (coral rectangle) rats were allowed to respond for water alone for one hour, then fed their daily rations in the operant chamber, and allowed to respond for water for a second hour. Etonitazene was gradually added to the water, to the concentration indicated above each colored rectangle. The dashed vertical line indicates the session after which food was no longer placed in the chamber during the two-hour session. Instead, rats were fed their daily food ration later in the day.



Dose-Dependence of etonitazene self-administration

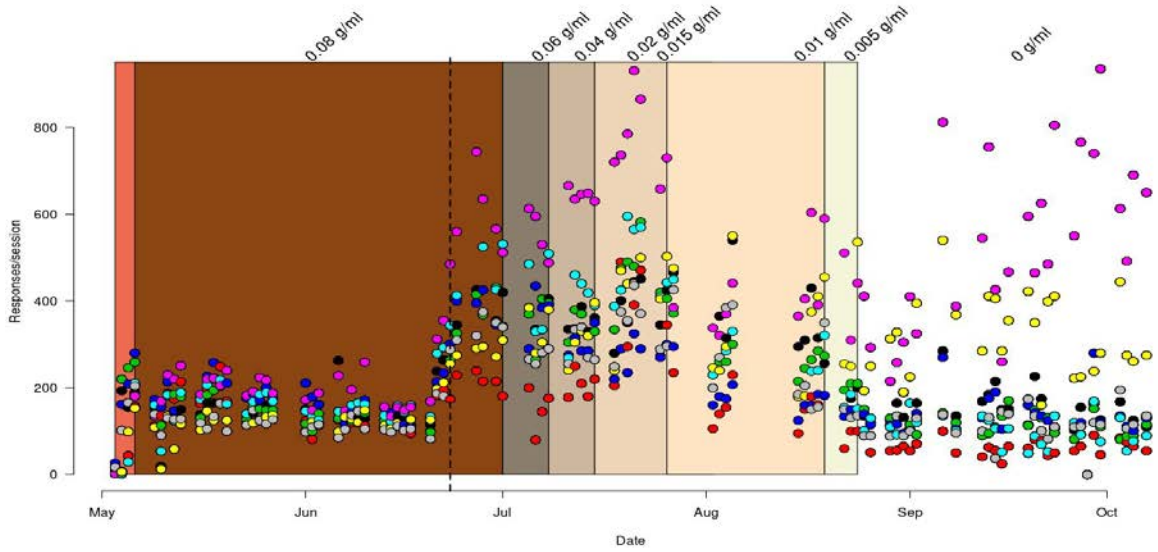
Etonitazene dose-dependently reinforced responding, as evidenced by increasing behavior as dose increased. Etonitazene dose-dependently increased responding in rats (n=8) with a history of responding for etonitazene in

water alone (no sucrose). Points in the figure represent the mean ± S.E.M. of responding maintained by the etonitazene concentration indicated. Concentrations of 3.0 and 5.0 µg/ml maintained greater responding than vehicle (water).



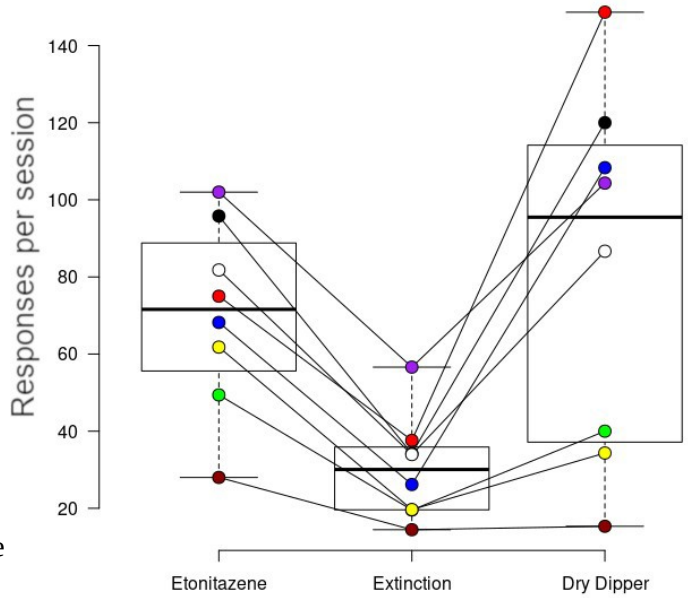
Etonitazene self-administration

Sucrose fading procedure: Responding during training in rats trained to respond for a sucrose/etonitazene solution. Total responses per each two hour session are shown for each subject, indicated by different colors (n=8). The leftmost orange rectangle indicates sessions where rats were given access to an 8% sucrose solution (no etonitazene). Each colored rectangle indicates a different sucrose concentration in the 5 µg/ml etonitazene solution. Sucrose concentrations are shown above each rectangle. The dashed vertical line shows the first session where rats were required to complete 5 lever responses to earn a 0.1 ml delivery of the solution.



