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EXAMINING THE BEHAVIORAL AND PHYISIOLGICAL EFFECTS OF D-METHAMPHETAMINE ADMINISTERED VIA E-CIGARETTE AEROSOLIZATION

Srikethan Mahavadi

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

Principal Investigator: Dr. Keith Shelton
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List of Abbreviations

Coc: Cocaine

d-Amph: d-Amphetamine

ICSS: Intracranial Self-Stimulation

IP: Intraperitoneal

Meth: Methamphetamine

PG: Propylene Glycol

SC: Subcutaneous

THC: Tetrahydrocannabinol

VG: Vegetable Glycerol

Abstract

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E-cigarettes have become increasing popular worldwide. They are primarily used as a means to deliver nicotine to users, but these devices may also be used to administer a wide range of other substances including psychomotor stimulants such as d-methamphetamine. Methamphetamine has been widely abused throughout the United States and across the world leading us to believe there may be a possibility of abuse potential resulting from aerosolization of methamphetamine via e-cigarettes. In the present thesis, methamphetamine aerosol's CNS-mediated behavioral effects were assessed using locomotor activity, sympathomimetics effects examined using physiological testing, and drug blood levels after aerosolized methamphetamine was assayed. Key user controllable parameters of e-cigarette which may impact abuse liability such as puff number and e-cigarette wattage were manipulated. The locomotor assay revealed dose-dependent effects on total distance traveled following

methamphetamine administered subcutaneously (at doses of 0.03, 0.1, and 0.3 mg/kg) as well as puff (1, 5, 10, 15, 20) and wattage (18 and 36 watt) dependent changes in locomotor after exposure to 30 mg/ml aerosolized methamphetamine. Physiological effects measuring blood pressure (systolic and diastolic), body temperature, and heart rate showed puff-dependent increases in blood pressure and body temperature; however, heart rate did not show similar results. Finally, methamphetamine plasma concentration levels were also puff dependent as the greater the number of puffs the higher the concentration of methamphetamine in plasma was detected. All of these results were similar or in some cases greater than our positive subcutaneously administered methamphetamine control suggesting that methamphetamine may have high abuse liability when administered using e-cigarettes.

Introduction

BACKGROUND AND SIGNIFICANCE

E-cigarettes also referred to in the scientific community as Electronic Nicotine Delivery Systems (ENDS) or as vapes to many users, are devices designed to deliver nicotine aerosol. These devices were invented in their current form in 2003 and have been modified since then across several successive generations of products. The nicotine aerosol produced by the devices is generated from a solution commonly composed of vegetable glycerin (VG) and propylene glycol (PG), drug (typically nicotine), and an optional flavoring agent, all of which are present in a variety of proprietary ratios. Aerosol generated from an e-cigarette is commonly referred to as "vapor" by some individuals. However, vapor refers to the gaseous state of a substance such as water vapor. Aerosol is a suspension of fine particles of liquid, solid, or both in a gaseous state (Chang, 2014). In this experiment, e-cigarette output is assumed and referred to as being aerosol containing methamphetamine as particulate matter; although, the states of matter from this "aerosol" was not tested to confirm this supposition as it was beyond the scope of the present project.

The usage of e-cigarettes has become widespread and has been on the rise over the last decade. In 2020, there were approximately 68 million e-cigarette users worldwide (Jerzyński 2021). In the United States alone, a study conducted in 2018 estimated that 8.1 million U.S. adults were e-cigarette users. (Creamer, 2018). Of particular concern is the use of e-cigarettes in younger users who never used tobacco products. In 2021, 2.06 million middle school and high school students had reported using e-cigarettes within the past 30 days. According to a study done by the National Youth Tobacco survey, 12th grade cigarette use (traditional tobacco

cigarettes) has been on a decline since 1999 while e-cigarette usage has increased since 2011 (Figure 1).

Although the potential long-term health risks of e-cigarettes are not as well documented as traditional cigarettes, e-cigarette usage has been correlated with chronic lung disease, poor mental health, lung cancer, and increased risk of myocardial infarction (Alqahtani MM, 2022; Bracken-Clarke D, 2020; T Alzahrani, 2018). While nicotine has been the primary drug used in these devices, e-cigarettes have the potential to be adapted for the use of other highly abused drugs. Abuse of illicit drugs such as psychomotor stimulants and potent opioids using e-cigarette technology has been hypothesized to potentially be an attractive means of use, especially compared to more invasive routes such as intravenous injection. E-cigarettes aerosolize drug and the exhaled aerosol typically has the odor of the flavor that is added to the e-liquid rather than the characteristic odors associated with smoked drugs. This would minimize telltale olfactory cues, making overt aerosolized drug use less detectable by law enforcement or the general public. As such anecdotal reports suggest that other perceived benefits might include the ability to use illicit drugs in a discrete manner in public settings.

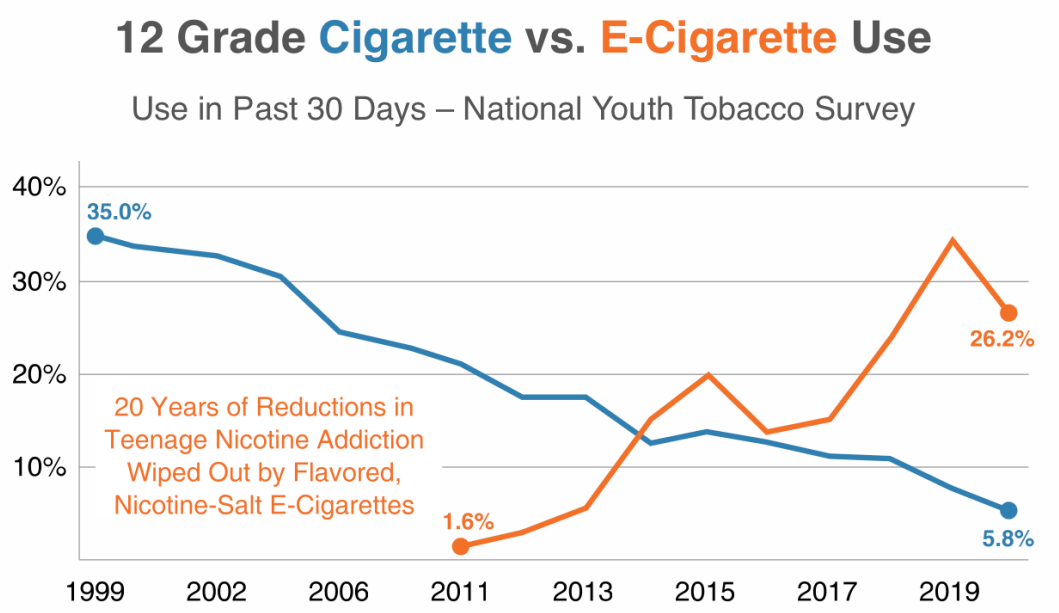

Figure 1: National Youth Tobacco Survey. 12th grade use in the past 30 days from 1999 to 2019. Traditional tobacco cigarette use is represented by the blue line while e-cigarette usage is represented by the orange line. (https://tobacco21.org/the-juul-epidemic/)

One particularly concerning drug, given current high levels of abuse by other routes is methamphetamine. In 2020, about 2.6 million people used methamphetamine in the past 12 months. Of those users, 1.5 million people are estimated as having a methamphetamine use disorder. (NIH, 2020). In addition to illicit use, amphetamine and methamphetamine are commonly prescribed drugs used to treat attention deficit hyperactivity disorder (ADHD). A study conducted in 2016 found that 6.1 million individuals under the age of 18 have been diagnosed with ADHD, of those 3.3 million are between the ages of 12-17 (CDC, 2021) and 62% of those are taking d-amphetamine (Adderall) as treatment. A study found that 61.7% of students were diverting their prescription d-amphetamine (Garnier, 2010). Given this concern

with abuse of methamphetamine and its derivatives, along with increased e-cigarette usage, especially among adolescents and young adults, there is potential risk of stimulants misuse and abuse using e-cigarettes.

METHAMPHETAMINE

Methamphetamine is a psychostimulant drug which exists in two stereoisomers; L-methamphetamine and D-methamphetamine. D-methamphetamine is a more potent stimulant with 3-5 times the CNS activity of L-methamphetamine. Methamphetamine is a lipophilic molecule which easily crosses the blood brain barrier. Methamphetamine stimulates the release and partially blocks the reuptake of newly synthesized catecholamines in the CNS. Methamphetamine binds to the dopamine transporter, norepinephrine transporter (NET), serotonin transporter (SERT), and vesicular monoamine transporter-2 (VMAT-2).

In vivo , the dopamine transporter utilizes Na^+ and Cl^- along their concentration gradient while coupling dopamine against its gradient resulting in dopamine in the synapse. NET is a co-transporter that couples the movement of Na^+ down its electrochemical gradient to the movement of norepinephrine against its gradient. Na^+ produces an outward-facing conformation while the absence promotes the inward facing conformation resulting in norepinephrine transport. VMAT-2 is driven by transmembrane pH and electrochemical gradient by vesicular H^+-ATPase in the vesicle membrane resulting in H^+ moving down its concentration gradient while the monoamine is moved against its gradient. SERT is driven by the symport of Na^+ and Cl^- along their concentration gradient with serotonin re-entering the presynaptic neurons. Methamphetamine reverses the endogenous function of each transporter

resulting in the monoamines being liberated into the cytosol and subsequently released into the synapse as opposed to being kept in storage vesicles. Methamphetamine also inhibits monoamine oxidase, attenuating the metabolism of monoamines which further enables the buildup of excess monoamines in the synapse.

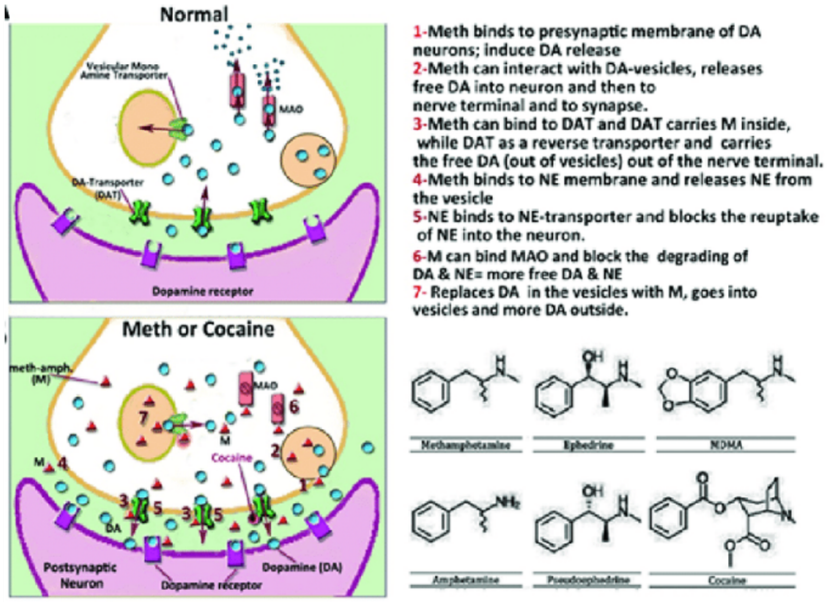

Figure 2: Methamphetamine mechanism of action. Mechanism of methamphetamine on multiple endogenous transporters. Top picture depicts normal function of transporters and bottom shows effects after the administration of methamphetamine (Sayn, 2019).

The monoamines released by methamphetamine act on major dopaminergic, adrenergic, and serotonergic pathways of the brain. Methamphetamine, specifically in the case of dopamine acting on D1 receptors, activates the mesolimbic pathway, mesocortical circuit, and the nigrostriatal pathways which have been related to the euphoric and locomotor effects observed following methamphetamine administration (Courtney, 2014). The medial forebrain,

hippocampus, prefrontal cortex represents adrenergic regions of interest with functions related to arousal, memory consolidation and cognitive processing. Affected serotonergic neurons are dispersed throughout the brain regulating functions such as respiration, pain perception, reward, and high order cognitive processing. The widespread distribution of monoamines throughout the CNS adds to the complexity of methamphetamine's effect on the monoamine systems (Courtney, 2014). Norepinephrine is increased by methamphetamine, stimulating the sympathetic nervous system producing effects such as increased blood pressure, metabolic rate and blood glucose as well as bronchial dilation, piloerection and other effects.

