A Multi-Level Analysis of Amphetamine Derivatives:

Repeated 3,4-Methylenedioxymethamphetamine Administration and Popular Methamphetamine Combinations in Mice and Humans

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Abstract

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Despite decades of research on amphetamine derivatives, a class of compounds sharing a structural foundation with amphetamine, crucial gaps remain in our understanding of these drugs in a variety of animal species and humans. This dissertation addresses three of these gaps through a multi-level approach involving studies in both humans and mice. Specifically, it focuses on investigating the lack of information regarding: 1) repeated dosing of 3,4-methylenedioxymethamphetamine in humans, 2) methamphetamine/alcohol combinations in humans and 3) methamphetamine/oxycodone combinations in mice. Study 1 involved administering three consecutive doses of 3,4-methylenedioxymethamphetamine to human volunteers at 12- and 24-hour intervals while physiological, behavioral, and subjective measures were collected. Study 2 reanalyzed Kirkpatrick and colleagues (2012a) data to evaluate repeated administrations of methamphetamine and alcohol. The reanalysis focused on quantifying the physiological and subjective effect differences between the first and second administrations, which occurred at a 12-hour interval on the same day, an aspect not previously analyzed or reported by the original authors. Study 3 utilized well-established animal models such as Conditioned Place Preference, Open Field Test, and Novel Object Recognition to evaluate the reward-like and aversive effects of methamphetamine and oxycodone combinations in mice.

Study 1 was the first to quantify the effects of multiple 3,4-methylenedioxymethamphetamine doses administered over a 36-hour period of time. Initially, acute 3,4-methylenedioxymethamphetamine produced dose-dependent increases in peak heart rate, blood pressure, and more positive than negative subjective effects. However, by the third dose, many of these effects dissipated, heart rate was no longer elevated, and residual mood effects were minor. Overall, the data do not support the general perception that 3,4-methylenedioxymethamphetamine produces dangerous cardiovascular and residual mood effects in humans following repeated administration. The results of Study 2, again a first in the field, discovered that contrary to expectations, heart rate increases produced by the methamphetamine/alcohol combination were not further increased with repeated dosing, but rather attenuated. In fact, methamphetamine offset alcohol-induced intoxication, even after repeated administration. Study 3 revealed that combining methamphetamine and oxycodone in mice increased reward as measured by Conditioned Place Preference, but not more than either drug alone. However, methamphetamine lengthened the duration of Conditioned Place Preference for the lower oxycodone dose and offset the oxycodone-induced disruptions in novel object recognition performance. One crucial cross-species observation was that methamphetamine mitigated adverse effects such as alcohol-related intoxication and oxycodone cognitive disruption, even after repeated administration. While seemingly beneficial, this observation raises concerns that individuals who combine these drugs may be at risk of underestimating their overall degree of impairment, potentially leading to hazardous activities like driving while intoxicated or engaging in risky behaviors. Sharing this insight is crucial to encourage informed, responsible behavior and safeguard public safety. In conclusion, these studies have significantly enhanced our understanding of two frequently used amphetamine

derivatives and their interactions with two commonly used psychoactive drugs—oxycodone and alcohol. Most importantly, we strongly advocate for robust empirical experimentation to counteract misinformation related to 3,4-methylenedioxymethamphetamine and methamphetamine. These endeavors are crucial for developing more precise assessments of the risks and benefits associated with these substances, and for improving drug policies and optimizing public health interventions.

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Acknowledgments

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Dedication

This dissertation is dedicated to my beloved late grandfather, Robert Hernandez, and my dear stepfather, Oscar Meyers. I've witnessed the struggles many face in expressing love towards non-biological family members. Yet, in our relationship, such barriers were nonexistent. The unconditional love you both bestowed upon me has been a driving force behind my success. As I move forward into this next chapter of my life, I am deeply committed to honoring your legacies and ensuring that your bloodlines thrive vicariously through me.

Chapter 1: General Introduction

Amphetamine derivatives are a class of compounds based upon the amphetamine structure. Structural modifications of the amphetamine molecule can yield a vast array of psychoactive compounds that have a broad pharmacological profile. Some notable examples are 3,4-methylendedioxymethamphetamine (MDMA), methamphetamine, and 3,4-methylendedioxyamphetamine (MDA) (**Figure 1**). Although numerous amphetamine derivatives have attracted popular attention, the current dissertation focuses on MDMA and methamphetamine because of their popularity among recreational users, their medical utility, and important gaps in our knowledge about their basic psychological and biological effects.

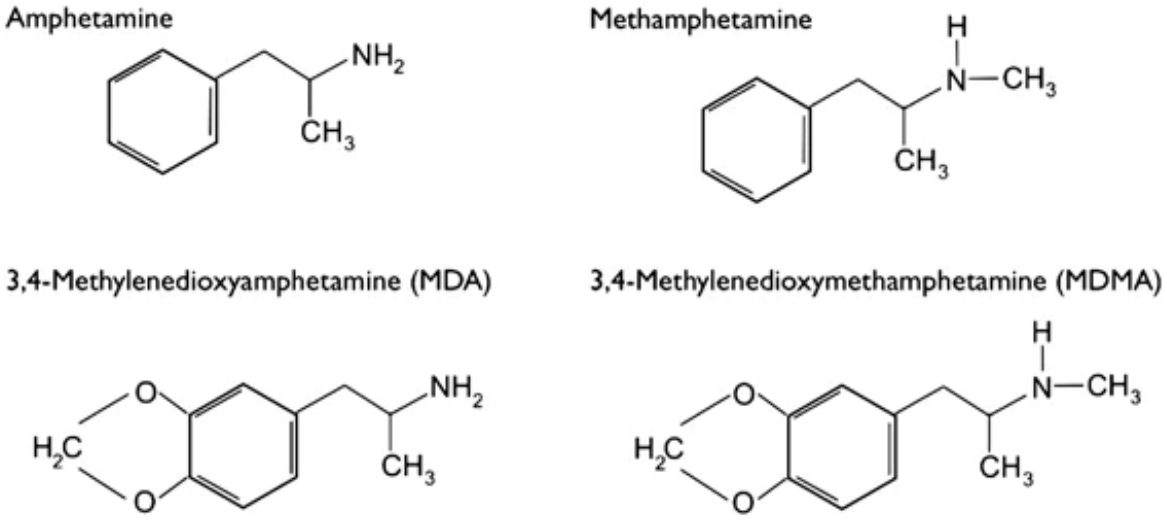

Figure 1: Chemical structure of amphetamine and amphetamine derivatives.
Adapted from "Is Cognitive Functioning Impaired in Methamphetamine Users? A Critical Review," by C. L. Hart, C. B. Marvin, R. Silver, and E. E. Smith, 2012*, Neuropsychopharmacology*, 37(*3*), p. 588.

The experiments included in this project played a crucial role in establishing a database on the *repeated* and *interactive* effects of the aforementioned amphetamine derivatives. The studies utilize human research volunteers and laboratory mice. The purpose of this dual approach was two-fold: First, it facilitated a holistic understanding of the repeated and interactive effects

of amphetamine derivatives by integrating insights from both human and animal studies, thus deepening comprehension of these phenomena. Second, it provided the opportunity to enhance the researcher's experience with two distinct animal groups. A total of three studies are included: the effects of repeated MDMA administration to humans, the interactive effects of methamphetamine and oxycodone in mice, and the interactive effects of methamphetamine and alcohol in humans.

1.1 Brief History of MDMA in the United States

In 1912, Merck chemist Anton Köllisch discovered MDMA as an intermediate compound during the synthesis of methylhydrastinine, a hemostatic agent (Freudenmann et al., 2006). However, MDMA remained relatively unknown until 1953 when the Central Intelligence Agency (CIA) initiated Project MK-ULTRA, a research program aimed at developing mind control drugs. As part of this program, toxicology tests of MDMA, along with several amphetamine derivatives, were conducted in various animals such as mice, rats, guinea pigs, dogs, and monkeys (Hardman et al., 1973; Passie & Benzenhofer, 2018). However, the question of whether MK-ULTRA carried out human studies on MDMA remains unanswered. If such studies were indeed conducted, they might have been classified. The full scope of human testing under the program is still a mystery, as many MK-ULTRA files were reportedly destroyed.

Despite the secret US Army's animal trials with MDMA in 1953, it appears that the effects of MDMA on humans were largely unknown before the 1970s. In 1976, the chemist Alexander Shulgin began self-experimentation with MDMA (Benzenhofer & Passie, 2010). His experiences led him to believe that MDMA could enhance communication, openness, and empathy, leading him to propose its use as an adjunct to psychotherapy (Karch, 2011). He shared his enthusiasm for MDMA with psychotherapist Leo Zeff, who played a key role in introducing

it to the psychotherapeutic community. Zeff administered MDMA to approximately 4000 individuals and trained over 150 therapists in MDMA-assisted psychotherapy, including Ann Shulgin, Alexander Shulgin's wife (Passie, 2018).

In 1983, Michael Clegg, a former theology student, founded a drug distribution organization in Texas, often referred to as the Texas Group (Passie, 2023a). This group played a significant role in large-scale MDMA production, leading to the drug's nickname "Ecstasy." While initially considering the name "empathy," the group opted for "ecstasy" due to its perceived marketability (Passie, 2023b). With a new marketable name, the Texas Group introduced MDMA into the Dallas nightlife, and the drug quickly became associated with dancing, making it popular among club-goers and party attendees.

To counter the growing popularity of ecstasy, on July 1st, 1985, the DEA used emergency scheduling provisions to temporarily classify MDMA as a Schedule I drug under the Controlled Substance Act (CSA: Rosenbaum & Doblin, 1991; Passie, 2023c). The CSA categorizes drugs into one of five "schedules" based on the federal government's assessment of their medical benefits and potential for abuse. Notably, these classifications are not rooted in pharmacology but are heavily influenced by law enforcement recommendations. Schedule I drugs, such as heroin and marijuana, are considered to have no medicinal purposes and a high potential for abuse, resulting in their complete prohibition. In contrast, Schedule II drugs (e.g., methamphetamine, oxycodone, cocaine) are recognized to have legitimate medical uses, but a high potential for abuse. As we move down the schedules, federal restrictions and recognized levels of abuse potential decrease.

Since the emergency Schedule I classification was temporary, public Federal court hearings were held to determine whether MDMA would be permanently designated as a

Schedule I drug (Rosenbaum & Doblin, 1991; Passie, 2023c). During these hearings, protests against the proposed scheduling emerged from psychiatrists and psychotherapists who firmly believed in MDMA's therapeutic potential. High-profile advocates of MDMA, including Alexander Shulgin, provided depositions in defense of the drug (Passie, 2023c). On May 22, 1986, the DEA's Administrative Law Judge Francis Young concluded that "MDMA indeed possessed a 'currently accepted medical use in treatment in the United States' and had not demonstrated a high potential for abuse." As a result, he recommended that the compound be placed in Schedule III, a classification that would permit therapeutic sessions to continue. However, the DEA rejected this Schedule III recommendation, dismissing the expert testimony of psychiatrists who discussed over 200 cases of MDMA-assisted therapy on the grounds that they had not been published in medical journals. Consequently, MDMA was categorized as a Schedule I drug, where it remains today (Rosenbaum & Doblin, 1991; Passie, 2023c).

1.2 Current Recreational and Clinical MDMA Use

Even with its Schedule I status, the use of MDMA continues worldwide. Recent data from the U.S. indicates that 389,000 people reported current (past month) MDMA use (SAMSHA, 2023a). However, stigmatization of illicit drug use may distort prevalence estimates, particularly in government-run face-to-face and telephone surveys, as individuals may feel hesitant to disclose their drug use (Chalmers et al., 2016). To overcome this limitation, the Global Drug Survey employs web recruitment to specifically target illicit drug users, providing insights into populations often challenging to reach through traditional methods. The survey found that 44.9% of respondents reported using MDMA at least once, a rate higher than any

other illicit drug except cannabis (Winstock et al., 2021). These findings highlight the persistent popularity of MDMA for recreational purposes, despite legal restrictions.

In addition to its illicit use, MDMA has garnered substantial interest as a potential therapeutic agent, as evidenced by the numerous clinical trials registered with ClinicalTrials.gov. To date, 64 clinical trials have been completed over the past 20 years, with an additional 14 actively recruiting participants and 13 more registered but not yet recruiting. The majority of these clinical trials were registered by researchers from the Multidisciplinary Association for Psychedelic Studies (MAPS), a nonprofit organization dedicated to evaluating the effectiveness of MDMA-assisted psychotherapy in treating post-traumatic stress disorder (PTSD). These research efforts have propelled MDMA into phase three clinical trials (For review: Sessa et al., 2019). Two trials currently registered with ClinicalTrials.gov (Identifiers: NCT03537014 and NCT04077437) have yielded encouraging results. In these randomized, double-blind, placebo-controlled studies, participants suffering from moderate to severe PTSD underwent three sessions of either MDMA-assisted therapy or placebo with therapy. The findings revealed a significant reduction in PTSD symptoms in the MDMA therapy group compared to the placebo group as gauged by the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5) score. These promising results offer support that MDMA might eventually be reclassified under Schedule II of the CSA, indicating a shift towards recognizing its therapeutic potential.

1.3 Repeated MDMA Administration Effects

When the DEA decided to ban MDMA in 1985, there was a dearth of empirical evidence on its effects. Consequently, the DEA reported that its decision was largely influenced by an unpublished study involving MDA, a structurally similar amphetamine derivative that was

already banned (Nauth & Corwin, 1985). In this now-published study, researchers showed that MDA produced neurotoxicity in laboratory rats (Ricaurte et al., 1985).

However, since then, there has been an improvement in our understanding of MDMA-related effects, especially in laboratory animals. For instance, Colado and colleagues (1993), administered a single intraperitoneal injection of MDMA (20 mg/kg) to rats daily for four consecutive days. Four days later, the rats were sacrificed through cervical dislocation and decapitation, and serotonin (5-HT) and 5-Hydroxyindoleacetic acid (5-HIAA: the primary serotonin metabolite) were assayed. The researchers reported that 5-HT and 5-HIAA concentrations were substantially reduced in the cortex and hippocampus, leading them to conclude that MDMA produced neurotoxic effects on 5-HT neurons. Similarly, Shankaran and Gudelsky (1999) administered a total of four intraperitoneal MDMA injections (10 mg/kg each) at 2-hour intervals. The rats were sacrificed by decapitation one week after the final administration and a decrease in serotonin tissue concentration was observed in the striatum, a brain region previously implicated in reward, cognition, and emotional regulation (Durston et al., 2003; Hare et al., 2005).

Comparable findings have been observed in non-human primates. For example, Ricaurte and colleagues conducted a series of studies examining the neurotoxic effects of MDMA in non-human primates (e.g., Ricaurte et al., 1988; 2000; Mueller et al., 2013). In one study, they administered subcutaneous MDMA doses (5 mg/kg b.i.d., for four consecutive days) to squirrel monkeys and, after an 18-month intervening period, the animals were sacrificed and their brains were assessed to determine the impact on 5-HT. The investigators reported that concentrations of 5-HT, 5-HT transporters, and 5-HIAA were all significantly reduced in the majority of brain

regions measured (Ricaurte et al., 1992). Collectively, these studies support the notion that MDMA-related serotonergic neurotoxicity may have lasting consequences.

It is important to highlight that training animals to self-administer the drug, which more closely aligns with the human MDMA experience, can prevent MDMA-related neurotoxicity. Self-administration models, unlike those where the experimenter administers the drug, simulate the voluntary drug-taking behavior seen in humans. In these models, animals actively choose to consume the drug, providing a more ecologically valid representation of drug use. For instance, Fantegrossi and colleagues (2004) trained rhesus monkeys to self-administer intravenous MDMA. After approximately 18 months of MDMA self-administration, the monkeys underwent a drug abstinence period lasting at least 2 months. Subsequently, they were anesthetized with ketamine and euthanized using a pentobarbital overdose (100-150 mg/kg). Their brains were then dissected, and brain tissue assayed. Self-administered MDMA intake ranged from approximately 120 to 250 mg/kg, yet no significant depletion of serotonin or its metabolites was found. These findings suggest that neurotoxicity can be avoided when animals have control over their MDMA intake, akin to human drug users.

Similarly, there is insufficient evidence to support the notion that MDMA, when administered by experimenters at doses comparable to those used by humans, induce serotonergic neurotoxicity in animal models (For review: Pantoni & Anagnostaras, 2019). As an example, Baumann and colleagues (2008) administered a total of three intraperitoneal doses of 1.5 mg/kg MDMA to rats at two-hour intervals. Two weeks later, the rats were decapitated and serotonin tissue concentration was assessed among various brain regions. Despite the cumulative dose reaching the higher end of the recreational dose range, approximately 300 mg within a span of six hours, no reductions in serotonin tissue concentration were observed.

It is important to note that the doses required to induce neurotoxicity in laboratory animals are difficult for humans to consume. For instance, a typical MDMA dose for a 155-pound human falls in the range of 75–125 mg, equivalent to approximately 1 to 2 mg/kg of MDMA. In contrast, the majority of animal studies administered doses between 10 to 20 mg/kg, equivalent to 700–1400 mg for a 155-pound human, which is about 10–20 times larger than a typical human dose. Notably, even commonly consumed vitamins, minerals and over the counter preparations like fluoride (Johnston & Strobel, 2020) can be neurotoxic at similarly high doses.

Even with the above caveats, the large database demonstrating MDMA-related neurotoxicity in laboratory animals raise concerns about whether human users are at risk. Accordingly, researchers have begun studying this issue using a variety of methods, including neuroimaging. One technique is Positron Emission Tomography (PET), during which selective, radiolabeled 5-HT receptor ligands are injected intravenously and with the use of a PET camera, the occupancy of these ligands can be accurately quantified, providing a measure of the location and density of the targeted brain receptor. Data from several PET-imaging studies demonstrate that individuals with a history of long-term MDMA use exhibit less serotonin transporter binding (e.g., McCann et al., 1998; Thomasius et al., 2003).

It is crucial to consider that the changes in radioactive binding detected in PET studies might reflect natural neural adaptations, such as receptor downregulation due to tolerance development rather than neurotoxicity (Hart & Grifell, 2018). Nonetheless, McCann and colleagues (2005) compared serotonin transporter density between abstinent MDMA users and non-users and found less serotonin transporter binding in MDMA users who had been abstinent for at least six months, suggesting the possibility of permanent neurotoxic effects in humans resulting from MDMA use.

It is important to note that if the reduced binding observed in the MDMA user group was indicative of neurotoxicity, we would expect a significant negative correlation between serotonin transporter binding and the extent of self-reported MDMA use. In simpler terms, more use should equate to more neurotoxicity, and, therefore, less binding potential. Yet, this was not the case (McCann et al*.,* 2005). Additionally, these studies were cross-sectional, meaning data were collected at a single time-point. Consequently, any differences between the MDMA and control groups in binding could be the result of differences that predated MDMA use. These considerations underscore the need for a cautious interpretation of findings from imaging studies on MDMA-related neurotoxicity in humans.

Shifting from these neurobiological considerations to psychological implications, serotonin is believed to play an important role in human mood. If recreational MDMA use damages serotonergic neurons, it *should* correspond to a decline in positive mood and, consequently, the cognitive performance of MDMA users. Several cross-sectional investigations have reported "deficits" in cognitive and emotional domains among MDMA users. For example, Rogers and colleagues (2009) conducted a systematic review and meta-analysis of data from over 100 cross-sectional comparison studies examining the cognitive and psychopathological effects of MDMA use. In general, compared to controls, MDMA users endorsed more depressive symptoms and performed worse on verbal and working memory tasks. However, assessing the clinical implications of these effects is challenging without knowledge of the expected performance range for a specific group. By utilizing normative data, which facilitates the comparison of individual or mean group scores against a database adjusted for variables like age and educational level, we can more accurately assess the clinical relevance of these effects. This approach is particularly important when researchers do not adequately "match" for critical

factors that may influence cognitive performance such as years of education and intelligence quotient. When the data from these individual studies were pooled and subjected to meta-analysis, the observed differences and effect sizes were relatively small. More importantly, the overall performance remained well within the normal range strongly suggesting that these "deficits" are not large enough to have clinical significance (Rogers et al., 2009). This highlights the importance of contextualizing results against a normal range, not only for an accurate assessment of clinical significance but also to prevent the unwarranted pathologizing of normal behavior.

There is also an important concern with these studies in that the researchers must rely on self-reports of drug use, which may be inaccurate due to initial elevation biases, imperfect memory, dishonesty, or unawareness (Shrout et al., 2018). Moreover, many MDMA users engage in polydrug use (Hopper et al., 2006). While some researchers attempt to address this concern by comparing MDMA users to controls with minimal drug use and including a polydrug group without MDMA use, the unpredictable composition of MDMA sold as ecstasy or Molly makes it challenging to attribute any observed neurotoxic or behavioral effects to MDMA alone.

We acknowledge that street drugs sold as ecstasy or Molly can vary greatly in purity and may even lack MDMA entirely. Consequently, in controlled human and animal studies, researchers often refer to the administration of the pure compound as 'MDMA.' However, in studies related to long-term recreational use, where dose and purity are frequently uncertain, the terms 'ecstasy' or 'Molly' are commonly employed. To avoid redundancy, we have chosen to use 'MDMA' consistently throughout this paper.

Another strategy used to study the effects of MDMA in humans is to assess a range of dependent measures before and after a single dose of pharmaceutical-grade MDMA under

carefully controlled conditions. De La Torres and colleagues (2000) conducted pooled analyses from three of their own MDMA clinical trials and two pilot studies, administering oral MDMA in doses ranging from 50 to 150 mg. Physiological measures were assessed before MDMA administration and at multiple time points afterwards. Compared to baseline, MDMA at 75 mg and above produced dose-dependent increases in heart rate, blood pressure, body temperature, and pupillary diameter (mydriasis) (De La Torre et al., 2000). These effects generally peaked between 60 and 120 minutes and persisted for approximately 4-8 hours after dosing. Importantly, it is worth noting that the physiological changes induced by a single dose of MDMA remain well below the thresholds associated with conditions such as tachycardia, hypertension, and hyperpyrexia (Kirkpatrick et al., 2016). In fact, these changes are comparable to those seen during moderate aerobic exercise.

It has been demonstrated that the subjective effects observed in laboratory settings follow a dose-dependent and predictable time course. These effects typically peak between 1 and 2 hours after drug administration and gradually return to baseline within several hours (for review see Kirkpatrick et al., 2016). The subjective experience is predominantly positive, marked by dose-dependent increases in drug euphoria, positive mood, and heightened arousal. However, some studies have reported occasional, albeit minor, increases in negative subjective effects like anxiety (Kirkpatrick et al., 2012b, 2014).

