2024

Naloxone and dexmedetomidine reversal of the effects of methamphetamine and fentanyl co-administration

Wesley R. Tackett
westackett31@gmail.com

NALOXONE AND DEXMEDETOMIDINE REVERSAL OF THE EFFECTS OF METHAMPHETAMINE AND FENTANYL CO-ADMINISTRATION

A thesis submitted to
Marshall University
in partial fulfillment of
the requirements for the degree of
Master of Science
in
Pharmaceutical Sciences
by
Wesley Ryan Tackett
Approved by
Dr. Michael Hambuchen, Committee Chairperson
Dr. Cynthia Jones
Dr. Eric R. Blough

Marshall University
December 2024

Approval of Thesis

We, the faculty supervising the work of Wesley Ryan Tackett, affirm that the thesis, *Naloxone and Dexmedetomidine Reversal of the Effects of Methamphetamine and Fentanyl Co-Administration*, meets the high academic standards for original scholarship and creative work established by the Department of Pharmaceutical Sciences and the Marshall University School of Pharmacy. The work also conforms to the requirements and formatting guidelines of Marshall University. With our signatures, we approve the manuscript for publication.

Dr. Michael Hambuchen, Department of Pharmaceutical Sciences	Committee Chairperson	11/1/2024 Date
Dr. Cynthia B. Jones, Department of Pharmaceutical Sciences	Committee Member	11/1/2024 Date
Dr. Eric R. Blough, Department of Pharmaceutical Sciences	Committee Member	11/1/24 Date

Dedication

I would like to dedicate this thesis to rats used to perform these studies. Their sacrifice contributed data to the field of substance use disorder and will hopefully improve human lives in the future.

Acknowledgments

Performing my thesis research and writing my thesis I have experienced many challenges and heartaches, but I have also learned a great many lessons. I was taught these lessons not only by myself but by the following people.

The greatest teacher of these lessons is a title only fit for my thesis advisor, Dr. Michael Hambuchen, for leading me through my master's program. His way of introducing me to tasks and objectives led me to be able to tackle these tasks effectively and accurately. His openness to my ideas has let me experience what a scientific collaboration should be and makes me excited and prepared for the field I am pursuing.

Others deserving acknowledgement include my committee members, Dr. Cynthia Jones and Dr. Eric R. Blough, for their mentorship, encouragement, and open doors for any questions.

Finally, I would like to show ultimate gratitude to my parents Angie and Jeremy Tackett and partner Corazon for helping me survive this daunting task of completing a thesis for they pushed me and provided non-stop support to my mental and physical health along the way.

Table of Contents

List of Tables

List of Figures

Abstract

Agitation is common amongst individuals who have taken methamphetamine (METH) when they arrive at the emergency room (ER). Fentanyl (FENT) and other opioids can mask this agitation which could then unexpectedly present after the reversal of these drugs by naloxone (NLX). Commonly used medications for agitation caused by METH include benzodiazepine sedatives (like alprazolam and similar drugs), but these drugs can produce dangerous sedation and inhibition of breathing when combined with opioids such as FENT. Therefore, the purpose of this MS thesis project was to use a rat model of METH-FENT overdose to test the ability of opioid antagonist NLX and d2 agonist sedative dexmedetomidine (DEXMED) which has minimal effect on breathing (even when combined with opioids) to safely and effectively inhibit the effects of both FENT and METH. Experiments tested for a reduction in agitation by measuring animal movement and safety by monitoring heart and breathing function. METH-induced locomotor activity was significantly reduced after the NLX administration at all DEXMED doses tested ($p<0.05$) to a level similar to rats not administered METH. Blood oxygenation was not reduced by NLX-adjunctive DEXMED in METH treated rats, but it was significantly reduced in the absence of METH. Heart rate was significantly reduced in both DEXMED treated groups, but METH partially attenuated this effect.

Chapter 1: Introduction

Background

Substance Use Disorder (SUD) is a significant issue which impacts public health, the economy, and societal well-being. The United States faces an extensive and growing SUD problem, with opioids being a major contributor. Regions like Appalachia and states like West Virginia are particularly hard-hit due to geographic isolation, economic hardship, and limited access to healthcare. These factors contribute to higher rates of illicit substance use and related deaths, highlighting the need for targeted interventions. The societal problems associated with SUD include a strain on public health systems, an immense economic burden, and numerous social consequences such as strained relationships and increased crime rates. Stigma and discrimination further complicate the issue, discouraging individuals from seeking help and affecting resource allocation for treatment programs (1).

Acute intoxication and with co-administered methamphetamine (METH) and opioids, particularly fentanyl (FENT), is increasing continentwide and is a significant societal concern (2). Although current treatments for opioid overdose involve the administration of naloxone (NLX) which is highly effective for reversing the effects of opioids it does not reduce METH-related effects such as agitation and aggressiveness. METH users frequently present danger to first responders and clinicians. In a qualitative study among first responders who handled METH-related events, there were many reports of fearing for their own safety and the safety of others in the surrounding area due to unpredictable and potentially violent behavior due to METH intoxication (3). Whether NLX in combination with other pharmacological agents could be used to attenuate the unpredictability in patient response after the reversal of acute opioid-METH intoxication has not been investigated to our knowledge.

Significance of the study

Overdoses involving the co-administration of opioids and METH are increasing nationwide. Although NLX is effective for reversing opioid overdose it does not prevent patient unpredictability or aggression against medical personnel (i.e., due to METH co-intoxication) which could negatively affect treatment outcomes and the safety of the patient and the treatment team. The purpose of this study was to investigate whether NLX in combination with DEXMED is associated with decreased patient agitation in a rat opioid-METH overdose model.

Hypothesis

The primary objective of this study was to determine the safety and efficacy of DEXMED as an adjunct to NLX to reduce METH-induced agitation unmasked by the NLX reversal of FENT-induced sedation in combination METH-FENT overdose. We hypothesize that co-administered NLX-DEXMED will reduce METH-induced locomotor activity in rats (i.e., a model of METH-induced agitation) after the NLX reversal of FENT-induced sedation without producing fatal reductions in cardiorespiratory function. To test this hypothesis, two specific aims were pursued:

Specific Aim 1. To determine if DEXMED will reduce METH-induced agitation unmasked by NLX reversal of FENT-induced sedation.

Hypothesis: Combination DEXMED-NLX will reduce METH-induced locomotor activity in rats after the reversal of FENT-induced sedation.

Specific Aim 2. To determine if NLX adjunctive DEXMED will adversely affect cardiorespiratory parameters after the reversal of METH-FENT co-intoxication.

Hypothesis: NLX adjunctive DEXMED will not fatally reduce blood oxygenation and heart rate in METH-FENT co-intoxicated rats.

Chapter 2: Review of the Literature

Introduction

A review of the literature pertinent to the present study is discussed in this chapter. The discussion includes characterization and severity of opioid use disorder, reasons behind the co-administration of FENT and METH, and the presentation and current management of acute METH intoxication and the complications of opioid involvement and the inadequacies of the current standard of care in the context of these co-administered substances. Additionally, we examine the issues associated with acute METH intoxication in the absence of opioids and the rationale for the use of DEXMED as a NLX adjunct to address the acute toxicities of co-administered METH and opioids.

Characterization and severity of opioid use disorder

The extent of the opioid crisis in the United States is alarming and continues to grow. According to the National Survey on Drug Use and Health (NSDUH), millions of Americans are affected by SUD, with opioids being a significant contributor. The opioid crisis has caused tens of thousands of overdose deaths annually (4), marking a severe public health emergency. Despite ongoing efforts to address the issue, opioid-related deaths have increased over recent years. The crisis is particularly severe in regions like Appalachia and states such as West Virginia, where economic hardship, high prescription rates, and limited access to healthcare exacerbate the problem (5). The widespread availability of synthetic opioids like fentanyl has further fueled the crisis, leading to more potent and dangerous overdoses. The opioid epidemic has profound

implications for public health, the economy, and social stability, necessitating comprehensive and sustained efforts to mitigate its impact.

Co-Administration of Opioids and METH: Incidence and Consequences

From 2015 to 2019, data from the NSDUH have shown a substantial increase in overdose deaths involving METH, and this increase is primarily attributed to its combination with opioids. This increase cannot simply be explained by increased METH use. Throughout this period, there was a 43% increase in METH use in adults aged 18 to 64 (disproportionately involving individuals with lower levels of education, lower income, and ongoing legal issues) alongside a staggering 180% rise in deaths involving METH (6). Another study found that from 1999-2021 METH-related mortality rate surged by a staggering 50-fold which was accompanied by a rising percentage of these deaths involving heroin or FENT, reaching a peak of 61.2% in the year 2021 (7). Interestingly, the trend in increasing co-usage of stimulants and opioids can also be observed in online spaces. A study of discussions on the popular internet forum, Reddit, which aligned with the findings in more traditional epidemiological data sources, provided additional insight showing an increase in METH use amongst patients being formally treated for opioid use disorder (8).

Indeed, the patterns of drug overdose in the US are dynamic and the geography of use is major factor. It has been shown that the percentage of US overdose deaths involving both FENT and stimulants have substantially increased in recent years (rising from 0.6% in 2010 to 32.3% in 2021, with a sharp increase starting in 2015) (9). Initially, FENT was most commonly found alongside prescription opioids, benzodiazepines, and alcohol. The pattern of use, however

differentially shifted to heroin-FENT co-involvement in the Northeastern states in the mid-2010s, and nearly universally to cocaine-FENT co-involvement by 2021 (which could also likely be addressed by our proposed intervention). In contrast, Western states shifted to predominantly METH-FENT co-involvement by 2021. The proportion of stimulant involvement in FENT-involved overdose deaths rose in virtually every state from 2015 to 2021. The rise in deaths involving stimulants in general must be understood in the context of a drug market dominated by illicit FENT, which has made polysubstance use commonplace and presents novel health risks and public health challenges.

In addition to national trends in the US, this pattern of METH-opioid overdose has been seen regionally in both urban and rural areas. For example, in Los Angeles County from 2012 to 2021, METH-related deaths involving opioids (particularly FENT) more than tripled (10). Additionally, more than a quarter of these deaths were caused by cardiovascular issues which could be due to the known cardiovascular toxicity of METH (11). From 2010 to 2022 in King County (i.e., Seattle area), 5,815 lives were lost due to overdose, with almost half of those deaths occurring more recently within the last 4 years (12). In the final year of the study, FENT and METH were predominantly involved in these deaths. In addition to direct drug toxicity, of the 149 homicides analyzed as a cause of death in this study in 2021, 35% involved a positive METH screen.

Poly substance use of METH and opioids is a major crisis in rural communities as well. In a survey of 144 participants in rural Oregon communities, 112 reported opioid use; out of 112, 96% also reported METH use (5). In West Virginia from 2013 to 2018, METH-combined with FENT overdose was involved in 337 deaths (13) while rates of fatal METH-related overdose attributed to METH alone remained stable, those involving both METH and FENT or FENT

analogs increased to a significant margin. Further reflecting the changes in the patterns of substance use, deaths involving FENT in the absence of METH decreased, while those involving both FENT and METH, as well as METH alone, increased. Rural communities have significant challenges dealing with polysubstance use including METH, FENT, and alcohol (1). While the public is generally aware of how to address acute opioid toxicity (i.e., NLX rescue), 40% of respondents found METH to be a major concern and a substance for which they were least prepared to address nor confront (as opposed to approximately 20% for FENT which was listed as the second most concerning illicit substance).

In addition, the acute toxicity of this combination disproportionately affects certain populations regardless of geographic location. For example, the 50 years old and above population, are disproportionately at risk of extreme complications with co-administration of opioids and METH (14). This is concerning as there was a steady increase in exposures to individuals 50 and above, from 2015 to 2021, with a notable uptick in METH exposures starting in 2018. Despite limitations in the data, such as lack of substance use history, these findings paired with the increase of METH overdose death rates may suggest a rise in new users of these substances among older adults which is a concern due to the increased toxicity in this population. Major effects or death occurred in nearly a 20% of exposures, with associations found between co-use of opioids and adverse outcomes. Considering biological sex, men statistically have a higher level of hospital admission and overdose death due to concurrent opioid and stimulant use compared to women (15). Using datasets spanning from 1992 to 2020, significant variations were found in treatment admissions and deaths across demographic groups (16). Also, these deaths notably surged among Native American individuals. These findings suggest the

immediate need for tailored interventions, especially considering the disproportionate impact on specific age, sex, and racial/ethnic groups.

The increased toxicity of the combination likely involves behavioral factors in addition to the direct toxicity of the METH-opioid combination. In Seattle, people who concurrently inject METH and opioids (which also had a higher likelihood of experiencing overdose related risks compared to either drug alone) reported both increased injection frequency and riskier injection practices (17). Similarly in Denver, individuals who co-inject METH and opioids are significantly more likely to report an overdose than those who inject either drug alone (i.e., a 2.8-fold greater incidence compared to those who injected heroin alone) (18).