Distribution studies with methamphetamine have indicated that the drug is rapidly absorbed into the brain which make it useful clinically for certain conditions. Methamphetamine is highly lipophilic (attributed to the methyl group) producing high tissue to plasma ratio. There are three main biotransformation pathways involved in methamphetamine clearance: 1) demethylation of methamphetamine to produce amphetamine 2) aromatic hydroxylation producing pholedrine and 3) beta-hydroxylation to produce norephedrine (Torre, 2012). The first two reactions are regulated by CYP2D6 (Lin, 1997) as can be seen in figure 3.

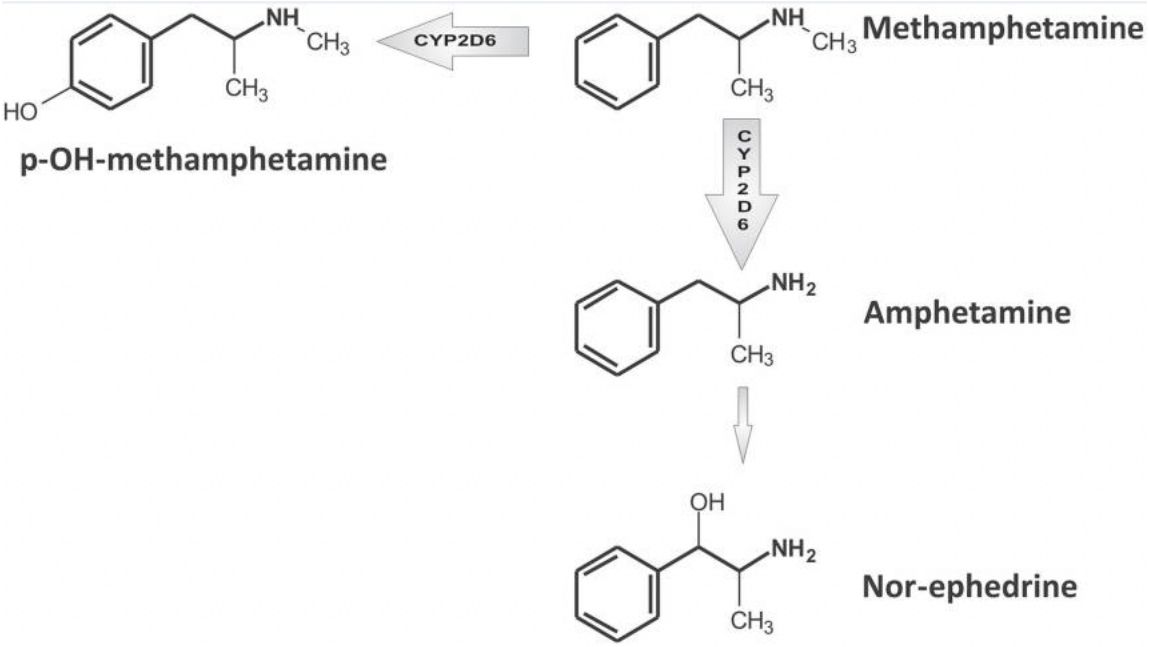

Figure 3: Simplified mechanism of methamphetamine main metabolic pathways. Methamphetamine is metabolized by the polymorphic cytochrome P450 isozyme CYP2D6 into either amphetamine or p-OH-methamphetamine.

METHAMPHETAMINE CLINICAL USAGE

Methamphetamine and its derivatives are used to treat attention deficit hyperactivity disorder (ADHD); however, prescription use has declined due to the drug's abuse liability resulting from its euphoric properties. Methamphetamine has also been used as a secondary treatment for narcolepsy and obesity (Rau 2016; Prakash 2017). For narcolepsy, methamphetamine has been prescribed at 60 mg a day and for ADHD up to 25 mg a day. At low to moderate doses (5-30 mg), methamphetamine produces sympathomimetic effects including accelerated heart rate, elevated blood pressure, increased temperature, etc. Higher doses

(>30mg), produce neuropsychological effects such as anxiety (Rau, 2016). The long-term effects of methamphetamine include lung, liver, and kidney damage, psychosis, depression, damage to brain structure, etc. (Yasaei, 2022). Short term effects of methamphetamine include appetite suppression, euphoria and nausea among others.

While methamphetamine abuse by the oral, injected and smoked routes is common, at this time little is known about the relative abuse liability of methamphetamine delivered by e-cigarette aerosol. There have, however, been numerous anecdotal reports on online forums discussing psychomotor stimulants, including methamphetamine, experimentation using e-cigarettes. One comment from a user stated that they could smoke methamphetamine in public places using an e-cigarette without raising suspicion. (Quora, 2021 https://www.quora.com/How-do-I-electronically-vape-meth). Other users discussed potential strategies for maximizing the subjective effects of drugs added to e-cigarette vaping solutions. These strategies included manipulating the e-cigarette output wattage or adding crushed methamphetamine to e-liquid. One particular user related their own experience of vaping methamphetamine with a Kangertech e-cigarette, a third generation high-output device, stating they normally had a "speedy clear high" from traditional smoked meth but when using the e-cigarette they reported a head buzz and blurred vision (Drug-forum, 2015 https://drugs-forum.com/threads/how-to-use-a-vaporizer-to-smoke-meth.280002/). The potential for illicit drug use has not escaped the lay press and several online publications have highlighted this possibility with titles such as "Why Some Meth and DMT users are Using Vapes" (https://www.vice.com/en/article/a3qbyj/why-some-meth-and-dmt-users-are-using-vapes) and "Meth Vape Pen. A 2021 Review of the Meth Vaporizer"

(https://www.upends.com/blogs/articles/meth-vape-pen-a-2021-review-of-the-meth-vaporizer) the latter of which provides a detailed how-to guide. Nicotine and methamphetamine have similar physiochemical characteristics that can possibly explain the ability to use both drugs in e-cigarette devices shown in Table 1. Unfortunately, the pharmacological effects and abuse liability of methamphetamine administered via e-cigarette aerosolization and the extent to which inhalation may impact abuse-related effects in contrast to use by more traditional routes has not been extensively explored.

Drug	Methamphetamine	Nicotine
Formula	$C_{10}H_{15}N$	$C_{10}H_{14}N_2$
Boiling Point	212 °C	247°C
Molecular Weight	149.23 g/mol	162.23 g/mol
pKa	9.99	8.11
Vapor Pressure	0.0054 mm Hg at 25 °C	0.038 mm Hg at 25°C

Table 1: Nicotine vs Methamphetamine physiochemical characteristics

ELECTRONIC CIGARETTES

The discussion of illicit drug use via Electronic Nicotine Devices (ENDS) requires some background on evolution of the product itself. Several laboratories, including our own (Shelton, 2022), have examined the effects of drugs aerosolized in e-cigarettes (Rom, 2015; Fadus, 2019;

DeVito, 2018). E-cigarettes are marketed as smoking cessation aids and there is a perception among many users that e-cigarette are harm-free or at least lower in potential harm than traditional tobacco cigarettes. This low perceived harm profile has led to their use by those who never smoked. It is hotly contested at the present time as to whether either of these two claims has scientific merit (Hart, 2018). E-cigarettes have been increasing in popularity especially among those under the age of 18 and adults since the devices first became available in the United States in 2007. All e-cigarettes operate in a similar manner; the device consists of a battery power supply and an atomizer which contains a coil and wicking system (Breland, 2016). The coil is composed of a metal resistance wire filament wrapped around a cotton or a silica wicking material which is immersed in e-liquid within a reservoir tank (Mulder, 2020). E-liquid is typically composed of propylene glycol (PG), vegetable glycerin (VG), a drug (typically nicotine), and an optional flavoring agent. Other less frequent components of e-liquids include smaller proportions of ethanol and water (Poklis, 2017). As the atomizer is heated, e-liquid is vaporized, condensing into an aerosol which is then inhaled by the user using the drip tip on top of the device (Krakowiak, 2019).

The first-generation e-cigarette devices, known as “cig-a-likes”, were disposable products mimicking the look and feel of traditional cigarettes and were designed to be discarded once the reservoir was empty. Users describe these devices as less satisfying and not working as well as traditional cigarettes, most likely because of low nicotine delivery yields (Hartmann-Boyce, 2018). Second generation e-cigarette devices were pen-shaped, containing rechargeable batteries onto which the user could place prefilled e-liquid cartridges. Third generation e-cigarettes were even more advanced and are often referred to as “mods” due to

the ability to customize the device in a variety of ways to suit the preference of the user. These devices allow the user to refill their own liquid, change coil and reservoir types as well as alter aerosol delivery parameters by adjusting wattage, voltage and the speed of coil heating using buttons or a touch screen interface. The most recent fourth generations, such as Juuls and NJOYs, are especially popular among those under the age of 18. These contain fixed voltage rechargeable batteries and a design that resembles a USB drive. Fourth generation devices utilize prefilled e-liquid pods that are readily available at any convenience store, gas station and grocery stores instead of dedicated vape shops. Both 3rd and 4th generation devices are currently in use but a larger number of those who vape now prefer the less complex and more transportable 4th generation devices (Pulvers, 2020). As the number of generations and modifications arise for e-cigarettes it is important to consider the advantages and disadvantages of e-cigarette as a delivery mechanism for psychoactive drugs.

ADVANTAGES OF INHALED DRUGS OVER OTHR ROUTES OF ADMINISTRATION

As previously noted, vaping could have a number of perceived advantages in promoting covert illicit substance use, but the route of administration itself could also have important implications to the abuse-liability of drugs abused as aerosols. Inhalation has been used as a route of drug administration for nearly 4,000 years (Rau, 2005). Inhalation allows drugs to be delivered to the lungs and be distributed into the periphery by oxygenated arterial blood flow which causes drug delivery to be more rapid in highly perfused tissues such as the brain. Some of the advantages associated with inhalation include the unquestionably faster onset of action

compared to oral administration as well as being less invasive and simpler than injection (Rau, 2005). Also, when compared with oral administration, inhalation avoids first pass metabolism which would be expected to result in higher bioavailability when administered as an aerosol as compared to being taken orally. (Herman, 2021). Previous studies have established the speed of drug onset has a positive relationship with the reinforcing effects of drugs, suggesting that inhalation may produce potent reinforcing effects rivaling those produced by intravenous drug delivery (Kimmel, 2007; Balster , 1973). Unfortunately, there has been a very limited number of studies conducted to explore the pharmacokinetics and pharmacodynamics of abused drugs administered as aerosols and the extent to which inhalation may impact abuse-related effects of drugs other than nicotine (Tafffe, 2021; Meng 1999; Paydar, 2019) which sets the predicate for some of the experiments conducted in the present thesis work.

VAPING IN ANIMAL MODELS

Multiple factors associated with e-cigarette use are unique to humans; however, there are a number of aspects that can be further investigated through the use of animal models such as potential abuse liability and health consequences. Due to the increasing popularity of electronic cigarette systems as mentioned earlier, there have been a number of recent studies examining the effects of several aerosolized drugs produced from these devices using animal models. A few recent studies used nicotine aerosol models to demonstrate that nicotine aerosol exposure alters temperature regulation and locomotor function in rats (Paydar, 2019 ;Lallai, 2021). The Javadi-Paydar (Paydar, 2019) group utilized sealed exposure chambers to

deliver nicotine dissolved in propylene glycol vehicle to rats at doses of 1, 10, or 30 mg/ml. Four 10 second aerosol puffs were delivered with 2 second interpuff intervals every 5 minutes. They observed that body temperature at the nicotine 30 mg/ml test condition was lower than the pre-treatment baseline and returned back to baseline in a time dependent manner by approximately 150 min after exposure was terminated. The group also examined locomotor activity following repeated 30 mg/ml nicotine inhalation sessions, each separated by an hour, in order to mimic a human's tendency to repetitively smoke throughout the day. They observed significant increases in activity relative to pre-aerosol baselines after nicotine inhalation. The Lallai group (Lallai, 2021) trained animals to press an active lever in an aerosol exposure chamber under conditions in which 5 lever presses resulted in liquid food delivery. After reaching stability animals then received e-cigarette aerosolized nicotine as opposed to receiving the food reward, this method was referred to as active delivery by the authors. The authors also utilized a passive nicotine administration which the subjects were exposed to one, 5 sec puff every 5 min for an hour. It was found that the active delivery methods resulted in comparable blood levels to that following intravenous nicotine self-administration leading the authors to conclude that the method has high translational relevance.