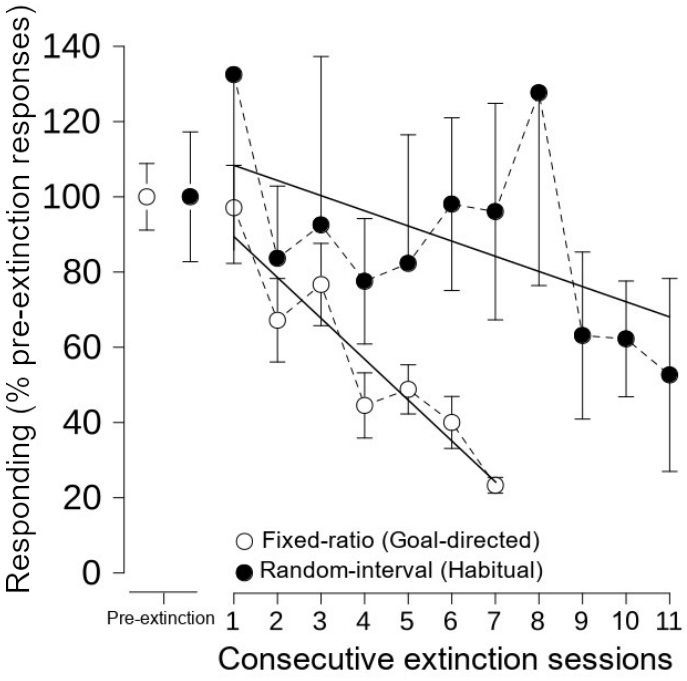
Extinction and Reinstatement

Extinguishing etonitazene reinforcement reduces responding, while exposure to an empty dipper (which previously contained etonitazene) reinstated responding. Responding declined when access to the solution was removed (extinction), demonstrating that rats were responding for solution access. Presenting the (empty) dipper that had provided the opioid solution reinstated responding**.** Responding in every rat declined when five lever responses no longer produced access to the etonitazene solution (left and middle panels). When responses subsequently produced exposure to the empty dipper (which had previously contained etonitazene solution), responding was reinstated (middle and right) in all but one rat (brown). This outcome has been related to drug-seeking or craving in other preclinical reports. Boxes represent the interquartile (25%-75%) range, whiskers represent the range, and the horizontal line represents the median.

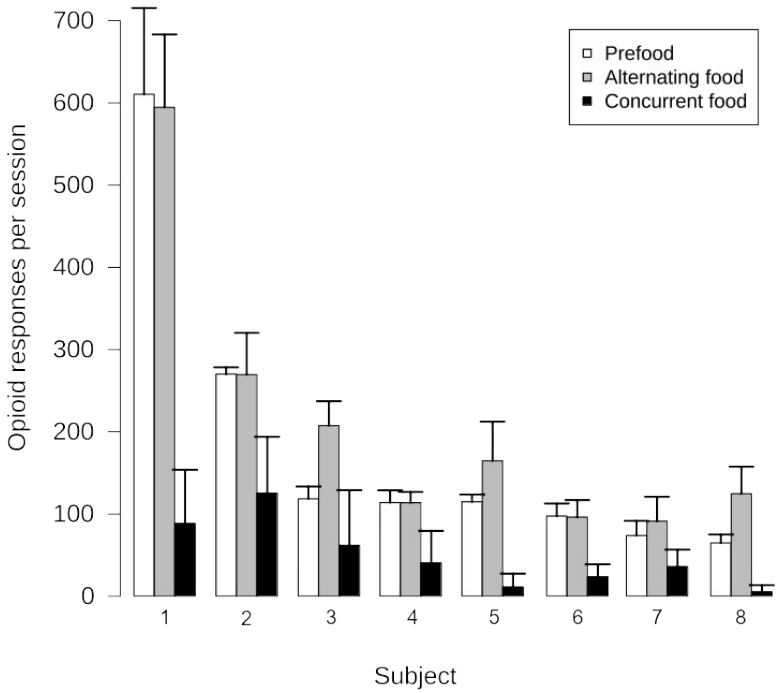


Habitual responding for opioid solution.

Extinction was slower in rats responding under a random-interval schedule compared with a fixed-ratio schedule. This slower decline in responding in extinction under the random-interval schedule has been considered to indicate responding that is relatively more habitual (versus goal-directed)[27]. Responding under a random-interval schedule (n=7 rats, RI30-sec) declines slower than responding under a fixed-ratio schedule (n=8 rats, FR5) in separate groups. Points above Consecutive extinction sessions represent mean (S.E.M.) responding per session as percent of the five sessions preceding extinction for each subject. Points above Pre-extinction represent the average (S.E.M.) for those five pre-extinction sessions for each subject. Solid lines represent the linear-mixed effects regression results for each group. These slopes were significantly different ($p<0.05$). This indicates random-interval schedules function similarly for oral opioid self-administration as for intravenous administration of other abused substances, and suggests this is a useful model of habitual oral opioid use.