In non-fatal overdoses there was a 669% increase in METH-FENT co-exposure from 2015 – 2021 as determined by National Poison Control data (19). While the increased mortality resulting from the combination of METH and opioids is a key concern, it is important to remember that acute toxicity adversely affects other health and quality of life outcomes as well. For example, METH and opioid combination use is associated with a 99% increase in overnight hospital stays, 46% increase in emergency room visits, 2.1 times higher rates of housing instability, 1.4 times greater use of social services, and 3.3 times more interactions with the criminal justice system compared to individuals using METH alone (20). In Toronto, Canada from 2014 to 2021, there was a significant rise in both amphetamine-related (largely METH) emergency department visits and inpatient admissions with a notable increase in opioid involvements (21). There was also a common co-occurrence of psychotic disorders upon presentation in these patients. This is not surprising considering the known risk of METH-induced psychosis which can occur at variable METH exposures due to genetic differences in sensitivity to this comorbidity (22). Taken together, these findings underscore the urgent need for

targeted interventions and comprehensive strategies to address the intertwined challenges of the acute toxicity produced by combined METH and opioid use in the context of the overdose crisis.

Use of METH and opioid in combination

The co-administration of METH and opioids is commonplace. One uninformed on this issue may be perplexed as to why this practice is so common. Overall, this can be explained by two basic factors: 1) a desire to modify or enhance the effects of one or both drugs; and 2) inadvertent administration of FENT due the adulteration of METH.

A qualitative study based on interviews in a neighborhood in Vancouver, Canada found that the motivation for deliberate co-administration of METH and opioids was to alter or enhance the effects of opioids, to alter or enhance the effects of METH, or to balance the effects of both drugs simultaneously (23). In a similar qualitative study of West Virginian patients who were predominantly focused on using opioids, METH was also co-administered to enhance the subjective pleasurable opioid effects and attenuate symptoms of opioid withdrawal, but the respondents also mentioned opportunistic co-administration of METH during social gatherings due to its low cost and being readily available (24). In a study which featured participants who were frequently binging on intravenous crystal METH, participants used heroin to attenuate the negative effects of the use pattern of METH in addition to co-administering to produce a prolonged more desirable high (25). Similarly, Smith showed that opioids can alter the behavioral effects of amphetamines (26). Therefore, there is a degree of motivation to co-administer both classes of drugs regardless of an individual's drug of choice.

Patients who use METH in an attempt to ward off opioid withdrawal report obtaining this knowledge through both lay knowledge and experimentation (27). This unfortunately often encourages opioid users to begin the co-administration of METH and opioids. Notably, in timeframes where there are shortages of opioids, individuals would often seek out a more available replacement to combat withdrawal, which was oftentimes METH (28). In addition to managing withdrawal, a qualitative study of patients in Dayton, Ohio found that participants commonly believed that METH could prevent or reverse opioid-related overdoses and many had personally used it for this purpose due to challenges accessing NLX and uncertainties about overdose symptoms (29). Similarly, survey respondents in rural Oregon actively shifted from heroin to METH use as a misguided form of harm reduction (5).

Other reasons individuals co-administer METH and opioids can be traced to the homelessness crisis in various regions of the U.S. Many unhoused individuals co-administer to endure harsh conditions without permanent housing, while many who co-use are more likely to become homeless than those who use METH or heroin alone (30). Co-usage is also commonly unintentional, as FENT is frequently found in street METH powder (31). However, many individuals who use METH are not concerned about the adulteration of substance with FENT (30). This is a major issue even compared to intentional combined METH-opioid use as a dedicated METH user will not be tolerant to the acute toxicity of opioids (i.e., respiratory depression) (32). Incidence of this phenomenon is a concern, in a study of emergency department patient presenting with non-fatal overdose, 84% of those who self-reported administering stimulants, benzodiazepines, or cannabis without opioids had positive urine screens for fentanyl (33). In addition, these patients were less likely to have or know how to acquire NLX than individuals deliberately using opioids. Furthermore, in a urine toxicology analysis of 41

individuals in Dayton, Ohio who deliberately were administering METH with what was thought to be heroin or FENT. There was an abundance of non-pharmaceutical FENT-type drugs which included the very potent carfentanil which may increase the unpredictability of this toxicity as well (29).

Current Management of Acute METH Intoxication and the Complications of Opioid Involvement

In a qualitative study amongst individuals who were administered NLX during an opioid overdose, NLX rescue was viewed as a horrible experience featuring both great confusion and heightened emotional responses (34). Regardless, agitation is uncommon with opioid use, and NLX rescue rarely produces agitation in scenarios involving acute opioid withdrawal in after administration at the hospital (35) or by law enforcement (36). The study of law enforcement found, however, some degree of irritability or combativeness when the opioid administered was FENT.

METH, however, more frequently presents danger to first responders and clinicians. In a qualitative study among first responders who handled METH-related events, there were many reports of fearing for their own safety and the safety of others in the surrounding area due to unpredictable and potentially violent behavior resulting from METH intoxication (3). An increased incidence of METH-related events from 2011-2012 and another from 2016-2017 found a large proportion of incidents to require police intervention and hospital transportation in Victoria, Australia (37). In addition to more frequent transportation to the hospital, METH use is

also related to prolonged ER stays and increased use of chemical restraint (38). Among patients with substance use disorders, METH use was associated with increased ER usage overall (39).

Various descriptions of stimulant-related (mostly METH or amphetamine-type stimulants) emergency department visits found that patients presented varying psychological and behavioral concerns such as psychosis, self-harming behaviors, hallucinations, and others. Medical concerns also included palpitations, nausea and vomiting, and significant physical injuries (40). However, those with METH use disorder presenting with psychosis had a high rate of emergency room and acute inpatient care services (41). A Swiss retrospective study on METH presentations in the ER found acute presentations within 72 hours of usage, which included agitation, hypertension, tachycardia, sleep disturbances, and aggression (42). There are biological sex dependent differences in this ER presentation as well. For example, women were less likely to become aggressive, but more likely to have psychiatric comorbidities while men were more likely to be admitted to the ICU (43). Overall, agitation is a major concern in acute METH intoxication as it is dangerous for both the healthcare provider and patient and interferes with patient care (3, 37, 40, 42).

Presentation with combined exposure to METH and opioids is made more challenging by the ability of opioids to mask the effects of METH (or vice versa depending on the dose of each agent) (44). For example, in a case report, a 30-year-old male was found to have a very high, normally fatal FENT blood concentration but was somewhat responsive due to the high concentration of METH in his plasma (45). Considering this, our concern is that METH-induced agitation and cardiovascular toxicity may be masked by concurrent opioid exposure and rapidly unmasked by NLX administration. Some related case studies demonstrate this phenomenon with

NLX administration resulting in ventricular tachycardia in a patient concurrently intoxicated on a stimulant (cocaine in these cases) and an opioid (46-48).

Currently METH-induced agitation is managed by the administration of benzodiazepines (49), but with the increasing trend of METH-opioid co-use, this therapeutic intervention may produce respiratory, depression, coma, or even death if the FENT dose administered is inadequately antagonized (50). Antipsychotics (e.g., haloperidol) have been used to manage METH-induced agitation and psychosis, but use of these agents causes patients to have an increased length of stay compared to not administering drugs of this class (51). High dose amphetamine produces additional locomotor activity when administered to rats pre-treated with haloperidol (52). ER management of individuals who are acutely intoxicated with METH can be resource intensive in general (53). Considering the additional challenges of treating METH-induced agitation in patients intoxicated with opioids, new therapeutic strategies are needed to manage these patients.

Dexmedetomidine (DEXMED) as a Naloxone (NLX) Adjunct for Co-intoxication with METH and opioids

DEXMED is a sedative which works by binding the alpha-2 adrenergic receptor and has other beneficial analgesic, antianxiety, and cardiovascular effects (Lee, 2009). In addition to being approved for use as a general anesthetic, it was also effective in (54, 55) and approved for (56) agitation in patients with bipolar disorder and schizophrenia. While not currently first line for METH-induced agitation, it has been shown to effectively treat METH-induced agitation in humans even when the agitation was not adequately controlled by the benzodiazepine standard

of care (57) and can do so while producing less delirium in patients than benzodiazepines (58). Clinically, DEXMED rarely causes respiratory depression in humans alone (59, 60) or when combined with opioids (58, 61). For example, in a study involving 69 female patients, the use of a combined propofol and remifentanil during a hysteroscopy resulted in 40% incidence of respiratory depression. When DEXMED was used in place of propofol, this figure dropped to 10% (61). The reduced risk of respiratory depression in combination with opioids may make this agent particularly suited for use in a patient co-intoxicated with METH and opioids.

Another advantage of this agent is that it is absorbed by a variety of routes of administration including the sublingual (55, 56), intramuscular and intranasal routes (62) which could be useful in a particularly agitated patient for which it is challenging or impossible to place an intravenous line. Unlike the standard of care benzodiazepines (DEA Schedule IV), DEXMED is not scheduled by the DEA (59) which improves the ease of both ordering and recordkeeping for clinicians. It also has physiochemical properties which allow for co-formulation with NLX (63) which may be useful in known cases of simultaneous METH-opioid intoxication.

The most reported adverse effects of DEXMED are cardiovascular (e.g., hypotension and bradycardia) (59), but considering the cardiovascular effects of METH (e.g., hypertension and tachycardia) (11), this may be beneficial in the treatment of METH-induced agitation alone or after the NLX reversal of opioid induced sedation masking these METH effects. The agent should be administered with care as while higher doses of DEXMED result in increased sedation, they also cause significant decreases in heart rate, cardiac output, and stoke volume (64).

In summary, there has been a rise in overdose deaths due to the combination of opioids with METH which is reflective of the rising trend of polysubstance abuse posing intense health

risks. METH and opioid co-administration is rampant despite the intense health risks due to the adulteration of METH with FENT and the motivation of individuals to alter the effect of one or both drugs. Treating acute toxicity of this co-administration is difficult due to the METH-induced agitation and cardiovascular toxicity. Benzodiazepines are currently indicated for METH-induced agitation, but when opioids are present, benzodiazepines could potentially produce additional respiratory depression. Therefore, DEXMED, which produces sedation with minimal risk of respiratory depression, is likely to show promise as a potential adjunct to NLX in the management of patients co-intoxicated with METH and opioids.

Chapter 3: Co-administration of NLX and DEXMED to simultaneously reverse acute effects of FENT and METH in rats

Abstract

Agitation is relativity rare after naloxone (NLX) reversal of opioid intoxication, but it is a common feature of methamphetamine (METH) intoxication. Since METH-induced agitation can be masked by opioid-induced sedation, NLX administration in the increasingly common occurrence of combined METH-opioid could potentially be dangerous for both patients and healthcare providers. This study examined the effectiveness of dexmedetomidine (DEXMED), an α2 agonist sedative, in reducing METH-induced agitation following NLX reversal of fentanyl (FENT)-induced sedation in rats. The results showed that DEXMED significantly reduced METH-induced locomotor activity without impacting blood oxygenation, although heart rate was reduced. These findings suggest that NLX-adjunct DEXMED may be a safe and effective treatment for combined METH-opioid intoxication, though clinical testing is necessary.

Introduction

METH-induced agitation is a major concern upon presentation in METH-intoxicated patients (3, 37, 40, 42), and opioids can mask these effects in humans (44). Opioid antagonist NLX can unmask these effects, as a result, we targeted the reduction in agitation for our major endpoint. We used METH-induced locomotor activity as an animal model of agitation based on previous studies (65-67) and the relevance to the pathophysiology of agitation. The severe restlessness and motion involved in agitation is produced by supraphysiological release of neurotransmitters such as dopamine, norepinephrine, acetylcholine, and glutamate with an

inadequate release of serotonin and GABA (68, 69). METH produces locomotor activity by producing supraphysiological release of both dopamine and norepinephrine (70).

Considering that we wanted to recreate the human phenomenon of opioids physiologically antagonizing the effects of METH (44), we chose rats rather than mice for this study. This is due to opioids producing increased locomotor activity in mice in contrast to producing sedation in rats (71) which is more comparable to the drug's effect in humans.

The subcutaneous (SC) route was chosen because METH is 100% bioavailable by this route in rats and is absorbed quickly enough to produce both a level and pattern of locomotor activity similar to that produced by an intravenous dose (72). This is clinically relevant because illicit METH is mostly administered through rapidly absorbed routes of administration in humans including inhalation by smoking, intranasal insufflation, and intravenous injection (73). The 1 mg/kg METH dose was chosen due to its consistency in producing a robust locomotor response in rats (72, 74). FENT was administered as the opioid in this study due to its increasingly common co-involvement in human METH overdose mortality (7, 10) and adulteration of the illicit METH supply (30, 31). The 0.1 mg/kg dose of FENT was chosen based preliminary studies which showed it to reliably inhibit the locomotor effects produced by up to 3 mg/kg SC administered METH in rats over the course of 15 minutes.