A study done by Nguyen et al. (Nguyen, 2016) used radiotelemetry to assess body temperature and locomotor responses to inhaled THC. Their method of aerosol exposure utilized sealed exposure chambers which delivered aerosolized drug to rats using e-cigarette cartridges. Animals were placed in pairs in the chambers for four, 10 second aerosol puffs delivered with 2 second interpuff intervals every 5 min for 40 min. Body temperature was not altered at the 10 min exposure test condition but had a significant reduction at both the 20 min

and 30 min conditions. It was observed that locomotor activity was affected in a time-dependent manner in their study.

An extremely relevant study to the current thesis was done by the same group (Nguyen, 2016). In this study the effect of aerosolized methamphetamine, mephedrone, and 3,4-methylenedioxypyrovalerone (MDPV) were explored. Rats were implanted with radiotelemetry devices to measure both physiological and behavioral effects. The same aerosol apparatus was used as in the THC experiment. Methamphetamine was administered in four 10 second aerosol puffs every 5 min for 40 min sessions. The inhalation of methamphetamine increased locomotor activity in at both the 25 mg/ml and 100 mg/ml test conditions over the PG control but was not significantly different between the two concentrations examined. Three different concentrations of MDPV increased activity compared with the PG vehicle condition. Mephedrone increased motor activity at the 200 mg/ml concentration but not the 100 mg/ml. Both methamphetamine and MDPV increased body temperature while mephedrone did not change body temperature (Nguyen, 2016). While these studies all demonstrated that aerosolized drugs of abuse have physiological effects, the durations of exposure were quite extended and may not mimic the type of exposures which would be likely in a human user engaging in experimentation. As such, exploration of more appropriate test conditions are necessary to further explore the abuse liability of aerosolized methamphetamine.

METHODS OF EXPOSURE OF RODENTS TO DRUG AEROSOLS

Humans inhale drug aerosols by sucking on the drip-tip of an e-cigarette, creating airflow that passes through the heated coil in the liquid reservoir. The depth of inhalation and the inhalation volume is therefore highly variable according to the preference of the user. Rodents do not have this capacity therefore other methods of inhalational exposure must be engineered. Several laboratories, including our own, continue to work toward developing models of vaping in rodent models. A few studies have utilized methods that attempt to mimic selective exposure to the orofacial region of rats but these experiments require extensive training in order to promote predictable and consistent exposure to drug aerosol (Shelton, 2022). One of the earlier studies, used sufentanil to assess a potential model of vaping in rodents (Jaffe, 1989). In this study, short bursts of sufentanil citrate aerosol generated by a nebulizer were delivered to rats in response to lever presses. Rats that were given access to sufentanili aerosol in the overnight training sessions reached an average of one reinforcement per hour on a fixed ratio 5 schedule sooner than rats that were receiving water aerosol.

In contrast, most studies of drug aerosol and aerosol exposure utilized a full-body exposure method in which the animal is placed in a chamber which is saturated with aerosolized drug. (Nguyen, 2016; Paydar, 2019; Gutierrez, 2021). Colloquially, this method of exposure is referred to as “hot boxing” which is a slang reference to smoking marijuana in an enclosed environment such as an automobile in order to maximize smoke exposure. While whole-body aerosol drug exposure does not fully recapitulate the short and repeated drug

puffs characteristic of human use it does allow a simple, reliable and quantifiable means of drug aerosol exposure.

A recent study used to validate a full body rat model for exploring the abuse-related effects of nicotine delivered by e-cigarette technology was done by the Javadi-Paydar group (Paydar, 2019). Rats were implanted with radio telemetry devices in order to monitor activity as well as temperature after exposure to aerosols under different test condition. Aerosol inhalation conditions included propylene glycol (PG) vehicle, nicotine (1, 10, 30 mg/ml) and delta-9-THC (12.5, 25 mg/ml). Four, 10 second aerosol puffs were delivered with 2 second intervals between puffs every 5 minutes in a 30-minute exposure session. Nicotine (0.4 mg/kg) or THC (5mg/kg) were injected intraperitonially were used as a control in order to assess activity and temperature following the more traditional injection route in rodents. The study reported that nicotine puff inhalation increased spontaneous locomotion and decreased body temperature in a manner similar to that of injection.

Collectively, these studies indicate that is it possible to study aerosol exposure in rodent models and drug-related behavior and effects can be reliably assessed. However, at the present time there is insufficient data to convincingly determine that e-cigarette delivered psychostimulant aerosols produce abuse-related effects when administered under conditions which would be likely to occur in human users. Given the prevalence of methamphetamine abuse in the United States, examining rodent models will be valuable in determining the abuse potential of methamphetamine aerosol delivered via e-cigarette.

LOCOMOTOR ACTIVITY

Exploration of the behavioral effects of psychoactive drugs can be achieved using a variety of techniques. One commonly used measure that is well validated and does not require extensive training of subjects is locomotor activity. Locomotor activity assays has been used to detect *in vivo* effects of a drug by measuring a number of different metrics including ambulation speed, time mobile, line crossings, rearing behavior and thigmotaxis among others. Perhaps the most widely utilized metric which provides a generalized behavioral endpoint is total distance traveled over the course of a behavioral test session (Nazari, 2020; Mihalčíková, 2019; Ortman, 2021). Typically, in locomotor assays the subject is placed in an open field arena where the animal is able to move freely. Scoring of activity can be done manually by observation but in most current systems it is automated using arrays of photobeams located in around the periphery of the arena or via software that captures and analyzes motion using a video camera system.

Locomotor activity is particularly useful for exploring the behavioral effects of psychomotor stimulants such as methamphetamine. The locomotor activating effects of psychomotor stimulants are dose-responsive and as such provide a convenient and sensitive surrogate behavioral measure of the CNS effects of drugs such as methamphetamine. The locomotor effects of stimulant drugs administered via injection have been extensively studied (Schindler, 2013; Mihalčíková, 2019; Ortman, 2021). One representative study done by Ortman et. al investigated the acute effects of methamphetamine on locomotor activity in both adolescent and adult mice. Both groups were given an intraperitoneal injection of saline, 2

mg/kg methamphetamine or 4 mg/kg methamphetamine (Ortman, 2021). The mice were then placed in an open field and distance travelled recorded for 45 min. Both adult and adolescent mice showed increases in locomotor activity after methamphetamine compared to the saline control condition. It was also observed that adult mice had a greater increase in distance travelled the 2 mg/kg condition compared with the adolescent while at the 4 mg/kg dose activity was greater in adolescent mice than adult mice.

Another study done by Mihalčíková et al (2019). focused on the locomotor activity of adult male rats after 1 mg/kg subcutaneous methamphetamine injection. It was found that locomotor activity, average speed, and distance traveled were all increased over a 60 min test session. These findings were consistent with other studies done on methamphetamine using similar doses (Mihalčíková, 2019). Other psychomotor stimulants also show effects in assays of locomotor activity. For instance, the effects of 3,4-methylenedioxymethamphetamine (MDMA) on locomotor activity have been examined using a radio transmitter locomotor transceiver. MDMA was administered subcutaneously at doses of 1, 3, 10 or 20 mg/kg. MDMA produced clear dose-dependent increase in total locomotor activity with significant effects observed at the 10 and 20 mg/kg doses (Schindler, 2013).

While these and many additional studies show that methamphetamine and other psychomotor stimulants increase locomotor activity following injection, far fewer experiments have been conducted examining the effects of psychomotor stimulants after aerosol administration. A recent study by Nguyen et. al conducted a study to validate a method for the delivery of stimulants via inhalation using an e-cigarette device. The study measured methamphetamine's behavioral effect in the locomotor assay examining activity counts using a

surgically-implanted radio transmitter. Briefly, animals were exposed two at a time in an exposure chamber for 40 min and placed back into their home cages where activity was recorded for 240 minutes (Nguyen, 2016). Four 10-s vapor puffs were delivered to animals every 5 min with a 2 second interval between puffs for the duration of the exposure session (40 min). This resulted in a total 32 puffs in 8 bouts of puffing with a total exposure duration of approximately 320 seconds. The investigators found that activity was increased over the vehicle condition when 25 mg/ml or 100 mg/ml methamphetamine aerosol were administered. It is important to note that the exposure parameters in this study were not typically representative of human inhalation patterns. A study done by St. Helen et. al (St. Helen, 2016) observed 17 healthy adult e-cigarette users and their puffing characteristics for 90 minutes. It was observed that the average number of puffs taken during the session was 64 ± 38 with an average inter puff interval of 2 min. The average puff duration was also much shorter at 3.5-4 seconds each.

PHYSIOLOGICAL EFFECTS OF ACUTE METHAMPHETAMINE ADMINISTRATION

In addition to CNS-mediated stimulant behavioral effects such as enhanced locomotor activity, methamphetamine also results in sympathomimetic peripheral effects including tachycardia, increase in body temperature, and hypertension. Given that the sympathomimetic effects methamphetamine can lead to significant adverse health consequences such as strokes and heart attacks, understanding the degree to which aerosolized methamphetamine produces

these effects after brief exposures likely to occur in human users is important. There have been a number of studies in rodents examining the sympathomimetic effects of methamphetamine after injection. For instance, a study done by Yoshida et al. (Yoshida, 1993) examined the effect of methamphetamine administered via *i.p.* injection versus intracerebroventricular injection on mean arterial blood pressure, body temperature, and heart rate. Rats were anesthetized and a battery-operated transmitter was inserted intraperitoneally into each rat to measure body temperature and mean arterial blood pressure. It was observed that 1 mg/kg methamphetamine *i.p.* injection significantly elevated mean arterial pressure, heart rate, and body temperature whereas a lower 0.1 mg/kg dose had no effects. The measurements were taken for 120 min in all animals with a peak in heartrate and mean arterial pressure withing the first 15 min. Body temperature peaked later at the 50 min time frame, returning back to basal level by 120 min. This dose range was chosen because higher doses (up to 10 mg/kg) used in previous studies (Clark and Lipton 1986) caused severe hyperthermia and circulatory collapse. Other stimulants also result in sympathomimetic effects. For instance, Schindler et. al, observed dose dependent effects in change in blood pressure after MDMA administration. MDMA increased heart rate at doses 3 and 10 mg/kg but not at the highest dose tested. The authors suggested that MDMA has a biphasic effect on heart rate (Schindler, 2013). We are aware of only one experiment that has examine the sympathomimetic effects of methamphetamine following aerosol administration (Nguyen, 2016). Change in body temperature were assessed using the implanted telemetry receiver. They found that body temperature was increased over the vehicle PG condition when 25 mg/ml or 100 mg/ml methamphetamine was administered but no other types of data were recorded. While

changes in body temperature are important following high dose stimulant administration as overdose of some drug such as MDMA can produce life threatening hyperthermia, other effects such as blood pressure which is relevant to the cardiac toxicity of methamphetamine are equally critical but have yet to be explored.

BLOOD LEVELS OF METHAMPHETAMINE

Exploration of the locomotor and sympathomimetic effects of methamphetamine aerosol can demonstrate that it is penetrating the CNS and producing relevant physiological effects. Although the effects of aerosol methamphetamine can be compared to those produced by a more traditional route of administration to infer relative tissue concentration a more direct measurement methamphetamine levels after aerosol exposure is important to determine the relative potency of aerosolized methamphetamine.

While little is known about methamphetamine concentrations following aerosol exposure, studies have been conducted exploring blood levels in rats that received methamphetamine intravenously. For instance, a study done in 2015 (Milesi-Hallé, 2015) measured methamphetamine blood levels in rats self-administering methamphetamine. Rats received a total of 27 doses of 0.048 mg/kg methamphetamine over 120 min. The total dosage administered to the animals was 1.3 mg/kg. Methamphetamine plasma concentration at 120 min was 106ng/mL in males and 139 ng/mL in females. It was observed that the self-administered drug taken within the first 20 min allowed most rats to attain a methamphetamine serum concentration which was maintained for the remainder of the

session. Unfortunately, there have a not been any studies examining methamphetamine blood levels following aerosol administration.