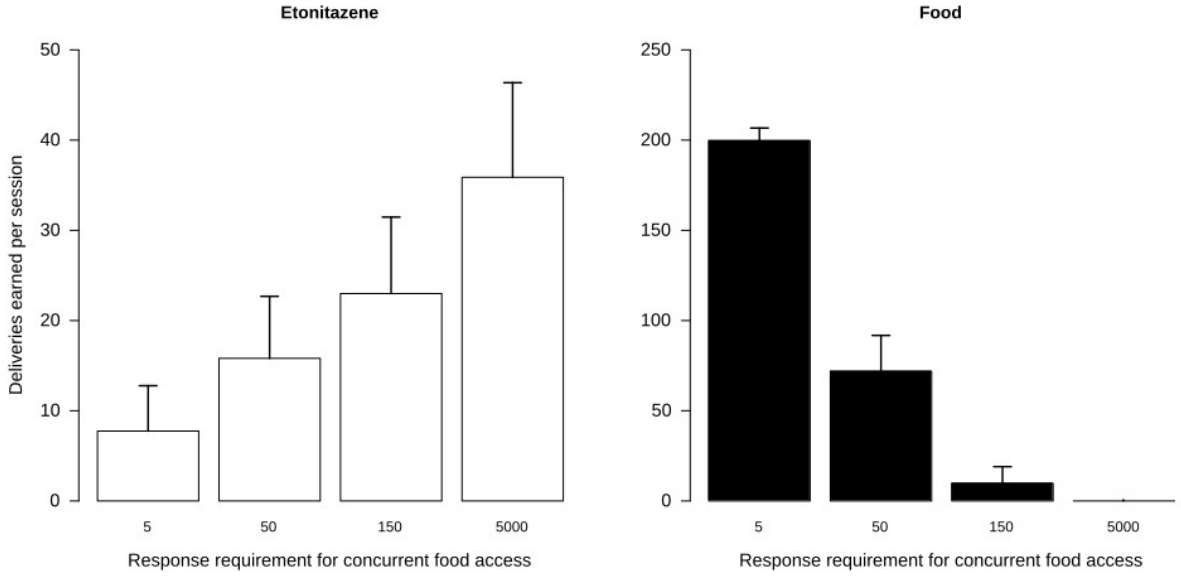


Impact of alternative reinforcement to opioid solution.



Providing alternative reinforcement on alternating days did not affect the amount of opioid consumed when opioid was available. However, providing alternative reinforcement concurrent with opioid availability reduced opioid use. This indicates that providing competing activities might help to reduce opioid use. Etonitazene responding before food access was provided during alternating operant sessions (white bars), during alternating daily sessions of food and etonitazene access (gray bars), and when food and etonitazene were concurrently available (black bars). Each bar represents the mean (± S.D.) for rats (n=8) over 5 sessions in each condition. Responding for etonitazene did not reliably change during alternating opioid and food sessions (white versus gray bars), but reliably declined in all subjects when food and opioid were concurrently available.