Groups of rats not administered METH (i.e., intoxication with FENT alone) were added to the study to test the tolerability of our intervention when METH is not present. This was important as while DEXMED has been shown clinically to attenuate METH-induced agitation (57), amphetamine is known to reverse the sedative effects of DEXMED in rats (75). After rats were injected with FENT ± METH, they were placed into a secondary chamber prior to NLX ± DEXMED reversal to minimize the disturbance of animals in the open field behavior chambers

where the locomotor activity data is collected. This decision was made based on the DEXMED in humans can produce unique type of sedation in which the patient is more easily awakened and is able to follow basic directions (60). After the locomotor studies, we performed an additional test to determine DEXMED's effect on cardiovascular (i.e., heart rate) and respiratory (i.e., blood oxygenation as SpO2%) parameters in this preclinical scenario (i.e., an aggressive dose of DEXMED with no respiratory support in the presence of FENT ± METH and potentially incomplete reversal of FENT by NLX).

Materials and Methods

Drugs and Chemicals

(+)-METH hydrochloride provided by Sigma Aldrich was dissolved in sterile normal saline to make a 1 mg/ml free base solution which could be administered easily in at 1 ml/kg (i.e., 300 µl solution administered to a 300 g rat). FENT (human grade) was provided in solution at 0.05 mg/ml free base by Hikma Pharmaceuticals (Berkeley Heights NJ). This required administration at 2 ml/kg to produce the desired 0.1 mg/kg dose. NLX hydrochloride (human grade) at 0.4 mg/ml was provided by Viatris (Canonsburg, PA) and Hikma Pharmaceuticals. For the non-DEXMED treated groups it was diluted in normal sterile saline alone to 0.1 mg/ml. For the DEXMED treated groups, it was co-formulated with DEXMED at this concentration prior to daily experiments. The veterinary grade DEXMED hydrochloride at 0.5 mg/ml free base was provided by Dechra (Cheshire, CT). It was diluted to 0.032, 0.056, or 0.1 mg/ml in combination with the 0.1 mg/ml NLX at the start of each experimental day. NLX ± DEXMED was administered at 1 ml/kg. All drugs were administered via the SC route.

Animals

Male Sprague Dawley rats weighing 212-251g and aged approximately 6-7 weeks old were obtained from Hilltop Lab Animals (Scottsdale, PA) n = 8 in all four experimental groups. These rats were housed two per cage in a climate-controlled room in the Robert C. Byrd Biotechnology Science Center at Marshall University under a 12 hr on/12 hr off light cycle, a temperature of 21-22°C, and humidity of 40-55%. These parameters were monitored by a Centron system (Raes Scientific, Trenton NJ). The animals were provided with *ad libitum* Rodent Diet 5001 (LabDiet, St Louis MO) and filtered water. Animals were handled by investigators with utmost care and gentle handling (see section below). The experiments conducted were in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health and was approved by the Marshall University Institutional Animal Care and Use Committee (protocol #821).

Detailed notes on animal handling

Excessive force was never used to restrain an animal. Rats were handled as gently as possible to maintain their calmness. If they became uncomfortable, the rats would let one know by vocalization (i.e., a squeak) or full body movement to escape restraint (typically both). We made all efforts to prevent these behaviors by focusing on rat comfort outside of any planned experimental intervention. We did this by handling the rats prior to the experiments, for at least 10 min beginning at least 2 days before conducting any experiments. When conducting the handling we use a standardized blue fabric surgical towel. This towel was wrapped around the entire body of the rat and no body parts exposed for the initial handling of the rat. Once wrapped the rat should be either held on a flat surface or the lap of a person. The superior handling position is debatable as the flat surface gives them stability and is used with the rat covered in a

towel to administer injections during the study, but a person's lap provides warmth and could possibly aid in comfort when first being held. A hand should always be placed on top of the rat during handling to apply slight pressure to simulate and prepare for restraint during injections. This also provides some warmth aiding in comfort. After at least one session of handling the rat, we give a slight pull of their skin on their backs to stimulate the start of an injection, as well as a very gentle pinch. The rat is never pinched too forcefully to prevent any aversion to the towel prior to the start of these experiments. Overall, we attempt to be mindful and make every effort to maintain the comfort of the animals and give them some leniency in movement while in the towel to not completely restrain them. In addition, since the MouseOx Plus pulse oximeter was used in these experiments, the rats were handled in the blue towel for five of the ten minutes wearing a training collar which simulates the collar detector which interfaces with the instrument. The rats gently chewed after they acclimated to the towel and sometimes let out an audible clicking. After the handling procedure, some rats even gently chewed on the gloves of the researcher handling them. Once the experiments began the rats were handled throughout, so there was no need for the researcher to spend dedicated time acclimating the rats.

Overall Experimental Design

All animal experiments were conducted in the Robert C. Byrd Biotechnology Science Center at Marshall University in a dedicated laboratory that was maintained at approximately 24°C. There were four experimental groups. All four groups were administered FENT as an intoxicant with two groups additionally being administered METH. Likewise, all four groups were administered NLX as a reversal agent with two of these groups being administer DEXMED.

Locomotor activity: day -3 (saline background activity), 0 (match pairing data), 1, 2, and 3
HR/SpO2: days 8 (baseline data) and 9

	Time of SC injection (mg/kg dose in parenthesis)							
	-15 min		0 min					
	Day -3	Days 0-3, 9	Day -3	Day 0	Day 1	Day 2	Day 3	Day 9
All rats	SAL	FENT (0.1)	-	NLX (0.1)	NLX (0.1)	NLX (0.1)	NLX (0.1)	NLX (0.1)
METH-SAL	SAL	METH (1)	-	-	-	-	-	-
METH-DEXMED	SAL	METH (1)	-	-	DEXMED (0.032)	DEXMED (0.056)	DEXMED (0.1)	DEXMED (0.1)
SAL-SAL	SAL	SAL	-	-	-	-	-	-
SAL-DEXMED	SAL	SAL	-	-	DEXMED (0.032)	DEXMED (0.056)	DEXMED (0.1)	DEXMED (0.1)

n=8 rats in all 4 experimental group with repeated trials on day -3 through 9
Body weight was measured on each day + 24 hrs post day 3 drug administration

Table 1 ***Experimental Schedule:*** *The overall experimental design displaying the experiments conducted on each day.*

Considering this, we have described the groups based on the agents administered. SAL being listed first denotes the administration of FENT alone as an intoxicant. SAL listed second denotes NLX alone as a reversal agent. METH being listed first or DEXMED being listed last denotes that they were added to FENT as an intoxicant or NLX as a reversal agent, respectively

- METH-SAL
- METH-DEXMED
- SAL-SAL
- SAL-DEXMED

See the above schematic for the drug exposures in each group on each experimental day.

Locomotor Activity Studies

On days 0-3, rats were SC administered 0.1 mg/kg FENT immediately prior to SC administration of 1 mg/kg METH or SAL at a separate site prior to being placed into an empty

rat cage as FENT produced sedation (note: bedding was removed to avoid obstruction of breathing in sedated rats). In addition, it prevented the disturbance of other rats in the primary chambers who had already been administered DEXMED since the drug produces a unique state of sedation with the potential for arousal by external stimuli when needed (60). A secondary camera was used to record this 15-minute session prior to reversal to later be scored by a blinded observer for detection of any body movement to verify the FENT-induced sedation.

Rats were SC administered 0.1 mg/kg NLX alone or co-formulated with 0, 0.032, 0.056, or 0.1 mg/kg DEXMED (on days 0, 1, 2, and 3, respectively) 15 min after FENT ± METH administration and placed into a 58 cm x 58 cm x 74 cm polyethylene open-field chamber. Five hrs of locomotor activity were measured with overhead cameras interfaced with the Ethovision 14 software automated behavioral analysis software (Noldus Information Technology, Inc., Sterling, VA). Data was reported both as total distance traveled and distance traveled in 5-minute bins to allow for the determination of pattern activity over time. Note that additional free-standing lights were added to minimize shadows in the behavioral chamber and improve body detection by the automated behavioral analysis system.

On day 0, rats were administered either 0.1 mg/kg FENT ± 1 mg/kg METH followed 15 min later by 0.1 mg/kg NLX prior to the measurement of locomotor activity. The total distance traveled within the FENT and FENT + METH treated groups was used to match pair the animals into the SAL-SAL/SAL-DEXMED and METH-SAL/METH-DEXMED groups, respectively (i.e., comparable average activity in the paired groups). Daily escalation of DEXMED doses to 0.032, 0.056, and 0.1 mg/kg co-formulated with 0.1 mg/kg NLX were administered on days 1, 2, and 3, respectively in the SAL-DEXMED and METH-DEXMED groups. The SAL-SAL and METH-SAL groups were repeatedly administered NLX alone on these days.

Three days before the first drug administration (i.e., day -3), all animals were SC administered saline in the place of FENT and METH and were held in the secondary pre-reversal chambers for 15 minutes. They were then placed into the open field behavioral chambers for the baseline locomotor activity measurements. The rats received 2 ml/kg of normal sterile saline and 1 ml/kg of normal sterile saline to mimic the same volumes that are used for the FENT and METH administrations, respectively. No injections were made to mimic the reversal agents NLX ± DEXMED as the rats would be under FENT-induced sedation and analgesia and not sense the reversal agent injection. Rat weight data was collected on all experimental days and 1 day after the final drug administrations in the locomotor study as well.

Heart Rate and Oxygen Measurement Studies

On experimental day 8 the rats were placed into the standard rat cages with bedding removed for the baseline measurements of the HR and SpO2 using the MouseOx Plus system (STARR Life Science Corp., Oakmont PA) interfaced with the collar sensor which can be used in conscious rats. The 0.1 mg/kg FENT ± 1 mg/kg METH administration from the locomotor study was repeated on day 9 followed by the rats being placed into the standard rat cage. The collar sensor was applied to the sedated rat approximately 10 min after FENT ± METH administration to start measurement of the cardiorespiratory parameters. The NLX ± DEXMED was administered at 15 minutes post FENT ± METH administration as the rat continued to lie in the cage. The HR and SpO2 measurements continued until either stable values were measured or 10 mins post reversal. If values were still increasing, the measurements at 10 mins were still used. The average values for both parameters from a stable 10 second span were reported as described by Raleigh et al. (76).

Note that we determined that the MouseOx Plus system would more effectively detect rat cardiorespiratory parameters (i.e., to start data collection within the desired time point) if the software was closed and re-opened between rats. The "optimization" box was checked until the red and yellow real-time signal was mostly aligned and produced a repetitive pattern which had a wavelength which only uses a fraction of the viewing area (i.e., approximately 10 – 20%). When this is achieved, the "optimization" box was unchecked. If this did not produce a reliable signal, we readjusted the collar around the rat's neck and/or verified that the cables were correctly connected to the system. Body temperature data was collected as an additional measure of safety just after the collection of the day 9 HR/SpO2 data.

Additional Detail on MouseOx Plus Operation:

Using MouseOx Plus (STARR Life Sciences Corp, Oakmont PA) for the HR and SpO2 data collection we followed a pattern of learning how to best utilize the equipment. The equipment was slowly tuned to give reliable and usable data. This was accomplished in the following ways:

When dealing with the fur of the rats, we shaved the rats on the locations in which the collar will interface with the animal. This insured that even though we used white rats for this experiment (black rats must be shaved to use this equipment properly), we were still able to get the infrared light to penetrate the skin and acquire the readings. We also maintained a rat housing chamber that was familiar to them and devoid of bedding to ensure we did not have any loss of SpO2 due to the nose of the rat from being obstructed and reducing breathing during sedation. When placing the collar onto the specimen the light of the collar should be placed on the right

lateral side of the rat. Once placed, one of the researchers involved can maintain a low amount of tension on the collar cord to make sure the movement of the specimen doesn't cause the collar to fall off or be moved out of position. The other researcher operating the computer restarted the program for each rat to ensure that the calibration doesn't carry over from the previous specimen. Once opened, it was ensured that the correct settings for the kind of test, specimen, and collar being used were selected (i.e. the large collar is marked). When beginning testing we lowered the sensitivity to movement slider down to be less sensitive to movement to better gather results. With a sedated rat, the slider is typically left in the center due to there being little to no movement from the specimen. The check box to calibrate the collar should be run first before gathering data. After calibration is complete there will be a constant pattern of peaks and values of moderate range which fit nicely in the pulse pattern screen. After this pattern is shown begin collecting results and time them accordingly for efficient bookkeeping. Re-calibration can be done during the collection of data if the collar of the specimen is removed and re-equipped. The system tries to collect more data than needed so it is recommended to uncheck the boxes of data that do not need to be collected to ensure the scientist can focus on the results of interest being collected. Stable readings can be a sign that the system is correctly collecting data but beware that the data may be shifted due to other elements such as the specimen hunching, moving, or performing erratic treatments.

Data and Statistical Analysis

Locomotor activity for each group was graphed over time in 5 min average bins + standard deviation. Mean bars surrounded by scatter plot data were used to report total distance traveled (locomotor activity during full 5 hr trial). Mean lines surrounded by scatter plot data

were used to report HR and SpO2 data. The percentage weight change (from day 0 pre-drug exposure weights) and temperature data were plotted as mean and standard deviation for each group. A one-way ANOVA was used to compare temperature data between groups. All other data was analyzed with two-way ANOVA tests using the experimental day/increasing DEXMED dose (when administered) as the within-subjects factor and the drug combination administered (i.e., experimental group) as the between-subjects factor. If significance was found with the ANOVA test, a Holm-Šídák's multiple comparisons test was performed. The statistical analysis was performed with GraphPad Prism V9 (La Jolla CA).