OTHER FACTORS INFLUENCING THE ABUSE RELATED EFFECTS OF DRUGS ADMINISTERED VIA E-CIGARETTE AEROSOLIZATION

While it is important to understand the abuse liability of drugs delivered via e-cigarettes this task is made more challenging by the fact that e-cigarettes lack standards of function and the characteristics of aerosol delivery may vary widely across the vast number of devices available. Therefore, assessing factors which appear to be particularly relevant to impacting drug aerosolization is an important goal of the present thesis project as it will provide insight into the abuse related effects aerosolized methamphetamine. For instance, e-liquid is generally comprised of both vegetable glycerin and propylene glycol although the ratio of the two varies in commercial products. A relevant study which highlights how vehicle might alter methamphetamine aerosol was done by Mulder et. al investigating the effect of e-cigarette modifications on particle size of aerosolized product (Mulder 2019). The authors used methamphetamine at 60 mg/ml in 50:50 PG:VG e-liquid. A 10 stage micro-orifice uniform deposit impactor drew samples through a cascading sequence of nozzles which deposits the aerosol onto plates used for particle size analysis. In the study, changes in battery voltage and coil resistance did not significantly the particle size distributions of the e-liquid although particle size distributions were not consistent across tests. The authors mention that

methamphetamine aerosol from e-cigarette system had a smaller size as opposed to traditional methamphetamine smoke. It was suggested that the smaller size of particles (less than 3 μm) might reach deep tissues of the lung and be absorbed more readily into the bloodstream thus leading to greater bioavailability increasing the potential for overdose.

Vehicle particles were also examined by the Mulder group, who found that pure vegetable glycerin (VG) achieved a substantial, but lower, number of particles and deep lung tissue penetration. A vehicle comprised of solely propylene glycol (PG) produced a larger particle size than a combination of both VG and PG or VG alone. A study done by Zervas et. al (Zervas, 2018), also looked at the particle size of both PG and VG alone and as mixtures. Propylene glycol alone emitted a higher particle concentration than VG; however, the particle concentration of the combination was in between the two values of VG and PG alone. Propylene glycol also emitted smaller particles than the VG vehicle alone, however; similar to the number of particles, the size of the mixture of both was in between the sizes of VG and PG alone. Thus, confirming the additive behavior of a mixture of both PG and VG.

Another potentially important parameter is the output power at which the e-cigarette is set. As described previously, third generation e-cigarettes have adjustable wattage which can be altered by the user. Mulder et al. (2019) came to the conclusion that varying voltage played no role in particle size distribution. Because voltage and wattage are directly related, particle size should also not be impacted by device power. While higher voltage or wattage may not impact particle size, higher wattages produce a greater volume of aerosol which we hypothesize will increase the amount of drug available for inhalation causing greater amount of

methamphetamine in the blood, greater locomotor activity, and more pronounced change in physiological effects in a wattage dependent manner.

OVERARCHING HYPOTHESIS

Our overarching hypothesis is that methamphetamine administered via 3rd generation e-cigarette aerosolization has potent behavioral and physiological effects similar to those produced when administered by the more traditional route of injection. If this hypothesis is confirmed it would lead us to conclude aerosolized methamphetamine is likely to have comparable abuse liability. Our hypothesis will be tested in three aims. The first aim will assess if methamphetamine administered via e-cigarette aerosolization produces CNS-mediated stimulatory effects by utilizing a locomotor assay. The second aim will be focused on testing sympathomimetic effects of methamphetamine aerosol by utilizing a CODA blood pressure monitor. Lastly, our third aim will be focused on measuring blood serum levels of methamphetamine administered via e-cigarette aerosolization and comparing those results to methamphetamine delivered by the more common injection route.

Methods

Aim 1: Effect of methamphetamine aerosol on locomotor activity.

As previously mentioned, locomotor assay is a conventional method of assessing the behavioral effects of psychoactive drugs such as stimulants and depressants (Schindler 2013,

MIHALČÍKOVÁ 2019, Ortman 2021). The overarching goal of Aim 1 is to determine if brief puffs of methamphetamine aerosol have locomotor stimulatory effects and to compare those effects to methamphetamine following the more common injected route of administration.

Subjects:

8 Sprague Dawley rats, 4 males and 4 females, from Charles River Laboratories were used as subjects. Animals were single housed in clear polycarbonate microisolator cages with corncob bedding on a 12/12-hour reversed light/dark cycle in a temperature and humidity-controlled vivarium. Rats were weighed twice-weekly and fed a daily amount of standard rodent chow in order to maintain healthy weights. Water was available to animals ad lib except during testing.

Aerosol Exposure Apparatus:

Aerosol exposures were conducted in an inhalation chamber constructed of 3/8" clear acrylic, measuring 24.4 X 17.5 X 25.5 cm with a total volume of 10888.5 cm^3. The door of the chamber contained an 8 X 8 X 2.5 cm, 24v DC fan with a mesh covered 3D printed self-closing louvered grate for exhausting the aerosol from the inside of the chamber into a fume hood. Also fitted to the front wall were 3D printed holding attachments located on the right and left side of the fan, respectively, for the e-cigarette (Smok MORPH) and an e-liquid atomizer tank (Innokin iSubV Vape Tank) fitted with an iSub Innokin SS BVC 0.5-ohm stainless steel vaporizer coil. The e-cigarette atomizer tank was modified with a hose barb so it could be pressurized by

a 12v DC diaphragm air pump. A clear length of 3/8" Tygon tubing was attached to the top of the atomizer and connected to a hose barb on the door of the exposure chamber.

The aerosol exposure system was controlled by an Arduino Uno single-board computer and three electromechanical relays. One relay each for the air pump, exhaust fan and e-cigarette. The voltage output to the fan and air pump were determined by individual variable voltage regulators. The relay to the e-cigarette was soldered to lead wires which bypassed the tactile firing switch. The air pump was calibrated to deliver a flow rate of 1 liter/min to the atomizer. The exhaust fan was driven at its rated voltage producing a free-air flow rate of 1.48 cubic meters/min. The timing of the activation of the exhaust fan, e-cigarette and air pump relays were controlled by a custom-written C++ program uploaded to the Arduino from a laptop PC computer.

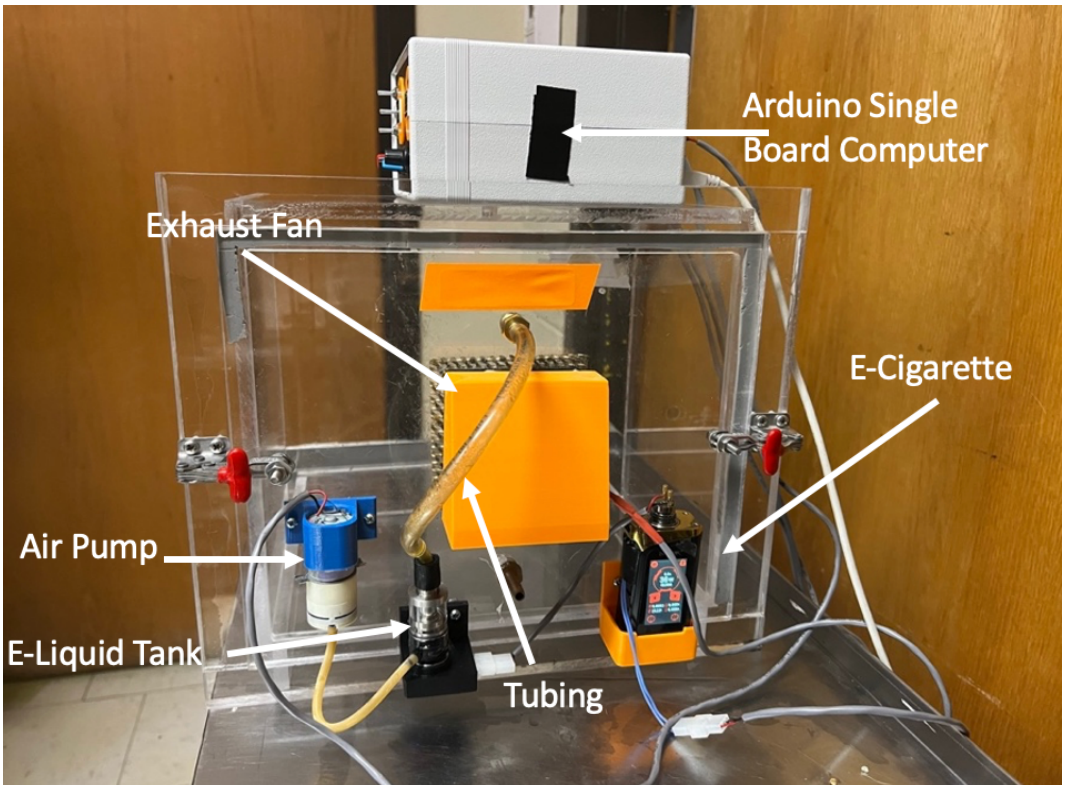

Figure 4: Clear acrylic inhalation chamber used to expose rats to e-cigarette aerosol. The experimental parameters were controlled by an Arduino board single computer located in the white box on top of the chamber. Also shown are the e-cigarette, e-liquid tank, exhaust fan, air pump and tubing which routed aerosol into the chamber.

Locomotor Activity Chamber:

Locomotor activity sessions were conducted in 4 metal framed, clear acrylic activity test chambers (Figure 5) measuring 43.2 X 43.2 X 30.5 cm (Med Associates model ENV-515). Each chamber was housed inside an individual sound-attenuating melamine cubicle with an exhaust fan in the upper left wall. Two horizontal strips of infrared photobeams were positioned at 1.5

above the chamber floor and a single strip of photobeams was positioned 6.0 cm from the chamber floor. Two 5-watt houselights in the upper left and right corners of the sound attenuating chamber provided dim illumination. Med Associates Activity Monitor software running on Windows PC was used to control the equipment and collect data. Total distance traveled in meters, time ambulatory, ambulatory movement, ambulatory counts, and jump time were recorded.

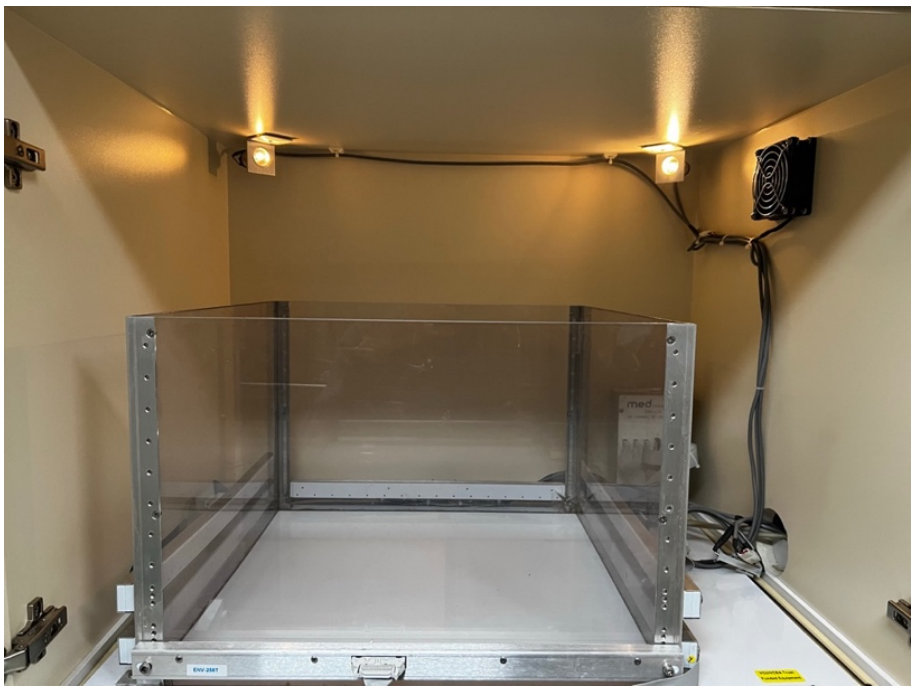

Figure 5: Locomotor Activity Chambers. Locomotor activity chambers housed within sound attenuating outer cubicles. Additional features depicted include the two 5-watt lights in the top coroners which provide dim illumination throughout the session and the ventilation fan located in the upper left wall of the cubicle.

Locomotor Activity Test Procedures:

Experiment 1: Effect of subcutaneous (s.c.) methamphetamine on locomotor activity.