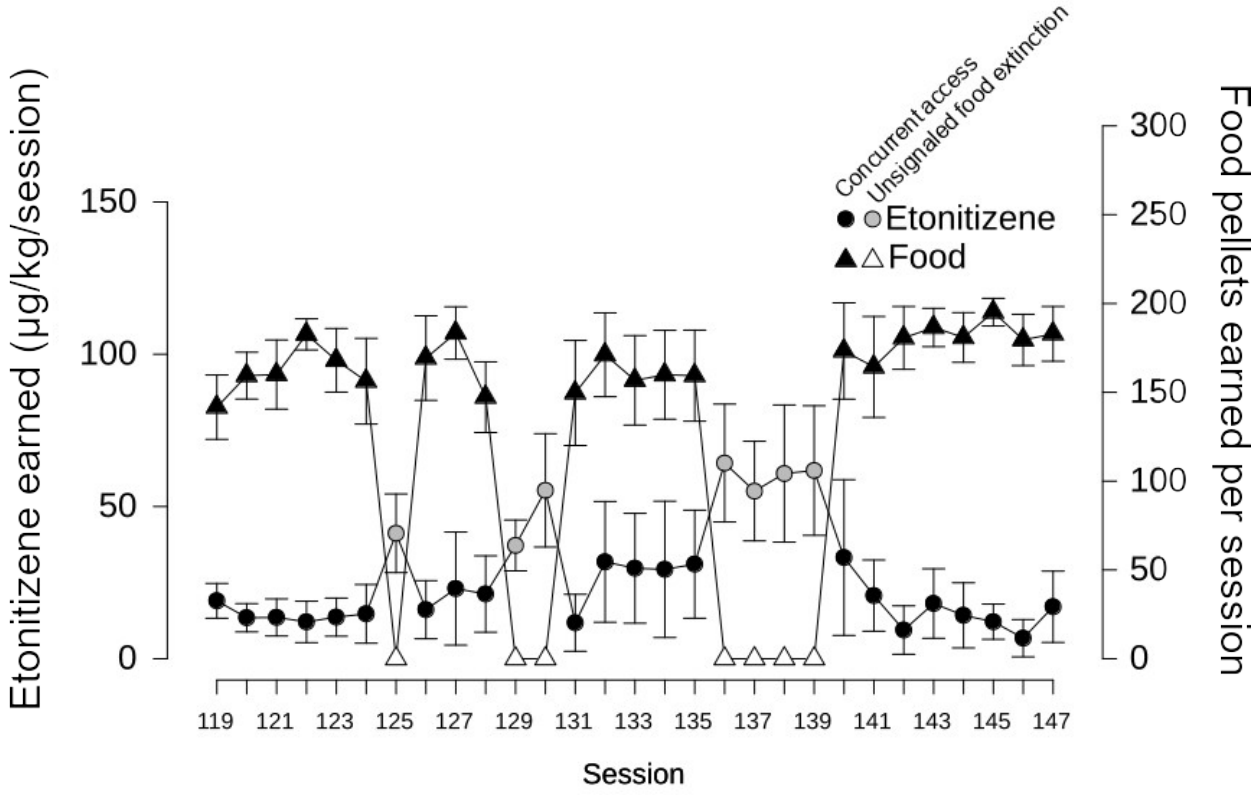
Effect of the availability of food on opioid responding. The amount of opioid use varied inversely with the availability of the alternative reinforcer (decreased alternative availability resulted in increased opioid use). The



number of deliveries of etonitazene (left) and food (right) as a function of the response requirement for concurrently available food. Bars represent the mean (± S.E.M.) number of deliveries of either etonitazene or food earned when food was available following the number of responses indicated on the x-axis by (n=7) rats.

The response requirement for etonitazene access remained fixed at 5 responses under each condition. Note the different scales indicated for the left and right panels. As the response requirement for food increased, the amount of etonitazene earned increased and the amount of food earned decreased, consistent with competition for drug use by the availability of alternative reinforcement.

Unexpected extinction of alternative reinforcement. Eliminating access to food resulted in increased opioid use, but this use did not exceed levels observed before food was made available.

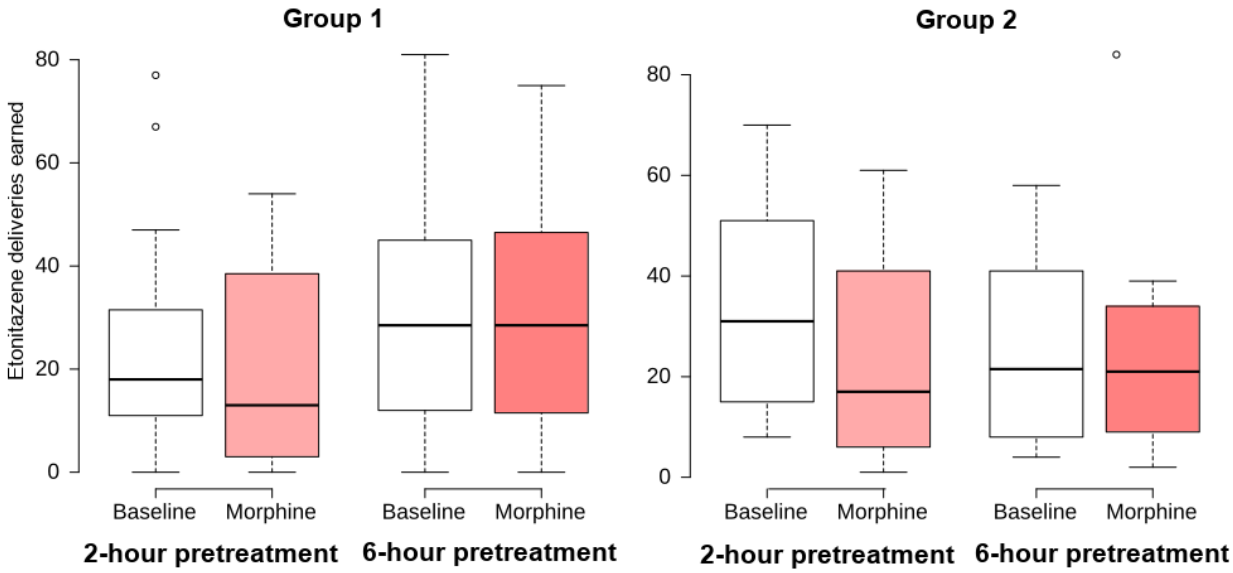


Filled symbols represent sessions in which both etonitazene and food were available following 5 responses on the appropriate lever. Grey symbols represent etonitazene earned when food was in extinction, Note that stimuli were not changed during any of these sessions. Points represent the mean (± S.E.M.) for each session.