Results

Locomotor Data

The treatment blinded scoring of animal body movements for ~15 min after FENT ± METH administration showed that there was minimal rat activity from the FENT-induced sedation before the reversal NLX ± DEXMED administration (Fig 1).

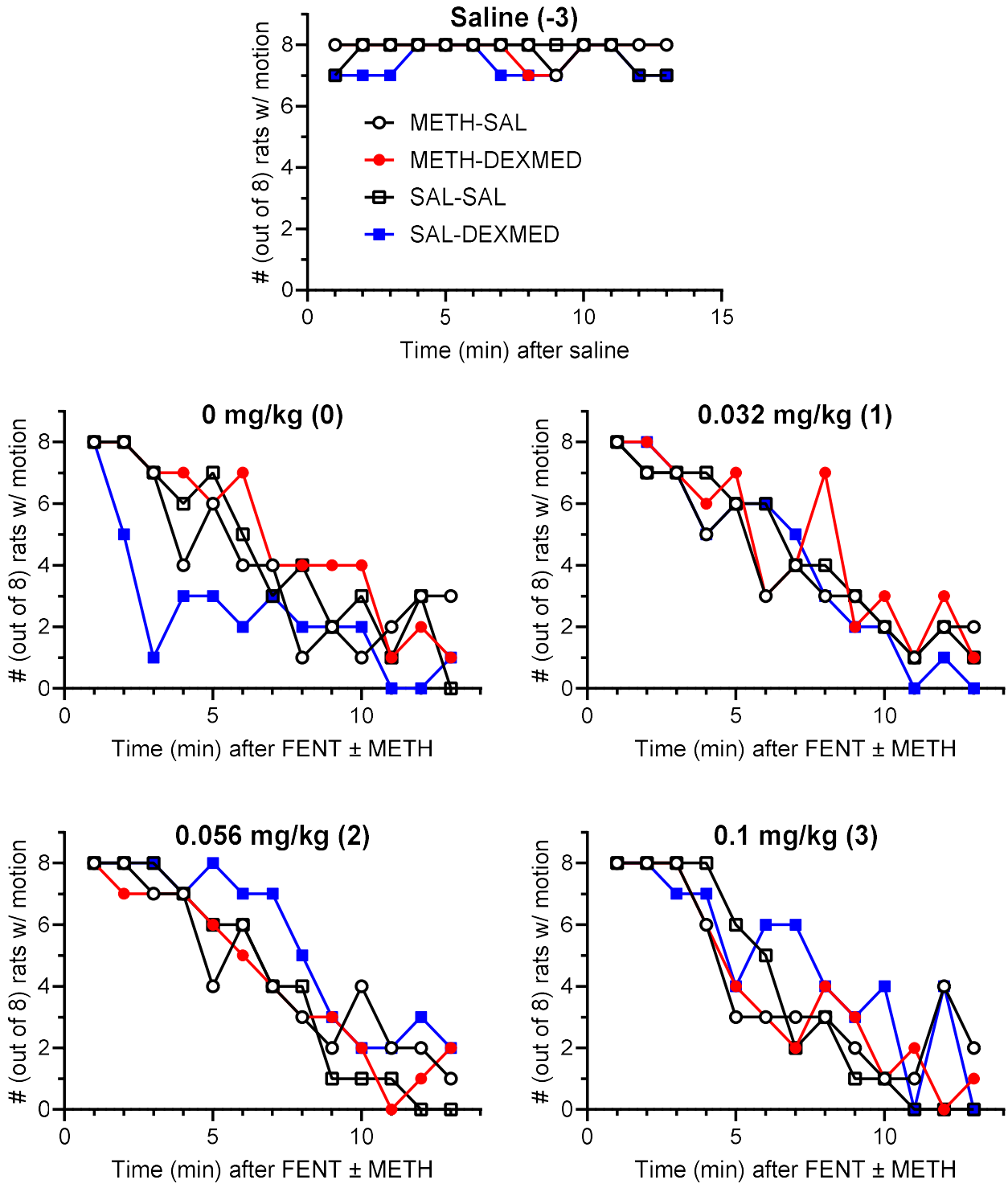

Figure 1: ***Movement scoring data.*** *Note that each graph is labeled with DEXMED dose (if administered) and experimental day in parenthesis.*

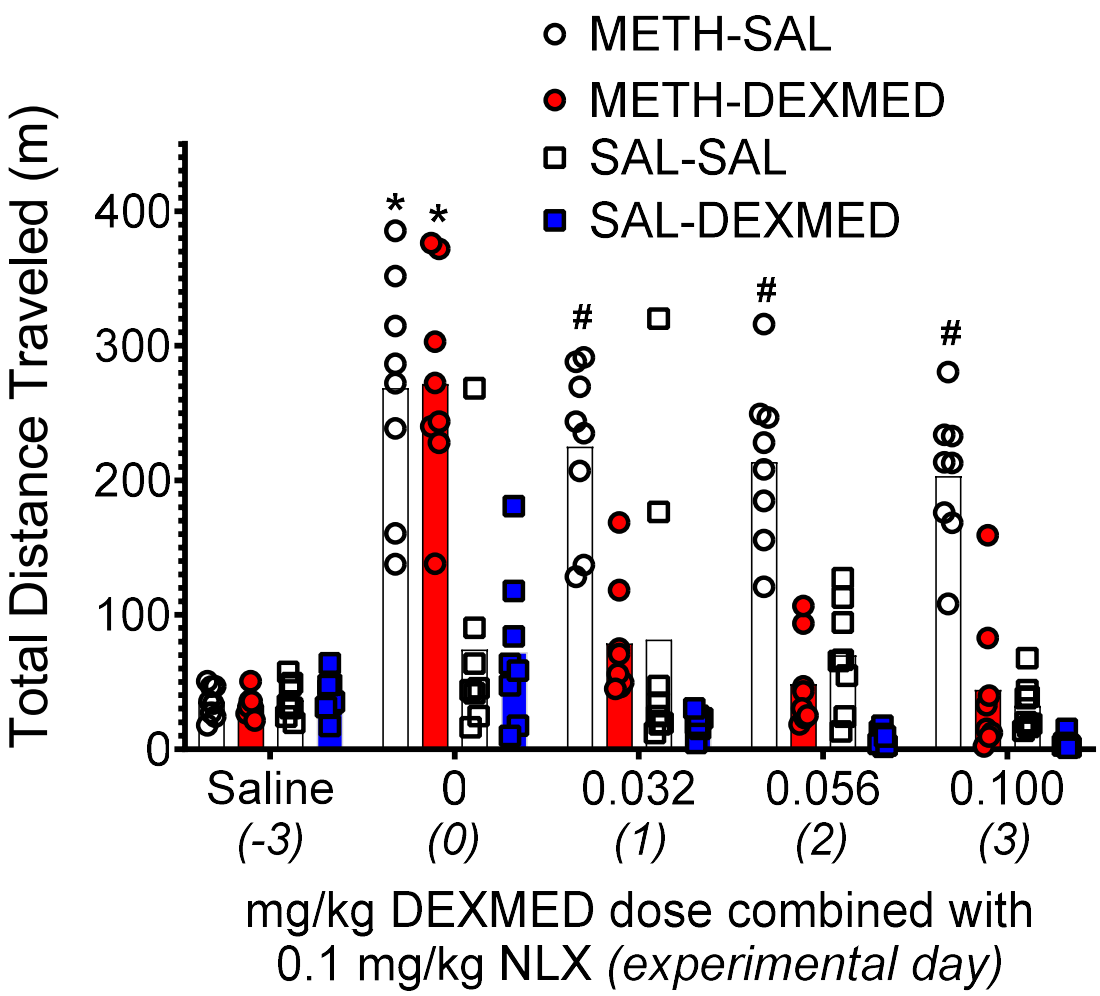

Figure 2: ***Average post- NLX ± DEXMED reversal total distance traveled data*** *(i.e., data from full 300 min trial). *Significant difference compared to SAL-SAL and SAL-DEXMED groups ($p < 0.05$). #Significant difference compared to SAL-METH, SAL-SAL, and SAL-DEXMED groups ($p < 0.05$).*

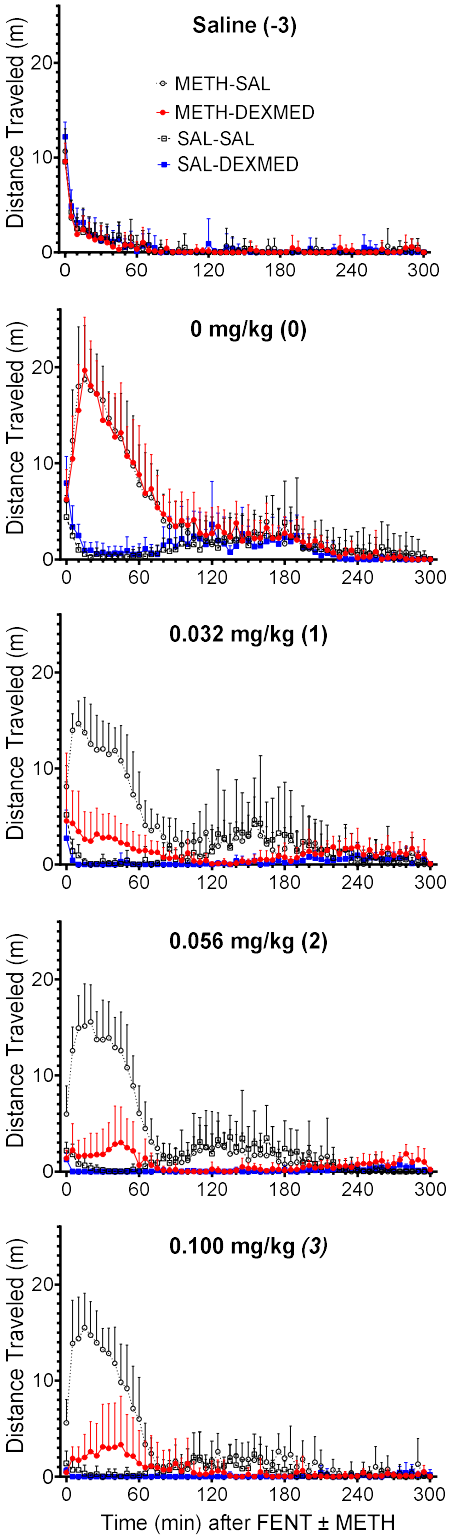

Figure 3: ***Locomotor activity over time.*** *Note that each graph is labeled with DEXMED dose (if administered) and experimental day in parenthesis.*

The rats were matched paired by using the data collected on day 0 into the METH-SAL and METH-DEXMED or SAL-SAL and SAL-DEXMED groups (Fig 2). Since there was a statistically significant interaction between the experimental day and treatment group [$F(12,112)= 13.1$, $p <0.0001$], a post hoc Holm-Sidak's multiple comparisons test was conducted to compare the different experimental groups on each of the day of the study. On day 0, both METH treated groups (METH-SAL and METH-DEXMED) had significantly ($p<0.05$) greater locomotor activity (as total distance traveled) compared to the groups not receiving METH (SAL-SAL and SAL-DEXMED). The activity in the METH-SAL group was significantly greater than that in all the other groups on days 1-3. Therefore, all administered doses of DEXMED reduced post-FENT reversal METH-induced locomotor activity to a level comparable to the groups not receiving the METH.

The average distance traveled in 5-minute intervals, (+SD) over time after the NLX ± DEXMED administration at 0 minutes illustrates the pattern of locomotor over time (Fig 3). On day 0 (i.e., prior to DEXMED administration on days 1-3), all the groups initially expressed increased locomotor activity after NLX was injected. In the METH-SAL and METH-DEXMED groups, activity increased over the 15 mins. In the SAL-SAL and SAL-DEXMED groups there was a decrease in activity over this period. Note that the mostly superimposable activity in the METH-SAL/METH-DEXMED and SAL-SAL/SAL-DEXMED groups on day 0 further demonstrates the effectiveness of our match pairing based on total distance traveled data from this day (Fig 3). Plotting the data in this manner also showed that there was a delayed two-hour period of lower level but detectable locomotor activity in the absence of DEXMED regardless of the presence of METH.

Cardiorespiratory/Weight/Temperature Data:

The blood oxygenation (SpO2), cardiovascular (HR), and temperature parameters were measured on day 9 with the highest DEXMED dose (0.1 mg/kg) as measures of safety (i.e., to determine if there were any unexpected adverse reactions between the any incompletely antagonized FENT and NLX co-administered DEXMED) (Fig 4).