Regarding psychomotor and cognitive functioning, MDMA generally shows little to no impact on conventional laboratory tasks. For example, Camí and colleagues (2000) administered 75 and 125 mg doses of MDMA to eight healthy research participants. Psychomotor performance was assessed using the Vienna Reaction Unit, a measure of reaction time, and the digit symbol substitution test (DSST), which evaluates visual spatial processing. The results

showed no significant differences in reaction time between the MDMA doses and placebo. However, when analyzing peak effects on the DSST, the larger MDMA dose produced a slight but statistically significant decrease in the number of correct responses compared to placebo (Camí et al., 2000). Notably, a subsequent study by the same research group did not replicate this effect (Farré et al., 2004). Interestingly, in some cases, researchers have even observed improvements in similar cognitive domains (Lamers et al., 2003). Overall, these findings do not provide substantial evidence to support the notion that acute MDMA causes cognitive disruption.

While acute, single-dose studies offer valuable insights into MDMA's safety profile, they often do not reflect real-world usage patterns. In practice, MDMA users frequently take multiple doses within a relatively short time period (Topp et al., 1999; Hammersley et al., 1999; Sterk et al., 2006). Researchers have suggested this practice can produce serious negative consequences (Dumont & Verkes, 2006). MDMA can inhibit its own metabolism through a process called metabolic inhibition (de la Torres et al., 2004). Therefore, MDMA plasma levels following an initial administration can be greatly increased with subsequent administrations and transported to sites of action. Accordingly, there is a concern that this dosing regimen can lead to harmful increases in heart rate, blood pressure and body temperature (Parrott, 2013). Therefore, conducting studies on repeated MDMA administration is crucial as they offer a more accurate reflection of real-world usage patterns, thereby improving the generalizability of findings to the public experience.

In line with this notion, de la Torre and colleagues assessed the effects of repeated MDMA administration (2-, 4-, and 24-hour inter-dose intervals with cumulative doses ranging between 150 and 200 mg) on multiple dependent variables, including cardiovascular activity and mood measures (Pacifici et al., 2001; Farré et al., 2004; Peiró et al., 2013). Their findings

indicated that repeated dosing did not potentiate peak (highest value recorded over multiple time points) physiological effects. Greater peak positive subjective-effect ratings (i.e., "stimulated") were observed, but only under the 24-hour inter-dose interval condition (Farré et al., 2004). These data suggest that under the dosing regimens employed, greater peak effects are generally not observed. However, only two drug administrations — with a maximum cumulative dosage of 200 mg — were assessed in this series of studies, which, by some accounts, is a relatively low number of administrations and small amount of drug (Parrott, 2013). Therefore, it is possible that a greater number of drug administrations and larger cumulative dose will lead to greater physiological effects.

This brief review of the historical aspects of MDMA use in the general population and attempts by scientists to study its effects highlight an important gap in our knowledge about the psychopharmacology of MDMA in humans. The gap pertains to the limited understanding of how MDMA affects humans under controlled conditions, especially with repeated administration. Therefore, the first study in this project addresses this gap by studying the effects of MDMA administered repeatedly to volunteer human research participants under carefully controlled conditions.

1.4 Brief History of Methamphetamine in the United States

In 1893, Nagai Nagayoshi, a Japanese chemist, pioneered the first synthesis of methamphetamine (Rasmussen, 2015). By 1938, methamphetamine had gained significant attention in Nazi Germany, where it was marketed under the brand name Pervitin (Vearrier et al., 2012). Initially, methamphetamine was promoted for medical and psychiatric uses. Notably, Adolf Hitler himself reportedly received methamphetamine injections from his physician to

manage his depression, fatigue, and Parkinson's disease symptoms. He also combined it with oxycodone, an opioid pain medication discovered by German chemists, to enhance pain relief and boost his confidence (Ohler, 2017).

During World War II, Nazi soldiers were given Pervitin tablets to enhance their endurance, alertness, and reduce fatigue (Ohler, 2017; Rasmussen, 2016). However, this practice was not exclusive to the Nazi party. Other nations, including Japan, the US, and the UK, also supplied their soldiers with methamphetamine or amphetamine for similar purposes (Rasmussen, 2011).

The 1960s saw a surge in concerns, primarily in San Francisco, about the intravenous use of methamphetamine. A liquid form of the drug was gaining popularity as a treatment for heroin addiction and was being diverted to the black market. Concurrently, heroin users were increasingly using intravenous methamphetamine in combination with opioids (Anglin et al., 2000). The emergence of this new and potentially more dangerous method of methamphetamine administration attracted national attention.

In response to concerns associated with methamphetamine use, it was classified as a Schedule II substance on July 7, 1971. This occurred just weeks after President Richard Nixon declared the "war on drugs." While this classification prohibited recreational use of methamphetamine, it remained available for medical purposes, including the treatment of conditions such as Attention Deficit Hyperactive Disorder (ADHD) and obesity.

1.5 Current Recreational and Clinical Methamphetamine Use

Despite rigorous regulatory measures, methamphetamine use persists today. Epidemiological reports indicate that illicit methamphetamine use has been gradually climbing in

recent years. Between 2015 and 2022, the number of current illicit methamphetamine users increased from approximately 900,000 (.3%) to 1,700,000 (.6%) (SAMSHA, 2023a). An increase has also been observed in Americans seeking treatment for their methamphetamine use. Between 2015 and 2021, the number of methamphetamine treatment seekers rose from 136,724 to 170,220 (SAMSHA, 2023b).

In addition to illicit use, methamphetamine continues to attract interest, albeit limited, as a therapeutic agent. For example, Desoxyn®, the only licit methamphetamine product, is FDA-approved for treating ADHD and obesity. However, it is rarely prescribed. In fact, the total per capita weight distribution of prescribed amphetamines (e.g., Adderall) is 4,000 times higher than Desoxyn® (Lopera et al., 2023). This vast contrast in distribution likely stems from the stigma associated with methamphetamine, as methamphetamine and *d*-amphetamine, the primary psychoactive ingredient in Adderall®, produce overwhelmingly similar effects (Kirkpatrick et al., 2012c).

The stigma of methamphetamine may extend into other professional domains. For example, over 300 clinical trials have been registered with ClinicalTrials.gov in connection with methamphetamine. However, the overwhelming majority of these trials focus solely on addressing methamphetamine use disorder. This disproportionate focus may inadvertently overshadow the potential broader medical applications of methamphetamine.

1.7 Interactive Effects of Methamphetamine and Alcohol

A substantial body of research conducted in laboratory animals suggests that methamphetamine may induce neurotoxicity, particularly impacting dopamine neurons, and disrupt several cognitive functions (Hart et al., 2012). However, it is crucial to acknowledge the inherent limitations of many of these animal studies when attempting to apply their findings to

humans. Similar to the critiques raised concerning the MDMA studies discussed earlier, the dosing regimens used in these animal experiments often do not mirror key aspects of human recreational methamphetamine use. For example, animals are typically administered extremely large doses without gradual dose escalation, which contrasts sharply with the more gradual dose increases typically seen in human recreational methamphetamine use. Notably, this gradual dose escalation can induce tolerance, which may protect against neurotoxic effects.

Moreover, if methamphetamine were indeed neurotoxic to humans, particularly to dopamine neurons — given the pivotal role of dopamine-rich brain areas in various essential human functions ranging from movement to learning and memory — we would anticipate deleterious effects on cognitive functioning. Hart and colleagues (2012) conducted a critical review of studies investigating the impact of recreational methamphetamine use on neuroimaging measures and human cognition. They identified statistically significant differences between methamphetamine users and control participants on some measures. While statistically significant, these differences may have limited clinical significance since cognitive functioning typically falls within the normal range when compared against normative data. Nonetheless, there is a tendency to interpret any differences in cognitive function and brain-related measures as indicative of clinically significant impairments or abnormalities, respectively.

To deepen our understanding of the effects of methamphetamine on humans, researchers have conducted controlled laboratory studies investigating the drug's physiological and psychological effects. In a series of studies led by Hart and his team, the effects of methamphetamine on humans were thoroughly investigated. Prioritizing participant safety, the team initially administered low oral doses of methamphetamine (5-10 mg: Hart et al., 2001). In subsequent studies, Hart and colleagues explored the effects of methamphetamine using the same

low oral doses administered repeatedly (b.i.d. for three consecutive days) (Comer et al., 2001) as well as larger doses administered intranasally (12, 25, and 50 mg/70 kg) (Hart et al., 2008). The effects of methamphetamine administration in humans vary based on factors such as the dose, route of administration, and individual degrees of tolerance. However, broadly speaking, the results of these studies indicated that methamphetamine led to dose-dependent increases in various physiological parameters, including heart rate, systolic blood pressure, and diastolic blood pressure. Additionally, beyond its physiological effects, it also reduced food intake and sleep but enhanced positive mood and arousal (Hart et al., 2001; Comer et al., 2001). Notably, repeated oral dosing induced rapid tolerance to the positive mood effects while sensitizing individuals to negative mood effects (Comer et al., 2001). Regarding cognitive function, methamphetamine improved reaction time and enhanced cognitive abilities such as vigilance and visuospatial processing (Hart et al., 2008). Despite these observed effects, methamphetamine administration at the dosing regimens employed in the studies was well tolerated with no significant drug-related adverse effects reported.

Despite the strides made in understanding the effects of methamphetamine when used alone, there are still notable gaps in our knowledge. Human drug administration studies have primarily focused on evaluating methamphetamine in isolation, inadvertently overlooking its potential interactions with other substances. This gap is crucial because in real-world scenarios, individuals often consume methamphetamine concurrently with other substances. Reasons for this co-consumption vary, but common motivations include enhancing the desired effects or mitigating the unwanted effects of either methamphetamine or the co-consumed drug. However, combining drugs can significantly increase the risk of adverse reactions, including overdose, toxicity, or unexpected side effects. Therefore, studying these combinations is paramount, as

researchers can identify potential safety concerns and develop strategies to mitigate the risks associated with polydrug use, thereby providing evidence-based guidance to individuals who engage in such practices.

Of particular concern is the combination of methamphetamine and alcohol, which is a prevalent practice among recreational methamphetamine users. Surveys indicate that approximately 80% of illicit methamphetamine users combine the drug with alcohol (Bujarski et al., 2014), and frequent alcohol consumers are roughly five times more likely to report methamphetamine use compared to non-drinkers (Furr et al., 2000). Despite its widespread use, this drug combination can have potentially dangerous consequences. The most recent data from the Drug Abuse Warning Network (SAMHSA, 2023c) revealed that alcohol has become the most common co-involved drug in methamphetamine-related Emergency Department visits. This phenomenon may, to some extent, be attributed to the physiological interactions of these substances. Methamphetamine is well-documented to elevate cardiovascular measures, and despite the common perception of alcohol as a sedative, it can also lead to increased cardiovascular activity at low to moderate doses (For review see Henler et al., 2011). Moreover, findings from epidemiological reports indicate that alcohol can intensify the cardiac response to methamphetamine, potentially leading to long-term cardiovascular issues (Won et al., 2013; Fleury et al., 2008). As a result, there is a concern about the possibility of dangerous synergistic cardiovascular effects when methamphetamine and alcohol are combined.

In the only acute study involving intravenous methamphetamine (0, 30 mg) and oral alcohol (0, 1 gm/kg), Mendelson and colleagues (1995) examined the physiological and psychological effects following the combined administration of these substances. The combination resulted in a greater increase in heart rate compared to methamphetamine alone,

although the observed increases were relatively modest and not considered clinically concerning. Interestingly, the combination also reduced feelings of alcohol-related intoxication. This finding raises a potential concern: some methamphetamine-using individuals might escalate their alcohol consumption to achieve their accustomed level of intoxication, possibly increasing the risk of alcohol toxicity.

Despite providing valuable insights into the acute effects of the methamphetamine-alcohol combination, critical research gaps persist. Specifically, in real-world scenarios, users of the methamphetamine-alcohol combination often consume multiple doses in a single day (Cho et al., 2001), yet this phenomenon remains poorly understood. It is conceivable that greater cardiovascular effects may occur following repeated administrations of the methamphetamine-alcohol combination, thereby increasing the risk of cardiotoxicity. Of particular interest is whether tolerance to the reduction in feelings of alcohol intoxication develops over time. If so, this may suggest that concerns regarding methamphetamine-alcohol combination users increasing their alcohol consumption in an attempt to achieve their accustomed level of intoxication are overstated. Consequently, our second aim was to directly measure the cardiovascular and subjective effects of repeated administration of a methamphetamine-alcohol combination.

1.6 Interactive Effects of Methamphetamine and Oxycodone

Recent data from the Drug Abuse Warning Network (SAMHSA, 2023c) reveals that opioids have become the most common co-involved class of drugs in methamphetamine-related Emergency Department visits, surpassing alcohol. Additionally, over the past decade, there has been a notable increase in overdose death reports mentioning both methamphetamine and opioids together (Spencer et al., 2023a). However, interpreting these overdose data are challenging due

to the lack of standardized toxicology verification and national death investigation protocols, complicating determinations of causality (Slavova et al., 2015). Nevertheless, a study analyzing drug mentions in social media posts found a similar upward trend in the co-mentions of methamphetamine and opioids during the same period (Sarker et al., 2022). These convergent findings, at the very least, suggest a growing trend in the popularity of methamphetamine-opioid combinations and underscore the need for further research into their use.

Fentanyl and its analogs, often referred to as "fentanyls," continue to be the primary opioids of concern in the majority of methamphetamine-opioid overdose death reports (Spencer et al., 2023b). However, in specific regions, like Oklahoma, oxycodone takes precedence (Bonk et al., 2020). Notably, there was a significant increase in methamphetamine-opioid overdose death reports in Oklahoma, from three cases in 2002 to 69 cases in 2017, with oxycodone being the most commonly mentioned opioid (Bonk et al., 2020). Moreover, even in cases where fentanyls are cited as a contributing factor in overdose death reports, it may be indicative of users seeking oxycodone. Counterfeit oxycodone pills have been increasingly found to contain fentanyls, sometimes in lethal doses (Hartmann & Sethi, 2023). Unfortunately, due to the scarcity of research on the methamphetamine-oxycodone combination, the public's understanding of this combination may be largely influenced by users not ingesting oxycodone as intended, but instead unknowingly consuming fentanyls, other drugs, or even no drugs at all. This highlights the urgent need for comprehensive and accurate information about the actual effects of combining methamphetamine and oxycodone.

To date, only one study has directly quantified methamphetamine-oxycodone combinations and the focus was to help develop treatments for co-occurring methamphetamine and opioid use disorder. In this *in vitro* experiment, Meyer and colleagues (2022) cultured

cortical neurons from Sprague Dawley rats and treated them with 100 mM methamphetamine and 100 mM oxycodone for 24 hours. The researchers then utilized quantitative mass-spectrometry proteomics, a method for measuring proteins in biological samples. The results unveiled significant differences in the concentrations of 94 proteins when compared to control cells. To comprehend the biological relevance of these protein changes, the researchers employed ClueGO analysis. Essentially, ClueGO serves as a reference dataset to ascertain the biological function of each protein and identify any interconnected functional themes. The analysis linked the list of 94 proteins to various molecular functions and biological processes, particularly those associated with neural plasticity. Additionally, Meyer and colleagues (2022) highlighted the downregulation of Striatin-1, a synaptic protein critical for regulating essential neurological functions, especially neural plasticity and synaptic structure. Based on this finding, Meyer and colleagues (2022) proposed Striatin-1 as a potential therapeutic target for mitigating the adverse effects of combined methamphetamine and oxycodone use.

However, it is essential to recognize that *in vitro* studies involve isolated cells or tissues that have been removed from living organisms, which may not fully capture the intricate interactions within a living body. Factors like drug metabolism, distribution, and elimination in animals can significantly impact a drug's pharmacological effects. Hence, living non-human animal studies are a logical progression from *in vitro* research. These studies allow us to assess behavioral effects, providing valuable insights that can guide future human research. Consequently, non-human animal studies often act as a bridge between *in vitro* and human studies.

On a more practical note, clinical reports can provide valuable insights into what motivates individuals to use methamphetamine-opioid combinations. For example, Ellis and

colleagues (2018) gathered both quantitative and qualitative data from patients entering drug treatment programs to explore the reasons underlying the popularity of methamphetamine and opioid combinations. A total of 300 participants completed an online survey that focused on their patterns and motivations related to using both methamphetamine and opioids simultaneously. Participants were specifically asked to explain why they used methamphetamine and opioids together, and the most common reason reported (51.0%) was the desire to experience a greater high resulting from their combined use. This was often described as a synergistic high, aimed at intensifying or optimizing the pleasurable sensations associated with both drugs.

Findings from non-human animal self-administration paradigms generally support these clinical findings. For instance, Ranaldi & Wise (2000) conducted an intravenous methamphetamine-heroin combination self-administration experiment using male Long-Evans rats. Catheters were inserted into their jugular veins to enable drug administration directly into their bloodstream. The rats then learned to press a lever for methamphetamine infusions. After learning to self-administer the drug they were then transitioned to a progressive ratio reinforcement schedule. Under this regimen, the number of lever presses required to obtain an infusion increased with each successive infusion until the rats reached a point where they ceased responding, referred to as the breaking point. Breaking points were determined across various methamphetamine doses (0.0625–2.0 mg/kg) alone or in combined with heroin (12.5 μg/kg). The results indicated that the combined administration of methamphetamine and heroin was associated with higher breaking points compared to methamphetamine alone across all tested doses. Notably, the most substantial difference between the combination and methamphetamine alone was observed at the lowest combination dose, where the breaking point for the combination was approximately four times higher than methamphetamine alone. Based on these

results, the authors concluded that this drug combination generates a more rewarding effect than methamphetamine alone.

While self-administration remains the gold standard in behavioral pharmacology, a diverse range of behavioral measures beyond self-administration are necessary to gain a comprehensive understanding of methamphetamine-opioid combinations. In addition to an enhanced rewarding effect, it is generally assumed that methamphetamine and opioids interact to lessen the adverse effects of one or both drugs. For example, amphetamines may acutely reduce the sedation associated with opioid administration and improve cognition (Jasinski & Preston, 1986; Dalal & Melzack, 1998). Conversely, opioids may reduce the intensity of methamphetamine-induced anxiogenic effects (Rhed et al., 2022). In such scenarios, the softened adverse impact of one drug due to the other could influence the appeal of the combination. However, these effects have been relatively overshadowed in preclinical models in favor of studying the combination's rewarding effects (Riley et al., 2019).

Animal studies present a valuable avenue for exploring drug combinations not yet evaluated in human drug administration studies. These preclinical investigations serve as a foundational platform for understanding the effects of such combinations on biology and behavior. By leveraging this understanding, researchers can discern potential benefits and risks, uncover unexpected effects, shape future human research, establish safety profiles, and guide regulatory decisions. Ultimately, insights from animal studies can be used to enhance safety measures and refine the design of human drug administration studies before implementation.

It is important to recognize that translating findings from animal studies to humans is a complex endeavor (Meyer et al., 2023). Species differences in biology and metabolism significantly impact drug effects, complicating cross-species extrapolations. Additionally,

assessing the effects of drugs on mood is crucial in human research. However, animals cannot verbally express their mood or emotional state, making it challenging to consider this factor in preclinical studies. Limited communication between preclinical and clinical researchers exacerbates these challenges, often leading to inadequate dose conversions and divergent experimental measures.

Taking into account these considerations, Study 3 represents a re-analysis of a previously unpublished study conducted by Keith (2014). In Keith's study, the behavioral effects of a methamphetamine-oxycodone combination in C57BL/6N mice were quantified using measures and doses comparable to those utilized in human studies. A variety of well-established paradigms, adapted for both animal and human use, were employed to characterize the behavioral effects of this drug combination. While methamphetamine-opioid combinations are typically associated with greater rewarding effects, this study also explored potential alterations in aversive effects induced by the combination. To address this, the study measures assessed both positive and potentially adverse effects of the methamphetamine and oxycodone interaction.

Keith (2014) utilized the Conditioned Place Preference (CPP) paradigm, allowing for the measurement of both conditioned place preference (indicating positive associations) and avoidance (indicating aversion) in response to an environment associated with pleasant or unpleasant stimuli. Next, the Open Field paradigm was employed to evaluate anxiety-like behavior, providing insights into both anxiolytic (anxiety-reducing) and anxiogenic (anxiety-inducing) effects. Finally, Novel Object Recognition was used to assess recognition memory and exploratory behavior, enabling the detection of enhancements or disruptions in task performance. Importantly, this unpublished study was the first to investigate the behavioral effects of the methamphetamine-oxycodone combination.

In Keith's (2014) study, CPP was extinguished for all drug conditions by the fourth day. However, it is possible that the results may differ if mice that did not develop an initial CPP are excluded from the statistical analysis. Literature suggests that CPP extinction studies should focus exclusively on animals that demonstrate an initial CPP, defined as spending at least 10% more time in the drug-paired chamber than the unpaired chamber (Voigt et al., 2011; Li et al., 2022). Furthermore, following drug administration, Keith (2014) reported no significant differences in Novel Object Recognition. However, it is possible that removing animals that do not interact with the objects or spend minimal time exploring them, as suggested by previous research, may lead to significant effects (Ennaceur & Delacour, 1988; Denninger et al., 2018).

Lastly, long-term novel object recognition locomotor activity data were not analyzed by Keith (2014). These unanalyzed data, along with the results of the data analysis suggested above, may be particularly informative in understanding the behavioral effects of the methamphetamine-oxycodone combination. Therefore, Study 3 represents a re-analysis of a prior unpublished study by Keith (2014), with more stringent guidelines for inclusion/exclusion criteria and the inclusion of unanalyzed locomotor activity data.

1.8 Scientific and Public Health Significance

These studies were undertaken to expand the database concerning the effects of amphetamine derivatives in mice and humans. Specifically, the present studies used a multilevel approach in order to better understand: 1) the repeated-administration effects of MDMA and 2) the interactive effects of two popular methamphetamine drug combinations.