In order to assess the locomotor effects of methamphetamine aerosol we first conducted an experiment validating prior work demonstrating that injected methamphetamine will increase locomotor activity. Animals were tested Monday – Friday between 9 AM and 5 PM. The rats first underwent five, consecutive, 60-minute habituation sessions preceded by a s.c. injection of saline. Animals were injected and returned to their homecage for a 10 min timeout before being placed in the locomotor chamber of a 60 min test session. Following habituation, a dose-effect curve was conducted with s.c. injected methamphetamine at doses of vehicle, 0.03, 0.1, and 0.3 mg/kg, presented in ascending dose order. Unlike during habituation, methamphetamine test sessions were separated by two days to reduce the possibility of locomotor sensitization which can occur following repeated tests with psychomotor stimulants. On the first of these two days the subjects remained in their home cage and were not tested. The following day a 60 min habituation measurement with no treatment was conducted. The day following the habituation session another dose of methamphetamine was examined and the process repeated for all methamphetamine test conditions.

Experiment 2: Effect of e-cigarette output wattage and number of puffs of methamphetamine aerosol on locomotor activity.

Following *s.c.* injection testing, we examined the effects of exposure to methamphetamine aerosol on locomotor activity. Animals were tested Monday-Friday between 9 AM and 5 PM. Test were conducted following 10, 15 and 20 puffs of 30 mg/ml methamphetamine aerosol at an e-cigarette output setting of 18 watts and after 1, 5 and 10 puffs of 30 mg/ml methamphetamine aerosol at an e-cigarette output setting of 36 watts. Each puff consisted of 10 second of aerosol generation, a 10 second hold in which the aerosol was allowed to dwell in the chamber, a 10 second exhaust fan activation to expel the aerosol out of the chamber and a 10 second time out before the cycle repeated. Control conditions included the 50:50 propylene glycol (PG): vegetable glycerin (VG) vehicle vapor alone and a no aerosol condition where the animal was placed in the exposure chamber for the duration of 10 puffs without exposure. Immediately following exposure, animals were placed in locomotor chambers for 60 min. Methamphetamine test sessions were separated by two days to reduce the possibility of locomotor sensitization. On the first of these two days the subjects remained in their home cage and were not tested. The following day a 60 min habituation measurement with no treatment was conducted.

Aim 2: Examine the sympathomimetic effects of methamphetamine administered via e-cigarette aerosolization.

Methamphetamine administration has pronounced sympathomimetic effects (Bexis, 2006; Nguyen, 2016; Schindler, 2013). The goal of Aim 2 was to determine if methamphetamine

aerosol puff exposure via e-cigarette aerosolization also has similar effects by measuring blood pressure, heart rate, and body temperature across a range of exposure conditions. The same 4 male and 4 female rats utilized for the locomotor study were also used to complete this aim.

Blood Pressure, Heart Rate, and Body Temperature Apparatus:

Blood pressure, heart rate, and body temperature were collected from animals using the Kent Scientific CODA Noninvasive Blood Pressure monitor, (Kent Scientific, Torrington Connecticut). The monitor was set up with an Occlusion-Cuff (O-Cuff), Volume Pressure Reading (VPR) cuff, and a heating pad which each attached to the monitor. An infrared thermometer was used to measure the animal's temperature while in the home cage and was recorded in order to measure the effect of test condition on animal's body temperature. The heating pad attached to the monitor was set to 42.0°C to allow animals tails temperature, when placed onto heating pad, to reach 32-35°C. This was done to cause proper blood flow to the tail so that accurate blood pressure measurements could be achieved. The monitor itself was automated and upon activation completed a test cycle in which the occlusion cuff was inflated to stop tail blood flow and then slowly deflated until blood flow returned. Presence and absence of blood flow was assessed by the VPR cuff. The system itself was in most respects comparable to an automated human blood pressure device. After the cycle completed a color LCD screen on the device provided output on systolic blood pressure, diastolic blood pressure, and heart of the animal. All measurements were recorded in a lab notebook and change between post-treatment measurement and initial measurement was calculated.

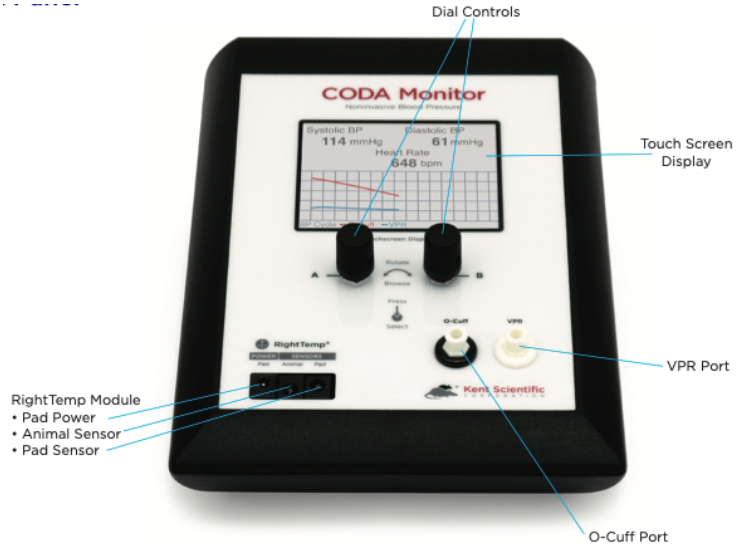

Figure 6: Kent Scientific CODA Monitor: Color LCD screen displays systolic, diastolic blood pressure, and heart rate of animal. The monitor has 5 ports one for pad power, pad sensor, animal sensor, volume pressure reading cuff, and occlusion cuff. The pad related ports provide power to the heating pad as well as monitors the temperature of the pad.

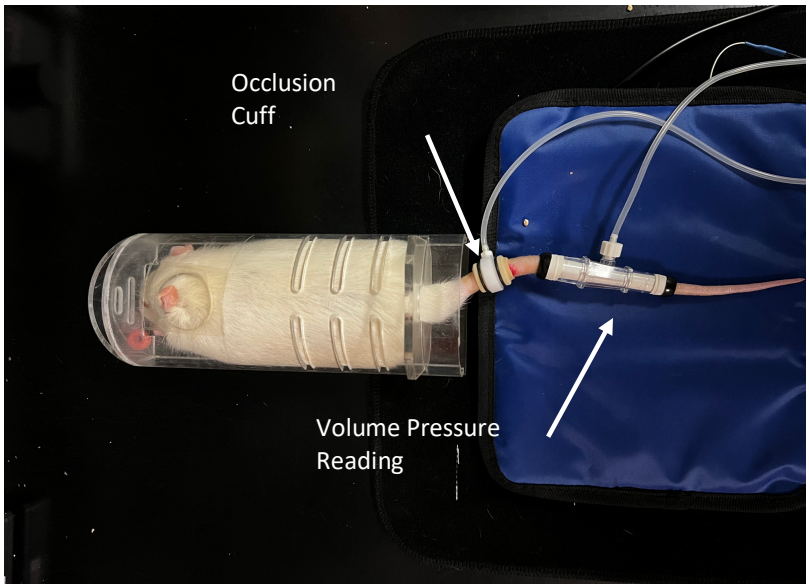

Figure 7: Animal in restraint tube. The heating pad, occlusion cuff and volume pressure transducer cuff are shown.

Experiment 3: Effect of methamphetamine on blood pressure, heart rate, and body temperature.

Animals were tested Monday-Friday between 9 AM and 5 PM. The rats underwent 5 habituation session prior to testing with methamphetamine. Each animal was placed into a commercially available or 3D printed animal holder. Once in place, the CODA monitor occlusion cuff placed 2/3 of the way on the animals tail and then the VPR cuff placed halfway on the

animal's tail. The animal's tail was then placed on the heating pad to bring the tail temperature to approximately 32-35°C to ensure adequate blood flow and an initial blood pressure and heart rate and body temperature determination was conducted. Each subject was then removed from the restraint tube and giving a 1 ml/kg s.c. saline and returned their home cage for 5 min. The animals were then placed back into the restraint tube, the sensors and heating pad applied and a second blood pressure, heart rate and body temperature reading was taken. After the habituation test days, exposure to methamphetamine was initiated. A total of 8 tests were performed. Tests included exposure to 1, 5, 10 or 15 puff of 30 mg/ml d-methamphetamine at an e-cigarette output setting of 36 watts in a manner identical to that described in the locomotor activity study. A test was also conducted examining an injection of 0.3 mg/kg s.c. d-methamphetamine. Three control tests were also performed. First, a control test was conducted in which subjects were not given an injection or placed into the exposure chamber. A second control test was conducted in which subjects were placed into the exposure chamber for a time identical to the 10-puff condition but no aerosol puffs were administered. Finally, a control condition was conducted in which the subjects received 10 puffs of 50/50 vegetable glycerol/propylene glycol vehicle. Methamphetamine test sessions were separated by two days as to prevent sensitization from occurring. On the first of these two days the subjects remained in their home cage and were not tested. The following day a habituation measurement with no treatment was conducted.

Aim 3: Correlating behavioral and physiological effects of aerosolized and injected d-methamphetamine with blood drug levels.

As a final aim we wished to correlate methamphetamine blood levels with the results from our behavioral and physiological measurements. 12 Long-Evans hooded male rats were used as subjects. Animals were single housed in clear polycarbonate microisolator cages with corncob bedding on a 12/12-hour reversed light/dark cycle in a temperature and humidity-controlled vivarium. Animals were tested between 9AM and 5 PM.

Experiment 4: Examine the blood plasma levels of methamphetamine administered via e-cigarette aerosolization and subcutaneous injection.

One group of 3 rats were administered 0.3 mg/kg s.c. methamphetamine, the same dose used in the prior studies exploring locomotor activity and physiological effects. Additional groups of 3 rats each were tested with 1, 5 or 10 puffs of 30 mg/ml methamphetamine aerosol. The e-cigarette was set at 36 Watts for each puff condition. Each puff was a 10 second aerosol emitted into the chamber, a 10 second hold in the chamber, a 10 second exhaust out of the chamber, and a 10 second interval between one puff and the subsequent one. Following exposure, animals were placed in their home cage for 15 min prior to being placed into a 30 cm X 16 cm X 16 cm anesthesia chamber. Animals were then exposed to 3.5% isoflurane in 100% oxygen for 5 min at a flow rate of 2L/min. After 5 min in the chamber, animals were placed on their back out of the chamber and a face mask was placed on the animal from which 3.5% isoflurane at a flow rate of 1L/min continued to be administered.

Once the animal was deeply anesthetized, confirmed by lack of response to a strong tail pinch, a cardiac puncture blood sample was obtained 20 min after test condition exposure. The approximate location of the animal's heart was noted. A 1ml syringe fitted with a 22-gauge needle was then inserted slightly below the lowest rib into the left ventricle and a 1 ml blood sample withdrawn. The filled syringe was removed from the needle and replaced with a syringe containing euthanasia solution. Approximately 0.3 ml of intracardiac euthanasia solution was administered which resulted in near instantaneous euthanasia. The blood was placed into a 1 ml sample tube containing EDTA to prevent clotting and gently rotated to mix the EDTA and sample. The tube was then placed into a cooling block until all samples obtained that day were collected. Blood tubes were then centrifuged for 7min at 7800 rpm to separate the blood plasma from cells. The plasma was withdrawn with a disposable pipet and placed into another 1 ml sample tube and stored in a -20°C freezer before being transferred to the VCU Pharm/Tox Analytical Core laboratory for identification and quantification of amphetamine & methamphetamine in plasma.