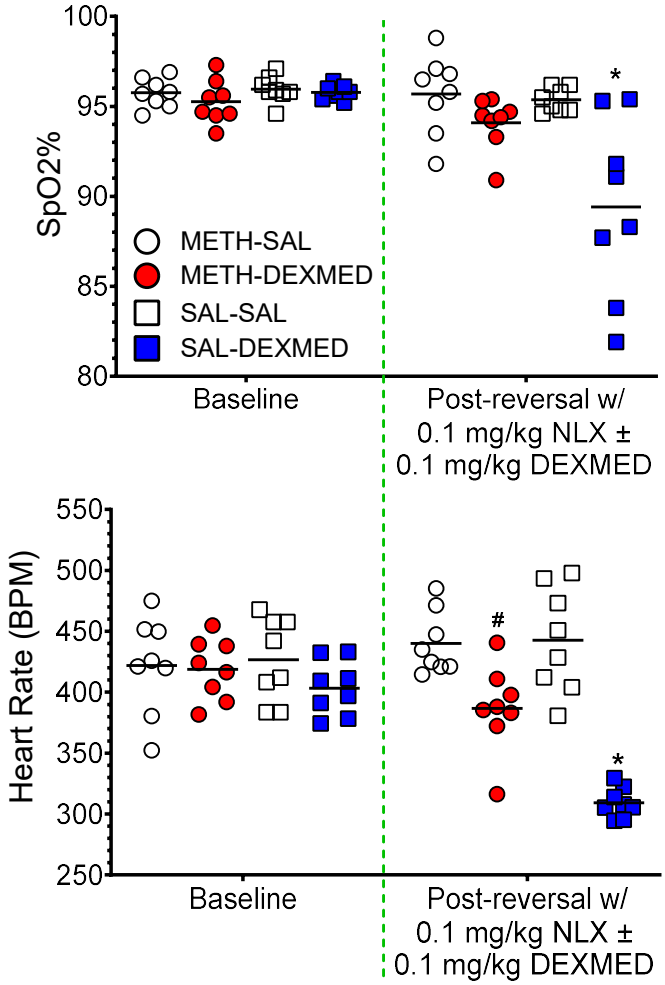

Figure 4: ***Baseline and post-NLX ± DEXMED reversal of fentanyl ± METH SpO2 and HR****. The solid lines depict average values within each group. *Statistically significant difference compared to METH-SAL, METH-DEXMED, and SAL-SAL groups ($p<0.05$). [#]Statistically significant difference compared to METH-SAL, SAL-SAL, SAL-DEXMED groups ($p<0.05$).*

A statistically significant interaction was found between experimental day and treatment was found in the SpO2 safety study [$F(3,28) = 7.505$, $p = 0.0008$]. Therefore, a post-hoc Holm-Šídák's multiple comparisons test was conducted to compare the experimental groups on both the baseline (day 8) and experimental (day 9) days of the study. There were no significant differences between the groups in day 8 baseline measurements despite match pairing being based solely on locomotor activity data. There were no significant differences between the SpO2 measurements in the METH-SAL, METH-DEXMED, and SAL-SAL groups. However, there was significantly lower SpO2 in the SAL-DEXMED group compared to the other three groups. The HR data similarly also showed a statistically significant interaction [$F(3,28) = 14.57$, $p < 0.0001$] and was followed up with a post-hoc analysis as well. As with SpO2, the SAL-DEXMED group displayed significantly lower HR than all the other groups. There was a significant reduction in HR in the METH-DEXMED compared to the SAL-SAL and SAL-METH (i.e., the non-DEXMED treated groups), but the HR in this group was significantly greater than the SAL-DEXMED group.

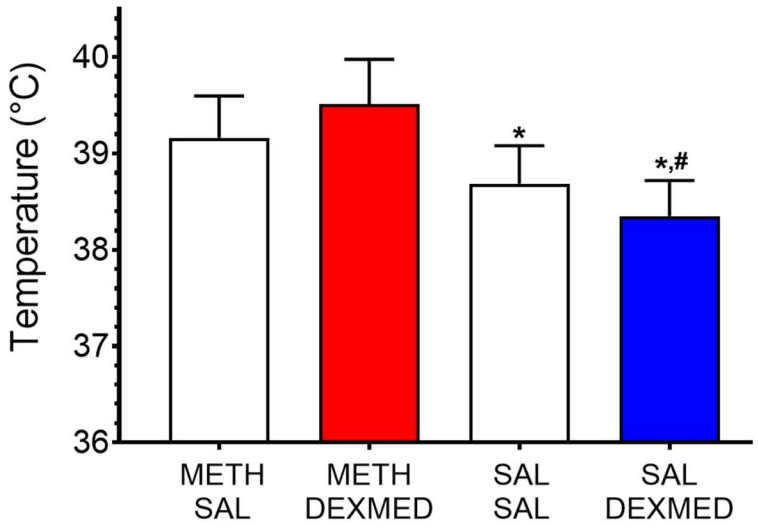

Figure 5: ***Post-SpO2/HR Temperature****. *Statistically significant difference compared to METH-DEXMED group (p<0.05). #Statistically significant difference compared to METH-SAL group (p<0.05).*

Since we detected significant differences in body temperature using a one-way ANOVA [$F(3,28) = 12.07$, $p < 0.0001$], we performed a post-hoc analysis and found that temperature was significantly lower in the SAL-SAL and SAL-DEXMED groups compared to the METH-DEXMED group and significantly lower in the SAL-DEXMED group compared to the METH-SAL group (Fig 5). These data provide some evidence that DEXMED may not attenuate the hyperthermic effects of METH.

In the percent weight change data (Fig 6), there was a statistically significant interaction between the experimental day/DEXMED dose and treatment [$F(9,84) = 2.333$, $p = 0.0212$], therefore we performed a post-hoc analysis. This analysis showed that by day 3 (i.e., 24 hrs after the 0.056 mg/kg DEXMED dose), the % weight change in the METH-DEXMED, SAL-SAL, and SAL-DEXMED groups was significantly greater than the METH-SAL group.

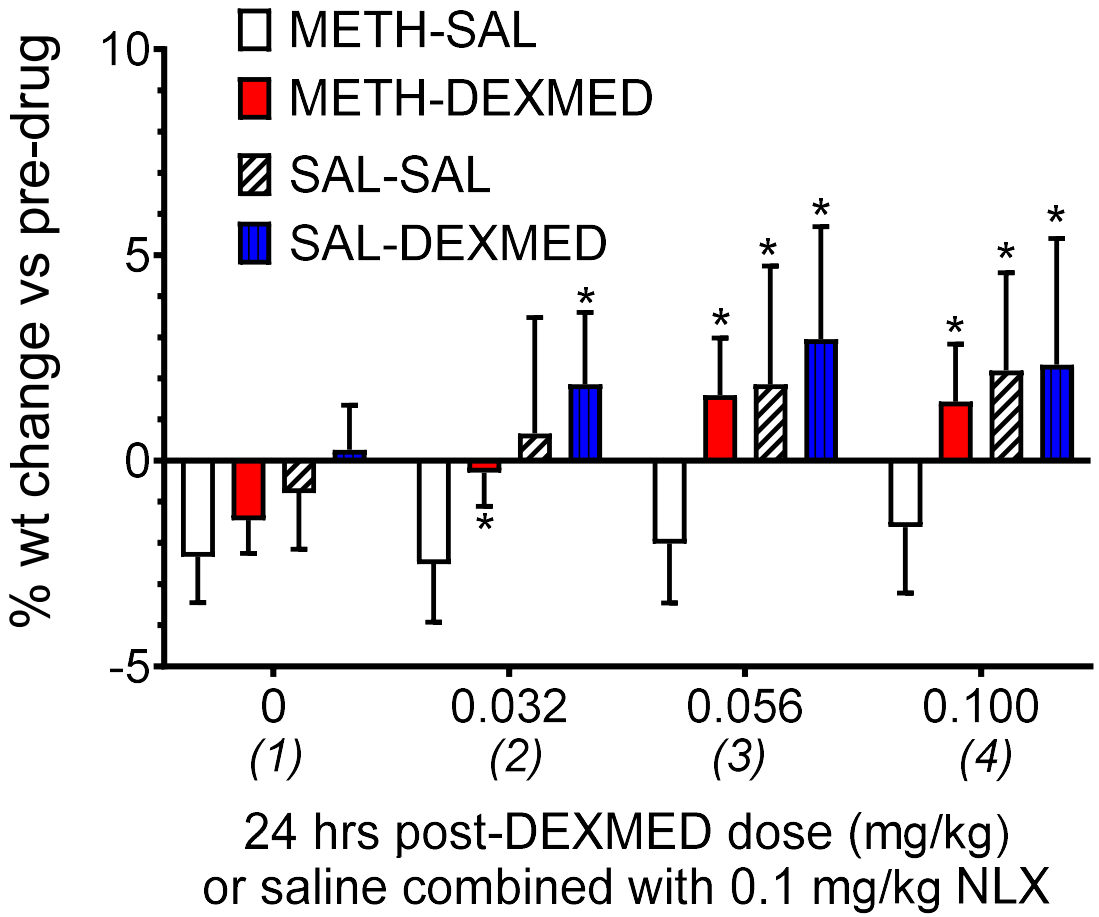

Figure 6: ***Percent weight change compared to pre-FENT ± METH baseline.*** *Note measurements were collected 24 hours after the listed DEXMED dose (if administered) on the experimental day shown in the parenthesis. *Statistically significant difference compared to METH-SAL ($p<0.05$).*

Discussion

Chronic co-exposure of opioids and METH worsens the NLX-induced withdrawal (77). Since agitation is a major concern in acute METH exposure in humans (3, 37, 40, 42), these enhanced withdrawal symptoms could further worsen agitation after NLX administration in those who are chronic users of both opioids and METH. Low doses of FENT enhance the low dose METH-induced locomotor activity (78), therefore incomplete reversal of FENT (i.e., which may be like low FENT concentrations) could potentially enhance agitation in humans as well.

We believe that our lab was the first to demonstrate NLX reversing the opioid-induced sedation masking the locomotor effects of METH in rats. Our study showed that DEXMED co-formulated with NLX could significantly reduce METH-induced locomotor activity (as a surrogate measure of agitation in humans) after the reversal of FENT-induced sedation in rats. Indeed, the DEXMED attenuation of post-NLX METH-induced locomotor activity was so considerable that activity in these rats was comparable to that of rats not treated with METH (Figs. 2 and 3). METH enhances the release of dopamine and norepinephrine which is involved as a major factor in the pathophysiology of agitation (68). When NLX-DEXMED is administered, the NLX antagonizes the FENT-induced sedation which masks METH-induced agitation. The DEXMED reduces this agitation by selectively activating the α_2 receptors in the locus coeruleus to reduce noradrenergic activity in sympathetic nervous system and elsewhere and to produce sedation which is more like normal sleep than sedation produced by more traditional sedatives (79, 80). While DEXMED has not yet been used for chemical restraint in adjunct with NLX in METH-opioid co-exposure in humans, it has been safely and effectively used for this METH-induced agitation in the absence of opioids in humans (57). In addition to providing additional controls, we included the non-METH treated groups to test the safety of

NLX-DEXMED when agitation is due to a non-stimulant cause. This is important as amphetamines are known to reverse the effects of DEXMED's sedative effects (Kato et al., 2021). While the animals in the SAL-DEXMED group were profoundly sedated for a prolonged period of time (Fig 3), there was no mortality in this or any of the experimental groups.

In our study we used an aggressive 0.1 mg/kg dose of DEXMED to determine if blood oxygenation would be severely impaired in combination with FENT if NLX reversal of FENT's effects was inadequate. During the cardiorespiratory data collection, it was shown that NLX-DEXMED did not significantly impair the recovery of blood oxygenation (i.e., SpO2%) in the presence of METH. There was, however, a significant yet non-fatal reduction in these values when METH was not present (Fig.4). This respiratory depression caused by DEXMED in rats is consistent with the literature (81-83). Intravenous doses of DEXMED produced reductions in SpO2 with lower doses than our experiments (0.005 and 0.05 mg/kg), but this could be due to DEXMED being administered via IV bolus or the rats being studied in chambers with controlled airflow (84) which differed with our study in which open chambers provided ample airflow to the animals. Regardless, 0.01 and 0.03 mg/kg doses of DEXMED administered intraperitoneally do not enhance alfentanil-induced respiratory depression in rats (85). In a study that used higher dose of SC DEXMED than our study (0.25 mg/kg) in combination with SC tramadol (low potency opioid), tiletamine (dissociative anesthetic), and zolazepam (benzodiazepine analogue), the four agents did not suppress blood oxygenation in rats that were also supplemented with 100% oxygen through a nose cone (86). Therefore, if DEXMED is used in a healthcare setting a simple safety measure such as supplemental oxygen may prevent this issue. In another study, a very high 1 mg/kg intraperitoneal DEXMED (plus ketamine) dose produced respiratory depression, which was reversible by potent, selective α_2 antagonist atipamezole (83). This

intervention could potentially be used clinically if a patient had severe DEXMED-induced respiratory depression due to excessive administration or patient sensitivity (i.e., in geriatric populations (62)) if oxygen supplementation did not suffice. Regardless, in the event of any future clinical trials of DEXMED in the scenario simulated by our preclinical study, clinicians should be adamant in monitoring respiratory parameters despite the low-risk respiratory depression with DEXMED. This is especially important as the combination of METH, FENT, and DEXMED in humans may produce unpredictable effects. In our future studies, we will evaluate the use of oxygen supplementation prevent DEXMED's effect on blood oxygenation with even more aggressive doses of DEXMED which might be needed for higher dose, age and more severe METH-induced agitation.