Chapter 2: Repeated MDMA Administration Effects

2.1 Introduction

There is a growing database describing the acute effects of a single MDMA dose administered to human research participants. In general, at doses ranging from ~75 to 145 mg, MDMA elevates heart rate and blood pressure, and enhances positive subjective effects (Clark et al., 2015; Kirkpatrick et al., 2014, 2016). The positive effects likely contribute to the popularity of MDMA at nightclubs and music festivals. However, studies also highlight that in recreational settings, users frequently consume multiple doses over a 48-hour period (Topp et al., 1999; Hammersley et al., 1999; Sterk et al., 2006). Researchers have suggested this practice can have serious negative consequences to users since MDMA can inhibit its own metabolism through a process called metabolic inhibition (de la Torres et al., 2004), thereby increasing plasma levels of MDMA that are transported to sites of action. Thus, the effects following an initial MDMA dose may increase with each successive administration. Consequently, there are significant concerns about the adverse effects associated with repeated MDMA use. Specifically, this dosing regimen may cause dangerous increases in heart rate, blood pressure, and body temperature (Parrott, 2013).

The impact of repeated MDMA dosing on physiological and behavioral outcomes remains poorly understood due to limited research. To bridge this gap, de la Torre and colleagues examined the effects of repeated MDMA doses on multiple dependent variables such as cardiovascular activity and mood. They utilized 2-, 4-, and 24-hour inter-dose intervals with cumulative doses ranging between 150 and 200 mg (Farré et al., 2004, 2015; Peiró et al., 2013). As expected, acute MDMA administration increased heart rate, systolic and diastolic blood pressure, and positive subjective-effect ratings (e.g., “good drug effect”). In general, repeated-

dosing did not potentiate physiological effects. Greater peak positive subjective-effect ratings (i.e., “stimulated”) were observed, but only under the 24-hour inter-dose interval condition (Farré et al., 2004). These data suggest that under the dosing regimens employed, greater peak effects are generally not observed.

Although de la Torres and colleagues did not observe greater physiological effects in response to repeated doses of MDMA, unanswered questions remain. For example, when 2- and 4-hour inter-dose intervals were utilized, area under the curve analysis revealed greater effects of repeated MDMA administration on systolic blood pressure. While these effects were small, only two drug administrations — with a maximum cumulative dosage of 200 mg — were assessed. This, by some accounts, is a relatively low number of administrations and small amount of drug over a weekend period (Parrott, 2013). Therefore, it is possible that a greater number of drug administrations and larger cumulative dose will lead to greater physiological effects.

Another concern is the lack of experimental studies in humans assessing the residual effects following repeated MDMA administration. Anecdotal evidence suggests that a short-term depressive state emerges in the days following MDMA use, a phenomenon commonly known as "mid-week blues" or "suicide Tuesdays." (Greer and Tolbert 1986; Curran and Travill 1997; Parrott 2014). It has been suggested that this phenomenon occurs one to two days after MDMA administration (Parrott & Lasky, 1998; Klitzman, 2006). Indeed, this depressed mood state could impact functioning in other domains, such as cognitive performance and sleep, and pose an unexplored potential health risk (Chepenik et al., 2007).

Added to the above, there is now considerable interest in the use of MDMA for medical purposes. The drug is currently being assessed in Phase 3 clinical trials for utility as a post-traumatic stress disorder (PTSD) treatment (Mithoefer et al., 2011, 2013, 2018; Oehen et al.,

2013; Mitchell et al., 2023). Mitchell and colleagues (2023) compared the effects of MDMA administration plus psychotherapy with placebo plus psychotherapy and found that the drug decreased PTSD symptom severity. Given that MDMA is administered repeatedly in these clinical trials, assessing drug-related repeated-dose effects in the human laboratory on a broad range of dependent variables could provide important complementary information about MDMA-related safety profile.

To date, no controlled laboratory study has investigated the repeated-dose and residual effects of MDMA administration on a broad range of physiological, subjective, and cognitive measures. Therefore, this 15-day residential study characterized the acute, repeated-dose and residual effects of MDMA (0, 50, 100 mg) with a maximum cumulative dose of 300 mg delivered over 36 hours. Multiple dependent measures, including blood pressure, heart rate, mood, and sleep, were assessed repeatedly.

2.2 Methods

The present report builds upon findings originally presented in the PhD thesis of Dr. Bajger (2014) entitled Acute, Repeated-Dose and Residual Effects of Amphetamines on Psychological Measures in Humans. Columbia University. For this current study, the data have been reanalyzed to incorporate the second administration, which was not included in the original analysis.

Participants

Participants were recruited through word-of-mouth referrals, as well as through advertisements in New York City newspapers, online platforms, and flyers. All potential participants underwent both an initial telephone screening and a subsequent in-person medical psychiatric evaluation. Eligibility criteria included a minimum of 21 years of age, and self-reported current (i.e., past 30 days) MDMA use. Exclusion criteria for the study included a

significant medical history, such as cardiovascular, neurological, or major psychiatric illnesses, or any other condition that could increase the risk associated with study participation. It is noteworthy that the subset of potential participants able to commit to extended stays on the residential unit was relatively small and possibly unique. This characteristic may limit the generalizability of the study's findings to a broader population.

Twelve research volunteers, with a mean age of 28.9 ± 7.2 (mean ± standard deviation), participated in this 15-day inpatient study. Among them, three identified as female at birth (one Black and two Latina), while nine identified as male at birth (three Black, three Latino, three White). Volunteers, on average, had completed 13.0 ± 1.7 years of traditional education.

Before enrolling in the study, participants successfully completed comprehensive medical and psychiatric evaluations (i.e., Structured Clinical Interviews for DSM-IV). Additionally, they fell within normal weight ranges based on the Metropolitan Life Insurance Company height/weight table, with a mean body mass index of 24.4 (± 3.8). All participants reported current MDMA use during an initial telephone interview (1.28 ± 0.95 times/week) and again during an in-person medical psychiatric evaluation. Additionally, nine reported current cocaine use (1-3 times/week), eleven reported current alcohol and cannabis use (0.25-5 drinks/week; 1-7 times/week respectively, with 3 being daily cannabis users), and five smoked five to 30 tobacco cigarettes/day. None of the participants met criteria for an Axis I disorder, and none were seeking drug use treatment.

Participants were informed that the purpose of the study was to evaluate the behavioral effects of one of the following drugs in a residential laboratory setting: 1) Amphetamine (speed, Adderall®, amp); 2) Methamphetamine (Tina, crystal, meth); 3) 3,4-Methylenedioxymethamphetamine (MDMA, XTC, ecstasy, Molly); or 4) Methylphenidate

(Ritalin®). Each participant, prior to enrollment, signed a consent form that had received approval from the Institutional Review Board of the New York State Psychiatric Institute. At the end of the study, participants were debriefed about the experimental and drug conditions and compensated at a rate of $35 per day. Additionally, those who completed the entire 15-day study received a bonus of $35 per day.

Pre-Study Training

Before the residential phase of the study began, participants underwent two 3-4 hour training sessions on the computerized cognitive and psychomotor tasks to be used during the study. This was intended to reduce learning effects on task performance. They were also oriented to the residential laboratory and study procedures. Separately, to screen for potential adverse reactions and acquaint them with the study drug, participants received the highest single dose of MDMA (100 mg) to be used in the study, although they were not informed of the dose until after the study concluded. No adverse effects were reported. No untoward effects were detected or reported.

Design

The study utilized a double-blind, placebo-controlled, within-participant design. It involved three groups, each with four participants, residing in a residential laboratory at the New York Psychiatric Institute for 15 days. As detailed in **Table 2.1**, the study was structured into three 5-day session blocks. Administrations occurred b.i.d., at 0900 hours in the morning and 2100 hours in the evening. The first day of each block served as a washout period during which only a placebo was administered. On the second day of each block, participants received the same active MDMA dose—either 0, 50, or 100 mg—in the morning and again 12 hours later in the evening. On the third day placebo was administered in the morning, then 24 hours from the

previous dose, the same MDMA dose was administered in the evening. The doses were counterbalanced both across and within participant groups. The subsequent two days (days 4-5) involved placebo administration at both dosing times. These consecutive days of placebo administration served a dual purpose: to examine any residual effects of MDMA and to act as a washout period.

Table 2.1 Study design and representative dosing schedule

100 mg MDMA block					
Time	Day 1	Day 2	Day 3	Day 4	Day 5
0900	*Placebo*	**100 mg**	*Placebo*	*Placebo*	*Placebo*
2100	*Placebo*	**100 mg**	**100 mg**	*Placebo*	*Placebo*
50 mg MDMA block					
Time	Day 6	Day 7	Day 8	Day 9	Day 10
0900	*Placebo*	**50 mg**	*Placebo*	*Placebo*	*Placebo*
2100	*Placebo*	**50 mg**	**50 mg**	*Placebo*	*Placebo*
Placebo only block					
Time	Day 11	Day 12	Day 13	Day 14	Day 15
0900	*Placebo*	*Placebo*	*Placebo*	*Placebo*	*Placebo*
2100	*Placebo*	*Placebo*	*Placebo*	*Placebo*	*Placebo*

Procedure

Participants were brought into the laboratory a day before the study began to help them acclimate to the unique environment, practice experimental procedures, and ensure abstinence from psychoactive substances. At 0900 and 2100 hours, participants were administered an active MDMA or placebo capsule. Performance and subjective-effects were evaluated on a computer when participants were alone, both at baseline and repeatedly after the first administration.

Subjective-effect measures were also assessed once following the second administration. Physiological measures were obtained at baseline and repeatedly after the first and second dose **(see Table 2.2)**. At 8:00 am daily, participants were provided with a box of food comprising a range of meal items, snacks, and beverages that could be consumed when not completing tasks. Frozen meals and other items from the food box were available continually upon request. However, meals requiring preparation were not permitted during task batteries. Water was available at all times. Participants could smoke cigarettes from 0800 to 2300 hours. Lights were turned out at 2400 hours, 3 hours after the second dose, for an 8-hour sleep period.

Table 2.2 Typical Experimental Day

Time	Activity
0820	Sleep questionnaire
	Physiological measures
	Task battery 1
0900	**Capsule administration**
0925	Physiological measures
0950	Physiological measures
1000	Task battery 2
	Drug effect questionnaire
1040	Physiological measures
1050	Task battery 3
1130	Physiological measures
1140	Task battery 4
1220	Physiological measures
1230	Task battery 5
1310	Physiological measures
1400	Physiological measures
1435	Task battery 6
1515	Task battery 7
1600	Task battery 8
1645	Task battery 9
2050	Physiological measures
2100	**Capsule administration**
2125	Physiological measures
2150	Physiological measures
2200	Physiological measures
2250	Physiological measures
	Visual analog questionnaire
	Drug-effect questionnaire
2400	Lights out

Subjective Effects

The computerized 44-item visual analog scale featured 100-mm lines, labeled 'not at all' on one end and 'extremely' on the other (Hart et al. 2003). The lines were presented one at a time and labeled with adjectives describing a positive (e.g., “Alert,” “Stimulated”) or negative (e.g., “Angry,” “Miserable”) mood, a drug effect (e.g., “Good effect,” “Bad effect”) or a physical symptom (e.g., “Nauseous,” “Noises seem loud”). The following is a list of all the adjectives included: “Alert,” “Angry,” “Anxious,” “Bad effect,” “Blurred vision,” “Can’t concentrate,” “Chills,” “Content,” “Clumsy,” “Confused,” “Depressed,” “Dizzy,” “Energetic,” “Forgetful,” “Friendly,” “Good effect,” “Headache,” “Heart pounding,” “High,” “Hungry,” “Irritable,” “Jittery,” “Mellow,” “Miserable,” “Muscle pain,” “Nauseous,” “Noises seem loud,” “On edge,” “Restless,” “Sedated,” “Self-confident,” “Sleepy,” “Social,” “Stimulated,” “Stomach pain,” “Sweating,” “Talkative,” “Tired,” “Unmotivated,” “Upset stomach,” “Want alcohol,” “Want cigarette,” “Want marijuana,” and “Withdrawn.” In addition, participants completed a drug-effect questionnaire (DEQ), during which they were required to rate “good effects” and “bad effects” on a five-point scale: 0 = “not at all” and 4 = “very much.” Additionally, ratings for drug strength and the desire to "take the drug again" were also gathered.

Cognitive/psychomotor tasks

The computerized cognitive/psychomotor task battery was selected in part because it had been used in multiple other studies assessing the effects of drugs on performance (e.g., Hart et al., 2008; Kirkpatrick et al., 2012c; Keith et al., 2017). This strategy facilitated the comparison of data collected in the current study with previous findings. Additionally, the task battery assesses a broad range of cognitive/psychomotor domains, which will help us better understand the acute and residual effects of MDMA on a broad range of cognitive/psychomotor domains. The battery

consists of five tasks administered in a fixed order: Digit Recall Task, Digit Symbol Substitution Task (DSST), Divided Attention Task (DAT), Rapid Information Task (RIT), and Repeated Acquisition Task (RA). Participants had already mastered these tasks during pre-study training sessions, mitigating potential order and learning effects on task battery performance within the study. The completion of the task battery typically takes around 30 minutes, with intervals between task batteries serving as rest periods.

The Digit Recall Task evaluated alterations in immediate and delayed recall (Hart et al., 2001). Participants were shown an eight-digit number for 3 seconds on a computer screen. They were asked to input the number accurately both while it was visible and after it vanished. Additionally, they were informed that they would be required to recall number approximately 30 minutes later.

The DSST gauged changes in visuospatial processing (McLeod et al., 1982). It involved nine random three-row, three-column squares (with one square blackened per row) displayed at the top of the computer screen. Each array corresponded to a number (1–9). A randomly generated number, indicating which array to replicate, appeared at the bottom of the screen. Participants were asked to reproduce as many patterns as possible using the nine-key keypad attached to the computer.

The DAT evaluated changes in vigilance and inhibitory control by integrating pursuit-tracking and vigilance tasks (Miller et al., 1988). Using a mouse, participants tracked a moving circle on a video screen and signaled whenever a small black square appeared at any of the screen's four corners. The accuracy of tracking the moving circle directly affected its speed, increasing proportionally with greater precision.

The RIT assessed changes in sustained concentration and inhibitory control (Wesnes & Warburton, 1983). During this task, digits were displayed at a speed of 100 per minute. Participants were required to quickly press a response button when they identified sequences of three consecutive odd or even digits. Points were given for each correct response ("hit") and subtracted for each incorrect response ("miss" or "false alarm"). The objective was to amass as many points as possible throughout the duration of the task.

The RA task assessed changes in acquisition efficiency, a measure of learning ability (Kelly et al., 1997). At the start of the task, participants were taught a ten-response sequence of button presses. Each correct press increased the position counter by one, which did not change after an incorrect response. Each complete and correct execution of the sequence added one point to the points counter. While the sequence was consistent for each run of the task, a new random sequence was introduced for every subsequent task battery. The goal for participants was to accumulate as many points as possible by accurately following the specified button sequence.

Sleep Monitoring

Sleep was measured objectively using the Actiwatch® Activity Monitoring System (Respironics Company, Bend, OR), which tracked gross motor activity to provide estimates of total sleep time, sleep onset latency, sleep efficiency (expressed as the percentage of total sleep time relative to time in bed), and the number of wake bouts (Kushida et al., 2001). Additionally, subjective sleep experience from the previous night was assessed each morning using a visual analog sleep questionnaire. This questionnaire utilized a series of 100-mm lines ranging from "not at all" to "extremely" and included prompts such as "I slept well last night," "I woke up early this morning," "I fell asleep easily last night," "I feel clear-headed this morning," "I woke

up often last night," and "I am satisfied with my sleep last night." Participants were also asked to estimate the number of hours they slept (Haney et al., 2001).

Drug

Tablets of MDMA hydrochloride, supplied by Dr. David Nichols of Purdue University, were repackaged by the Pharmacy Department at the New York State Psychiatric Institute. Each white #00 opaque capsule was filled with the designated amount of MDMA hydrochloride and lactose as a filler. The placebo capsules, also white and #00 in size, contained only lactose.

The three drug conditions were: Placebo, 50 mg MDMA, and 100 mg MDMA. The doses selected were based on previous studies indicating that 100 mg of MDMA reliably increased cardiovascular activity and positive mood (Bedi et al., 2009, 2010; Kirkpatrick et al., 2012b).

Data Analysis

Acute cognitive/psychomotor performance data were analyzed using two-factor repeated-measures analysis of variance (ANOVA) for every outcome: the first factor was drug condition (placebo, 50, and 100 mg MDMA) and the second factor was time. For each physiological and subjective-effect measure, we identified the peak effect by selecting the maximum value obtained over multiple time-points. Subsequently, we conducted a single-factor (condition) repeated-measures ANOVA on these peak effects. Sleep effects, encompassing objective measures from the Actiwatch® Activity monitoring System (e.g., total sleep time and sleep onset latency), and subjective measures from the sleep questionnaire (e.g., estimated hours of sleep) were analyzed individually using single-factor ANOVAs; the factor was drug condition. Residual drug effects, commonly referred to as hangover effects, on physiological, cognitive, and subjective measures were examined using a repeated-measures two-factor ANOVA. One factor was drug condition, and the other factor was day (Days 3, 4, and 5). Planned contrasts (i.e.,

paired samples t-tests) were employed to compare these effects on days 3-5 following MDMA administration with the corresponding days following a series of placebo administrations. Planned contrasts (i.e., paired samples *t*-tests) were employed to compare these effects on days 3-5 following MDMA administration with the corresponding days following a series of placebo administrations.

Repeated administration physiological effects and subjective ratings were analyzed using a two-factor ANOVA with drug condition as the first factor and drug administration (Administrations 1, 2, and 3) as the second factor. Physiological effects were assessed by comparing peak effects produced by administrations 1-3 within each block. Subjective effects were evaluated using planned contrasts that compared outcomes ~150 minutes after the first drug administration to those observed after the second and third administrations across the same block.

For all analyses, ANOVAs supplied the error terms necessary to calculate planned comparisons between drug conditions (placebo vs. active MDMA doses, 50 mg MDMA vs. 100 mg MDMA) and within (administration 1 vs. administration 2, administration 3, administration 2 vs. administration 3). To address the issue of multiple comparisons and reduce the chance of Type I errors, we utilized the Bonferroni correction. Specifically, we adjusted the significance threshold for *p* values to be less than 0.01. Huynh-Feldt corrections were used when appropriate. All analyses were computed using the statistical package Superanova (1.11, ABACUS Concepts, Berkeley, California).

2.3 Results

Acute Effects

Table 2.3 shows peak acute physiological and subjective effects as a function of MDMA condition. Both MDMA doses (50 and 100 mg) increased peak heart rate and blood pressure ($p<0.01$, for all comparisons). There were no significant effects on oral temperature. Both active doses produced increases of positive-subjective effects (e.g., "High," "Stimulated") and negative subjective-effects ratings (e.g., "Can't Concentrate," "Dizzy"). Finally, cognitive performance was not significantly altered as a function of MDMA dose.

Table 2.3 Acute MDMA Effects on Subjective Measures

	Placebo	**50 mg MDMA**	**100 mg MDMA**
Peak 'positive' mood ratings			
Friendly	72.7 ± 4.7	86.4 ± 4.3 *d* = 3.0*	88.5 ± 5.0 *d* = 3.3*
Good Drug Effect	23.5 ± 8.5	84.6 ± 6.7 *d* = 8.0*	92.8 ± 4.0 *d* = 10.4*
High	19.7 ± 7.2	82.8 ± 5.3 *d* = 9.9*	88.2 ± 5.3 *d* = 10.8*
Self-Confident	82.2 ± 4.5	86.4 ± 4.3 *d* = 1.0	88.8 ± 4.2 *d* = 1.5*
Social	74.7 ± 5.3	83.6 ± 5.3 *d* = 1.7	87.3 ± 5.7 *d* = 2.3*
Stimulated	21.0 ± 7.7	81.0 ± 7.5 *d* = 7.9*	89.9 ± 5.6 *d* = 10.2*
Peak 'negative' mood ratings			
Anxious	3.6 ± 2.0	31.1 ± 11.8 *d* = 3.2*	40.0 ± 10.1 *d* = 4.9*
Can't Concentrate	43.3 ± 8.1	65.1 ± 8.6 *d* = 2.6*	63.8 ± 8.6 *d* = 2.5*
Clumsy	22 ± 9.7	33.3 ± 10.5 *d* = 1.1	48.6 ± 10.1 *d* = 2.7*
Confused	15.0 ± 7.1	30.4 ± 12.7 *d* = 1.5	39.1 ± 9.5 *d* = 2.9*
Forgetful	27.1 ± 9.4	34.6 ± 11.0 *d* = 0.7	54.4 ± 9.6 *d* = 2.8*§
Jittery	12.7 ± 6.9	32.3 ± 10.0 *d* = 2.3	52.6 ± 10.8 *d* = 4.4*§
Peak physical symptoms			
Blurred Vision	8.4 ± 6.6	30.1 ± 10.5 *d* = 2.5	42.9 ± 10.1 *d* = 4.0*
Chills	14.5 ± 8.1	54.0 ± 12.0 *d* = 3.8*	54.1 ± 11.9 *d* = 3.9*
Heart Pounding	14.7 ± 7.3	49.3 ± 11.8 *d* = 3.5*	50.2 ± 11.8 *d* = 3.6*
Nauseous	8.5 ± 4.8	23.8 ± 9.0 *d* = 2.1	38.7 ± 11.3 *d* = 8.6*§
Noises Seem Loud	11.9 ± 7.2	35.2 ± 10.9 *d* = 2.5*	49.3 ± 12.1 *d* = 3.8*§
Sedated	9.5 ± 5.5	41.1 ± 12.0 *d* = 3.4*	41.9 ± 10.5 *d* = 3.9*
Sweating	9.0 ± 5.0	34.3 ± 10.3 *d* = 3.1*	48.2 ± 10.4 *d* = 4.8*
Upset stomach	6.7 ± 5.0	26.3 ± 9.7 *d* = 2.6	32 ± 10.7 *d* = 3.03*
Drug-effect questionnaire ratings			
Good Effect	0.6 ± 0.3	2.3 ± 0.3 *d* = 5.6*	3.4 ± 0.2 *d* = 11.2*
Like	0.3 ± 0.5	2.6 ± 0.2 *d* = 6.0*	3.3 ± 0.3 *d* = 7.6*
Strong	0.6 ± 0.3	2.6 ± 0.2 *d* = 7.8*	3.6 ± 0.2 *d* = 2.2*§
Take Again	1.2 ± 0.5	2.8 ± 0.3 *d* = 3.9*	3.4 ± 0.3 *d* = 5.4*
Bad Effect	0.1 ± 0.1	0.3 ± 0.1 *d* = 2.0	0.8 ± 0.4 *d* = 2.6*

Note: Scores range from a minimum of 0 to a maximum of 100; All values are means ± sem; *d* = Cohen's d; * = $p < .01$, significantly different from placebo; § = $p < .01$, significantly different from 50 mg MDMA; *df* = 2,4

Repeated Administration Effects

Figure 2.1 shows peak heart rate, blood pressure, and oral temperature as a function of MDMA dose and administration number. After the first administration, MDMA produced significant heart rate and blood pressure elevations relative to placebo (p<0.01 for all comparisons). As compared to administration 1, the second administration produced greater effects on oral temperature in the larger dose condition (p<0.01 for all comparisons). By the third administration, only blood pressure remained significantly elevated (p<0.01).