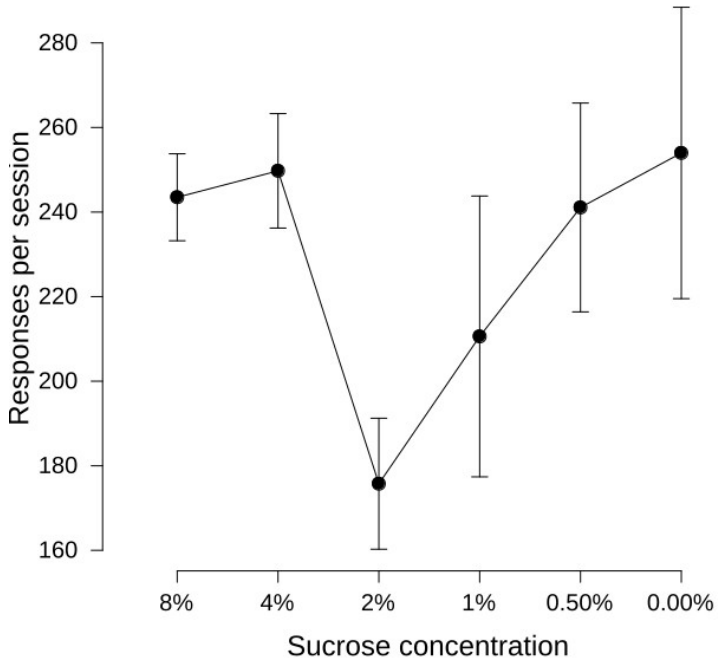
This indicates that "frustration" does not result in excessive substance use due to dysphoria or stress, but rather substance use follows from allocation of behavior within the time available to the individual.

Supplemental opioid effects on responding for etonitazene.
Morphine exposure 2 hours, but not 6 hours, before etonitazene access decreased etonitazene self-administration. Withdrawing supplemental morphine did not have any apparent effect on responding for etonitazene or food (responding remained at pre-supplement levels, not shown). Supplemental opioid (32 mg/kg morphine) administered 2 or 6 hours before operant sessions does not significantly affect responding maintained by etonitazene solution. Rats in Group 1 were able to respond on an alternative lever for concurrently available food (FR150). Morphine administration did not reliably affect responding on the food lever in these rats, which was at low levels (not shown). Rats in group 2 had a history of responding for etonitazene only. In this boxplot, each box represents the interquartile range for etonitazene deliveries earned, whiskers represent the 95% confidence interval for each condition. Filled boxes represent data from 3 sessions following morphine pretreatment (as indicated) for each Group (n=8 and n=7, respectively). White boxes are matched sessions in the

week preceding morphine administration for each subject. A trend of decreased etonitazene earned was present in both groups after the 2-hour pretreatment, though this difference was not significant.