In both groups administered DEXMED (i.e., METH-DEXMED and SAL-DEXMED), the average HR was significantly reduced compared to both groups not administered DEXMED (Fig 4). There was a more substantial reduction in the SAL-DEXMED group (i.e., without METH) which was significantly reduced compared to the METH-DEXMED group. Amphetamines are known to reverse DEXMED-induced sedation (75). The reduction in DEXMED's suppression of cardiovascular and respiratory function in the presence of METH shows that amphetamines interact with additional DEXMED effects. Indeed, DEXMED's inhibition of the sympathetic nervous system (79, 80) is known to reduce HR and/or blood pressure in both rats (81, 87) and humans (64, 88). In a case in which DEXMED was administered to patients with METH-induced agitation after it was inadequately treated with the benzodiazepine standard of care, hypotension and bradycardia were a transient issue which was resolved simply by lowering the rate of infusion (57). Since high blood pressure and HR are common problems upon clinical presentation in METH intoxication (49), the cardiovascular

effects of DEXMED may even be beneficial for patient health. Like with respiratory monitoring, any future clinical trials of NLX-DEXMED for the treatment of acute combination METH-opioid intoxication should carefully monitor cardiovascular parameters. We will also continue investigating DEXMED and its interactions with METH and FENT on cardiovascular function.

Based on the continuously monitored rat body weight data DEXMED has a protective effect against the anorexiant effects of METH in rats. The METH-SAL group maintained a reduction in rat weight compared to the weights prior to drug exposure and was significantly lower than the METH-DEXMED group while not significantly different than the SAL-SAL and SAL-DEXMED groups by 24 hours after the first DEXMED dose (Fig 6). These results are likely due to the DEXMED inhibiting satiety via the α_2 receptor (89). In addition to showing the overall tolerability of DEXMED in this preclinical scenario, our data also show that adjunctive α_2 agonists may help reduce weight loss when stimulants are used for attention deficit hyperactive disorder (ADHD). Further investigation is needed to determine if this effect is unique to DEXMED or it could be produced by other α_2 agonists already indicated for ADHD.

Conclusions

In the scenario of FENT-METH co-exposure in rats, DEXMED co-formulated with NLX inhibited the rat model of METH-induced agitation (i.e., METH-induced locomotor activity) which was unmasked by NLX reversal of FENT induced sedation. The NLX-DEXMED combination also produced potentially useful significant reductions in HR without significantly affecting blood oxygenation compared to non-DEXMED administration controls. DEXMED can potentially produce respiratory depression (62) and commonly produces hypotension and

bradycardia (64, 88), but these side effects in a hospital setting would have reduced risk due to the availability of supportive measures (e.g., oxygen supplementation, epinephrine, or even respirators). These adverse effects likely do however make DEXMED an undesirable option for a combination with NLX outside of professional clinical support. In a hospital or other controlled clinical setting, DEXMED-NLX may be an effective sedative for chemical restraint in patients presenting with certain acute METH-opioid intoxication. DEXMED could also potentially be used separately after NLX reversal unmasks METH-induced agitation as well. Regardless, proper cardiorespiratory monitoring and support should be implemented in this clinical scenario.

The results of this study are encouraging for this possible indication for NLX adjunctive DEXMED, but additional studies are still needed to ensure pre-clinical safety and efficacy of NLX-DEXMED for the purpose of reversing FENT-METH intoxication. Future directions are discussed in Chapter 4.

Chapter 4: Conclusions and Future Directions

Conclusions

In this study, we determined that co-administered NLX-DEXMED safely and effectively reduced METH-induced agitation unmasked by NLX reversal of FENT-induced sedation in rats without fatally reducing measures of cardiorespiratory function.

Specifically, our data suggest the following:

1. DEXMED co-formulated with NLX reduced METH-induced locomotor activity which was unmasked by NLX reversal of FENT induced sedation.
2. When METH was present, NLX-DEXMED also reduced HR without significantly affecting blood oxygenation.

Taken together, these data suggest that the addition of DEXMED with NLX will reverse the effects of FENT while also reducing the METH-induced agitation level of the patient. HR reductions may also be beneficially introduced, reducing tachycardia.

Future Directions

While the project described provides valuable proof of concept data in support of dexmedetomidine (DEXMED) as a naloxone (NLX) adjunct to treat agitation in combination opioid-methamphetamine (METH) overdose, additional preclinical studies are needed. These studies are needed to determine the sex differences in the safety and efficacy of this intervention, pharmacokinetic characterization of the concurrently administered agents, and consideration of the common fentanyl (FENT) adulterant xylazine (XYL).

NLX-DEXMED reversal of FENT-METH needs to be tested in female rats to determine if there are any sex differences in the safety and efficacy of this novel intervention especially due to the known sex-dependent differences in the effects of both FENT and METH. For example, both female humans (90) and female rats are more sensitive to METH-induced stimulant effects (91) than the males of these species. In addition, both female humans (92, 93) and female rats (94) are more sensitive to opioid-induced respiratory depression as well. This study will be performed in male rats as well which will facilitate the addition of experimental endpoints and the determination of dose dependent effects (i.e., all doses administered in our study were effective, the follow-up study will determine the effects of a broader range of doses). The weight data in this future study may be of particular interest with female metabolism being different than that of males (95).

Pharmacokinetic characterization of these complex drug exposures (i.e., both initial intoxication and subsequent reversal) is needed. We are particularly interested in a potential pharmacokinetic interaction between METH and DEXMED since α_2 agonists are known to increase tubular filtration (96) and METH clearance in both rats and humans has an unchanged renal component (97). Indeed, in DEXMED treated rats in our study we crudely detected the possibility of an increase in glomerular filtration as substantially greater volumes of urine were observed in the open field behavioral chambers compared to rats not treated with DEXMED. Since METH is actively transported into the urine of both humans (97) and rats (98), the increase in glomerular filtration could result in enhanced unchanged METH clearance by this route of elimination (99).

Our novel intervention also needs to be studied in the context of FENT and XYL co-administration (i.e., FENT-METH-XYL co-intoxication). XYL is a common low potency (i.e.,

considerably lower potency than DEXMED) α_2 agonist which is therapeutically used as an anesthetic in veterinary medicine and is commonly an adulterant of illicit FENT (100, 101). XYL is not currently approved for human use but is still commonly involved in illicit drug use both purposely and accidentally (i.e. adulteration of other drug supplies) (102). Our concern is that a dose of XYL too low to mask METH-induced agitation, may have a deleterious interaction with DEXMED. Future studies will include rats co-intoxicated with XYL-FENT-METH prior to reversal with NLX-DEXMED. The XYL dose will be titrated to a with a XYL dose which will not attenuate METH-induced locomotor activity.

DEXMED may also be used as an additional treatment with current stimulant treatments for ADHD in low doses to help combat weight loss. Further studies will be conducted with both male and female rats with chronic stimulant exposure (i.e. as per clinical treatment of ADHD) to track weight changes.

Addendum

Description of Miscellaneous Techniques Not Used in Thesis project

Mouse Handling

Very minimal force was used when working with mice. Due to the standard handling procedure, it is better to let the mice back into the cage once they loosen the handler's grip to regain proper position. This is precautionary due to the high likelihood that once loose, the mice will try and bite the handler. To begin a standard mice restraint, the handler should quickly but gently grab the tail approximately half of its length from the base and raise the mouse into suspension. Then the handler should place the wire cage topper back on to the cage to prepare for the next step. The mouse should then be lowered onto the top of the wire cage topper in a manner that forces the forelimbs to grab the cage first. It is recommended to position the forelimbs on a wire that is perpendicular to the body with wires also running at a slight incline from head to tail. The grip on the tail should be used to pull the body of the mouse into a stretched position. This will allow the handler to begin running their less dominant forefinger and thumb from the base of the back up to the section behind the ears to scruff the mouse (starting at the base is a means of training for the handler, once more proficient, the handler can make a quick movement towards the head to scruff the mouse). Once scuffed, the handler should then place their middle finger at the base of the hindlimb to hold the hindlimbs in an open position. This is done to avoid limb interference when performing intraperitoneal (IP) injections.

Mouse IP injections begin after standard restraint is achieved as described above. The handler is to grab the syringe with an unoccupied hand, placing the ring finger on one side of the syringe shaft and the middle finger on the other, creating a stopper for the base of the syringe,

leaving the thumb ready to push the injection. Make sure the meniscus is on the correct value for the planned injection and that there are no air bubbles in the syringe. Place the sharp side of the syringe on the skin fist with the bevel up, in either of the two lower quadrants of the mouse's abdomen, ensuring alternating injection sites with multiple injections. Push the needle in with no variation in angle and make a short pull of the plunger outward to ensure blood is not pulled. After observing no blood in the syringe, push the full dose and remove the needle at the same angle it was inserted.

Tail Vein Bleed (Rat)

When collecting blood from the tail vein of a rat, the rat should be properly anesthetized with isoflurane. Then the rat should be continuously supplied the isoflurane with a nose cone and placed on a heating block to prevent hypothermia. The rat should be placed on its lateral side and the tail vein should be visible through the skin at this step. The skin surface of the tail should be cleaned thoroughly with rubbing alcohol and then wiped down with a gauze prior to inserting the needle to collect blood. It is recommended to have the tail lay partially off the heating block to give the handler a better angle for inserting the needle. The type of syringe used is the handler's choice, as different needle sizes have different aspects of them which alter their feel of the pull. I personally recommend a lower gauge needle (e.g., 26 G), my theory being that a smaller needle can more easily fit in the vein. Place the syringe bevel facing upwards on top of the tail vein, starting at a low point of the tail directly on the vein. Insert the syringe at a very high angle, almost 180º, to slide the needle slightly into the skin and reach the vein. If using the tail hanging off the edge of the heating block approach, the syringe angle will be significantly less and require alternative hand placement to stabilize the tail and syringe after beginning the pull.

Create a vacuum on the plunger of the syringe for a flash of blood to appear once the needle appropriately penetrates the vein. Maintain the location of the needle once the flash of blood appears and continue pulling the plunger of the syringe until an appropriate amount of blood has been collected. Hand placement is the researcher's preference, but should be placed where the handler can maintain the syringe in a stable location until the collection is complete.

Lab Design Overview

Figure 7: Lab Layout

Lab Layout

The lab was rearranged to ensure proper experimental flow. The center tables were rotated to be presented in a horizontal pattern aligning with the retired lab refrigerator. This increased the available floor space from the back wall to the first tabletop. The new space also allowed for optimal space for the use of the computers on either side of the lab (i.e., for the Noldus Ethovision automated behavioral analysis and MouseOx Plus pulse oximeter systems). Shelf space was created by storing old lab equipment allowing for the use of the shelves and tabletops during the experiment days. The tabletops beside the Ethovison computer were used to place the home cages which allowed handlers to promptly acquire and measure the weights of the specimen of the experiment prior to any formulation of doses. The center tables are covered in an organizational chuck pad that has been labeled with the number designation of each rat, what drugs they will receive, and in which order.

The cages on the center tables are the pre-reversal cages used to house the specimen after receiving the initial drugs and before receiving the reversal drugs. These cages are also being recorded with a camera placed beside the computer on the same side of the lab as the pre-reversal cages. Sharps containers and trash containers are placed below the center tables beside the chair that the scientist used during the experiment for prompt disposal of any sharps or trash.

The single cage without any bedding or wire top beside the MouseOx computer is used to house the specimen before, during and after the reversal for the safety measures of heart rate and oxygen saturation to be measured.

The behavioral chambers near the window side of the lab are placed below cameras that are mounted on the ceiling tiles. The chambers are adjusted for alignment with these cameras.

This alignment is made easier with the addition of markers such as tape on the floor to place the chambers in the correct position if moved or disturbed. This is a fast adjustment, but finer adjustments may still be needed to ensure accurate readings by the camera and software.

The sink is used during the planned euthanasia days by placing the guillotine on the countertop to collect trunk blood for further analysis. The veterinary isoflurane vaporizer is located proximal to the guillotine to assure deep sedation prior to its use. The tools are also cleaned using this sink and are then placed on a chuck pad on the countertop to dry. Beside the fume hood there is a small countertop that is accompanied by a small lamp light. This area is used for dissection and tail vein bleeds. Sharps containers and trash cans are under the countertop. A chair is utilized in front of this countertop for the researchers' comfort when performing dissections.