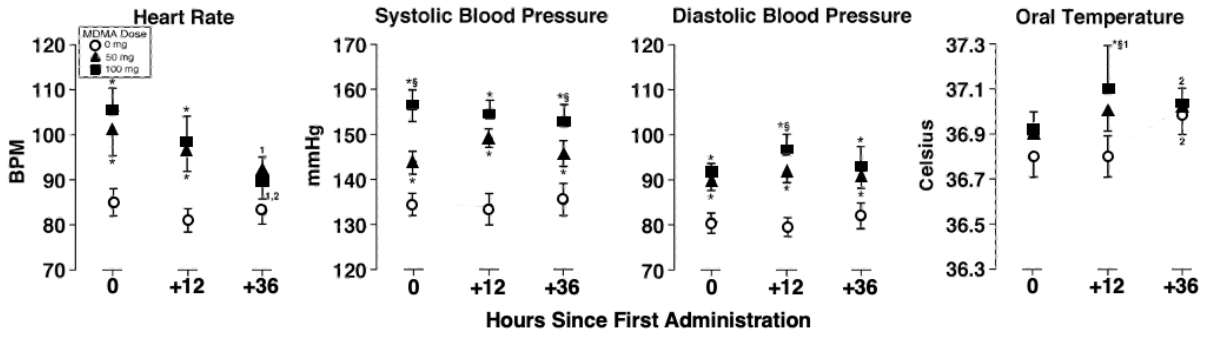

Figure 1.1. Repeated-dose effects on peak physiological measures as a function of MDMA dose and hours since last administration.
Error bars represent 1 standard error of the mean (SEM). Overlapping error bars were jittered for clarity. * MDMA significantly different from placebo (p < 0.01). § 100 mg MDMA significantly different from 50 mg MDMA (p < 0.01).). 1 indicates administration is significantly different from administration 1 (p < 0.01). 2 indicates administration significantly different from administration 2 (p < 0.01).

Figure 2.2 shows selected subjective-effect ratings as a function of MDMA dose and administration number. After the first administration, MDMA, compared with placebo, produced marked elevations on several subjective effect ratings (e.g., "Stimulated," "High," "Good Drug Effect," "Clumsy": p<0.01 for all comparisons). As compared to the first administration, administration 2 produced greater effects on some positive subjective-effect ratings (i.e., "Stimulated," "High," "Good drug effect": p<0.01 for all comparisons). By the third administration, these effects were attenuated, although still significantly elevated as compared to placebo (p<0.01 for all comparisons). **Table 2.4** shows additional significant subjective effects.

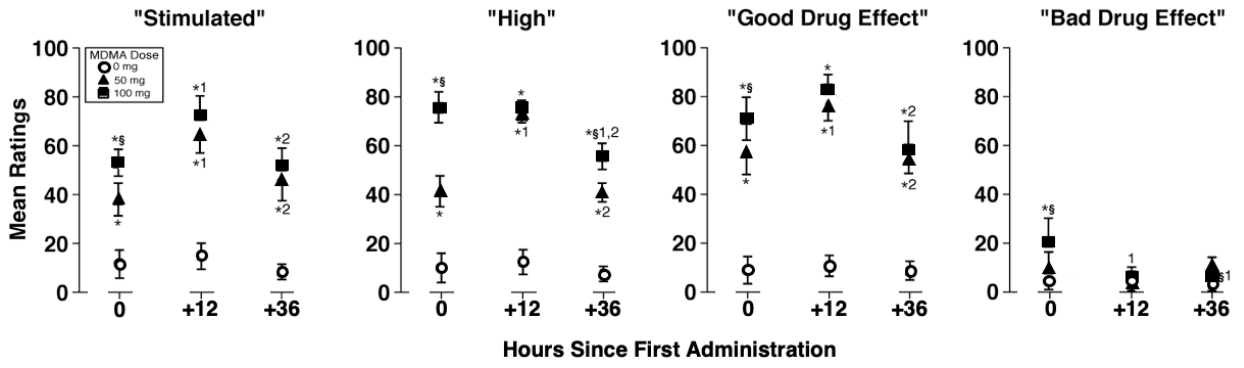

Figure 2.2 Selected repeated-dose effects on subjective-effect ratings as a function of MDMA dose and hours since last administration.

Error bars represent 1 SEM. Overlapping error bars were omitted for clarity. * MDMA significantly different from placebo ($p < 0.01$). § 100 mg MDMA significantly different from 50 mg MDMA ($p < 0.01$). 1 indicates administration is significantly different from administration 1 ($p < 0.01$). 2 indicates administration significantly different from administration 2 ($p < 0.01$).

Table 2.4 Repeated MDMA Administration Effects on Subjective Measures

	Placebo			50 mg MDMA			100 mg MDMA		
	0	**+12 hours**	**+36 hours**	**0**	**+12 hours**	**+36 hours**	**0**	**+12 hours**	**+36 hours**
Positive subjective effects									
Alert	57.0 ± 7.9	45.7 ± 10.4	39.8 ± 9.0	50.8 ± 9.9 *d* = 0.2	55.3 ± 10 *d* = 0.3*	62.8 ± 10.2 *d* = 0.7	43.8 ± 7.5 *d* = 0.5	51.6 ± 9.0 *d* = 0.0	52.2 ± 8.8 *d* = 0.4
Social	58.8 ± 7.8	53 ± 9.7	49.6 ± 10.3	55.9 ± 9.4 *d* = 0.1	79.7 ± 5.9 *d* = 1.0*	70.5 ± 7.5 *d* = 0.7	58.8 ± 8.9 *d* = 0.0	80.1 ± 6.7 *d* = 1.0*	68.1 ± 7.1 *d* = 0.6
Talkative	50.0 ± 8.0	43.9 ± 9.5	43.0 ± 10.6	57.9 ± 7.3 *d* = 0.3	77.5 ± 6.2 *d* = 1.3*	69.3 ± 8.1 *d* = 0.9	45.1 ± 11.1 *d* = 0.2	69.7 ± 10.6 *d* = 0.8	60.0 ± 7.2 *d* = 0.6
Negative subjective effects									
Can't concentrate	11.7 ± 5.2	9.0 ± 5.6	18.0 ± 9.6	28 ± 10.8 *d* = 0.6	26.5 ± 6.2 *d* = 0.9	11.9 ± 5.6 *d* = 0.2	43.1 ± 10.0 *d* = 1.1*	30.0 ± 6.7 *d* = 1.0	24.1 ± 7.1 *d* = 0.2
Forgetful	7.9 ± 5.0	8.8 ± 5.1	11.3 ± 7.1	17.3 ± 8.3 *d* = 0.4	22.0 ± 9.0 *d* = 0.5	12.0 ± 6.6 *d* = 0.0	34.4 ± 9.1 *d* = 1.0*	22.9 ± 8.9 *d* = 0.6	21.8 ± 7.7 *d* = 0.4
Jittery	0.3 ± 0.3	5.3 ± 4.2	1.8 ± 1.2	17.1 ± 6.5 *d* = 1.0	20.8 ± 10 *d* = 0.6	20.5 ± 8.9 *d* = 0.9	31.1 ± 10.4 *d* = 1.2*	34.3 ± 11.5 *d* = 1.0*	18.7 ± 8.9 *d* = 0.7
Restless	9.5 ± 6.5	10.3 ± 7.1	10.0 ± 6.5	21.0 ± 9.7 *d* = 0.4	26.7 ± 9.3 *d* = 0.6	24.3 ± 9.7 *d* = 0.5	26.5 ± 7.1 *d* = 0.7	35.3 ± 9.7 *d* = 0.8*	24.2 ± 8.2 *d* = 0.6
Physical symptoms									
Blurred Vision	3.5 ± 3.3	2.8 ± 2.6	3.4 ± 3.2	7.9 ± 7.5 *d* = 0.2*	21.3 ± 9.7 *d* = 0.8*	8.2 ± 6.5 *d* = 0.3	25.8 ± 10.5 *d* = 0.8*§	23.5 ± 7.6 *d* = 1.0*	24.2 ± 9.9 *d* = 0.8*
Chills	10.8 ± 7.2	10.4 ± 7.2	6.3 ± 4.4	38.8 ± 12 *d* = 0.3*	20.4 ± 9.5 *d* = 0.3	12.7 ± 8.4 *d* = 0.3	32.6 ± 11.7 *d* = 0.7	37.1 ± 11.7 *d* = 0.8*	33.2 ± 11.1 *d* = 0.9*
Heart pounding	6.7 ± 4.3	6.4 ± 6.4	1.8 ± 1.8	30.7 ± 9.6 *d* = 0.9*	27.8 ± 9.8 *d* = 0.8*	31.1 ± 9.1 *d* = 1.3*	43.5 ± 11.1 *d* = 1.3*	38.2 ± 10.5 *d* = 1.1*	32.8 ± 11.4 *d* = 1.1*
Noises seem loud	7.4 ± 5.5	9.3 ± 7.2	10.3 ± 6.3	16.6 ± 8.6 *d* = 0.4	21.8 ± 10 *d* = 0.4	19.8 ± 9.7 *d* = 0.3	39.8 ± 11.2 *d* = 1.1*§	33.0 ± 10.6 *d* = 0.8*	26.2 ± 9.5 *d* = 0.6
Sedated	3.0 ± 3.0	4.8 ± 4.8	6.7 ± 4.4	16.6 ± 7.9 *d* = 0.7	28.2 ± 11 *d* = 0.8*	12.1 ± 7.7 *d* = 0.9	27.1 ± 9.1 *d* = 1.0*	19.2 ± 7.9 *d* = 0.7	16.0 ± 5.3 *d* =.55
Sweaty	2.2 ± 1.6	1.3 ± 1.0	1.1 ± 1.1	12.2 ± 6.9 *d* = 0.6	20.7 ± 8.2 *d* = 1.0	8.8 ± 5.1 *d* = 0.6	15.6 ± 6.9 *d* = 0.8	38.5 ± 10.0 *d* = 1.5*[1]	15.3 ± 4.9 *d* = 1.2[2]

Note: All values are means ± sem; *d* = Cohen's d; * $p < .01$, significantly different from placebo; § = $p < .01$, significantly different from 50 mg MDMA; 1 $p < .01$, significantly different from first administration; 2 $p < .01$, significantly different from first administration

Residual Effects

No significant residual physiological effects were observed. **Table 2.5** shows subjective-effect ratings as a function of dose and day. On day 3, 50 mg MDMA, compared with placebo, produced increases on "Can't Concentrate," (p<0.01 for all comparisons). On day 4, 100 mg MDMA, compared with placebo, produced increases on "Muscle Pain": p<0.01 for all comparisons). On day 5, subjective-effect ratings were not significantly altered as a function of MDMA dose.

Table 2.5 Residual Subjective Effects of Repeated MDMA Administration

	Placebo	**50 mg MDMA**	**100 mg MDMA**
Day 3			
"Can't Concentrate"	18.7 2.6	30.24 ± 3.27 d = 3.8*	22.43 ± 2.79 d = 1.3
Day 4			
"Muscle Pain"	3.3 ± 2.0	11.13 ± 2.35 d = 4.3	14.65 ± 2.52 d = 5.9*

Note: All values are means ± sem; d = Cohen's d; * = p < .01, significantly different from placebo

Figure 2.3 shows residual performance effects on the Rapid Information Task as a function of MDMA dose and day. On day 3, 4, and 5 performances on the Rapid Information Task was improved for both dosing conditions as measured by the number of "false alarms" (p<0.01). On day 4, performance was improved for the larger dose condition as measured by "hits" (p<0.01 for all comparisons). No other significant cognitive performance effects were observed.

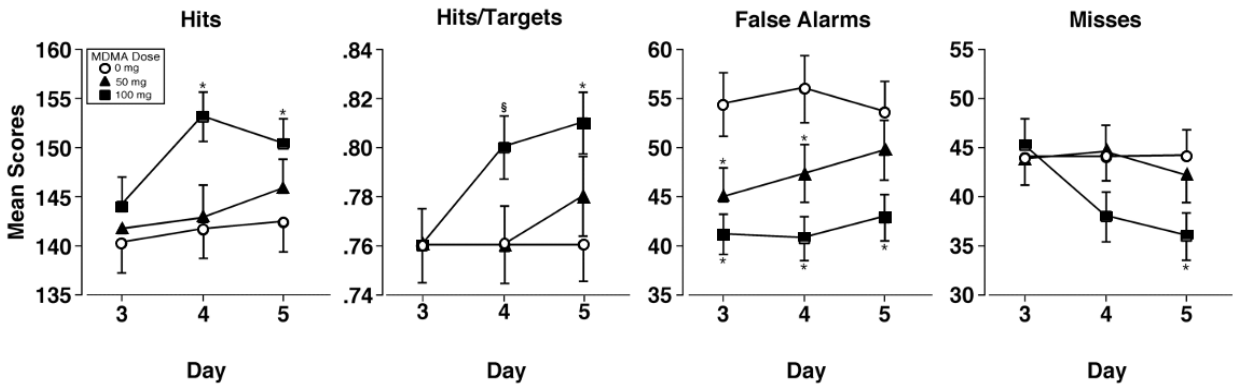

Figure 2.3 Residual effects of repeated MDMA administration on the Rapid Information Task as a function of MDMA dose and day.

Error bars represent 1 SEM. Overlapping error bars were omitted for clarity. * = significantly different from placebo ($p < 0.01$). § = 100 mg MDMA significantly different from 50 mg ($p < 0.01$).

Sleep Effects

Table 2.6 shows significant effects produced by MDMA on objective and subjective sleep measures. Objective measurements obtained from the sleep period immediately following administration of active MDMA at 0900 and 2100 hours (Day 2), show that sleep onset latency was increased and ratings of "slept well" were decreased under the larger dose condition ($p<0.01$ for all comparisons). The number of hours participants estimated they had slept were reduced under both dosing conditions although this effect did not reach statistical significance ($p<0.01$ for all comparisons). No significant MDMA-related sleep effects were observed for days 3, 4, and 5 sleep.

Table 2.6 Sleep Effects of Repeated MDMA Administration (After First Two Doses)

	Placebo	50 mg MDMA	100 mg MDMA
Objective sleep measures			
Hours slept	6.7 ± 0.2	5.8 ± .5 $d = 2.4$	5.5 ± 0.2 $d = 3.2$
Subjective sleep measures			
Fell asleep easy	46.5 ± 8.5	23.8 ± 7.8 $d = 2.8$	18.8 ± 6.3 $d = 3.7$*
Hours slept	5.7 ± 0.3	5.3 ± 0.4 $d = 1.1$	4.8 ± 0.4 $d = 2.5$
Sleep onset latency	96.7 ± 12.8	101.2 ± 17.3 $d = 0.3$	172.5 ± 14.8 $d = 5.5$*§

Note: All values are means ± sem; d = Cohen's d; * = $p < .01$, significantly different from placebo; § = $p < .01$, significantly different from 50 mg MDMA; $df = 2,8$

2.4 Discussion

Concerns have been raised about potentially greater effects produced by multiple MDMA administrations that are not apparent after single acute doses. The data present in this study do not support these concerns as the administration of three MDMA doses within 36-hours did not produce greater peak heart rate effects than observed following the initial dose. Furthermore, there were few residual effects on mood, performance, and sleep. These are the first data assessing residual effects up to three days following repeated MDMA administration. Overall, these data add to a lean database assessing MDMA-related effects following repeated administration over 1.5 days.

As expected, following acute administration, both MDMA doses increased physiological measures (Clark et al., 2015; Kirkpatrick et al., 2012b, 2014, 2016). Repeated-dose administration of MDMA generally did not produce greater physiological effects than observed after the initial dose. The exception was a greater increase in oral temperature for the larger MDMA dose following the second administration. However, we observed a similar increase

from the second to third administration in the placebo condition. Importantly, previous repeated-administration MDMA studies have not observed greater increases in peak oral temperature (Farré et al., 2004, 2015; Peiró et al., 2013). Thus, the observed temperature increase may be due to natural variance in our measurement methods.

Cardiovascular elevations produced by the first administration of MDMA were not further increased by the second or third administrations of the drug. In fact, the third MDMA administration, under both active dosing conditions, failed to elevate peak heart rate above placebo rates, potentially reflecting the development of tachyphylaxis (i.e., decreased drug effectiveness with repeated administrations). Peak heart rate attenuations were unexpected. Although previous researchers did not observe greater peak effects, a heart rate attenuation has not been previously reported (Farré et al., 2004, 2015; Peiró et al., 2013). One possible reason for these incongruent findings is that the maximum cumulative dose administered in earlier studies was 200 mg, in the present study it was 300 mg. Also, the number of drug administrations in previous studies was two; three administrations were used in the current study. Another possible reason is that heart rate was assessed at different times of day. For example, the first administration occurred in the morning while the second and third occurred in the evening. It is possible that day-to-day variations in cardiovascular activity influenced the current results. However, this is unlikely considering there was not a similar decrease in heart rate in the placebo condition. Still, our observations are consistent with previous repeated-dose studies showing greater peak physiological effects are not observed (Farré et al., 2004, 2015; Peiró et al., 2013).

The second MDMA administration produced greater effects on some subjective-effect measures than the initial dose. On the other hand, these effects were dampened following the third administration. This pattern deviates from data investigating the consequences of multiple-

dose MDMA administration (Peiró et al., 2013; Farré et al., 2015). However, one limitation associated with our measurement of subjective effects is that we only obtained ratings at one time-point (+150 minutes) following the third administration, whereas previous studies included multiple time-points. Thus, it is possible a different pattern of effects might emerge if measurements were collected at a broader range of time-points.

When active MDMA was administered at both 0900 and 2100 hours, sleep time, as measured by the Actiwatch® Activity Monitoring System, was decreased by ~60 minutes, although this effect did not reach statistical significance after a Bonferroni correction. Additionally, after consuming the larger 100 mg dose, participants reported not falling asleep as easily and taking longer to fall asleep (i.e., subjective measures of "fell asleep easy" and "sleep onset latency", respectively). There were no significant effects on sleep measures the next day. These findings are congruent with results from an earlier study showing that a single MDMA dose (2 mg/kg) administered five hours prior to the sleep period decreased sleep time without increasing next day sleepiness (Randall et al., 2009).

Residual mood effects were limited following repeated MDMA administration. For instance, ratings of depressed mood state were not increased in the days immediately following MDMA use. This finding is consistent with evidence from other controlled laboratory studies (Kirkpatrick et al., 2012b; Sessa et al., 2022). Still, it is possible that depressed mood subsequent to MDMA use might be observed in people with a history of depression. Such individuals were excluded from the current study, perhaps limiting generality the present data.

Ratings of "Can't concentrate", on the other hand, increased from 18.77 out of a possible 100) in the placebo condition to 30.24 under the 50 mg condition on the day following two MDMA administrations. Ratings of "Muscle pain" increased from 3.32 in the placebo condition

to 14.65 in the larger dose condition on the day following the third administration. These differences, however, were no longer significant by day 5. Ratings of positive mood were unaltered on all three residual effect days.

Cognitive performance measurements were generally unaltered on days 3, 4, and 5. One exception was that under the 100 mg condition performance on the rapid information task was improved on the days following MDMA use. It is difficult to compare these findings with other data because virtually no other study has assessed residual cognitive performance following repeated doses of MDMA.

These data should be interpreted within the context of several limitations. First, the inter-dose intervals employed here are but a small number of possible dosing regimens. Clearly, there exists a broader range of inter-dose intervals in the natural ecology. Second, a related limitation is that the study design did not incorporate other factors that might contribute to MDMA-related problems in the natural ecology, such as heightened physical activity and sleep deprivation (Kuypers et al., 2007, 2008). Nonetheless, it is important to have a better understanding of a broad range of MDMA inter-dose intervals, as well as to clarify the pharmacological effects of MDMA independent of environmental factors. Third, the sample population consisted of fewer female than male participants, which precluded an analysis of sex differences. This point is particularly important given recent findings that show more pronounced MDMA-related effects in women (Bedi & de Wit 2011; Papaseit et al., 2018). Future studies should address this limitation by actively recruiting a larger number of female participants so that hypotheses related to sex differences can be tested. Finally, the sample size of 12 was small, however, due to the within-participant design, it was sufficient to allow the detection of a broad range of drug effects.

In conclusion, the current findings indicate that clinically significant physiological

elevations were not observed following repeated doses of MDMA. By the third dose, tolerance developed for several effects. Limited residual mood effects were observed. These data contribute to a limited database assessing the consequences of repeated MDMA administration. Future research should assess different dosing regimens because it is possible we missed the critical window where dangerous effects might be observed.

Chapter 3: Interactive Effects of Methamphetamine and Alcohol

3.1 Introduction

Surveys show that methamphetamine users frequently use the drug in combination with alcohol (Bujarski et al., 2014). For example, roughly 80% of methamphetamine users report commonly combining the drug with alcohol (Leslie et al., 2017). Additionally, frequent alcohol drinkers are roughly five times more likely to report methamphetamine use as compared to non-drinkers (Furr et al., 2000). Despite the relatively common use of this drug combination, it may produce dangerous consequences. For example, findings from epidemiological reports indicate that alcohol can intensify the cardiac response to methamphetamine and potentially produce cardiovascular issues (Won et al., 2013; Fleury et al., 2008). Accordingly, alcohol was the most common drug involved in methamphetamine-related emergency department visits (SAMSHA, 2023c). Nonetheless, very few studies have investigated the combined effects of alcohol and methamphetamine.

The combined effects of alcohol and other prototypical stimulants such as cocaine, on the other hand, are well documented (For review: Althobaiti & Sari et al., 2016). The cocaine-alcohol combination generally increases heart rate and subjective measures of euphoria more than either drug alone and offsets alcohol-specific feelings of intoxication (Althobaiti & Sari et al., 2016; Foltin et al., 1993; Higgins et al. 1993; McCance-Katz et al. 1998, 2005). A similar pattern of effects has been observed following administration of alcohol combined with d-amphetamine (Perez-Reyes et al. 1992), 3,4-methylenedioxymethamphetamine (MDMA) (Hernández-López et al., 2002; Kuyupers et al., 2006), and mephedrone (Papaseit et al., 2020; Farre et al., 2016). Therefore, alcohol-stimulant combination users may combine the drugs to increase euphoria while offsetting alcohol-specific feelings of intoxication.