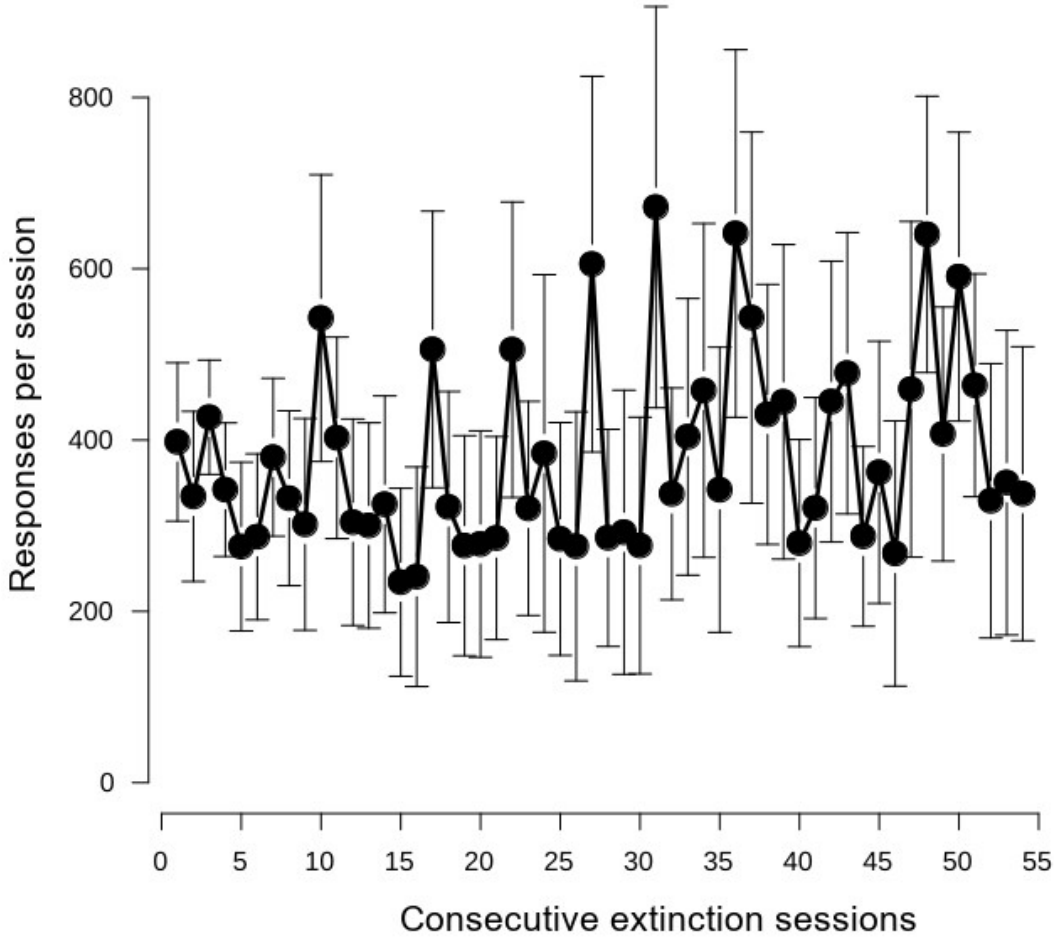


Mice can be trained to respond for etonitazene. Responding for etonitazene (5 µg/ml) in decreasing concentrations of sucrose in water (w/v) by mice (n=8). Each point represents the mean (± S.E.M.) of responding for each solution. Reducing the sucrose concentration had transient effects on the amount of responding the solution maintained, though responding for etonitazene alone was not different from responding for the same concentration of etonitazene in 8% sucrose.



Responding for etonitazene by mice rapidly became habitual. Responding by mice for etonitazene was insensitive to extinction, indicating behavior had become habitual. Although mice robustly responded for the etonitazene solution (with no sucrose), this behavior rapidly became habitual. Eliminating solution delivery did not affect responding for at least 55 consecutive sessions. This insensitivity to devaluation of the opioid indicates the responding had become habitual. Future studies might utilize this to better understand how to reduce habitual (rather than goal-directed) opioid use. Circles indicate the mean (± S.E.M.) number of responses per session for n=8 mice across sessions in which responding did not provide etonitazene access.

The overarching goal of this project was to develop an animal model of oral opioid use, as this is the most



common route of non-prescription opioid use. Oral preparations are long-lived and allow for better modeling of the chronic use common in opioid addiction. We initially attempted to train oxycodone self-administration, but had trouble establishing the behavior, likely due to a combination of poor bioavailability in the rat and low potency (which requires a higher concentration and stronger flavored solution). In the interest of keeping the project on track, we switched to etonitazene. Etonitazene is a mu-opioid agonist with a high potency, similar to high-potency opioids which are increasingly prevalent among opioid users. We were able to train etonitazene self-administration in rats using two different methods; sucrose fading and postprandial drinking induction. Both procedures yielded similar results. Etonitazene maintained responding in a dose-dependent manner, with

doses up to 5 µg/ml maintaining increasing amounts of responding. We found that responding declined more rapidly in extinction (when opioid access was withheld) when rats were trained on a fixed-ratio schedule (opioid delivered after a fixed number of responses), compared with rats trained on a random-interval schedule (where opioid delivery occurred upon a response after variable periods of time had elapsed). This has been reported for other abused drugs, and is considered to indicate that the random-interval schedule produces more habitual responding (which is insensitive to opioid devaluation).

Allowing rats to respond for an alternative reinforcer (food) during alternating daily sessions in the same environment did not affect opioid use, however providing concurrent access to food did reduce opioid use, and this reduction depended on the response requirement for food. Unexpected loss of food access (unsignaled extinction) can be considered stressful, similar to other frustrations common in daily life (i.e. job loss or divorce). We found that unsignaled extinction of food reinforcement did result in a resurgence of opioid use, consistent with such common life stressors precipitating relapse. However, opioid use returned to levels observed before concurrent food access was introduced, indicating that 'frustration stress' is likely better interpreted as a loss of behavioral control over drug use by other contingencies in the environment. This work has been published and emphasizes the importance of maintaining alternative reinforcement, especially during recovery from problematic opioid use, as these alternative activities are highly effective at preventing a return to drug use. Further, rapidly restoring alternative reinforcement can restore behavioral control over drug use, perhaps preventing a lapse from becoming a relapse.