References

1. Carpenedo Mun C, Schuler H, Baker R, Byrne F, Bresani E, Meyers K. Rural communities face more than an opioid crisis: Reimagining funding assistance to address polysubstance use, associated health problems, and limited rural service capacity. J Rural Health. 2023;39(4):795-803.
2. Palis H, Xavier C, Dobrer S, Desai R, Sedgemore KO, Scow M, et al. Concurrent use of opioids and stimulants and risk of fatal overdose: A cohort study. BMC Public Health. 2022;22(1):2084.
3. Jones R, Jackson D, Woods C, Usher K. Complexity, safety and challenges: Emergency responders' experience of people affected by methamphetamines. Nurs Health Sci. 2022;24(3):535-44.
4. Compton WM, Jones CM. Epidemiology of the U.S. opioid crisis: the importance of the vector. Ann N Y Acad Sci. 2019;1451(1):130-43.
5. Baker R, Leichtling G, Hildebran C, Pinela C, Waddell EN, Sidlow C, et al. “Like Yin and Yang”: perceptions of methamphetamine benefits and consequences among people who use opioids in rural communities. J Addict Med. 2021;15(1):34.
6. Han B, Compton WM, Jones CM, Einstein EB, Volkow ND. Methamphetamine use, methamphetamine use disorder, and associated overdose deaths among US adults. JAMA Psychiatry. 2021;78(12):1329-42.
7. Hoopsick RA, Andrew Yockey R. Methamphetamine-related mortality in the United States: co-involvement of heroin and fentanyl, 1999–2021. Am J Public Health. 2023;113(4):416-9.
8. Sarker A, Al-Garadi MA, Ge Y, Nataraj N, Jones CM, Sumner SA. Signals of increasing co-use of stimulants and opioids from online drug forum data. Harm Reduct J. 2022;19(1):51.
9. Friedman J, Shover CL. Charting the fourth wave: Geographic, temporal, race/ethnicity and demographic trends in polysubstance fentanyl overdose deaths in the United States, 2010–2021. Addiction. 2023;118(12):2477-85.
10. Shover CL, Friedman JR, Romero R, Buhr R, Chu B, Tang A, et al. Longitudinal changes in co-involved drugs, comorbidities, and demographics of methamphetamine-related deaths in Los Angeles County. J Subst Use Addict Treat. 2023:209101.
11. Kevil CG, Goeders NE, Woolard MD, Bhuiyan MS, Dominic P, Kolluru GK, et al. Methamphetamine Use and Cardiovascular Disease. Arterioscler Thromb Vasc Biol. 2019;39(9):1739-46.
12. Harruff RC, Yarid NA, Barbour WL, Martin YH. Medical examiner response to the drug overdose epidemic in King County Washington: "Real-time" surveillance, data science, and applied forensic epidemiology. J Forensic Sci. 2023;68(5):1632-42.
13. Dai Z, Abate MA, Groth CP, Rucker T, Kraner JC, Mock AR, Smith GS. Fentanyl and other opioid involvement in methamphetamine-related deaths. Am J Drug Alcohol Abuse. 2022;48(2):226-34.
14. Choi NG, Choi BY, Marti CN, DiNitto DM, Baker SD. Substance use and medical outcomes in those age 50 and older involving cocaine and metamfetamine reported to United States poison centers. Clin Toxicol (Phila). 2023;61(5):400-7.

15. Butelman ER, Huang Y, Epstein DH, Shaham Y, Goldstein RZ, Volkow ND, Alia-Klein N. Overdose mortality rates for opioids and stimulant drugs are substantially higher in men than in women: state-level analysis. Neuropsychopharmacology. 2023;48(11):1639-47.
16. Jones AA, Shearer RD, Segel JE, Santos-Lozada A, Strong-Jones S, Vest N, et al. Opioid and stimulant attributed treatment admissions and fatal overdoses: Using national surveillance data to examine the intersection of race, sex, and polysubstance use, 1992-2020. Drug Alcohol Depend. 2023;249:109946.
17. Glick SN, Klein KS, Tinsley J, Golden MR. Increasing heroin-methamphetamine (goofball) use and related morbidity among Seattle area people who inject drugs. Am J Addict. 2021;30(2):183-91.
18. Al-Tayyib A, Koester S, Langegger S, Raville L. Heroin and Methamphetamine Injection: An Emerging Drug Use Pattern. Subst Use Misuse. 2017;52(8):1051-8.
19. Palamar JJ, Cottler LB, Goldberger BA, Severtson SG, Grundy DJ, Iwanicki JL, Ciccarone D. Trends in characteristics of fentanyl-related poisonings in the United States, 2015-2021. Am J Drug Alcohol Abuse. 2022;48(4):471-80.
20. Howell BA, Bart G, Wang EA, Winkelman TN. Service involvement across multiple sectors among people who use opioids, methamphetamine, or both, United States-2015-2018. Med Care. 2021;59(3):238.
21. Tardelli VS, Johnstone S, Xu B, Kim S, K. Kim H, Gratzer D, et al. Marked increase in amphetamine-related emergency department visits and inpatient admissions in Toronto, Canada, 2014–2021. Can J Psychiatry. 2023;68(4):249-56.
22. Glasner-Edwards S, Mooney LJ. Methamphetamine psychosis: epidemiology and management. CNS Drugs. 2014;28(12):1115-26.
23. Ivsins A, Fleming T, Barker A, Mansoor M, Thakarar K, Sue K, McNeil R. The practice and embodiment of "goofballs": a qualitative study exploring the co-injection of methamphetamines and opioids. Int J Drug Policy. 2022;107:103791.
24. Ondocsin J, Holm N, Mars SG, Ciccarone D. The motives and methods of methamphetamine and 'heroin' co-use in West Virginia. Harm Reduct J. 2023;20(1):88.
25. Palmer A, Scott N, Dietze P, Higgs P. Motivations for crystal methamphetamine-opioid co-injection/co-use amongst community-recruited people who inject drugs: a qualitative study. Harm Reduct J. 2020;17(1):1-6.
26. Smith MA, Ballard SL, Ballesteros CF, Bonge SA, Casimir AT, Childs LM, et al. Interactions Between Opioids and Dextroamphetamine on Locomotor Activity: Influence of an Opioid's Relative Efficacy at the Mu Receptor. Front Psychiatry. 2021;12:790471.
27. Silverstein SM, Daniulaityte R, Getz K, Zule W. "It's Crazy What Meth Can Help You Do": Lay Beliefs, Practices, and Experiences of Using Methamphetamine to Self-Treat Symptoms of Opioid Withdrawal. Subst Use Misuse. 2021;56(11):1687-96.
28. Lopez AM, Dhatt Z, Howe M, Al-Nassir M, Billing A, Artigiani E, Wish ED. Co-use of methamphetamine and opioids among people in treatment in Oregon: a qualitative examination of interrelated structural, community, and individual-level factors. Int J Drug Policy. 2021;91:103098.
29. Daniulaityte R, Silverstein SM, Getz K, Juhascik M, McElhinny M, Dudley S. Lay knowledge and practices of methamphetamine use to manage opioid-related overdose risks. Int J Drug Policy. 2022;99:103463.

30. Daniulaityte R, Ruhter L, Juhascik M, Silverstein S. Attitudes and experiences with fentanyl contamination of methamphetamine: exploring self-reports and urine toxicology among persons who use methamphetamine and other drugs. Harm Reduct J. 2023;20(1):54.
31. Wagner KD, Fiuty P, Page K, Tracy EC, Nocera M, Miller CW, et al. Prevalence of fentanyl in methamphetamine and cocaine samples collected by community-based drug checking services. Drug Alcohol Depend. 2023;252:110985.
32. Algera MH, Olofsen E, Moss L, Dobbins RL, Niesters M, van Velzen M, et al. Tolerance to Opioid-Induced Respiratory Depression in Chronic High-Dose Opioid Users: A Model-Based Comparison With Opioid-Naïve Individuals. Clin Pharmacol Ther. 2021;109(3):637-45.
33. Pappalardo FS, Krieger M, Park C, Beaudoin FL, Wightman RS. Patients presenting to the ED with nonfatal drug overdose: Self-reported history of overdose and naloxone use. Am J Emerg Med. 2024;82:21-5.
34. Kahn LS, Wozniak M, Vest BM, Moore C. "Narcan encounters:" overdose and naloxone rescue experiences among people who use opioids. Subst Abus. 2022;43(1):113-26.
35. Isoardi KZ, Parker L, Harris K, Rashford S, Isbister GK. Acute opioid withdrawal following intramuscular administration of naloxone 1.6 Mg: a prospective out-of-hospital series. Ann Emerg Med. 2022;80(2):120-6.
36. Gooley B, Weston B, Colella MR, Farkas A. Outcomes of law enforcement officer administered naloxone. Am J Emerg Med. 2022;62:25-9.
37. Jones R, Usher K, Woods C. Crystal methamphetamine's impact on frontline emergency services in Victoria, Australia. Australas Emerg Care. 2019;22(4):201-5.
38. Edmundson J, Skoblenick K, Rosychuk RJ. Flow through the Emergency Department for Patients Presenting with Substance Use Disorder in Alberta, Canada. West J Emerg Med. 2023;24(4):717-27.
39. Armoon B, Fleury MJ, Griffiths MD, Bayani A, Mohammadi R, Ahounbar E. Emergency Department Use, Hospitalization, and Their Sociodemographic Determinants among Patients with Substance-Related Disorders: A Worldwide Systematic Review and Meta-Analysis. Subst Use Misuse. 2023;58(3):331-45.
40. Redona Jr PT, Woods C, Jackson D, Usher K, Ushee K. Rates and patterns of Australian emergency department presentations of people who use stimulants: a systematic literature review. Cureus. 2022;14(10).
41. Zito MF, Fei Z, Zhu Y, Clingan SE, Marder SR, Mooney LJ. Psychosis among individuals with methamphetamine use disorder is associated with elevated rates of hospitalizations and emergency department visits across an academic health care system. J Subst Use Addict Treat. 2023;151:209033.
42. Romann V, Illgen M, Derungs A, Klukowska-Rötzler J, Ricklin ME, Exadaktylos A, Liakoni E. Presentations with reported methamphetamine use to an urban emergency department in Switzerland. Swiss Med Wkly. 2021;151:w30099.
43. Weng TI, Chen LY, Chen HY, Yu JH, Su YJ, Liu SW, et al. Gender differences in clinical characteristics of emergency department patients involving illicit drugs use with analytical confirmation. J Formos Med Assoc. 2022;121(9):1832-40.
44. Glidden E, Suen K, Mustaquim D, Vivolo-Kantor A, Brent J, Wax P, et al. Characterization of Nonfatal Opioid, Cocaine, Methamphetamine, and Polydrug Exposure and Clinical Presentations Reported to the Toxicology Investigators Consortium Core Registry, January 2010–December 2021. J Med Toxicol. 2023;19(2):180-9.

45. Mata DC, Coleman D. Unusually High Concentration of Fentanyl in a Driver: A Case Report. J Anal Toxicol. 2021;45(8):847-50.
46. Lameijer H, Azizi N, Ligtenberg JJ, Ter Maaten JC. Ventricular tachycardia after naloxone administration: a drug related complication? Case report and literature review. Drug Saf Case Rep. 2014;1:1-4.
47. Hunter R. Ventricular tachycardia following naloxone administration in an illicit drug misuse. J Clin Forensic Med. 2005;12(4):218-9.
48. Merigian KS. Cocaine-induced ventricular arrhythmias and rapid atrial fibrillation temporally related to naloxone administration. Am J Emerg Med. 1993;11(1):96-7.
49. Methamphetamine: acute intoxication [Internet]. 2024 [cited January 11, 2024].
50. FENT [package insert]. Berkeley Heights, NJ: Hikma Pharmaceuticals USA Inc. 2020.
51. Herbst C, O'Connell M, Melton BL, Moeller KE. Initiation of antipsychotic treatment for amphetamine induced psychosis and its impact on length of stay. Journal of Pharmacy Practice. 2023;36(6):1324-9.
52. Kuczenski R, Segal DS. Sensitization of amphetamine-induced stereotyped behaviors during the acute response. J Pharmacol Exp Ther. 1999;288(2):699-709.
53. Hicks S, Miller BD. Emergency department management of methamphetamine toxicity. Emerg Med Pract. 2023;25(11):1-20.
54. Preskorn SH, Zeller S, Citrome L, Finman J, Goldberg JF, Fava M, et al. Effect of Sublingual Dexmedetomidine vs Placebo on Acute Agitation Associated With Bipolar Disorder: A Randomized Clinical Trial. Jama. 2022;327(8):727-36.
55. Citrome L, Preskorn SH, Lauriello J, Krystal JH, Kakar R, Finman J, et al. Sublingual Dexmedetomidine for the Treatment of Acute Agitation in Adults With Schizophrenia or Schizoaffective Disorder: A Randomized Placebo-Controlled Trial. J Clin Psychiatry. 2022;83(6).
56. Smith CM, Santalucia M, Bunn H, Muzyk A. Sublingual Dexmedetomidine for the Treatment of Agitation in Patients with Schizophrenia and Bipolar Disorder. Clin Psychopharmacol Neurosci. 2023;21(2):215.
57. Lam RPK, Yip WL, Wan CK, Tsui MSH. Dexmedetomidine use in the ED for control of methamphetamine-induced agitation. Am J Emerg Med. 2017;35(4):665. e1-. e4.
58. Wang L, Zhang T, Huang L, Peng W. Comparison between dexmedetomidine and midazolam for sedation in patients with intubation after oral and maxillofacial surgery. Biomed Res Int. 2020;2020:1-6.
59. Lee S. Dexmedetomidine: present and future directions. Korean J Anesthesiol. 2019;72(4):323-30.
60. Venn RM, Hell J, Michael Grounds R. Respiratory effects of dexmedetomidine in the surgical patient requiring intensive care. Crit Care. 2000;4:1-7.
61. Park S, Choi S-L, Nahm FS, Ryu J-H, Do S-H. Dexmedetomidine-remifentanil vs propofol-remifentanil for monitored anesthesia care during hysteroscopy: Randomized, single-blind, controlled trial. Medicine (Baltimore). 2020;99(43).
62. Weerink MA, Struys MM, Hannivoort LN, Barends CR, Absalom AR, Colin P. Clinical pharmacokinetics and pharmacodynamics of dexmedetomidine. Clin Pharmacokinet. 2017;56(8):893-913.
63. Y-site compatibility of dexmedetomidine hydrochloride with selected parenteral medications. [Internet]. TriPharma. 2005 [cited 10/23/24].