There are only two published studies on the effects of methamphetamine–alcohol combinations in humans. Mendelson and colleagues (1995), assessed acute physiological and psychological effects following combined administration of intravenous methamphetamine (0, 30 mg) and oral alcohol (0, 1 gm/kg). Relative to either drug alone, the drug combination significantly increased heart rate and ratings of euphoria. Furthermore, methamphetamine decreased ratings of alcohol-related intoxication. Although the above study provides valuable information about the acute effects of the methamphetamine-alcohol combination, additional questions remain. For example, the increases in heart rate observed were only modest and not clinically concerning. However, in the natural ecology, methamphetamine-alcohol combination users often take multiple doses over the course of an evening and/or several days, which may significantly increase the likelihood of experiencing cardiovascular issues (Cho & Melega, 2001). Therefore, a more concerning pattern of effects may emerge following repeated-administrations of the methamphetamine-alcohol combination.

In an attempt to address some of the above questions, Kirkpatrick et al. (2012a) assessed the consequences of repeated-administrations of the methamphetamine–alcohol combination on a range of physiological, behavioral, and subjective measures. During this 20-day within-subjects residential laboratory study, participants received oral methamphetamine (10 mg) combined with alcohol (0.375, 0.75 g/kg) or either drug alone (10 mg methamphetamine or 0.75 g/kg alcohol) on three consecutive occasions over a 2-day period (i.e., the morning of Day 2, the evening of Day 2, and the evening of Day 3). In line with findings from Mendelson and colleagues (1995), acutely the methamphetamine-alcohol combination increased heart rate and ratings of "good drug effect" more than either drug alone, and attenuated ratings of alcohol intoxication. However, these effects were significantly diminished with repeated administrations, suggesting

that participants developed tolerance to the cardiovascular and subjective effects of the combination.

Nevertheless, it is crucial to acknowledge that they may have missed the critical window where greater cardiovascular effects would be observed. The statistical comparisons were exclusively focused on the first and third administrations, leaving the second administration unanalyzed. Given the dearth of scientific data on the methamphetamine-alcohol combination, these data may be particularly informative. There are two important aspects to consider regarding these unanalyzed data. First, the methamphetamine plasma half-life is roughly 6-12 hours (Cook et al., 1992, 1993). Since the inter-dose interval between the first and second administrations was only 12-hours, less of the first dose was likely to have been eliminated. Therefore, it is possible that an increased amount of methamphetamine plasma is bound to sites of action and exerting its effects, potentially increasing the risk of cardiotoxicity. Second, these data may elucidate the timeline of tolerance (or enhanced effect) development. In particular, if tolerance to the reduction in feelings of alcohol intoxication develops quickly, this may indicate that concerns regarding experienced users increasing their alcohol consumption or underestimating their impairment when using the drug combination are overblown. To address these concerns, we conducted a secondary analysis of the data generated and published by Kirkpatrick et al., 2012a, and focused exclusively on statistical comparisons between the first and second administration. The purpose of this secondary data analysis was to characterize the interactive effects of methamphetamine and alcohol on cardiovascular activity and subjective feelings of intoxication following repeated-administrations at a 12-hour inter-dose interval.

3.2 Methods

Participants

The participants were previously described by Kirkpatrick and colleagues (2012a). Briefly, nine healthy male research volunteers completed this study. "Healthy" denotes that all volunteers successfully passed comprehensive medical and psychological evaluations during the screening process prior to study participation. Each had used amphetamines (D-amphetamine, methamphetamine, or MDMA) in the past year and reported alcohol use within the last 30 days. Three of the participants met DSM-IV criteria for cocaine dependence. None of the participants were seeking drug use treatment at the time of the study. The original research published by Kirkpatrick and colleagues (2012a) provides individual participant demographic information.

Pre-Study Training

Prior to starting the residential portion of the study, participants were trained on the computerized tasks. On a separate day, participants received the largest dose of oral methamphetamine (10 mg) and alcohol (0.75 g/kg) to be tested inpatient in order to provide them with experience with the drug combination in a laboratory setting and to monitor any adverse reactions. Participants were not informed of the dose until the study was completed.

Design

Three groups of three participants each successfully completed the study at a residential laboratory located within the New York Psychiatric Institute. The study comprised four 5-day blocks where participants filled out subjective-effect scales and underwent physiological assessments. Twice daily, at a 12-hour interval, participants received either an active methamphetamine or placebo capsule, along with a liquid containing either placebo or active alcohol—once in the morning and once in the evening. The first day of each block served as a

washout period with only placebo administered. On the second day, active drugs were given during both the morning and evening sessions, with these two administrations being the primary focus of the study. The third day involved a morning placebo and an evening dose of the active drug(s), consistent with the previous day's dosage. This was followed by two consecutive days (days 4 and 5) of placebo administration at both dosing times, serving as additional washout periods. The dosing order was counterbalanced across participants to prevent from order effects.

Procedure

The study used a limited set of measures (described below) from Kirkpatrick and colleagues (2012a). The Visual Analog Questionnaire was selected for its extensive use in multiple studies evaluating the effects of drugs on mood (e.g., Comer et al., 2001; Kirkpatrick et al., 2012b). This consistency allows for direct comparison of data from the current study with prior research findings. Briefly, an experimental day was structured as follows: Participants began with breakfast at 0800 hours. At 0900 hours, they received either a methamphetamine or placebo capsule. Then, at 1000 hours, participants completed the Visual Analog Questionnaire and were given either an active alcohol dose or a placebo liquid. Cardiovascular measurements were taken at .75, 1.5, 2.25, 3, 4, 5, and 6 hours following capsule administration. The Visual Analog Questionnaire was administered at 1, 1.75, 2.5, 3.5, 6.25, 7, 7.75 hours post-capsule administration.

At 2100 hours, participants received either an active methamphetamine or a placebo capsule. An hour later, at 2200 hours, they completed the Visual Analog Questionnaire and were given either active alcohol or a placebo liquid. Cardiovascular measurements were taken at 0.75, 1.5, 2, and 3 hours after the capsule administration. Additionally, the Visual Analog

Questionnaire was administered at 1, 1.5, and 2.5 hours post-capsule. The day concluded with lights off at midnight, marking the start of an 8-hour sleep period.

Subjective Effects

The computerized Visual Analog Questionnaire featured a series of 100-mm lines. Each line was labeled with an adjective describing a specific drug effect, such as "Good effect" or "Drunk," and ranged from "not at all" at one end to "extremely" at the other. The lines were presented sequentially (Hart et al., 2003).

Drug

The alcohol dose for each participant was calculated based on estimated total body water (Watson et al., 1981). To mitigate alcohol expectancy effects, all beverage volumes were standardized at 500 ml. The alcoholic beverage was a mix of Canada Dry Tonic® water and Ocean Spray Cranberry Juice Cocktail in a 3:1 ratio, with 100 proof Absolut® vodka added. The placebo beverage mirrored this mix, excluding the vodka. Both drinks were isocaloric, differing by no more than 10 calories, and were enhanced with 1 ml of vodka and a drop of peppermint oil for uniformity. Participants consumed their beverages within 10 minutes.

The New York State Psychiatric Institute Pharmacy Department repackaged methamphetamine hydrochloride (Desoxyn®, Abbott Laboratories, North Chicago, IL) tablets into white no. 00 opaque capsules with lactose filler. The placebo capsules contained only lactose.

The study included four drug conditions: a larger methamphetamine-alcohol combination (10 mg methamphetamine with 0.75 g/kg alcohol), a smaller methamphetamine-alcohol combination (10 mg methamphetamine with 0.375 g/kg alcohol), methamphetamine alone (10 mg methamphetamine with a placebo liquid), and alcohol alone (a placebo capsule with 0.75

g/kg alcohol). These alcohol doses were chosen because they are known to significantly affect breath alcohol levels, subjective effects, and behavioral intoxication measures in current alcohol users (e.g., Evans and Levin 2003).

Data Analysis

Peak cardiovascular effects and subjective ratings were analyzed using a two-factor ANOVA with drug condition (MA/P, P/A2, MA/A1, MA/A2) as the first factor and drug administration (Administrations 1 and 2) as the second factor. The analysis focused on comparing the peak effects produced by the first and second administrations within each block.

For all analyses, the ANOVAs provided the necessary error terms to perform planned comparisons between different drug conditions (MA/P vs. MA/A1, MA/A2; P/A2 vs. MA/A2) and within the same drug condition across different administrations (Administration 1 vs. Administration 2). Huynh-Feldt corrections were used when appropriate. All analyses were computed using the statistical package RStudio.

3.3 Results

Cardiovascular Effects

Figure 3.1 shows peak heart rate and blood pressure as a function of drug condition and administration number. After the first and second dose administrations, both methamphetamine–alcohol combinations increased heart rate more than either drug alone ($p < .05$). However, heart rate increases were significantly less pronounced following the second drug administration as compared with first administration ($p < .05$). Following the first administration, both methamphetamine–alcohol combinations increased systolic blood pressure as compared to alcohol alone ($p < .05$). However, by the second administration there were no significant differences between drug conditions. The larger methamphetamine-alcohol combination and

both drugs alone increased systolic blood pressure following the second administration as compared to the first administration ($p < .05$). Following the first administration, the larger methamphetamine–alcohol combination increased diastolic blood pressure more than either drug alone and the smaller methamphetamine-alcohol combination increased diastolic blood pressure more than methamphetamine alone ($p < .05$). However, by the second administration there were no significant differences between drug conditions. Furthermore, both drugs alone increased diastolic blood pressure following the second administration as compared to the first administration ($p < .05$).

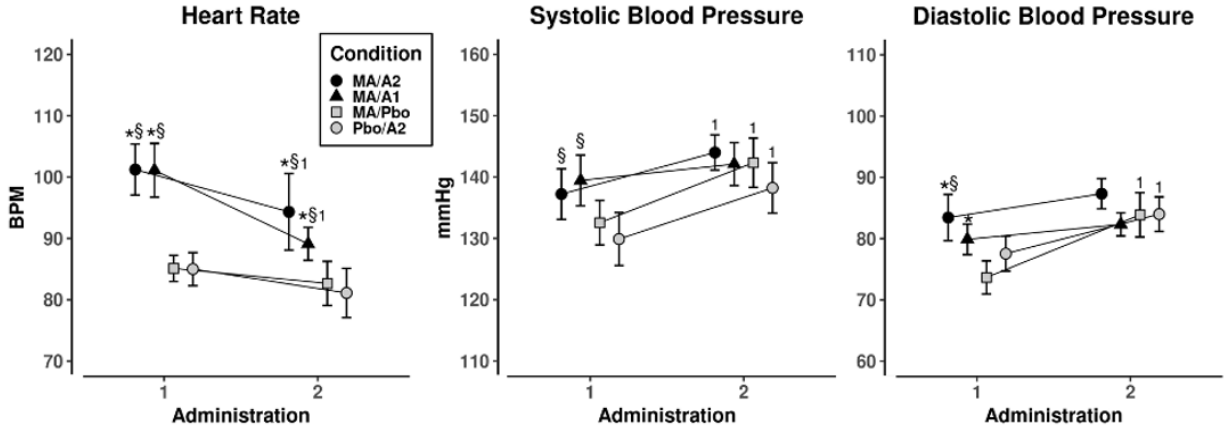

Figure 3.1 Repeated-dose effects on peak cardiovascular measures as a function of drug condition and administration number.

Graphs are based on a reanalysis of the Kirkpatrick et al. (2012a) data. Error bars represent 1 standard error of the mean (SEM). Overlapping error bars were jittered for clarity. * Significantly different from MA/Pbo ($p < 0.05$). § Significantly different from Pbo/A2 ($p < 0.05$). # Significantly different from MA/A1 ($p < 0.05$). [1] Significantly different from administration 1 ($p < 0.05$).

Subjective Effects

Figure 3.2 shows selected subjective-effect ratings as a function of drug condition and administration number. As can be seen in the top panels, alcohol alone significantly increased ratings of "Drunk" and "Sedated" across both administrations compared to all other drug conditions ($p < .05$). The methamphetamine-alcohol combination attenuated these effects ($p < .05$). Following the first administration, the methamphetamine-alcohol combination increased

ratings of "Good Drug Effect" more than either drug alone ($p < .05$). However, following the second administration, the combination only increased ratings of "Good Drug Effect" more than methamphetamine alone ($p < .05$). Ratings of "High" followed a similar pattern (**Figure 3.2**).

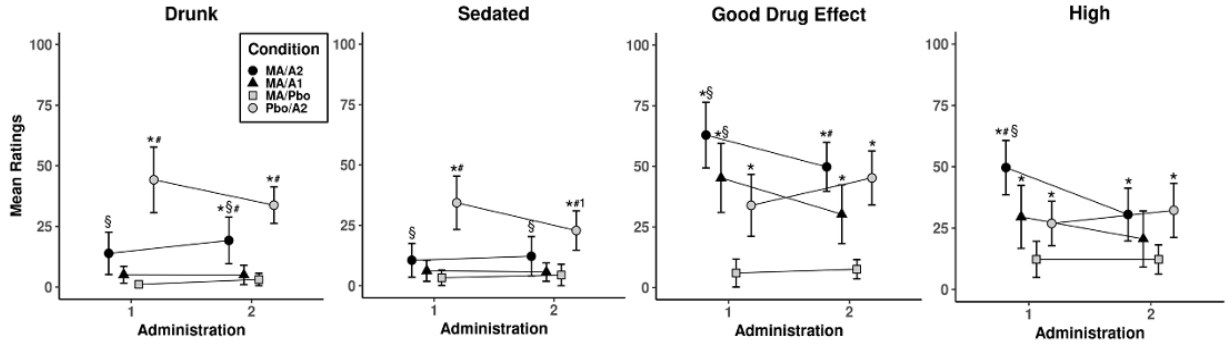

Figure 3.2 Repeated-dose effects on peak subjective effect ratings as a function of drug condition and administration number.

Error bars represent 1 standard error of the mean (SEM). Overlapping error bars were jittered for clarity. * Significantly different from MA/Pbo ($p < 0.05$). § Significantly different from Pbo/A2 ($p < 0.05$). # Significantly different from MA/A1 ($p < 0.05$). [1] Significantly different from administration 1 ($p < 0.05$).

3.4 Discussion

The present reanalysis of the Kirkpatrick et al., 2012a data revealed that cardiovascular activity following repeated administrations of the methamphetamine-alcohol combination were mixed. For example, heart rate elevations produced by co-administration of methamphetamine and alcohol were attenuated when this drug combination was administered 12-hours later. Feelings of euphoria, as measured by ratings of "Good Drug Effect" and "High," showed a similar pattern. Conversely, systolic blood pressure was significantly increased from the first- to the second-drug administration under the methamphetamine-alcohol (10 mg-0.375 g/kg) condition. As expected, the alcohol alone conditions increased subjective ratings of drunkenness (i.e., "Drunk" and "Sedated"). Methamphetamine substantially reduced these effects and this diminution remained stable from the first- to the second-drug administration. In general, the pattern of effects observed here are consistent with previous data suggesting that tolerance

develops to the heart rate elevations and subjective ratings of euphoria produced by repeated administration of the methamphetamine-alcohol combination (Kirkpatrick et al., 2012a).

A key aim of this secondary data analysis was to determine if there was potential dangerous heart rate elevations following repeated co-administration of alcohol and methamphetamine. Such effects were not supported as our analysis show that heart rate increases failed to reach clinically significant levels, with an average peak heart rate of 105 beats per minute that subsided to below 100 beats per minute within 90 minutes (data not shown). It is true that the methamphetamine-alcohol combination increased heart rate more than either drug alone, even following repeated administration, which is consistent with the findings of McCance-Katz et al. (1998), who reported similar results with repeated administration of cocaine and alcohol combined at a 60-minute inter-dose interval. However, unlike McCance-Katz and colleagues, the heart rate elevation produced by co-ingestion of alcohol and methamphetamine was not further augmented by a subsequent administration. Instead, beats per minute were considerably reduced, indicating the potential development of tolerance, or a reduced drug effect following repeated administrations. Findings from the original manuscript support this conclusion. When comparing the first and third administration, Kirkpatrick and colleagues (2012a) found that beats per minute decreased to the extent that only the larger methamphetamine-alcohol combination was elevated as compared to either drug alone, providing further evidence that tolerance develops to the heart rate effects of this drug combination with repeated administration.

In the present reanalysis, repeated administration of the methamphetamine-alcohol (10 mg-0.375 g/kg) combination produced a greater increase in systolic blood pressure than observed following the initial dose. However, repeated administration of both alcohol alone and methamphetamine alone produced similar systolic blood pressure elevations, suggesting that

each drug alone drove the increase rather than their combination. It is worth noting that the systolic blood pressure levels achieved were not clinically concerning. For instance, the average peak systolic blood pressure under the methamphetamine-alcohol condition, was 144 mmHg, which is lower than levels achieved with moderate exercise (Sabbahi et al., 2018) and this effect subsided within 30 minutes (data not shown). Additionally, following administration two, systolic blood pressure produced by the methamphetamine-alcohol combination was not significantly different from either drug alone, in line with the findings of McCance-Katz et al. (1998), who reported similar results following a second administration of cocaine and alcohol combined at a 60-minute inter-dose interval. When taken together with our heart rate results, these findings indicate that repeated administration of the methamphetamine-alcohol combination does not lead to clinically significant cardiovascular effects.

Methamphetamine substantially reduced alcohol-induced feelings of drunkenness and the reduction remained stable from the first to second administration. This attenuation of alcohol specific feelings of intoxication is consistent with findings from studies on the acute effects of alcohol combined with various stimulants, including methamphetamine, cocaine, and mephedrone (Mendelson et al., 1995; Hernández-López et al., 2002; Kuyupers et al., 2006; Dumont et al., 2008, 2010; Higgins et al., 1993; McCance-Kats et al., 1998; Papaseit et al., 2020; Farré et al., 2016). However, findings from the original study indicate that this effect dissipates following a third administration at a 24-hour inter-dose interval (Kirkpatrick et al., 2012a). The obvious reasons for this incongruence are the extra drug administration and larger cumulative dose in the original study. Nonetheless, our current result suggests a potential concern because at specific inter-dose intervals some methamphetamine-using individuals might increase their alcohol consumption in an attempt to achieve the accustomed level of alcohol intoxication.

Acutely, the methamphetamine–alcohol combination increased ratings of euphoria more than either drug alone, which is consistent with the acute effects of alcohol combined with multiple stimulants, including methamphetamine, cocaine, MDMA, and mephedrone (Mendelson et al., 1995; Higgins et al., 1993; McCance-Katz et al., 1998; Hernández-López et al., 2002; Papaseit et al., 2020; Farré et al., 2016). By the second administration, the methamphetamine–alcohol combination only increased ratings of euphoria more than methamphetamine alone. This finding is generally consistent with McCance-Katz et al., (1998), who reported that the repeated administration of the cocaine–alcohol combination significantly increased ratings for "feel good" relative to cocaine alone. Overall, the subjective effect findings indicate that repeated administration of the combination does not enhance the subjective effects.

These data should be interpreted within the context of several limitations both concerning the broader context of secondary data analysis and the original study conducted by Kirkpatrick and colleagues (2012a), which served as the source for our secondary analysis. First, the data we reanalyzed were originally collected for research objectives distinct from our own. In the original study by Kirkpatrick and colleagues (2012a), a primary aim was to compare the effects of the methamphetamine-alcohol combination with the effects of each drug alone. Therefore, a placebo/placebo condition was not included. However, a placebo/placebo condition would have been particularly informative for our secondary data analysis. For example, the original study's scheduling of the first drug administration in the morning and the second in the evening introduces the possibility that diurnal variations in cardiovascular activity and mood may have influenced our findings (Keith et al*.,* 2013; Hart et al*.,* 2003). Having a placebo/placebo condition would have allowed us to control for any potential day-to-night variations and their impact on the observed results, if any such variations did exist.

Additionally, anecdotal evidence indicates that methamphetamine is frequently consumed at inter-dose intervals that are shorter than the 12-hour inter-dose interval employed in the original study by Kirkpatrick and colleagues (2012a). Time-course data indicates that plasma concentrations and the subjective and cardiovascular effects of oral methamphetamine begin to dissipate around 2.5-3 hours after ingestion (Kirkpatrick et al., 2012c). As a result, methamphetamine users may self-administer another dose to enhance or sustain desired effects. It is possible that administering a subsequent dose at this time, when the effects of methamphetamine are beginning to dissipate, could result in greater effects. Data from studies assessing the repeated-administration effects of the cocaine-alcohol combination may inform this situation. Intranasal cocaine plasma levels peak around 30-45 minutes after insufflation (Cone, 1995), and it was around this time (60 minutes) that McCance-Katz et al. (1998, 2005) administered a subsequent dose of intranasal cocaine combined with alcohol, resulting in greater heart rate and euphoric effects. This might suggest a similar situation following repeated-administrations of methamphetamine combined with alcohol if we shorten the inter-dose interval to under 3-hours.

Another limitation is the all male sample, which precluded an analysis of sex differences (Bolger et al., 2019). This point is particularly important given findings that show different effects in women, compared with men, in response to alcohol, stimulants (e.g., methamphetamine, cocaine), or co-ingestion of alcohol and cocaine (Lex et al., 1988; Lukas et al., 1996, 2005; Mayo et al., 2019; McCance-Katz et al., 1998, 2005).

In conclusion, repeated administration of the methamphetamine-alcohol combination in male participants did not result in clinically significant cardiovascular elevations or greater feelings of euphoria, but instead, it reduced feelings of alcohol-related intoxication. This unique

profile of effects may partially explain why methamphetamine is repeatedly taken in combination with alcohol, to counteract alcohol-specific feelings of intoxication rather than amplifying euphoria. This notion is consistent with anecdotal reports of repeated stimulant use, such as cocaine, for the purpose of sobering up from alcohol (Pakula et al., 2009). However, it is important to consider that inexperienced users may consume excessive amounts of alcohol in an effort to attain their accustomed level of drunkenness, in turn, elevating the risk of alcohol-related toxicity.