Identification and Quantification of Amphetamine & Methamphetamine in Plasma:

Plasma samples were collected and stored in a -20°C freezer until analyzed. Seven-point calibration curves ranging from 10 to 1000 ng/mL of amphetamine and methamphetamine (Cerilliant, Round Rock, Texas). Quantity controls specimens at 30, 300 and 750 ng/mL, negative control and blank controls were prepared in plasma with each analytical run. The Internal standard (ISTD) containing 100 ng/mL of ampetamine-d11 and methamphetamine-d11 (Cerilliant, Round Rock, Texas) in methanol were added to aliquots of 100 µL of plasma of each

calibrator, control and sample except the negative control. Added to each sample was 100 µL of concentrated ammonium hydroxide (Macron Fine Chemicals, Center Valley, PA) and 2.0 mL of n-butyl chloride (Burdick and Jackson, Muskegon, MI). Samples were then mixed for 5 min and centrifuged for 5 min. The n-butyl chloride layer was transferred to a clean borosilicate test tube (12 x 75 mm). A 100 µL of 10% hydrochloric acid in methanol was added prior to drying the samples down under a gentle stream of nitrogen at room temperature. The extracts were then reconstituted with 100 µL of water transferred to the auto-sampler. The analysis was performed using a Sciex ExionLC 2.0+ liquid chromatography system attached to a Sciex 6500 QTRAP system with an IonDrive Turbo V source for TurbolonSpray® controlled by Analyst software (Sciex, Ontario, Canada). Chromatographic separation was performed on an Agilent Poroshell 120 EC-C18 column 2.1 x 50 mm x 2.7 µm (Agilent Technologies, Santa Clara, CA). Mobile phase A consist of 10 mM ammonium formate and 0.1% formic acid in water and mobile phase B consisted of acetonitrile. The flow rate was 1 mL/min with an injection volume of 4 µL. The gradient stated with mobile phase B at 2% and was held for 0.5 min. Mobile phase B was than increased to 98% over 3.7 min and held for 1 min before returning to the start conditions. The source temperature was set at 750 °C with a curtain gas at a flow rate of 40 mL/min. The ionspray voltage was 5500 V with the ion source gases 1 and 2 flow rates of 30 mL/min. The acquisition mode used was multiple reaction monitoring (MRM) in a positive mode. The following transition ions (m/z) with their collision energies (eV) in parentheses were monitored; amphetamine 136>91 (20) & 136>119 (14): amphetamine-d11 147>98 (20) & 147>130 (14); methamphetamine 150>97 (22) & 150>119 (16); and methamphetamine-d11 161>97 (22) & 161>127 (16). The total run time was 7.4 min. A linear regression of the peak

area of ratios of the quantification and the ISTDs transition ion were used to construct the calibration curves. The limit of quantification was determined to be 1 ng/mL based on a signal to noise ratio of greater than 10%. The precision for all the controls, analyzed in triplicate, were determine to have coefficient of variation (CV) of less that 13% and bias of less than 19%. No carryover was observed in the negative or blank controls.

Statistical Analysis:

For locomotor studies total distance travelled over the 60 min test session was collected for each subject as well as total distance travelled for each of the four 15 min bins (0-15, 15-30, 30-45 and 45-60 min) of each test session. Statistical analysis was performed using GraphPad Prism Version 9 for Macintosh (GraphPad Software, LLC). Locomotor tests examining the interaction of post-exposure time x treatment were assessed by two-way mixed model analysis of variance (ANOVA) followed by Fishers post-hoc tests when main effects or interactions were statistically significant at the $P < 0.05$ level. Statistical analysis on locomotor data examining only total distance travelled over the 60 min test session were conducted by one-way ANOVA followed by Fisher's post-hoc tests on statistically significant ($p < 0.05$) effects. Physiological data (heart rate, blood pressure and body temperature) were analyzed by calculating a change value between the initial measurement taken prior to exposure to methamphetamine and the measurement taken after exposure to methamphetamine. These change values were subjected to 1-way ANOVAs followed by Fisher's post-hoc tests on statistically significant main effects. Total blood methamphetamine and amphetamine concentration in ng/ml were analyzed by two independent 1-way ANOVA. Statistically significant main effects ($p < 0.05$)

were subsequently assessed by Fisher's post-hoc tests comparing all test conditions to the 1 puff condition and then all test conditions to the 0.3 mg/kg s.c. methamphetamine control condition. Sex differences were not explored due to limited statistical power of the sample size.

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Results

Aim 1: Effect of methamphetamine aerosol on locomotor activity.

Experiment 1: Effect of subcutaneous (s.c.) methamphetamine on locomotor activity.

Following five consecutive habituation 60-minute habituation session and *s.c.* saline injection, animals underwent *s.c.* injected methamphetamine testing at doses of 0.03, 0.1, and 0.3 mg/kg, presented in ascending dose order. The effects of *s.c.* methamphetamine injections were tested to validate prior published studies as well as to serve as a comparison in the present study to the effects of methamphetamine aerosol. Methamphetamine was administered *s.c.* 10 min prior to the session. In Figure 8, data is presented as a mean (+/- SEM) distance traveled in cm across all 8 subjects for the entirety of the 60 min session. Methamphetamine injection had dosage dependent effects on distance traveled (Figure 8). Methamphetamine given at a dose of 0.3 mg/kg had the most pronounced effects showing greatest mean distance traveled. There was a significant effect of dose [$F(1.4, 9.6) = 9.69$, $P = 0.008$]. Post-hoc Holm Sidak's tests revealed that all three doses of methamphetamine (0.03,

0.1, and 0.3 mg/kg) significantly increased distance traveled compared to the saline injection control condition.

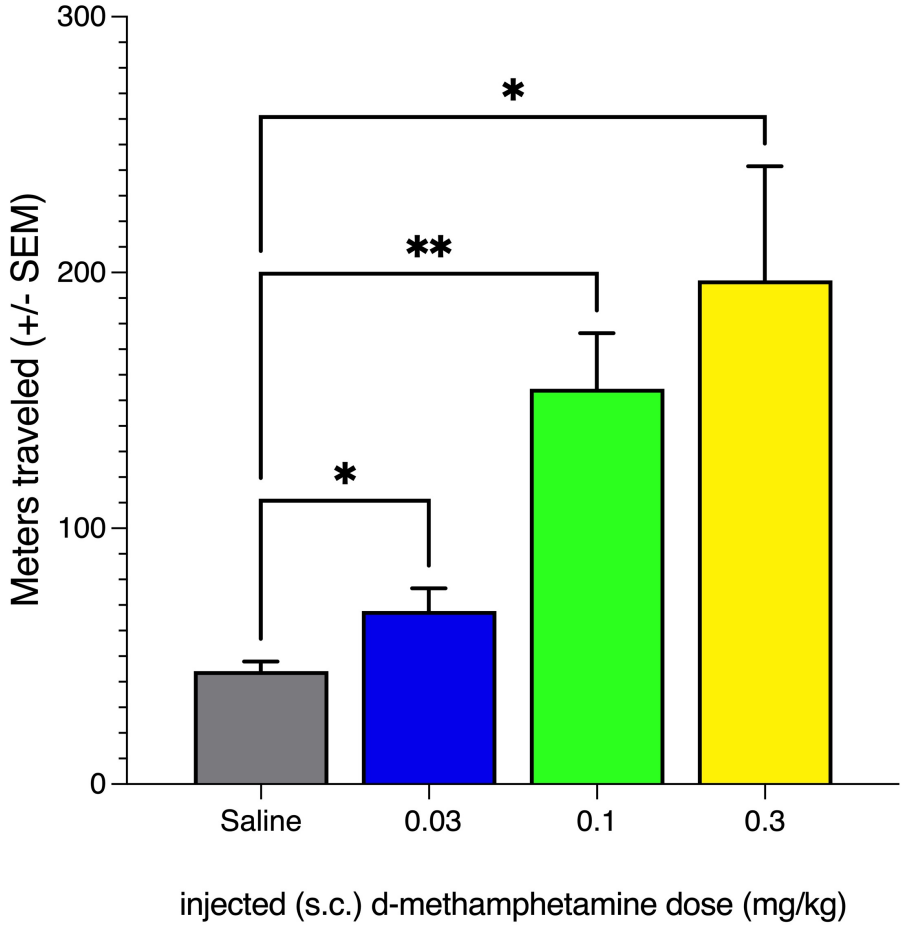

Figure 8: Average meters traveled (+/- SEM) over the total 60 min test session for 8 subjects after subcutaneously injected d-methamphetamine. Methamphetamine was administered 10 min prior to the start of each 60 min locomotor activity session at doses of 0.03 mg/kg (blue),

0.1 mg/kg (green), and 0.3 mg/kg (yellow). * indicates statistically significant differences (P < 0.05) between each test condition compared to the *s.c.* saline (gray) control condition.

Experiment 2: Effect of e-cigarette output wattage and number of puffs of methamphetamine aerosol on locomotor activity.

After *s.c.* methamphetamine demonstrated locomotor stimulatory effects, we examined the effects of exposure to methamphetamine aerosol on locomotor activity. Subjects were tested following 10, 15, and 20 puffs of 30 mg/ml methamphetamine aerosol at an e-cigarette setting of 18 Watts. Each puff consisted of 10 second of aerosol generation, a 10 second hold in which the aerosol was allowed to dwell in the chamber, a 10 second exhaust fan activation to expel the aerosol out of the chamber and a 10 second time out before the cycle repeated. Following aerosol exposure, animals were immediately placed in locomotor chambers for 60 min. In Figure 9, data is presented as a mean (+/- SEM) distance traveled for 8 subjects across each of the 4 successive 15 min time periods. There were significant main effects of number of puffs [$F(4,28)=7.82$, $P=0.0002$] and time [$F(3,21)=25.19$, $P < 0.0001$] as well a significant puff number X time interaction [$F(12,84)=2.719$, $P = 0.004$). Post-hoc Holm Sidak's tests revealed that all three puff conditions significantly increased distance traveled compared to the vehicle aerosol control condition in all 4 successive 15 min time period. There was a general trend showing greatest distance traveled in the first 15 min time interval in the 10-puff condition and the 0.3 mg/kg *s.c.* methamphetamine injection. Both the 15 and 20 puff condition had similar mean distance traveled in the first two 15 min intervals. The 15 and 20 puff test conditions also

appeared to result in a more sustained increase in locomotor activity compared to the 10 puff and 0.3 mg/kg s.c. methamphetamine test conditions.

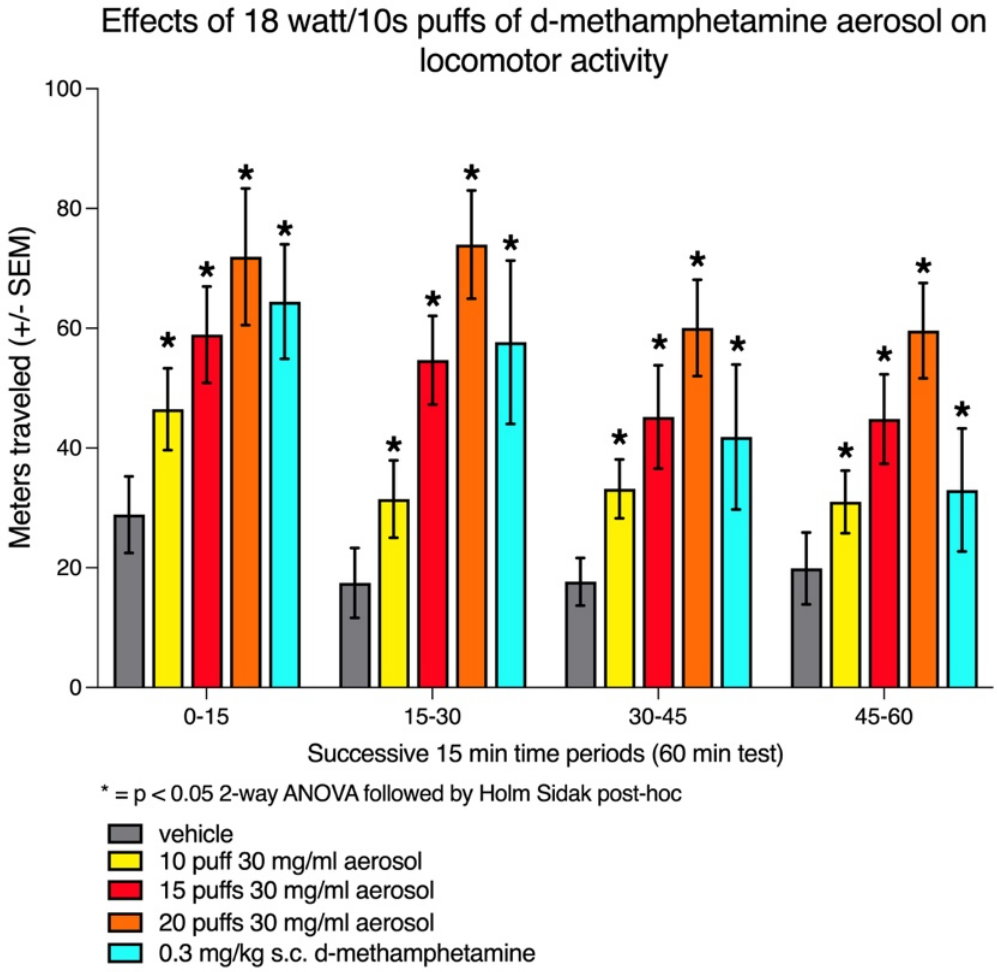

Figure 9: Average meters traveled (+/- SEM) of 8 subjects after increasing numbers of puffs of 30 mg/ml methamphetamine aerosol at an e-cigarette output setting of 18 watts. Data are shown across 4 successive 15 min time periods. Methamphetamine aerosol exposure tests were conducted following 10 (yellow), 15 (red), and 20 (orange) puffs at an e-cigarette output setting of 18 Watts. A dose of 0.3 mg/kg *s.c.* methamphetamine (blue) served as a positive control. Average distance traveled of all 8 subjects in each session was compared to 50:50 PG:VG vehicle aerosol (gray). * indicates statistical significance ($p < 0.05$)]