Finally, we found that in rats with a long (~1 year) history of etonitazene self-administration, supplemental opioid administration (morphine, 32 mg/kg, 2 or 6 hours before etonitazene access) did not significantly disrupt responding, despite these morphine treatments having profound behavioral effects consistent with opioid agonist administration, including lethargy and tail rigidity. This is consistent with responding for etonitazene becoming increasingly habitual and thus insensitive to devaluation by a substitute agonist. This suggests that habitual opioid use may be less responsive to pharmacological interventions that blunt the effects of opioid agonists, and emphasizes the importance of developing behavioral therapies that can speed the decline of habitual drug use. Because problematic opioid use is almost necessarily habitual, a shift in focus from pharmacotherapeutic development to therapies that rapidly gain control over drug use is warranted.

5.0 CONCLUSIONS

We succeeded in completing the proposed studies as well as some additional, related research. Our primary Aim was to develop an oral opioid self-administration procedure in rats that can subsequently be used to understand opioid dependence among military personnel and their families. To this end, we successfully:

- Trained rats to respond for and consume an oral opioid solution (oxycodone)
- Trained rats to respond for another opioid solution (etonitazene) which is easier to train, and is more similar to new, high-potency opioids, such as fentanyl, responsible for increasing numbers of overdoses.
- Higher concentrations of etonitazene (up to 5 µg/ml) maintained greater amounts of responding.
- Consumption produced observable signs of opioid intoxication, including tail rigidity, stereotypies, and flat body posture.
- Responding declined when access to the solution was removed (extinction) demonstrating that rats were responding for solution access.
- Responding was reinstated by presenting the empty solution dipper contingent upon lever responses.

We also determined that the way opioid access is provided can increase the likelihood that opioid use becomes habitual. This has implications for how opioids are used therapeutically. Future studies might investigate whether different prescription strategies might reduce the likelihood of patients developing subsequent habitual opioid use.

- Extinction was faster in rats responding under a fixed-ratio schedule compared to rats responding under a random-interval schedule. This slower decline in responding in extinction under the random-interval schedule is characteristic of habitual responding.
- This indicates random-interval schedules function similarly for oral opioid self-administration as for intravenous administration of other abused substances, and further, suggests this is a useful model of habitual oral opioid use.
- Mice might also be trained to self-administer etonitazene, which could help identify genetic factors that produce vulnerability to opioid abuse. However, mice were insensitive to extinction, suggesting habitual responding might develop faster in mice than in rats.

We found that providing supplemental opioid can reduce opioid use, but this effect is transient and depends on the time since the supplemental opioid exposure.

- Supplemental opioid slightly decreased responding for etonitazene when administered 2 hours, but not 6 hours, before etonitazene access.
- Withdrawing supplemental morphine did not have any apparent effect on responding for etonitazene or food (responding returned to pre-supplement levels).
- This suggests that substitute agonist pharmacotherapy (e.g. methadone) might be less useful among habitual opioid users (compared to those who maintain goal-directed use either for the analgesic or the reinforcing effects of the abused drug).

We demonstrated that, as shown for other abused substances, opioid use can be mitigated by providing alternative reinforcement, however this alternative must be concurrently available. Providing alternative reinforcement in the same context where opioid use occurs, but at different times did not affect opioid use.

- Providing alternative reinforcement on alternating days did not affect the amount of opioid consumed when it was available.
- However, providing alternative reinforcement concurrent with opioid availability reduced opioid use. This indicates that providing competing activities might help to reduce opioid use.
- The amount of opioid use varied inversely with the availability of the alternative reinforcer (decreased alternative availability resulted in increased opioid use).

We found that unexpected loss of alternative reinforcement can produce a resurgence of opioid use, though the amount used in this situation did not exceed the levels observed before alternative reinforcement was available. This indicates that sudden loss of alternative reinforcement that is controlling opioid use could threaten sobriety among those who had previously used opioids.

- Unexpected extinction of alternative reinforcement resulted in increased opioid use, but this use did not exceed levels observed before food was made available in the operant chamber. This indicates

that “frustration” does not result in excessive substance use due to dysphoria or stress, but rather substance use follows from allocation of behavior within the time available to the individual.