Moreover, it should be noted that the current results may not be applicable to women as research shows they generally have more pronounced responses to alcohol and methamphetamine (Mayo et al., 2019; McCance-Katz et al., 2005). Thus, future studies should investigate the effects of a wide range of methamphetamine-alcohol dosing regimens on a broad range of behavioral measures, including subsequent choice to consume alcohol, in both women and men. Overall, these data add to a scant database assessing repeated-administrations of the methamphetamine-alcohol combination.

Chapter 4: Interactive Effects of Methamphetamine and Oxycodone

4.1 Introduction

Polysubstance use involving opioids (e.g., oxycodone, heroin) and stimulants (e.g., methamphetamine, cocaine) is prevalent among specific substance-using populations. Epidemiological studies show that most individuals seeking treatment for opioid use also report using stimulants (Ellis et al., 2018; Leri et al., 2003). Furthermore, intravenous drug users frequently combine opioids and stimulants, with an estimated 30% reporting coadministration in the past month and 13% within the last 24 hours (Brener et al., 2022). Despite their relatively common use, concerns have been raised about the safety profile of stimulant-opioid combinations as they have been linked to several recent high-profile deaths such as Shock G, Mac Miller, and Coolio (Salas-Rodriguez, 2023).

Clinical reports suggest that stimulant-opioid combinations produce a more rewarding high than either drug alone (for review see Leri et al., 2003). Findings from non-human animal self-administration paradigms somewhat support this notion. For example, combining cocaine with opioids, such as heroin, remifentanil, and alfentanil, resulted in increased reinforcer potency, such that low doses of either drug that were initially ineffective in maintaining self-administration behavior became effective when combined (Rowlett & Woolverton, 1997; Duvauchelle et al., 1998). Notably, although less common, larger dose combinations of cocaine and heroin, as well as methamphetamine and heroin, produced enhanced reinforcing effectiveness (Lacy et al., 2014; Ranaldi & Munn, 1998; Ranaldi & Wise, 2000). While self-administration remains the gold standard in behavioral pharmacology, a diverse range of behavioral measures beyond self-administration are necessary to gain a comprehensive understanding of stimulant-opioid combinations.

In addition to an enhanced rewarding effect, it is generally assumed that stimulants and opioids interact to lessen the adverse effects of one or both drugs. For example, amphetamine and related drugs may acutely reduce the sedation associated with opioid administration and improve cognition (Jasinski & Preston, 1986; Dalal & Melzack, 1998). Conversely, opioids may reduce the intensity of methamphetamine-induced anxiogenic effects (Rhed et al., 2022). In such scenarios, the softened adverse impact of one drug due to the other could influence the appeal of the combination. However, these effects have been relatively overshadowed in preclinical models in favor of studying the combination's rewarding effects (Riley et al., 2019).

One stimulant-opioid combination that warrants further investigation is methamphetamine and oxycodone. Only one study on the combination has been published in the scientific literature. In this *in vitro* study on primary neurons, researchers found that the methamphetamine-oxycodone combination downregulated Striatin-1, a protein involved in synaptic functioning (Meyer et al., 2022). However, the combined effects were not compared to either drug alone, and behavioral assessments were not conducted. Thus, although their findings are interesting from a neurobiological perspective, it is essential to investigate their clinical relevance and behavioral implications through further research.

Therefore, the primary goal of the present study was to quantify the behavioral effects of the methamphetamine-oxycodone combination in C57BL/6N mice. To accomplish this, we employed a series of well-established measures to characterize this drug combination's behavioral effects. First, we used the Conditioned Place Preference (CPP) paradigm, a widely used measure of reward in rodents (Tzschentke, 2007). Next, we employed the Open Field paradigm to evaluate anxiety-like behavior and locomotor activity (Walsh & Cummins, 1976). Finally, we utilized the Novel Object Recognition test to assess recognition memory.

Our hypotheses are based on reverse translation as they were generated based on findings from studies in humans (Jasinski & Preston, 1986; Dalal & Melzack, 1998; Ellis et al., 2018; Rhed et al., 2022). We hypothesized that the methamphetamine-oxycodone combination would *enhance* the positive effects of each drug while tempering their adverse effects. Specifically, the combination would produce a more rewarding effect on conditioned place preference than either drug alone. Oxycodone would counteract methamphetamine-related anxiety-like behavior in the open field, and methamphetamine would offset oxycodone-related novel-object recognition memory disruptions.

4.2 Methods

The present report is based on animals tested and results initially reported in the PhD thesis of Dr. Keith (2014) entitled Marijuana, Methamphetamine, and Oxycodone: A multilevel approach to understanding drug effects. Columbia University. For the present work, some of the data were reanalyzed to take into account that in some tests animals that did not meet inclusion criteria for specific behavioral tests had not been removed from the analysis.

Animals and Housing

Subjects were male C57BL/6N mice, aged 7-9 weeks (Jackson Laboratory, Bar Harbor, ME). They were individually housed in transparent polycarbonate cages measuring 27 × 16.5 × 12 cm, within sound-attenuating, ventilated chambers (Phenome Technologies Inc. Lincolnshire, IL). The room conditions were maintained at a constant 23 ± 2°C and 72% humidity, with water and standard mouse chow (Purina, St. Louis, MO) available ad libitum.

Prior to the start of the experiments, the mice underwent an acclimatization period of 14-16 days to a 12:12 light-dark cycle (200 lux), with lights off at "midnight" (zeitgeber time 12, ZT 12) and on at "dawn" (ZT 0). Behavioral experiments were conducted during the daytime,

specifically between ZT 2 and ZT 8. Animal care protocols strictly adhered to the guidelines set by the Columbia University Institutional Animal Care and Use Committee and complied with all relevant Animal Welfare regulations. At the end of the study, euthanasia was humanely carried out using carbon dioxide (CO2).

Drugs

Stock solutions of methamphetamine (MA) hydrochloride and oxycodone (OXY) hydrochloride (Sigma-Aldrich Inc., St. Louis, MO) were prepared in a saline vehicle to maintain consistent injection volumes across groups: MA =0.125 mg/mL methamphetamine; OXY1 = 0.0625 mg/mL oxycodone; OXY2 = 0.125 mg/mL oxycodone; MAOXY1 =0.125 mg/mL methamphetamine and 0.0625 mg/mL oxycodone; MAOXY2 =0.125 mg/mL each of methamphetamine and oxycodone. Final volumes yielded a dose of 1 mg/kg MA, 0.5 mg/kg, and 1 mg/kg OXY, based on the mean initial body weight of 22 g/mouse. Drugs and saline were administered via intraperitoneal injection. The doses were chosen as they are near the threshold for inducing conditioned place preference (Der-Avakian et al., 2007; Liu et al., 2009; Suzuki et al., 1996; Tokuyama et al., 1996).

Experimental groups

Animals were organized into six experimental groups, each comprising eight mice: 1) MA, 2) OXY1, 3) OXY2, 4) MAOXY1 5) MAOXY2, and 6) SAL. Behavioral assessments were conducted over a three-week period (**see Table 4.1**). All mice were kept in the same experimental group throughout the study.

Table 4.1: Representative timeline of experimental procedure for methamphetamine-alone

Week	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
1	CPP (Pre-Conditioning) SAL	CPP (Pre-Conditioning) SAL	CPP (Conditioning) MA	CPP (Conditioning) SAL	CPP (Conditioning) MA	CPP (Conditioning) SAL	CPP (Conditioning) MA
2	CPP (Conditioning) SAL	CPP (Post-Conditioning)	CPP (Post-Conditioning)	CPP (Post-Conditioning)	CPP (Post-Conditioning)	Open Field MA	Novel Object Recognition (Acute) MA
3							Novel Object Recognition (Long-Term) SAL

CPP= Conditioned Place Preference; SAL= Saline; MA= Methamphetamine (1 mg/kg)

Measures

All behavioral tests were conducted in a separate, quiet testing room, with illumination provided by overhead lighting (approx. 100 lux). Mice were transported to the test room in their home cages 45 minutes before testing and were tested one at a time. Each apparatus was cleaned between tests using 70% ethyl alcohol. During testing, the experimenter was absent from the room.

Conditioned place preference

The experiment utilized a 3-chamber conditioned place preference apparatus (46.5 × 12.7 × 12.7 cm; MED-CPP-MSAT, Med Associates, Vt., USA), featuring automatic metal guillotine doors to separate the chambers. One chamber (16.8 × 12.7 cm) had black walls with a stainless-steel grid rod floor comprised of 3.2 mm rods set 7.9 mm apart. The opposite chamber (16.8 × 12.7 cm) had white walls and a stainless-steel mesh floor. The central chamber (7.2 × 12.7 cm) was neutral grey with a smooth, removable PVC floor. Corncob bedding was added beneath the floors of both end chambers for uniformity, and a caffeine-free chamomile tea bag (Trader Joe's) was placed within the bedding as an olfactory cue.

Photo-beam signals from the chambers were transmitted to a desktop PC equipped with MED-PC for Windows software (Med Associates, VT., USA). Mouse movement within the

apparatus was detected by six infrared photo beams positioned 2.8 cm apart and 1.0 cm from the end wall in the black and white chambers and three spaced 2.8 cm apart in the center chamber. The photobeams, positioned 1.8 cm above the apparatus floor, captured horizontal locomotor activity. The absence of vertical photobeams limited the ability to detect upward movements such as rearing.

Briefly, the conditioned place preference procedure consisted of pre-conditioning, conditioning, and post-conditioning phases, as detailed in **Figure 4.1.** For each day of the pre-conditioning phase, mice were weighed, injected with saline, placed in the central compartment, and allowed 30 minutes of free exploration of all three chambers. The first pre-conditioning day was meant to habituate the mice to the apparatus and familiarize them with the handling and injection procedures. The following day, time spent in each chamber was measured to obtain individual baseline preference and determine apparatus bias. The mean percentages of time spent in the black chamber (M = 41.01%, SE = 2.14) and white chamber (M = 58.99%, SE = 2.39) were compared using a paired-sample t-test. A significant difference was observed, $t(34) = 3.46$, $p = .001$, indicating apparatus bias and an overall preference for the white chamber.

Despite the apparatus bias, an unbiased procedure was used to assign mice to a drug-paired side of the apparatus. Therefore, the black or white chamber assignment as the drug-paired chamber was counterbalanced across mice. The unbiased design was chosen to balance the initial chamber preference (Cunningham et al., 2003) and circumvent the interpretational problems associated with the fully biased design (see review, Tzschentke, 1998). Drug conditioning spanned six days. Mice were injected with a single dose of either drug or saline before being confined to the corresponding side of the apparatus for a period of 30 minutes. Half of the mice received the drug on the first conditioning day, and half received saline. Drug and

saline were injected on alternating days. However, control mice received saline in both conditioning chambers, with one chamber designated as the "drug" paired chamber solely for data analysis. Post-conditioning spanned four days, during which mice received a saline injection, were placed in the central chamber, and allowed 30 minutes of free exploration of all chambers. The following behavioral measurements were scored: time in chambers (s), distance in arena (mm), and velocity (mm/s).

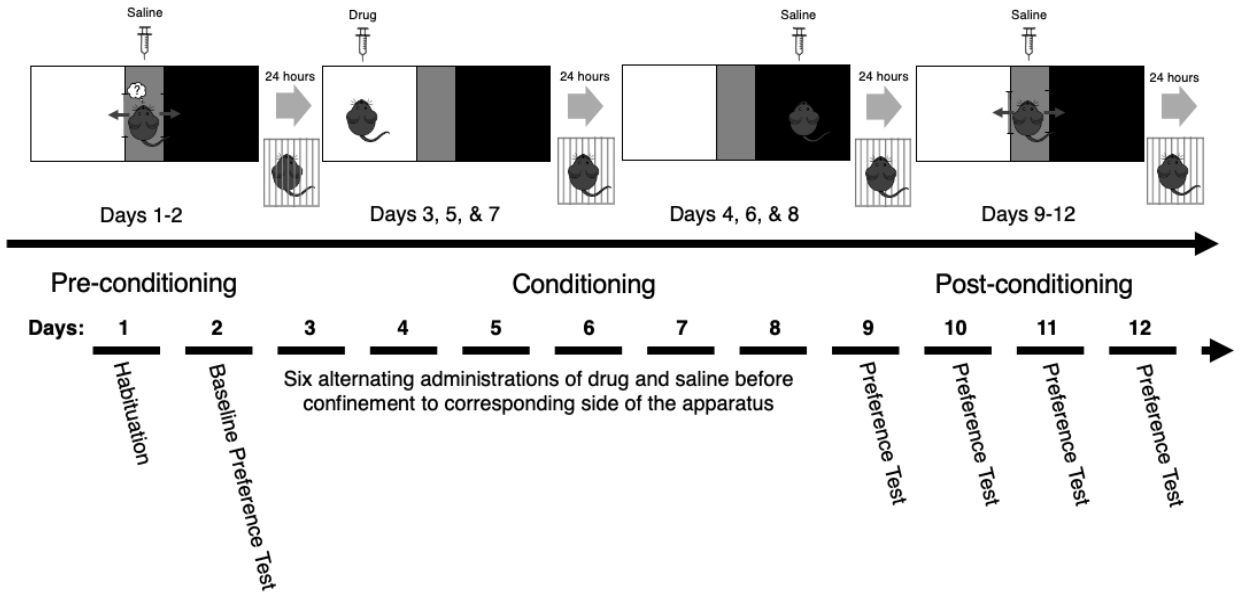

Figure 4.1 Representative timeline of Conditioned Place Preference procedure.
The procedure involved pre-conditioning, conditioning, and post-conditioning phases. In pre-conditioning, mice were acclimated to the apparatus. The next day, baseline preferences were established. During the six-day conditioning phase, mice received alternating drug or saline injections and were confined to the corresponding apparatus side. In post-conditioning, mice were given saline, placed in the central compartment, and allowed free exploration.

A place preference ratio was calculated by dividing the time spent in the drug-paired chamber by the total time spent in both the black and white chambers and multiplying the result by 100. A baseline preference ratio was calculated and subtracted from the preference ratios obtained for each post-conditioning day to account for initial chamber bias. A final ratio of zero

or below indicates no preference for the drug-paired chamber, while a final ratio greater than zero indicates conditioned place preference.

Mice that did not demonstrate a conditioned place preference of at least 60% were excluded from the data analysis for days two through four (Voigt et al., 2011; Li et al., 2022). This criterion was not included in Keith (2014). The rationale for setting this criterion was based on literature suggesting that CPP extinction studies should only include animals that develop an initial CPP (at least 10% more time spent in the drug-paired chamber than the unpaired chamber: Voigt et al., 2011; Li et al., 2022). The number of mice included in each analysis can be found in the Statistical Analysis section and figure captions.

Behavioral sensitization was assessed during the conditioning phase by comparing locomotor activity between the first and third drug administrations. Behavioral sensitization was established if locomotor activity during the third conditioning session was significantly elevated compared to the first session.

Open Field

The open field arena (43.2 cm x 43.2 cm x 30.5 cm) has a white plastic base with transparent plexiglass walls (ENV-515; Med Associates, Vt., USA). Data were captured using a Canon DC210 video camcorder mounted on a tripod positioned above the testing arena. The TopScan 2.0 program (Clever Sys Inc., Reston, VA) delineated areas within the arena and tracked locomotor activity. The inner 50% of the arena was designated as the center.

The open field test was conducted on day 13 and involved a 10-minute session. Mice were injected with drug or saline and returned to the home cage (located in the testing room) for 30 minutes. Mice were placed in the center of the arena and given 10 minutes to explore the

apparatus. The following behaviors were scored: time in the center zone (s), time outside of the center zone (s), distance in arena (mm), and velocity (mm/s).

Novel Object Recognition

The novel object recognition arena was identical to the arena used in the open-field test. Each novel object recognition test comprised a set of three objects, each available in duplicate. The first set of objects comprised a blue plastic box (10 cm x 8.5 cm x 3 cm), a clear plastic funnel (7 cm in diameter, 13 cm tall), and a grey cylindrical pipe (5 cm in diameter, 7.5 cm tall). The second set featured a pipette-tip box in white and yellow (12 cm x 8.5 cm x 4 cm), a clear Pyrex bottle of 100 mL capacity with an orange screw cap (5 cm in diameter and 10 cm in height), and a white plastic Coplin staining jar with a circular top and base, along with a square-shaped body (5.5 cm, 3.5 cm x 3.5 cm). Pilot experiments confirmed that all objects elicited equal levels of exploration (data not shown). All mice were tested with both sets of objects.

Data were recorded on a video camcorder (Canon DC210) affixed to a tripod above the testing arena. The TopScan 2.0 program (Clever Sys Inc., Reston, VA) was used for arena mapping and locomotor activity tracking. The regions surrounding both the familiar and novel objects were defined as a 1 cm radius around the outer edge of each object.

The novel object recognition procedure was conducted according to the method described by Denny et al. (2012) with minor modifications. This included five 5-minute exposures with 3-minute intervals between each (**refer to Figure 4.2**). The tests, conducted on days 14 and 21, assessed the effects of acute and saline injections respectively on short-term memory. The first four exposures were learning and habituation sessions, with two symmetrical objects placed about 7 cm from the arena walls. Mice were returned to their home cages between exposures,

during which time the arena and objects were cleaned. These 3-minute inter-trial intervals allowed for memory consolidation of the objects.

During the fifth exposure, occurring approximately 32 minutes after injection, one of the two previously familiar objects was substituted with a novel object for a 5-minute test session. The selection of the novel object, the retained familiar object, and their placements were counterbalanced across mice. During the fifth exposure (5-minute test), the duration of exploration for each object was measured. Object exploration was defined as a mouse's nose directed toward an object within approximately 1 cm (Bevins & Besheer, 2006). Climbing or sitting on objects was not scored as object exploration. The following behaviors were scored: time exploring familiar object/s (s), time exploring novel object (s), distance in arena (mm), and velocity (mm/s).

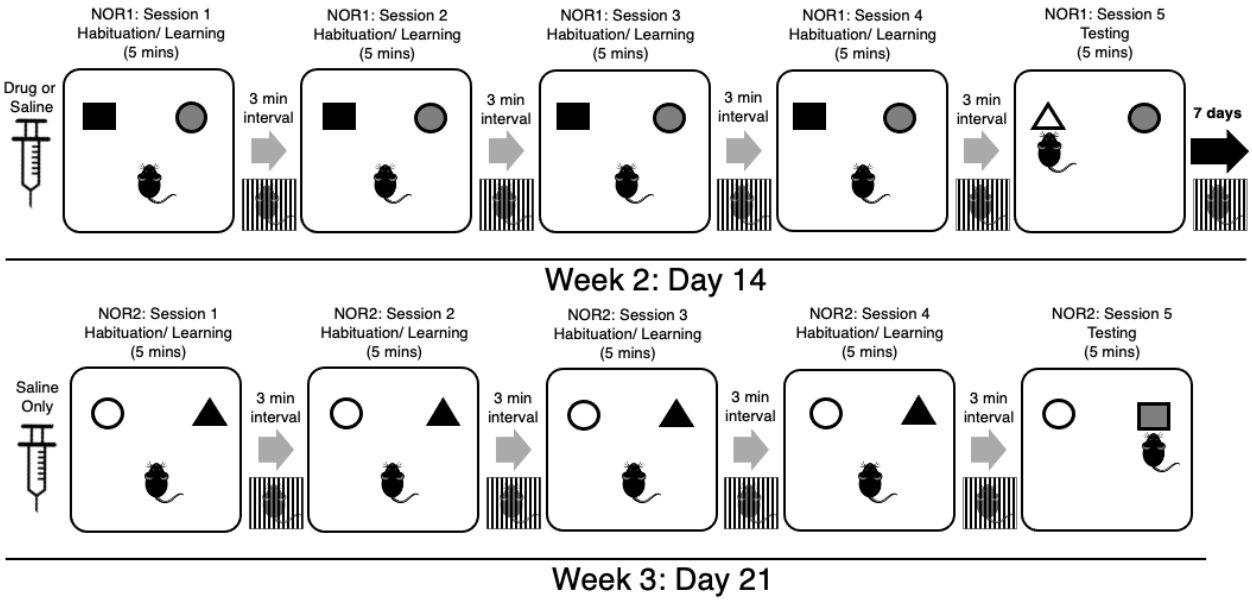

Figure 4.2: Representative timeline of Novel Object Recognition procedure.
The NOR procedure comprised five 5-minute exposures with 3-minute intervals in between. Sessions 1-5 were performed twice: on day 14 after a drug injection to assess short-term memory (top panel) and on day 21 after a saline injection to evaluate long-term memory effects (bottom panel). The initial four exposures were habituation/learning sessions with two objects positioned symmetrically about 7 cm from the wall. Mice were returned to their home cage during the 3-minute inter-trial intervals. In the fifth exposure, a novel object replaced one of the familiar ones, and exploration duration for each object was measured.

Mice that did not reach a total exploration time of 15 seconds exploring the objects were excluded from the data analysis (Ennaceur & Delacour, 1988; Denninger et al., 2018). Notably, Keith (2014) elected to conduct the study using the entire sample size and did not establish a minimum criterion for interaction with objects. This may have contributed to added variance and a lack of significant findings. In such procedures, it is recommended that mice with minimal or zero interaction with the objects during testing are excluded from the data analysis (Ennaceur & Delacour, 1988; Denninger et al., 2018), which was the focal point of this part of the dissertation. The number of mice included in each analysis can be found in the Statistical Analysis section and figure captions.

A novel object recognition ratio was calculated by dividing the time spent exploring the novel object by the total time spent exploring both the novel and familiar object and multiplying the result by 100. A novel object recognition ratio of 0.5 indicates no preference for the novel object, while a final novel object recognition ratio greater than 0.5 indicates a preference for the novel object.

Data Analysis

Data were analyzed using R-Studio (Version 4.3.0). The following sections describe the statistical analyses conducted for each experimental task. Due to procedural errors, data from two mice, one from the SAL group and one from the OXY2 group, were excluded from all analyses. Scores that were more than two standard deviations from the group mean were considered outliers and removed from statistical analysis. *Post hoc* tests were performed when appropriate to investigate statistically significant main effects and interactions further. Mean comparisons were restricted to those appropriate for evaluating specific hypotheses, rather than

testing for all possible differences, to reduce type I error. The alpha level was set at 0.05 for all analyses.

Conditioned Place Preference

To assess the effects of drug condition on conditioned place preference during post-conditioning day 1, a one-way analysis of variance (ANOVA) was conducted. After removing an outlier from the MAOXY1 group, conditioned place preference ratios were averaged for each group. Group sizes were: SAL = 7, OXY1 = 8, OXY2 = 7, MA = 8, MAOXY1 = 7, MAOXY2 = 8. Drug condition was treated as a between-subjects factor with six levels: SAL, OXY1, OXY2, MA, MAOXY1, MAOXY2.