We subsequently examined the consequences of increasing e-cigarette wattage on locomotor activity. Subjects were tested following 1, 5 and 10 puffs of 30 mg/ml methamphetamine aerosol at an e-cigarette output setting of 36 watts, double that in the first experiment. Following aerosol exposure, animals were immediately placed in locomotor chambers for 60 min. In Figure 10, data is presented as mean (+/- SEM) distance traveled for 8 subjects across each of the 4 successive 15 min time periods. There were significant main effects of puff number [$F(4,28)=7.70$, $P=0.0003$] and time [$F(3,21)=20.14$, $P< 0.0001$) as well as a significant puff number X time interaction [$F(12,84)=8.84$, $P<0.0001$) Post-hoc Holm Sidak's tests revealed that 5 and 10 puff and 0.3 mg/kg *s.c.* injection significantly increased distance traveled compared to the vehicle aerosol control condition in all four 15 min time periods. In contrast the 1 puff condition was only significantly greater than vehicle at the 45-60 min time point. There was a general trend indicating that the 10-puff test condition has a more sustained increasing effect on locomotor activity than the 0.3 mg/kg methamphetamine injection or 5 puff condition the latter of which were highest in the first 15 min and declined each subsequent time interval.

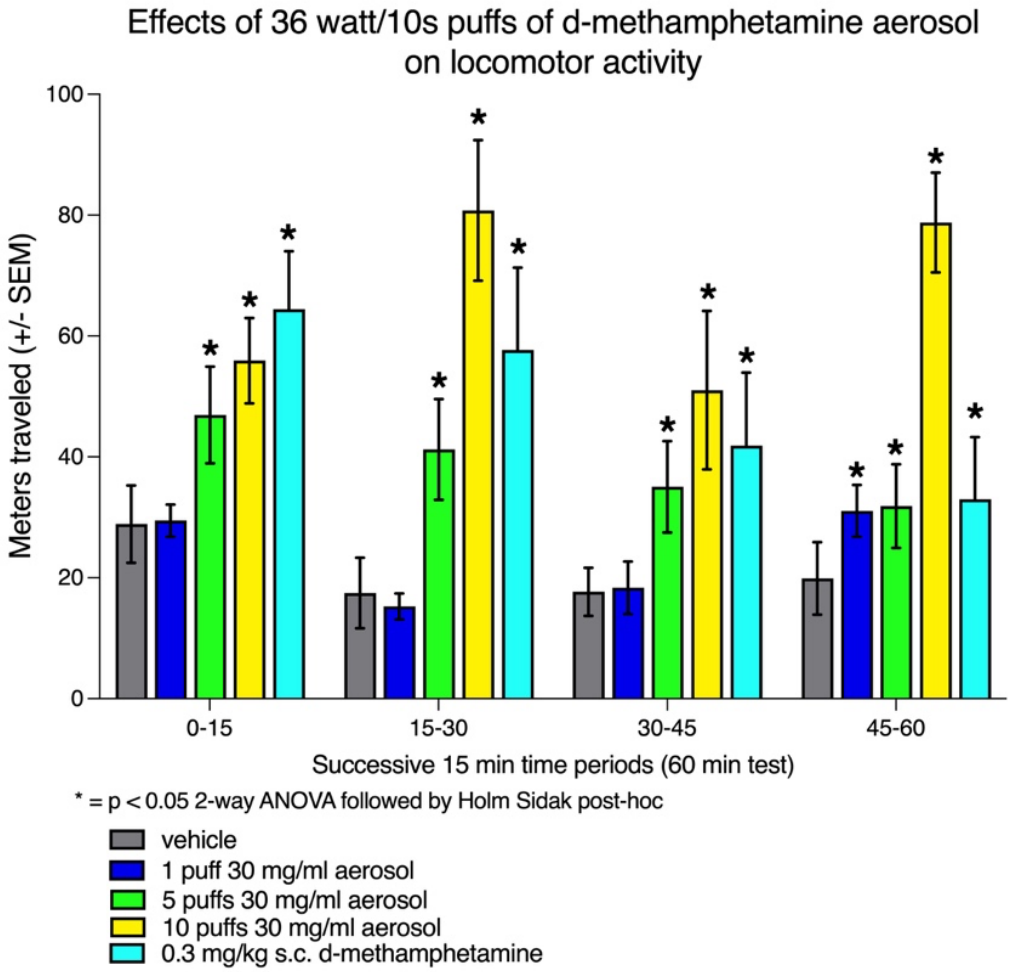

Figure 10: Average meters traveled (+/- SEM) for 8 subjects after 1, 5, and 10 puffs of 30mg/ml methamphetamine aerosol at an e-cigarette output setting of 36 watts across 4 successive 15 min time periods. Activity was assessed following 1 (blue), 5 (green), and 10 (yellow) puffs at an e-cigarette output setting of 18 Watts. Methamphetamine 0.3 mg/kg *s.c.* injection (teal) was also tested as positive control. * Indicates statistical significance differences (P< 0.05) compared to 50:50 PG:VG vehicle aerosol (gray).

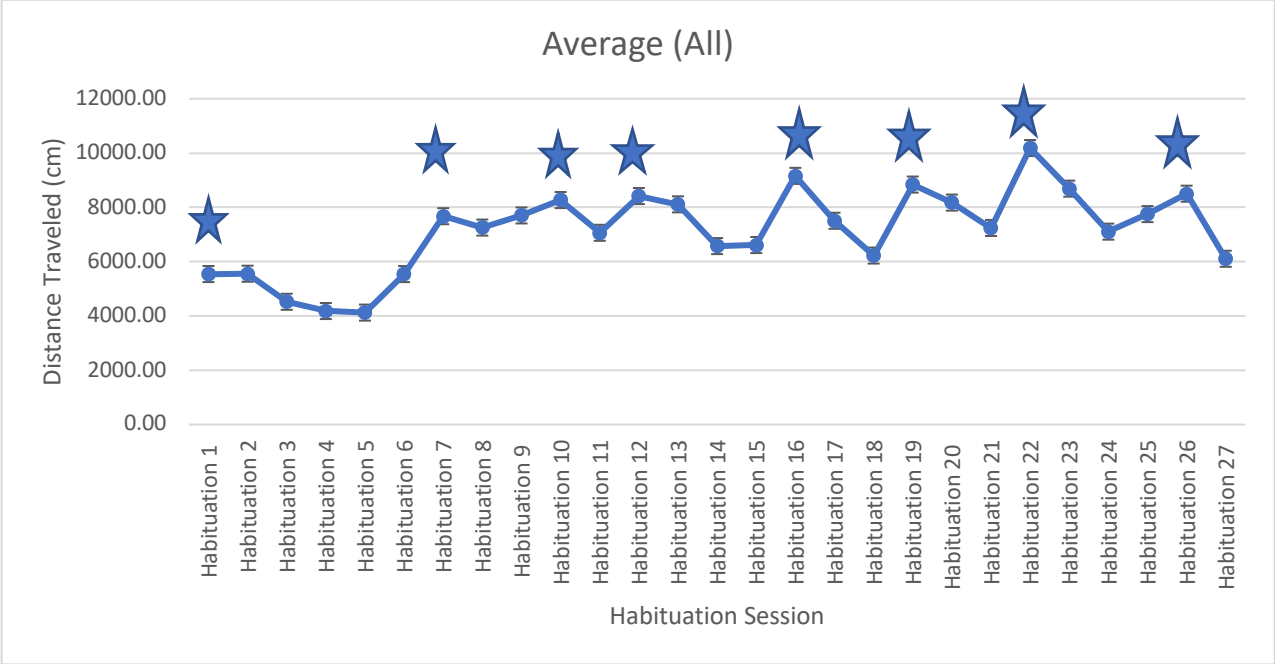

Figure 11: Mean Distance Traveled in Habituation Sessions: The average distance traveled in all subjects prior to treatments sessions including the 5 consecutive sessions prior to any treatment. Monday sessions are denoted with stars.

Five habituation sessions were conducted prior to testing and continued throughout duration of experimentation of locomotor studies on days between drug tests. Methamphetamine test sessions were separated by two days to reduce the possibility of locomotor sensitization. On the first of these two days the subjects remained in their home cage and were not tested. The following day a 60 min habituation measurement with no treatment was conducted. In Figure 11, data is presented as a mean (+/- SEM) across all habituation sessions. Locomotor activity during the first 5 habituation sessions before any

treatment was administered decreased for the first 3 session and then stabilized. Locomotor activity then increased on habituation days following the first two drug treatment sessions (sessions 7 and 8) and generally remained stable for the remainder of the experiment. It was also noted that habituation sessions conducted on Mondays (2 days following a test session) were higher than the mid-week habituation sessions.

Aim 2: Examine the sympathomimetic effects of methamphetamine administered via e-cigarette aerosolization.

Methamphetamine administration has pronounced sympathomimetic effects (Bexis, 2006; Nguyen, 2016; Schindler, 2013). The goal of Aim 2 was to determine if methamphetamine aerosol puff exposure via e-cigarette aerosolization also has similar effects by measuring blood pressure, heart rate, and body temperature across a range of exposure conditions. The same 4 male and 4 female rats utilized for the locomotor study were also used to complete this aim.

Experiment 3: Effect of methamphetamine on blood pressure, heart rate, and body temperature.

Animals tested were exposed to 1, 5, 10, or 15 puffs of 30 mg/ml d-methamphetamine at an e-cigarette output setting of 36 watts in a manner identical to locomotor activity studies. The same 0.3 mg/kg *s.c.* methamphetamine injection was used as a positive control. Data was analyzed by calculating a change value between the initial measurement taken prior to exposure and measurement taken post exposure. Pretreatment with methamphetamine

aerosol had a puff dependent effect on systolic blood pressure (Figure 12). There was a significant main effect of puff number [$F(5,35)=14.15$, $P<0.0001$]. Post hoc Fisher's test revelated that the 5, 10, 15 puff condition significantly increased systolic blood pressure compared to the vehicle aerosol control. The 5, 10 and 15 puff condition appear to have a comparable increase in systolic blood pressure compared to the positive control of 0.3 mg/kg s.c. methamphetamine. There was no significant difference between the no treatment, air control and 1 puff condition compared to vehicle.