6.0 RECOMMENDATIONS

- Oral self-administration of opioid solutions in rodents is feasible and represents a route of administration more common in people, particularly service members.
  - This model incorporates both the direct reinforcing effect of the opioid, and also appears to capture the habitual nature of opioid use disorder as well.
  - Rodents allow testing of novel pharmacotherapeutics, behavioral therapies, and combinations which could help optimize and individualize treatment.
  - More research on how best to model each of these treatment modalities is needed.
- Both sucrose-fading and postprandial training resulted in robust, stable opioid self-administration.
  - Sucrose-fading introduces a history of responding for sucrose which may influence the choice to use this procedure
  - Postprandial training eliminates the history of sucrose exposure, and may result in shorter training time.
  - Postprandial training is the recommended training procedure, from these studies.
- Alternative reinforcement can reduce opioid use, but only when it is concurrently available (rather than available in separate instances in the same environment).
  - Patients on longer courses of opioid treatment should be provided alternative activities that can compete with the rewarding aspect of the opioids.
  - The availability of alternative reinforcement can protect against excessive opioid use.
  - Unsignalled elimination of the alternative reinforcement can produce a resurgence in opioid use (if opioid is available in the same environment), and thus should be avoided, to the extent possible.
- Substitute agonist pharmacotherapy may be less useful once opioid use becomes more habitual, and less driven by the direct rewarding effects of the opioid.
  - Timing of the agonist administration relative to the typical time opioid is used is important.
  - Combination of a substitute agonist with alternative reinforcement is likely more effective at reducing non-medical opioid use than either alone.

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APPENDIX A - PUBLICATIONS AND PRESENTATIONS

Presentation: “Unsignalled extinction of alternative reinforcement weakens control of alternative reinforcement over drug use.” College on Problems of Drug Dependence, 2017 annual meeting, June 2017

Manuscript: Ginsburg, B.C., Lamb, R.J., 2018. Frustration stress (unexpected loss of alternative reinforcement) increases opioid self-administration in a model of recovery. Drug Alcohol Depend 182, 33–39. https://doi.org/10.1016/j.drugalcdep.2017.09.016

APPENDIX B - ABSTRACTS

Unsignalled extinction of alternative reinforcement weakens control of alternative reinforcement over drug use.
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Drug use is controlled by both contingencies on its availability and those on its alternatives. When access to these alternatives is suddenly restricted (which might be perceived as stressful), drug use can surge. This may be an important factor in relapse. Others have modeled this using reinstatement. However, it is unclear from these studies if the resurgent drug-seeking leads to resumption of drug consumption that matches or exceeds levels seen before drug use was extinguished. Here, we examine the impact that unsignaled restriction of alternative reinforcement has on resumption of drug use using a model of recovery. Rats were trained to respond when a stimulus light was illuminated for dipper delivery of an etonitizene solution (FR5; 0.05 mg/0.1ml) during 2-hr sessions. Once trained, rats earned 0.06 ± 0.02 mg/kg/day. Subsequently, rats were trained to respond for food on an alternate lever when another light was illuminated (FR5; 45mg pellet) in 2-hr sessions on alternating days. Etonitizene consumption during sessions when it was available remained unchanged (0.06 ± 0.02 mg/kg/day). Once responding for food stabilized (258 ± 32 pellets), both lights were illuminated, and both etonitizene and food were available under independent FR5 schedules. During this period, both etonitizene and food consumption fell relative to sessions where only one was available (Etonitizene: 0.01 ± 0.01 mg/kg/day; Food: 165 ± 10 pellets). Unsignalled extinction of food responding under this condition increased etonitizene consumption (0.04 ± 0.01 mg/kg/day), which returned to the previous level when food was again available (0.02 ± 0.01 mg/kg/day) the following day. Drug consumption increased during subsequent exposures to unsignalled food extinction (0.06 ± 0.02 mg/kg/day after 7 such sessions). Drug consumption when food was again concurrently available was also greater after these exposures (0.03 ± 0.01 mg/kg/day), compared to before. However, over time, drug consumption eventually returned to previous levels (0.01 ± 0.01 mg/kg/day). These results indicate that unsignalled extinction of alternative behavior can transiently increase drug consumption, and that repeated exposure to extinction might weaken control alternative reinforcement has over drug use which may increase the time to recovery once alternative reinforcement is again available..

The experiments reported herein were conducted according to the principles set forth in the National Institute of Health Publication No. 80-23, Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966m as amended.

APPENDIX C - LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS

AFMSA/SGE-C - Air Force Surgeon General's Research Oversight and Compliance Division

FR - fixed ratio schedule of reinforcement: Reinforcement is delivered after the number of responses indicated (e.g. FR5 = five responses)

RI - Random interval schedule of reinforcement: Reinforcement is delivered after a the first response after a random amount of time, this interval averages the duration as described for the entire session (e.g. RI-30sec means on average the first response after 30-sec is reinforced)..

RT - Random time schedule of reinforcement: Reinforcement is delivered after a random amount of time, averaging the duration as described for the entire session (e.g. RT30-sec means delivery occurs on average every 30-sec).

S.E.M. - Standard error of the mean

S.D - Standard deviation