A two-way mixed factor repeated-measures ANOVA was used to investigate the persistence of conditioned place preference. After excluding drug-treated mice lacking conditioned place preference, ratios were averaged for each group. Group sizes were: SAL = 7, OXY1 = 5, OXY2 = 5, MA = 6, MAOXY1 = 5, MAOXY2 = 5. The between-subjects factor was drug condition, and the within-subjects factor was post-conditioning day, consisting of four levels: Post-conditioning days 1, 2, 3, and 4.

A two-way mixed factor repeated-measures ANOVA was performed to assess whether sensitization occurred. After removing two outliers, one from MA and another from MAOXY2, locomotor activity was averaged for each group. Group sizes were: SAL = 7, OXY1 = 8, OXY2 = 7, MA = 7, MAOXY1 = 8, MAOXY2 = 7). Drug condition served as a between-subjects factor, and drug administration number during the conditioning phase was treated as a within-subjects factor with two levels: Administrations 1 and 3 (48 hours apart).

Open Field

To examine the effects of drug condition on time spent in the center zone a single-factor ANOVA was conducted. After removing two outliers, one from OXY2 and another from MAOXY1, center zone time was averaged for each group. Group sizes were: SAL = 7, OXY1 = 8, OXY2 = 6, MA = 8, MAOXY1 = 7, MAOXY2 = 8. Drug condition served as the between-subjects factor.

Single-factor ANOVAs were used to examine the effects of drug condition on distance traveled and velocity. No outliers were identified. Group sizes were: SAL = 7, OXY1 = 8, OXY2 = 7, MA = 8, MAOXY1 = 8, MAOXY2= 8). Distance traveled and velocity were averaged for each group. Drug condition served as the between-subjects factor.

Novel Object Recognition

To assess the effects of drug condition on acute novel object recognition a single-factor ANOVA was conducted. After removing one outlier from MAOXY1 and excluding mice that failed to interact with the objects, novel object recognition ratios were averaged for each group. Group sizes were: SAL = 7, OXY1 = 8, OXY2 = 6, MA = 6, MAOXY1 = 5, MAOXY2 = 4. Drug condition served as a between-subjects factor.

Single-factor ANOVAs were used to examine the acute effects of drug administration on distance traveled and velocity. After removing two outliers from MAOXY1, distance traveled and velocity were averaged for each group. Group sizes were: SAL = 7, OXY1 = 8, OXY2 = 7, MA = 8, MAOXY1 = 6, MAOXY2 = 8. Drug condition served as the between-subjects factor. Due to procedural errors, data from two SAL mice were excluded from all long-term analyses.

A single-factor ANOVA was used to assess the effects of drug condition on long-term novel object recognition. After removing three outliers, one each from MA, OXY1, and OXY2, novel object recognition ratios were averaged for each group. Group sizes were: SAL = 5, OXY1

= 7, OXY2 = 6, MA = 7, MAOXY1 = 8, MAOXY2 = 8. Drug condition served as a between-subjects factor.

Single-factor ANOVAs were used to examine the long-term effects of drug condition on distance traveled and velocity. After removing two outliers, one from MA and another from MAOXY2, distance traveled and velocity were averaged for each group. Group sizes were: SAL = 5, OXY1 = 8, OXY2 = 7, MA = 7, MAOXY1 = 8, MAOXY2 = 7. Drug condition served as the between-subjects factor.

4.3 Results

Conditioned Place Preference

Conditioned place preference was evaluated over four days following the conditioning phase. A significant main effect of drug condition was observed **(Fig. 4.3b)**, $F(5, 25)=7.75$, $p < .001$. On day 1, all drug conditions demonstrated significant place preference compared to saline **(Fig. 4.3a)**. This preference persisted through days 2 and 3 for all groups. On day 4, only the lower oxycodone dose failed to maintain significant place preference. Neither combination showed greater place preference than either drug alone. However, the sustained preference in the smaller combination group suggests that methamphetamine may have strengthened the persistence of the smaller oxycodone dose.

Sensitization

Locomotor activity was measured by the distance traveled during the conditioning phase. A main effect of drug condition was found $F(5,38)=25.86$, $p < .001$, and an interaction between drug condition and administration number was found, $F(5,38)=4.58$, $p = .002$. Methamphetamine and both combination groups traveled a greater distance than saline and oxycodone alone. Sensitization, defined as the increased distance traveled from the first to third drug

administration, was observed in the methamphetamine alone and combination groups **(Fig. 4.3c)**, MA: $t(38)=-2.54$, $p = .02$; MAOXY1: $t(38)=-2.61$, $p = .01$; MAOXY2: $t(38)=-2.56$, $p = .01$.

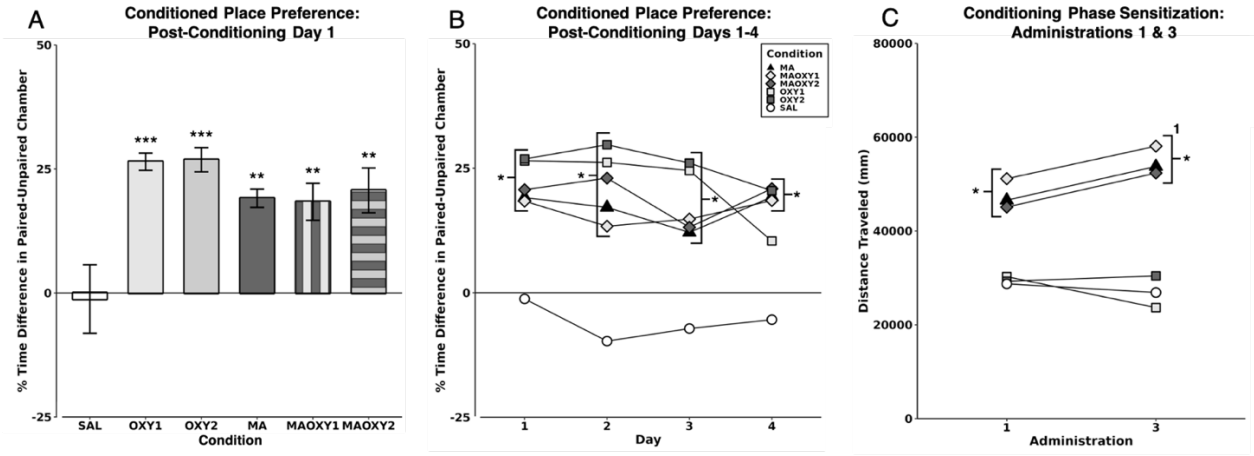

Figure 4.3 Conditioned Place Preference & Sensitization.
Column graph (A) displays conditioned place preference on the first day of post-conditioning tests (n = 45: SAL = 7, OXY1 = 8, OXY2 = 7, MA = 8, MAOXY1 = 7, MAOXY2 = 8). ** indicates a significant difference from SAL at $p < 0.01$, while *** indicates a significant difference from SAL at $p< 0.001$. The line graph (B) shows conditioned place preference over the four days of post-conditioning tests for each drug condition (n = 33: SAL = 7, OXY1 = 5, OXY2 = 5, MA = 6, MAOXY1 = 5, MAOXY2 = 5). * denotes a significant difference from SAL ($p < 0.05$). Error bars were removed for clarity. The line graph (C) displays sensitization data by showing the change in drug-induced locomotor activity from drug administration 1 to 3 (n = 44: SAL = 7, OXY1 = 8, OXY2 = 7, MA = 7, MAOXY1 = 8, MAOXY2 = 7). Error bars were removed for clarity. * signifies a significant difference from SAL, OXY1, and OXY2 ($p < 0.001$). Moreover, **1** indicates a significant increase from administration 1 ($p < 0.05$).

Open field

Anxiety-like behavior was assessed by measuring the time spent in the center of the arena and less time in the center was interpreted as increased anxiety-like behavior. A main effect of drug condition was observed $F(5,38)=8.35$, $p < .001$). Compared to saline, all drug conditions reduced center time, indicating that each drug and their combination increased anxiety-like behavior. Furthermore, the smaller combination led to a greater reduction in center time than oxycodone alone. Conversely, the larger combination resulted in a greater reduction in center time than either methamphetamine or oxycodone alone, indicating that the highest level of anxiety-like behavior was observed in this condition.

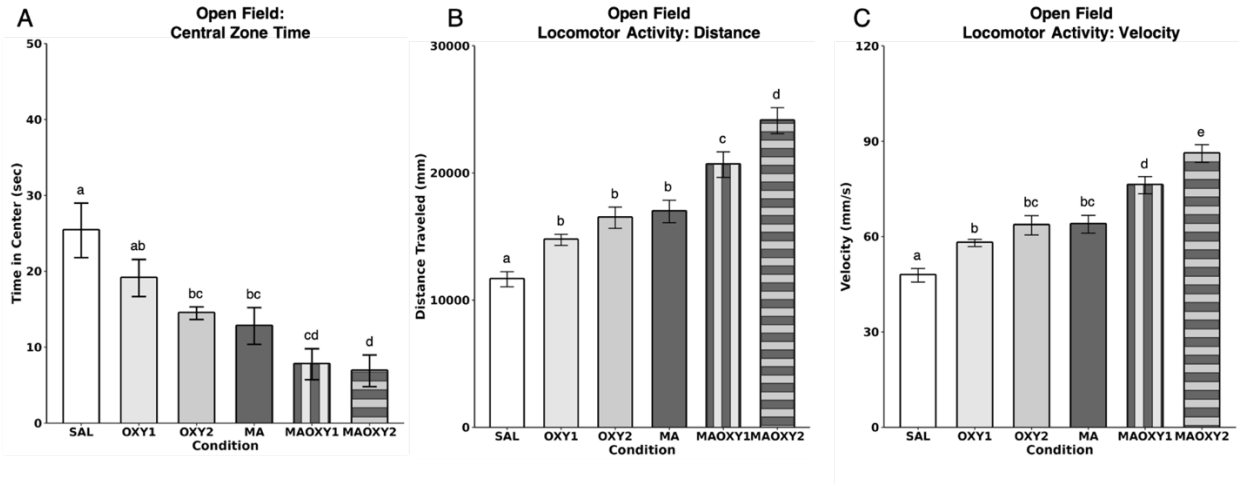

Figure 4.4 Open Field Test

Column graphs display measures in the open field paradigm: (A) central zone time (n = 44: SAL = 7, OXY1 = 8, OXY2 = 6, MA = 8, MAOXY1 = 7, MAOXY2= 8), (B) distance traveled (n = 46: SAL = 7, OXY1 = 8, OXY2 = 7, MA = 8, MAOXY1 = 8, MAOXY2= 8), (C) velocity (n = 46: SAL = 7, OXY1 = 8, OXY2 = 7, MA = 8, MAOXY1 = 8, MAOXY2= 8). Letters that differ indicate significant differences between groups ($p < .05$ for all significant post hoc tests).

Locomotor activity, determined through distance traveled and velocity, was increased in all drug conditions: Distance: $F(5, 40)=27.61$, $p < .001$; Velocity: $F(5,40)=28.91$, $p < .001$. Both combinations increased locomotor activity more than either drug alone. These increases were dose-dependent, as the larger combination produced significantly greater increases than the smaller combination.

Novel Object Recognition

Novel object recognition was assessed post-drug administration (acute) and one week later (long-term) without an active drug. Acute administration resulted in a significant main effect of drug condition **(Fig. 4.5a)**, $F(5,30)=3.07$, $p = .02$. Oxycodone reduced novel object exploration, but this effect was nullified when combined with methamphetamine. One week later (day 7), no main effect on novel object recognition was observed **(Fig. 4.5d)**, $F(5,35)=0.37$, $p = .87$.

For acute novel object recognition, locomotor activity was determined through distance traveled and velocity. Drug condition had a main effect on both measures **(Fig. 4.5b)**: Distance: $F(5,38)=32.08$, $p < .001$; **(Fig. 4.5c)**; Velocity: $F(5,38)=52.39$, $p < .001$. *Post-hoc* independent samples *t*-tests revealed that methamphetamine and the smaller combination increased locomotor activity compared to saline and oxycodone. However, the larger combination produced a dose-dependent increase greater than either drug alone.

Regarding long-term novel object recognition, a trending effect of drug condition on distance traveled was noted, $F(5,36)=2.22$, $p = .07$. Methamphetamine increased the distance traveled compared to saline; this effect was still attenuated when combined with oxycodone.

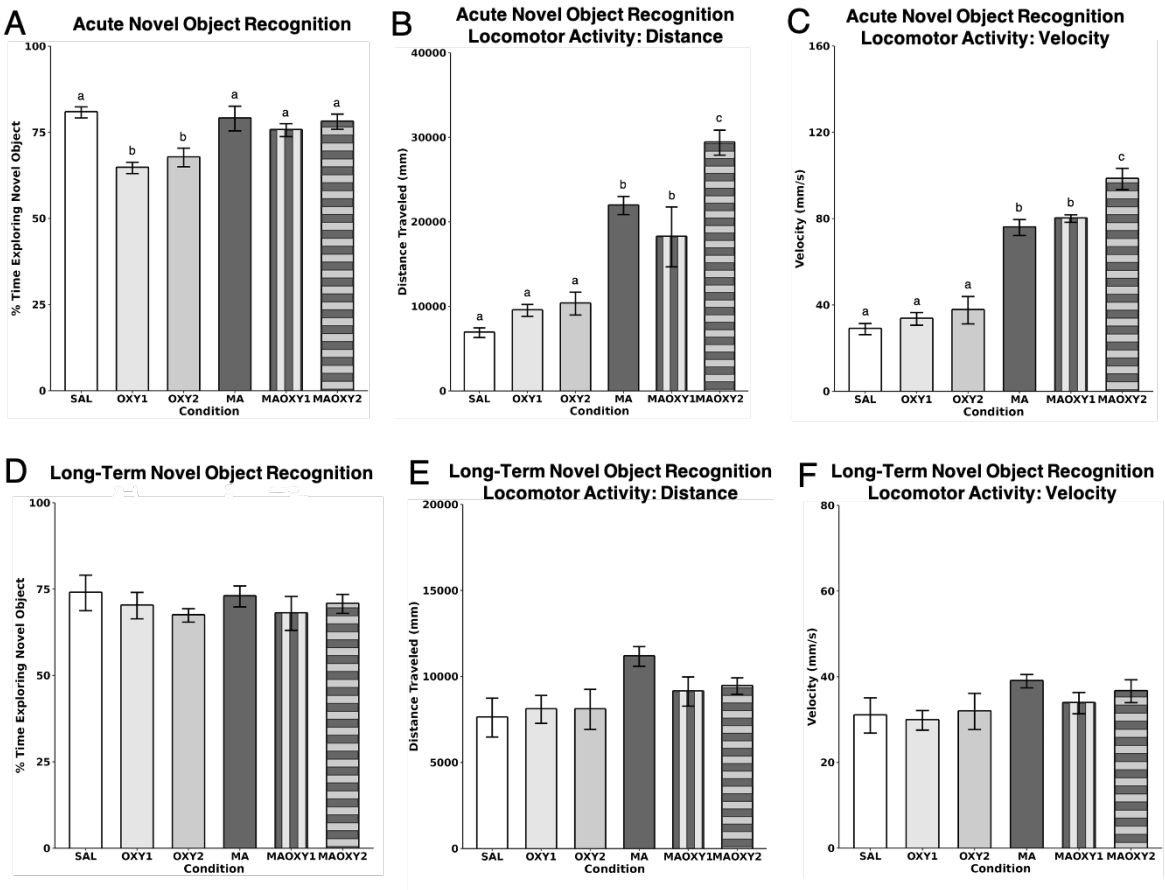

Figure 4.5 Novel Object Recognition

Column graphs illustrate both acute (A, B, C) and long-term (D, E, F) impacts of drug administration as measured by Novel Object Recognition: (A) acute novel object recognition (n = 36: SAL = 7, OXY1 = 8, OXY2 = 6, MA = 6, MAOXY1 = 5, MAOXY2 = 4), (B, C) acute distance traveled & velocity (n = 44: SAL = 7, OXY1 = 8, OXY2 = 7, MA = 8, MAOXY1 = 6, MAOXY2 = 8), (D) long-term novel object recognition (n = 41: SAL = 5, OXY1 = 7,

OXY2 = 6, MA = 7, MAOXY1 = 8, MAOXY2= 8), (E, F) long-term distance traveled & velocity (n = 42: SAL = 5, OXY1 = 8, OXY2 = 7, MA = 7, MAOXY1 = 8, MAOXY2 = 7). Letters that differ indicate significant differences between groups ($p < .05$ for all significant post hoc tests).

4.4 Discussion

My results differed slightly from Keith (2014) in CPP and acute Novel Object Recognition. One of our aims was to assess not only conditioned place preference, but its duration through an extinction model. In Keith (2014), CPP was extinguished for all drug conditions by the fourth day. However, I found that the preference generally remained throughout all four days. The reason for this discrepancy lies in my decision to exclude mice that did not develop an initial CPP from the statistical analysis. This criterion was based on literature suggesting that CPP extinction studies should focus exclusively on animals that demonstrate an initial CPP, defined as spending at least 10% more time in the drug-paired chamber than the unpaired chamber (Voigt et al., 2011; Li et al., 2022). The rationale behind this criterion is straightforward: it is illogical to examine extinction in animals without the conditioned behavior.

Following drug administration, Keith (2014) reported no significant differences on Novel Object Recognition. To reduce variance, I elected to use the criteria noted by Ennaceur & Delacour (1988) and Denninger et al., (2018) and remove animals that did not interact with the objects or spend only minimal time exploring them (Ennaceur & Delacour, 1988; Denninger et al., 2018). After implementing these criteria, I found that following oxycodone administration, novel object recognition was disrupted. Although this finding differs from Keith (2014), when you look at those findings, although it is not statistically significant, you can see a small dip in the oxycodone groups. Therefore, the new results are in line with the pattern from the original Keith (2014) plot, but are now significant.

Our results clearly show that the combination of methamphetamine and oxycodone can affect the individual effects of either drug. The outcomes vary depending on the dose and behavioral measure used. Specifically, methamphetamine strengthened the persistence of conditioned place preference for the smaller oxycodone dose and nullified the disruptions in novel object recognition. Conversely, the combination increased anxiety-like behavior and locomotor activity more than either drug alone. The interpretation of increased locomotor activity is nuanced, yet it could be considered positive, given methamphetamine's ability to offset opioid-induced lethargy in humans (Ivsins et al., 2022). Our study expands upon previous research on stimulant-opioid combinations by offering the first empirical investigation into the behavioral effects of the methamphetamine-oxycodone combination. (David et al., 2001; Lan et al., 2009; Martin et al., 2006; Masukawa et al., 1993; Pereira et al., 2006; Ranaldi & Munn, 1998; Rowlett et al., 2005, 2007; Rowlett & Woolverton, 1997).

In the present study, all drug conditions significantly increased place preference, suggesting drug-induced reward. This observation aligns with previous research that found methamphetamine and opioids consistently produce conditioned place preference in rodents (Der-Avakian et al., 2007; Liu et al., 2009; Suzuki et al., 1996; Tokuyama et al., 1996). We proposed that the common use of stimulant-opioid combinations among drug users might be due to enhanced rewarding effects when combined, a hypothesis partially supported by our results.

We found that the conditioned place preference induced by methamphetamine was not enhanced by oxycodone, suggesting that the initial reward of the combination is not greater than either drug alone. It is possible that significant enhancement would occur if we examined a broader range of doses. In a previous study, Lan and colleagues (2009) compared place preference across three doses each of methamphetamine (.375, .75, 1.5 mg/kg), morphine (2.5, 5,

10 mg/kg), and their combination. While all drug conditions produced place preference, a significant enhancement was observed at only one of the combination doses (MA: 0.75 mg/kg, morphine: 5 mg/kg). It is important to note that conditioned place preference is commonly considered a binary, "all or none" phenomenon (Mueller & DeWitt, 2011). In support of this notion, conditioned place preference experiments rarely produce dose-dependent or linear pharmacological effects and are often criticized for these reasons (Bardo & Bevins, 2000; Cunningham et al., 2006; Tzchentke et al., 2007; Rutten et al., 2011). Therefore, exploring other doses may lead to a significant enhancement, but this would not necessarily indicate a greater reward.

Nevertheless, the duration of conditioned place preference could serve as an alternative measure of greater rewarding effects. Consistent with our hypothesis, the sustained preference of the smaller combination group over all four post-conditioning days, compared to only three days in the smaller oxycodone dose, suggests that methamphetamine may enhance the duration of oxycodone's place preference. This finding is consistent with results from Lan and colleagues (2009), which also found a slower decline of conditioned place preference from a combination of morphine and methamphetamine compared to the corresponding doses alone. Despite this, it is important to note that place preference for opioids and methamphetamine can endure for weeks and even months (Lien et al., 2004; Mueller & DeWitt, 2011; Rutten et al., 2011; Voigt et al., 2011; He et al., 2004). Absence of preference on a particular day does not necessarily imply a complete loss of preference. Conventionally, the criterion for extinction is three consecutive days without preference (Rutten et al., 2011). Thus, future studies with extended extinction protocols are warranted to corroborate our findings and those of Lan et al. (2009). Nonetheless, our results hint that users might combine methamphetamine and oxycodone for a longer duration of effects

rather than heightened euphoria, a notion supported by anecdotal reports indicating that a substantial proportion of methamphetamine-opioid users consume one drug to extend the euphoric effects of the other (Rhed et al., 2022).

As expected, methamphetamine administration increased time outside the center zone during the open-field test when administered alone, suggesting potential anxiogenic effects. This finding aligns with previous research demonstrating anxiety-like behavior in rodent models subjected to the open-field and elevated plus maze measures following acute methamphetamine administration (Cancela et al., 2001; Hayase et al., 2005; Lapin, 1993).

Oxycodone's increase in anxiety-like behavior was unexpected, given the well-documented anxiolytic effects of opioids (Glover & Davis, 2008; Kõks et al., 1999; Rex et al., 1998; Rezayof et al., 2009; Shin et al., 2003). One explanation may be oxycodone's agonism at kappa-opioid receptors, as kappa-opioid agonists can produce anxiety in humans. However, buprenorphine, a kappa-opioid receptor antagonist, also increased anxiety-like behavior in mice during the open-field test (Lelong-Boulouard et al., 2006). Conversely, when tested with an elevated plus maze in rats, buprenorphine and oxycodone decreased anxiety-like behavior (Bruijnzeel et al., 2022; Etaee et al., 2017). Thus, the observed anxiogenic effect of oxycodone might be more dependent on the experimental measure and species used than its kappa-opioid receptor activity.