64. Ebert TJ, Hall JE, Barney JA, Uhrich TD, Colinco MD. The effects of increasing plasma concentrations of dexmedetomidine in humans. Anesthesiology. 2000;93(2):382-94.
65. Coenen A, Drinkenburg W, Hoenderken R, Van Luijtelaar E. Carbon dioxide euthanasia in rats: oxygen supplementation minimizes signs of agitation and asphyxia. Lab Anim. 1995;29(3):262-8.
66. Masini CV, Holmes PV, Freeman KG, Maki AC, Edwards GL. Dopamine overflow is increased in olfactory bulbectomized rats: an in vivo microdialysis study. Physiol Behav. 2004;81(1):111-9.
67. Powell CM, Miyakawa T. Schizophrenia-relevant behavioral testing in rodent models: a uniquely human disorder? Biol Psychiatry. 2006;59(12):1198-207.
68. Miller CW, Hodzic V, Weintraub E. Current understanding of the neurobiology of agitation. West J Emerg Med. 2020;21(4):841.
69. Siddiqui W, Gupta V, Huecker MR. Agitation: Treasure Island (FL); 2023.
70. Tseng LF, Loh HH. Significance of dopamine receptor activity in dl-p-methoxyamphetamine- and d-amphetamine-induced locomotor activity. J Pharmacol Exp Ther. 1974;189(3):717-24.
71. Uddin O, Jenne C, Fox ME, Arakawa K, Keller A, Cramer N. Divergent profiles of fentanyl withdrawal and associated pain in mice and rats. Pharmacol Biochem Behav. 2021;200:173077.
72. Gentry WB, Ghafoor AU, Wessinger WD, Laurenzana EM, Hendrickson HP, Owens SM. (+)-Methamphetamine-induced spontaneous behavior in rats depends on route of (+) METH administration. Pharmacol Biochem Behav. 2004;79(4):751-60.
73. Pro G, Hayes C, Montgomery BEE, Zaller N. Demographic and geographic shifts in the preferred route of methamphetamine administration among treatment cases in the US, 2010-2019. Drug Alcohol Depend. 2022;237:109535.
74. Rivière GJ, Byrnes KA, Gentry WB, Owens SM. Spontaneous locomotor activity and pharmacokinetics of intravenous methamphetamine and its metabolite amphetamine in the rat. J Pharmacol Exp Ther. 1999;291(3):1220-6.
75. Kato R, Zhang ER, Mallari OG, Moody OA, Vincent KF, Melonakos ED, et al. d-Amphetamine rapidly reverses dexmedetomidine-induced unconsciousness in rats. Front Pharmacol. 2021;12:668285.
76. Raleigh MD, Baruffaldi F, Peterson SJ, Le Naour M, Harmon TM, Vigliaturo JR, et al. A fentanyl vaccine alters fentanyl distribution and protects against fentanyl-induced effects in mice and rats. J Pharmacol Exp Ther. 2019;368(2):282-91.
77. Kaka G, Rahmanzade R, Safee F, Haghparast A. Naloxone induces frequent jumping after chronic morphine and methamphetamine co-administration in rats. Basic Clin Neurosci. 2014;5(1):42.
78. Paz-Ramos MI, Violante-Soria V, Browne Jr T, Cruz SL. Effects of fentanyl and the adulterant levamisole on the rewarding and locomotor effects of methamphetamine in rats. Drug Alcohol Depend. 2024:111098.
79. Gertler R, Brown HC, Mitchell DH, Silvius EN, editors. Dexmedetomidine: a novel sedative-analgesic agent. Proceedings / Baylor University Medical Center; 2001: Taylor & Francis.
80. Scott-Warren V, Sebastian J. Dexmedetomidine: its use in intensive care medicine and anaesthesia. BJA Education. 2016;16(7):242-6.

81. Fernandes FC, Ferreira HC, Cagido VR, Carvalho GM, Pereira LS, Faffe DS, et al. Effects of dexmedetomidine on respiratory mechanics and control of breathing in normal rats. Respir Physiol Neurobiol. 2006;154(3):342-50.
82. He F-Y, Feng W-Z, Zhong J, Xu W, Shao H-Y, Zhang Y-R. Effects of propofol and dexmedetomidine anesthesia on Th1/Th2 of rat spinal cord injury. Eur Rev Med Pharmacol Sci. 2017;21(6).
83. Wellington D, Mikaelian I, Singer L. Comparison of ketamine–xylazine and ketamine–dexmedetomidine anesthesia and intraperitoneal tolerance in rats. J Am Assoc Lab Anim Sci. 2013;52(4):481-7.
84. Kitajima Y, Hashizume NS, Saiki C, Ide R, Imai T. Effect of dexmedetomidine on cardiorespiratory regulation in spontaneously breathing adult rats. PLoS One. 2022;17(1):e0262263.
85. Furst SR, Weinger MB. Dexmedetomidine, a selective alpha 2-agonist, does not potentiate the cardiorespiratory depression of alfentanil in the rat. Anesthesiology. 1990;72(5):882-8.
86. Limprasutr V, Sharp P, Jampachaisri K, Pacharinsak C, Durongphongtorn S. Tiletamine/zolazepam and dexmedetomidine with tramadol provide effective general anesthesia in rats. Animal Model Exp Med. 2021;4(1):40-6.
87. Savola J-M, Virtanen R. Central α2-adrenoceptors are highly stereoselective for dexmedetomidine, the dextro enantiomer of medetomidine. Eur J Pharmacol. 1991;195(2):193-9.
88. Kallio A, Scheinin M, Koulu M, Ponkilainen R, Ruskoaho H, Viinamäki O, Scheinin H. Effects of dexmedetomidine, a selective α2-adrenoceptor agonist, on hemodynamic control mechanisms. Clin Pharmacol Ther. 1989;46(1):33-42.
89. Wellman PJ, Davies BT, Morien A, McMahon L. Modulation of feeding by hypothalamic paraventricular nucleus α1-and α2-adrenergic receptors. Life Sci. 1993;53(9):669-79.
90. Mayo LM, Paul E, DeArcangelis J, Van Hedger K, de Wit H. Gender differences in the behavioral and subjective effects of methamphetamine in healthy humans. Psychopharmacology (Berl). 2019;236:2413-23.
91. Milesi-Hallé A, Hendrickson HP, Laurenzana EM, Gentry WB, Owens SM. Sex-and dose-dependency in the pharmacokinetics and pharmacodynamics of (+)-methamphetamine and its metabolite (+)-amphetamine in rats. Toxicol Appl Pharmacol. 2005;209(3):203-13.
92. Dahan AM, Sarton E, Teppema L, Olievier C. Sex-related differences in the influence of morphine on ventilatory control in humans. Anesthesiology. 1998;88(4):903-13.
93. Sarton E, Teppema L, Dahan A. Sex differences in morphine-induced ventilatory depression reside within the peripheral chemoreflex loop. Anesthesiology. 1999;90(5):1329-38.
94. Marchette RC, Carlson ER, Frye EV, Hastings LE, Vendruscolo JC, Mejias-Torres G, et al. Heroin-and fentanyl-induced respiratory depression in a rat plethysmography model: potency, tolerance, and sex differences. J Pharmacol Exp Ther. 2023;385(2):117-34.
95. Quirós Cognuck S, Reis WL, Silva M, Debarba LK, Mecawi AS, de Paula FJA, et al. Sex differences in body composition, metabolism-related hormones, and energy homeostasis during aging in Wistar rats. Physiol Rep. 2020;8(20):e14597.
96. Strandhoy JW. Role of alpha-2 receptors in the regulation of renal function. J Cardiovasc Pharmacol. 1985;7:S34.

97. Kim I, Oyler JM, Moolchan ET, Cone EJ, Huestis MA. Urinary pharmacokinetics of methamphetamine and its metabolite, amphetamine following controlled oral administration to humans. Ther Drug Monit. 2004;26(6):664-72.
98. Kitaichi K, Morishita Y, Doi Y, Ueyama J, Matsushima M, Zhao Y-L, et al. Increased plasma concentration and brain penetration of methamphetamine in behaviorally sensitized rats. Eur J Pharmacol. 2003;464(1):39-48.
99. Chapron A, Shen D, Kestenbaum B, Robinson-Cohen C, Himmelfarb J, Yeung C. Does secretory clearance follow glomerular filtration rate in chronic kidney diseases? Reconsidering the intact nephron hypothesis. Clin Transl Sci. 2017;10(5):395-403.
100. Leconte CE, Sethi R. The appearance of xylazine in the United States as a fentanyl adulterant. Prim Care Companion CNS Disord. 2023;25(6):49730.
101. Quijano T, Crowell J, Eggert K, Clark K, Alexander M, Grau L, Heimer R. Xylazine in the drug supply: emerging threats and lessons learned in areas with high levels of adulteration. Int J Drug Policy. 2023;120:104154.
102. Tan M, Nassau T, Kuncio D, Higgins D, Teixeira da Silva D, Tomlinson D, Brady KA. Xylazine Use Among People Who Inject Drugs, Philadelphia 2022. J Addict Med. 2024;18(2):194-200.

Appendix A: IRB Approval Letter

MARSHALL UNIVERSITY
www.marshall.edu

Office of Research Integrity

October 4, 2023

Wes Tackett
1538 Charleston Ave
Office Suite 353
Huntington, WV 25701

Dear Wes,

This letter is in response to the submitted thesis abstract entitled *"Naloxone and Dexmedetomidine Reversal of the Effects of Methamphetamine and Fentanyl Co-Administration."* After assessing the abstract, it has been deemed not to be human subject research and therefore exempt from oversight by the Marshall University Institutional Review Board (IRB). The Institutional Animal Care and Use Committee (IACUC) has reviewed and approved the study under protocol #821 submitted by Dr. Michael Hambuchen. The applicable human and animal federal regulations have set forth the criteria utilized in making this determination. If there are any changes to the abstract, you provided then you would need to resubmit that information to the Office of Research Integrity for review and a determination.

I appreciate your willingness to submit the abstract for determination. Please feel free to contact the Office of Research Integrity if you have any questions regarding future protocols that may require IRB review.

Sincerely,

Bruce F. Day, ThD, CIP
Director

MARSHALL UNIVERSITY
www.marshall.edu

Animal Resource Facility

DATE:	April 19, 2023
TO:	Michael Hambuchen, PharmD, PhD
FROM:	Marshall University IACUC
IACUC #:	821
PROJECT TITLE:	[2026675-2] Preclinical development of pharmacotherapy for rescue from combination methamphetamine-opioid overdose
SUBMISSION TYPE:	New Project
ACTION:	APPROVED
APPROVAL DATE:	April 19, 2023
EXPIRATION DATE:	April 18, 2026
REVIEW TYPE:	Full and Designated Member Review

Thank you for your submission of Revision materials for this research project. The Marshall University IACUC has APPROVED your submission. All research must be conducted in accordance with this approved submission.

This submission has received Full and Designated Member Review.

Please note that any revision to previously approved materials must be approved by this committee prior to initiation. Please use the appropriate revision forms for this procedure.

Please report all NON-COMPLIANCE issues regarding this project to this committee.

This project requires Continuing Review by this office on an annual basis. Please use the appropriate renewal forms for this procedure.

If you have any questions, please contact Monica Valentovic at (304) 696-7332 or valentov@marshall.edu. Please include your project title and reference number in all correspondence with this committee.

Monica A. Valentovic

Monica A. Valentovic, Ph.D.
Chairperson, IACUC

Appendix B: Abbreviations

DEXMED- Dexmedetomidine

FENT- Fentanyl

IP- Intraperitoneal

METH- Methamphetamine

NLX- Naloxone

SUD- Substance Use Disorder

XYL- Xylazine