We hypothesized that drug users might combine methamphetamine and opioids to mitigate the adverse effects of each drug. Specifically, we believed that oxycodone would alleviate the anxiety-like behavior induced by methamphetamine in the open-field test. However, our findings showed that this combination increased anxiety-like behavior more than either drug alone. Our results align with a study by Etaee et al. (2017), which examined the effects of acute

buprenorphine and methamphetamine combined in the elevated plus maze. However, they diverge from human studies such as Jasinski & Preston (1986), which found that morphine and amphetamine combinations did not further intensify feelings of nervousness.

We faced several challenges in drawing conclusions from our open-field test results. One of the main issues was hyperlocomotion across all drug conditions, which negatively correlated with center zone time. The methamphetamine-oxycodone combination resulted in the most locomotor activity and, consequently, the most anxiety-like behavior (least center zone time). However, there could be alternative explanations, such as a stimulant effect or even a reduction of anxiety-induced inhibition of movement, which could actually be interpreted as an anxiolytic effect (Boerngen-Lacerda & Souza-Formigoni, 2000). It's important to note that anxiety-like behavior is often observed when established anti-anxiety drugs are given to rodents in the open-field test. A comprehensive literature review showed that about half of open-field studies with well-established anxiolytic drugs reported increased anxiety-like behavior (Thompson et al., 2015). These findings highlight the limitations of using center zone time as a primary indicator of anxiety-like behavior, especially when increased movement is involved.

Acute methamphetamine did not affect short-term memory, as measured by novel object recognition. This finding aligns with research in humans showing that methamphetamine administration does not disrupt cognitive performance (e.g., Breitenstein et al., 2004; Hart et al., 2008). Nevertheless, in rodents, acute methamphetamine can disrupt multiple cognitive domains (Marshall et al., 2007). It is important to note that researchers in these studies often administered substantial doses of methamphetamine, much larger than humans typically use (Hart et al., 2012). Hence, future studies in non-human animals could administer more ecologically relevant doses to improve the translatability of their findings.

As predicted, acute oxycodone disrupted the recognition of novel objects, aligning with previous research on the acute effects of opioids on memory in rodents (Macht & Mora, 1920; Hepner et al., 2002). However, although our study found a statistically significant effect of oxycodone on novel object recognition, mice still preferred the novel object over the familiar one, indicating their recognition memory remained mainly intact. Thus, our findings are consistent with human studies showing that low doses of acute oxycodone either have no impact or cause only slight reductions in memory measures (Cherrier et al., 2009; Friswell et al., 2008; Schoedel et al., 2010; Zacny & Gutierrez, 2003).

As hypothesized, methamphetamine prevented oxycodone-induced memory disruptions as measured by the novel object recognition test. The dearth of studies on the acute effects of amphetamine-opioid combinations makes it difficult to relate our findings to previous literature. Nonetheless, our results are consistent with the findings of Forrest et al. (1977), who found that amphetamine attenuated the slight disrupting effects of morphine on several measures of cognitive performance. Our results suggest that users might combine methamphetamine with oxycodone to improve cognition, a notion supported by clinical reports indicating that opioid users often combine the drug with methamphetamine to function more effectively (Ellis et al., 2018).

Our findings indicate that although acute oxycodone reduces novelty preference, the impact does not persist long-term. This result is consistent with a recent study on tat-transgenic mice (Salahuddin et al., 2020) and epidemiological research suggesting no concerning long-term cognitive risks of prescription opioid use (Zacny, 1995; Akhurst et al., 2021).

Regarding methamphetamine, we observed no long-term effects on novel object recognition. Previous studies on the impact of repeated methamphetamine administration on

recognition memory have produced mixed results. Some studies showed no significant differences (Corrone et al., 2023), while others reported memory disruption (Kamei et al., 2006). However, even when memory disruption occurred, it eventually returned to normal (Clemens et al., 2007; Kamei et al., 2006). For example, Belcher et al. (2006) administered a sensitizing regimen of 3 mg/kg methamphetamine injections twice daily for ten injections. The mice showed significant memory disruptions a week after their last drug injection, but their memory returned to normal after three weeks. Our study also used a sensitizing regimen of methamphetamine, but we did not observe long-term memory disruption. The difference in results may be due to the dose Belcher et al. (2006) used, equivalent to roughly 210 mg administered ten times in humans. For context, prescribed therapeutic doses of Desoxyn® (brand name for methamphetamine) usually do not exceed 60 mg per day (Desoxyn® Label, 2022). It is worth noting that tolerance can protect against memory disruptions and can be developed through an escalating dose regimen (Hart et al., 2012). Indeed, our study is consistent with the findings of Clark et al. (2007), who administered a 13-day escalating-dose regimen followed by a methamphetamine binge that did not result in long-term memory disruptions.

Our study found no evidence of long-term effects on novel object recognition from the combination of methamphetamine and oxycodone. Again, limited research on the behavioral impacts of amphetamine-opioid combinations makes it difficult to compare our results to previous studies. Nevertheless, our findings align with a comprehensive literature review by Hart et al. (2012), who investigated the impact of methamphetamine use on human cognition. Notably, many reviewed studies included opioid and other drug use that was not controlled for (e.g., Johanson et al., 2006). Statistically significant differences in cognitive performance were observed on only a few measures between polydrug methamphetamine users and control

participants. However, these differences may not have significant clinical implications as cognitive functioning generally fell within the normal range compared to normative data, which, for the most part, is consistent with our findings.

In our exploratory analyses, mice conditioned with methamphetamine showed elevated locomotor activity even though they had not received the drug before the task (see **Figure 4.3.E**). This increase could be attributed to the unintended conditioning of locomotor activity to the drug-associated environment. When reintroduced to the same environment without the drug, the inadvertently reinforced increase in locomotor activity may have been triggered (Skinner, 1998; Huston et al., 2013; Saunders et al., 2014). This effect was not observed in the combination groups, perhaps because oxycodone's subtle disruption of recognition memory prevented retention of the unintended hyperlocomotion conditioning. Therefore, the conditioning of locomotor activity may not have been retained, which inhibited the later increase in locomotor activity.

We observed that acute oxycodone had differential impact on locomotor activity depending on the test environment. Specifically, we found that oxycodone increased locomotor activity only in the open-field test, a stressful environment (refer to **Figures 4.2.B & 4.2.C**). This finding suggests that the stress caused by the open-field test was responsible for the increase. Further supporting our interpretation, a study on rats treated with 5 mg/kg methadone found little locomotor activity in a dry box but hyperlocomotion when placed in a stressful environment, such as the same box filled with water (Cummins et al. 2012). Thus, oxycodone's effect on locomotor activity likely depends on the environment.

Several limitations of the present study should be acknowledged. First, we exclusively used male mice. Given that responses to experimental treatments can vary between sexes due to

inherent biological distinctions (Mitre et al., 2017; Bolger et al., 2019) future studies must be conducted in females to ensure our findings can be generalized across biological sex. Second, we examined the effects of a single methamphetamine and two oxycodone doses. These doses were selected as they are close to the threshold required to trigger conditioned place preference (Der-Avakian et al., 2007; Liu et al., 2009; Suzuki et al., 1996; Tokuyama et al., 1996). However, methamphetamine-related hyperlocomotion might have influenced the outcomes of multiple behavioral measures. Future research should either utilize methodologies less sensitive to methamphetamine-induced hyperlocomotion or examine lower doses that do not increase locomotor activity. Third, our study utilized a within-task design, such that the behavioral tests were consistently administered in the same order without counterbalancing. While this approach prevented us from evaluating potential carry-over effects, it did have two key benefits. First, it reduced the number of mice required for sacrifice. Secondly, it simulated the varied and often random patterns of self-administered drug use observed in humans, providing a translational advantage over testing with drug-naive mice. Future investigations into the behavioral effects of this drug combination should carefully consider the advantages and disadvantages of within-task and between-subjects designs.

Finally, a drawback of the current study is that we used conditioned place preference as an *indirect measure* of drug reward. However, reward is a subjective experience and can only be measured directly in humans. As a result, the face validity of conditioned place preference as a reward measure in rodents is often disputed, making it a controversial measure in behavioral pharmacology (Bardo & Bevins, 2000; Mueller & DeWitt, 2011). Additionally, there is uncertainty surrounding the open field test for measuring anxiety and the novel object recognition test for evaluating memory (Thompson et al., 2015; Snyder et al., 2021; Ennaceur,

2010). Relying on these single tests to characterize such wide-ranging domains complicates the interpretation of our results even further. We could offer a more comprehensive analysis by employing a series of tests covering each domain. For instance, reward-related behavior might be assessed through self-administration and conditioned place preference paradigms across both non-human animals and humans.

In conclusion, our research supports our hypothesis that combining methamphetamine and oxycodone can enhance the positive and decrease the adverse effects of either drug. Specifically, we found that methamphetamine prolonged oxycodone's conditioned place preference. It also appeared to counteract the subtle short-term memory disruption caused by oxycodone without any long-term negative effects. However, due to the hyperlocomotion observed across all drug conditions in the Open Field Test, we were unable to draw any firm conclusions regarding anxiety-like behavior. Overall, our findings suggest that the appeal of combining methamphetamine and oxycodone may be due to a prolonged euphoric experience and cognitive enhancement rather than merely intensifying euphoria. Clinical reports support this notion, as many opioid users report self-administering methamphetamine to both extend the euphoric effects of opioids and function more effectively (Rhed et al., 2022; Ellis et al., 2018). Nevertheless, our study has limitations, including the short extinction protocol, the potential influence of methamphetamine-induced hyperlocomotion, and our reliance on optimal translation of test scenarios. Given these constraints, our findings should be considered preliminary and in need of further validation. Future research could assess doses that do not produce hyperlocomotion, less hyperlocomotion-susceptible methodologies, and a more diverse range of behavioral tests.

Chapter 5: General Discussion

Concerns persist regarding the illicit use of amphetamine derivatives with significant gaps in our knowledge. These studies addressed two key gaps: 1) the effects of repeated MDMA administration, and 2) the interactive effects of methamphetamine with commonly used drugs (i.e., alcohol and oxycodone).

5.1 Repeated MDMA Administration Effects

An important gap addressed by the current study is the lack of empirical evidence regarding the effects of repeated-MDMA administration. The major scientific contribution of the current study is that it is the first study to quantify the repeated-administration (12 and 24-hour inter-dose intervals), and residual effects of MDMA on a wide range of physiological, behavioral, and subjective mood measures in MDMA-experienced human volunteers.

In recreational settings, people report taking multiple doses of MDMA within one or two days. This pattern of use has raised concerns among health care providers due to the potential for adverse effects. One reason for this concern is that MDMA can hinder its own metabolism through a process known as metabolic inhibition (de la Torres et al., 2004). This inhibition can lead to an increase in MDMA plasma concentrations in the blood stream, which can subsequently be transported to sites of action. Consequently, the effects following an initial MDMA dose may intensify with each subsequent administration, which has prompted concerns about the potential for harmful increases in heart rate, blood pressure, and body temperature. However, the data from our study do not support these concerns. In fact, our findings suggest the occurrence of a reduced effect following repeated administration. For instance, after the first MDMA administration, heart rate showed a dose-dependent increase. Yet, by the third

administration, which was 36 hours from the first and 24 hours from the second, these elevations were attenuated and no longer significantly elevated as compared to placebo. One potential explanation for this phenomenon is related to MDMA's mechanism of action, which involves displacing monoamine neurotransmitters from their vesicular storage sites. This process could deplete monoamines to a level where a subsequent dose within a short timeframe may not be able to displace as much neurotransmitter, hence why we observed a reduced cardiovascular effect.

Another pressing concern associated with MDMA is the potential emergence of a depressed mood state in the days following use. Some scientists and health professionals have even speculated that this post-MDMA depressed mood could lead to suicide (Parrott, 2013; Kim et al., 2011). This concern is tied to the intensely criticized "serotonin hypothesis (for review: Moncrieff et al., 2023)," which posits that depression is caused by low levels of serotonin. As noted above, MDMA can potentially lead to a drop in serotonin levels after use. When combined with the public's tendency to associate depression with serotonin, this interaction could conceivably foster pessimistic expectations about MDMA's residual mood effects. Indeed, drug expectancies often influence subjective responses to drugs (Lundahl et al., 2006; Yamamoto et al., 2007). Importantly, Kirkpatrick and colleagues (2012b) conducted a double-blind, placebo-controlled study design. They found no evidence of a depressed mood in the days immediately following a single MDMA administration (Kirkpatrick et al., 2012b). Importantly, the current study not only replicates this finding but also builds upon it by investigating the effects of repeated MDMA administration. In the present study, we did not observe a depressed mood state in the days following repeated MDMA administrations.

These findings have substantial implications for public health. Currently, MDMA is undergoing Phase 3 clinical trials to assess its effectiveness in treating post-traumatic stress disorder (PTSD) (Sessa et al*.,* 2019). However, despite promising results, MDMA remains classified as a Schedule I drug with no recognized medical use, primarily due to DEA concerns about its safety and abuse liability discussed in the introduction. Nevertheless, the current data could provide a basis for advocating a more rational approach to MDMA-related legal and medical practices. It is essential to note that our study, while contributing valuable information, was not a clinical trial and did not include the assessment of therapeutic measures. Consequently, it does not directly provide evidence of the therapeutic efficacy of MDMA.

While the empirical database on the effects of repeated-MDMA administration in humans is growing, unanswered questions still remain. For instance, it is possible that life threatening cardiovascular and residual mood effects attributed to MDMA are a result of non-pharmacological factors. MDMA, often referred to as a 'Rave drug,' is popular in nightlife settings. Consequently, factors such as vigorous aerobic activity, elevated body temperature, dehydration, and sleep deprivation, all prevalent in nightlife culture, may be the cause of these effects. Conversely, MDMA might interact with these non-pharmacological factors, complicating our understanding of its effects even further. Future studies on this topic should consider the incorporation of these additional variables to provide a more comprehensive understanding of repeated-MDMA administration effects

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5.2 Interactive Effects of Methamphetamine and Alcohol

Another important gap addressed by the current studies was the dearth of information about the effects of methamphetamine combined with alcohol. The major scientific contribution

of Study 2 is that this was the first investigation of repeated administrations of methamphetamine in combination with alcohol at a 12-hour dosing interval.

Recent data from the Drug Abuse Warning Network (SAMHSA, 2023) highlights a concerning trend: alcohol has emerged as the most frequently co-involved substance in methamphetamine-related Emergency Department visits. This phenomenon may, to some extent, be attributed to the physiological interactions of these substances. Methamphetamine is well-documented to elevate cardiovascular measures, and despite the common perception of alcohol as a sedative, it can also lead to increased cardiovascular activity at low to moderate doses (For review see Henlder et al., 2011). This combination raises the possibility of potentially life-threatening synergistic cardiovascular effects when methamphetamine and alcohol are used together. It is important to note that prior research has indeed shown that the combination of these two substances results in a more substantial increase in heart rate compared to either drug alone (Mendelson et al., 1995). However, these heart rate elevations are generally modest and do not typically reach levels of clinical concern. Nonetheless, we were concerned that potentially dangerous heart rate effects may appear following repeated doses of the combination. However, our study did not substantiate this concern, as we observed a reduction in heart rate elevations following repeated administrations.

On a related note, the combination not only reduced alcohol-induced feelings of drunkenness but also increased feelings of euphoria compared to either drug alone. Importantly, both of these effects were sustained with repeated administration. These findings offer insights into why these drugs may be used in combination repeatedly: to mitigate the undesired effects of alcohol while maintaining euphoria. Nonetheless, two potential concerns arise from the observation that methamphetamine continued to offset alcohol-related feelings of intoxication

with repeated administration. First, it is possible that some individuals who use methamphetamine may increase their alcohol consumption in an attempt to attain their accustomed “state” of alcohol intoxication. It's noteworthy that subjective ratings of "want alcohol" were not heightened by the methamphetamine-alcohol combination. However, in line with the concept of the intention-behavior gap (Inauen et al., 2016), craving alone may not reliably predict alcohol self-administration (Tiffany et al., 1998). Consequently, future studies should explore the choices individuals make regarding alcohol self-administration following methamphetamine-alcohol combination use. Second, there's a possibility that methamphetamine-alcohol users might underestimate their level of alcohol-related impairment after consuming the combination. This underestimation of impairment could lead to an increase in risky behaviors, such as driving while intoxicated. While this remains speculative, it is a more plausible cause of Emergency Department visits than the cardiovascular effects resulting from the interaction of these two drugs. Therefore, future studies should explore the impact of this drug combination on cognitive measures, particularly those of higher complexity, such as the metacognition of agency task, which evaluates the ability to assess one's own level of physical control (Kirkpatrick et al., 2008), and driving simulators, following repeated administrations.

5.3 Interactive Effects of Methamphetamine and Oxycodone

Our study has addressed a significant gap in the existing knowledge base by providing empirical evidence of the effects of methamphetamine when used in combination with oxycodone. Notably, this research represents the first study of the behavioral consequences of combining methamphetamine and oxycodone. This is important because public perception of methamphetamine combined with oxycodone largely stems from anecdotal reports. These

reports can be unreliable, as users may unknowingly consume more potent or adulterated substances. For instance, counterfeit oxycodone pills can contain lethal amounts of fentanyl or other drugs, posing severe dangers since they closely resemble legitimate oxycodone pills. In some instances, these pills may not contain any active substances, highlighting the urgency of understanding the actual behavioral effects of the methamphetamine-oxycodone combination. In this context, animal studies serve as a valuable tool for investigating drug combinations, offering a foundational platform for identifying potential benefits, risks, and unexpected effects. Such research is particularly important when it comes to understanding the biological and behavioral effects of under studied drug combinations.

Our findings demonstrate that combining a CNS stimulant and an opioid analgesic may result in a generally positive experience, though this is clearly dose-related. Consistent with our initial hypothesis, the combination of methamphetamine and oxycodone potentially enhanced positive effects while mitigating adverse ones associated with either drug alone. Specifically, we observed that methamphetamine extended the conditioned place preference for oxycodone. Furthermore, methamphetamine appeared to counteract the subtle short-term memory disruptions caused by oxycodone, with no apparent long-term negative consequences. Overall, these results suggest that the allure of combining methamphetamine and oxycodone may be attributed to a desire to prolong euphoria and enhance cognitive performance. Clinical reports support this notion, as many opioid users report self-administering methamphetamine to both extend the euphoric effects of opioids and function more effectively (Rhed et al., 2022; Ellis et al., 2018). Nevertheless, a major limitation of our study is our reliance on the optimal translation of our test measures to the human condition. Given this constraint, our findings should be considered preliminary and require further validation in human drug self-administration studies.

However, our results broadly align with human stimulant-sedative administration studies, which generally demonstrate overwhelmingly positive effects. Moreover, these human administration studies show that stimulant-sedative combinations can be safely administered under controlled laboratory settings. Hence, we are inclined to speculate that adverse effects associated with the methamphetamine-oxycodone combination may be more aptly explained within the context of an illicit drug supply tainted with adulterants. Unfortunately, animal studies yielding findings not easily translatable to human drug use have skewed our understanding of drug effects, fueling public concerns. Ironically, these very concerns have prompted lawmakers to pass legislation that paradoxically exacerbates drug-related harms. For instance, by imposing harsher penalties for pure methamphetamine than for adulterated versions, the Methamphetamine Trafficking Penalty Enhancement Act of 1998 may have inadvertently encouraged drug dealers to adulterate their methamphetamine, resulting in a potentially more dangerous product.

In conclusion, a noteworthy challenge in the scientific community, especially in the realm of substance use research, is the limited communication between preclinical and clinical researchers. A glaring example of this divide lies in the utilization of doses and measures in animal studies that may not readily translate to human drug use. While these studies may provide valuable insights, it is problematic to extrapolate these results to human drug use. While our study aimed to use doses and measures that are applicable to humans, it is still of paramount importance that our results are not overly extrapolated and do not overshadow or overly influence the interpretation of results from human studies.

5.4 Concluding Thoughts

The studies presented in this chapter have addressed key gaps in our understanding of the effects of amphetamine derivatives, particularly repeated MDMA administration and

methamphetamine’s interactions with the commonly used drugs alcohol and oxycodone. These findings have important implications for both the scientific community and public health.

One of the primary contributions of these studies is the examination of repeated MDMA administration, a pattern often observed in recreational settings. While concerns exist regarding potential adverse effects of repeated use, our results did not support these concerns. Contrary to expectations, we observed tachyphylaxis, indicating rapid tolerance development to MDMA's cardiovascular effects following repeated administration. Additionally, a post-MDMA depressed mood state was not observed in our study. These findings challenge prevailing assumptions about MDMA's risks and could inform more rational approaches to its legal and medical regulation.

Our reassessment of data on methamphetamine's interactive effects with alcohol addressed a growing cardiovascular concern. While the combination of these two substances did lead to modest increases in heart rate, they did not reach levels of clinical concern, and were attenuated with repeated administration. One notable finding to consider is methamphetamine's ability to offset alcohol-induced feelings of intoxication, even after repeated-administrations. This effect could potentially lead to an underestimation of impairment, subsequently increasing the likelihood of engaging in risky behaviors such as driving while intoxicated.

Our study on the methamphetamine-oxycodone combination has provided valuable insights into a drug combination that has primarily been characterized by anecdotal reports. Our findings suggested that this combination may enhance positive effects while mitigating adverse ones, offering a more comprehensive understanding of why individuals might use this combination. Nevertheless, our results should be considered preliminary with further validation in human drug administration studies needed.

In conclusion, these studies have made significant contributions to the understanding of two frequently used amphetamine derivatives and their interactions with two commonly used psychoactive drugs—oxycodone and alcohol. However, our findings also underscore the need for additional research and collaboration between preclinical and clinical researchers (Meyer et al., 2022). While our data from the mouse model aligns well with the effects observed in humans, a cautious approach is still recommended when extrapolating results from animal studies to human drug use. Above all, we advocate for robust empirical experimentation to be used as a major tool to combat ignorance, anecdotal evidence and misguided information (Altimus et al., 2020) about complex issues surrounding MDMA and methamphetamine. These efforts are not only critical for developing more precise assessments of the risks and benefits associated with these substances but also for improving drug policies and optimizing public health interventions.

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