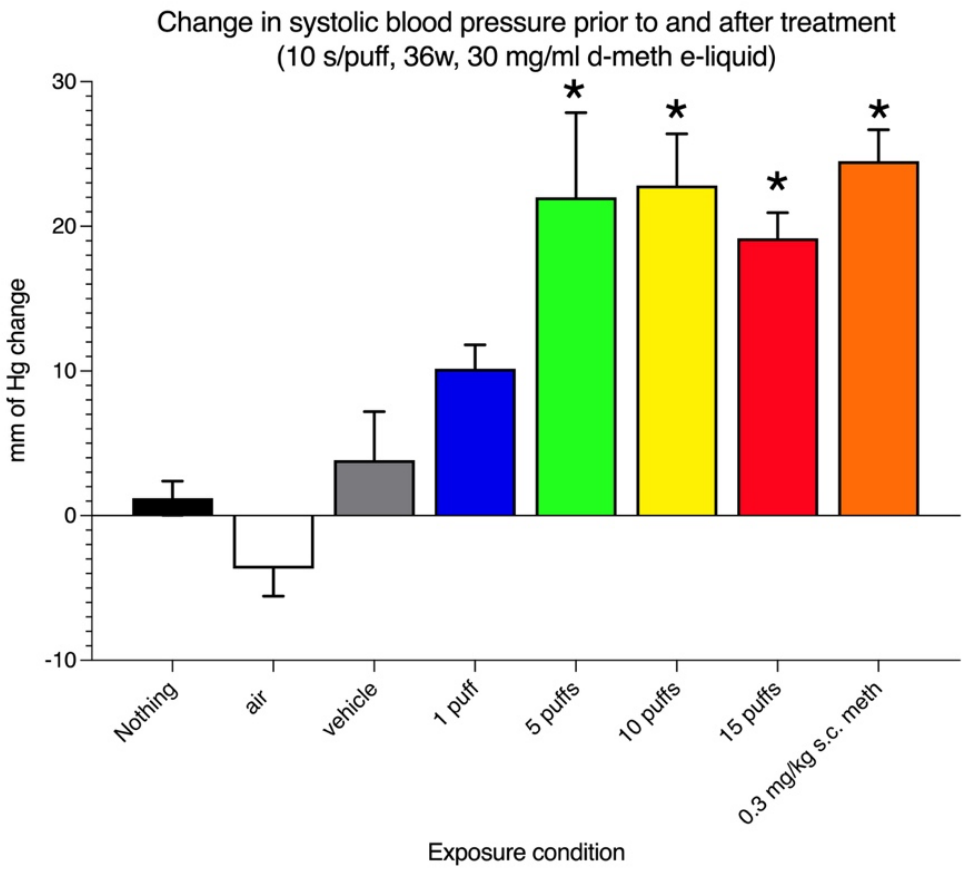

Figure 12: Mean (+/- SEM) change in systolic blood pressure (mm of Hg) of 8 subjects after exposure to 30 mg/ml d-methamphetamine aerosol at an e-cigarette output setting of 36 Watts. Shown are 1 (blue), 5 (green), 10 (yellow), and 15 (red) puffs. Methamphetamine 0.3 mg/kg *s.c.* injection (orange) was used as a positive control. The nothing condition (black) represented a baseline measurement with no aerosol exposure. The air condition (white) represented the animal being in the exposure chamber for the duration of 10 puffs with no aerosol.).* Indicates statistically significant ($p<0.05$) changes in systolic blood pressure compared to 50:50 PG:VG vehicle aerosol (gray)

Effect of methamphetamine puffs on diastolic blood pressure was also assessed (Figure 13). Pretreatment with methamphetamine aerosol had an inverted U-shaped puff dependent effect on change in diastolic blood pressure. There was a significant main effect of methamphetamine puff number [$F(7,35)=6.14$, $P=0.0001$)]. Post hoc Fisher's test revealed that 10 and 15 puff conditions resulted in a significant increase in diastolic blood pressure compared to the vehicle vapor control. Unlike systolic blood pressure, the 1 and 5 puff condition were not significantly greater than vehicle vapor. Mean change in diastolic blood pressure was greatest in the 10-puff condition but mean change for all aerosol exposure conditions were less than the mean change produced by the positive control of 0.3 mg/kg s.c. methamphetamine. There were no significant differences between the vehicle control condition, the no treatment condition and the air exposure condition.

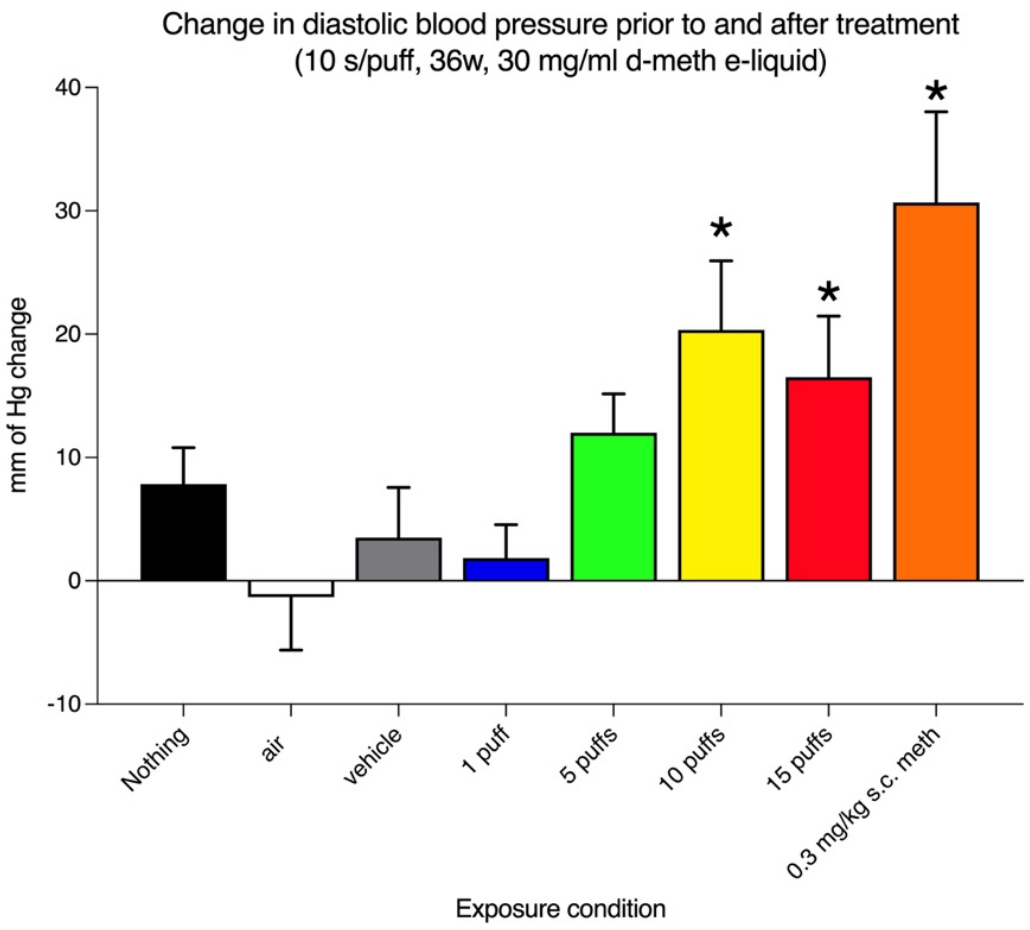

Figure 13: Mean (+/- SEM) change in diastolic blood pressure (mm of Hg) of 8 subjects after exposure to 30 mg/ml d-methamphetamine aerosol at an e-cigarette output setting of 36 Watts. Shown are 1 (blue), 5 (green), 10 (yellow), and 15 (red) puffs. Methamphetamine 0.3 mg/kg *s.c.* injection (orange) was used as a positive control. The nothing condition (black) represented a baseline measurement with no aerosol exposure. The air condition (white) represented the animal being in the exposure chamber for the duration of 10 puffs with no aerosol.).* Indicates statistically significant ($p<0.05$) changes in diastolic blood pressure compared to 50:50 PG:VG vehicle aerosol (gray).

Figure 14 shows the mean (+/- SEM) change in body temperature as the number of puffs of methamphetamine 30 mg/ml aerosol at an e-cigarette output setting of 36 watts. There was a puff dependent increase in body temperature as the number of puffs increased (1, 5, 10, 15 puffs). There was a significant main effect of puff number [$F(7,35)=7.01$, $P<0.0001$] on body temperature. Post hoc Fisher's test revealed that the 5, 10, and 15 puff conditions and the 0.3 mg/kg *s.c.* injection significantly increased body temperature compared to the vehicle aerosol control. The 5 and 15 puff conditions appear to have a comparable change in body temperature with the positive control 0.3 mg/kg. The maximum change in body temperature occurred at the 10-puff condition; however, the 15-puff condition appeared to have a lower effect on body temperature compared with the 10-puff condition suggesting an inverted U-shaped effect.

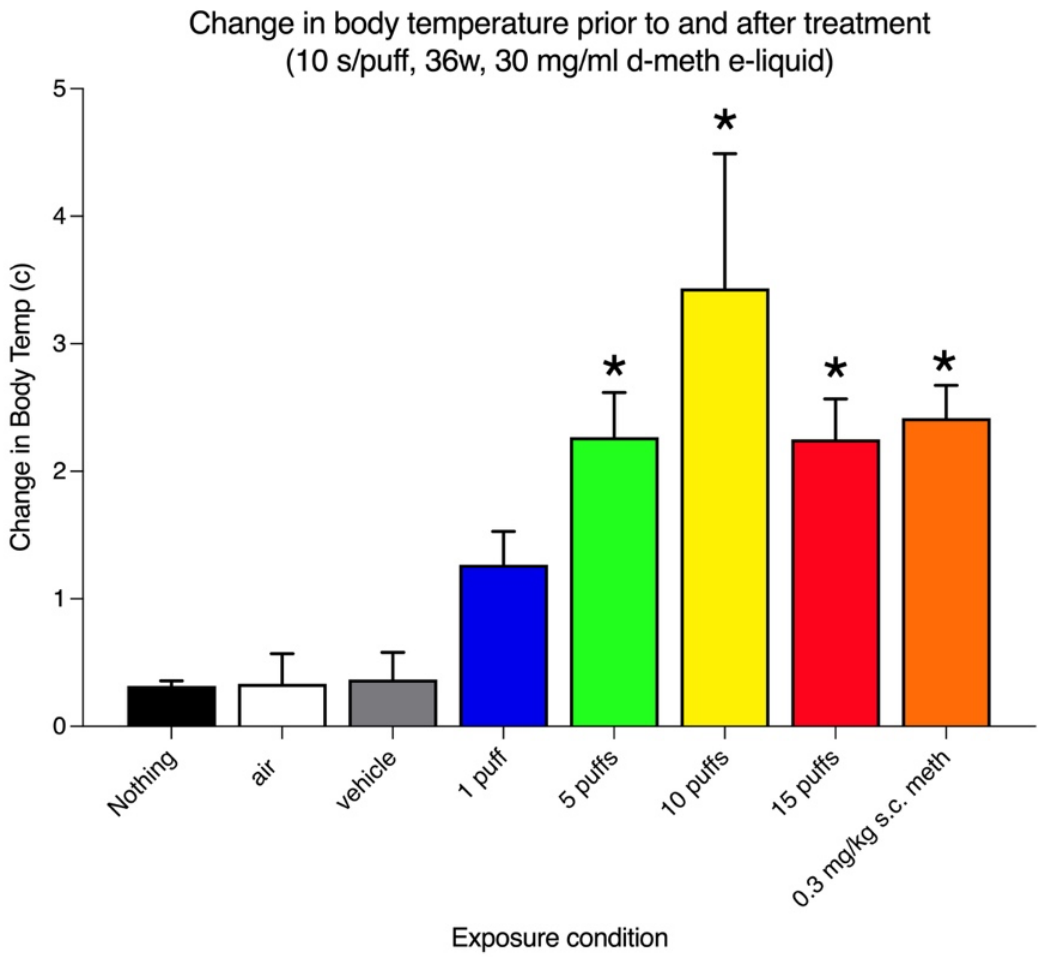

Figure 14: Mean (+/- SEM) change in body temperature of 8 subjects after exposure to 30 mg/ml d-methamphetamine aerosol at an e-cigarette output setting of 36 Watts. Shown are 1 (blue), 5 (green), 10 (yellow), and 15 (red) puffs. Methamphetamine 0.3 mg/kg *s.c.* injection (orange) was used as a positive control. The nothing condition (black) represented a baseline measurement with no aerosol exposure. The air condition (white) represented the animal being in the exposure chamber for the duration of 10 puffs with no aerosol.).* Indicates statistically significant ($p<0.05$) changes in body temperature compared to 50:50 PG:VG vehicle aerosol (gray).

The results of change in heart rate as a result of increasing methamphetamine aerosol puffs of 30 mg/ml methamphetamine at an e-cigarette output setting of 36 watts increased are shown in figure 15. There was no main effect of puff number on heart rate [$F(7.35)=1.88$, $P=0.10$]. A subsequent paired t-test demonstrated that the 0.3 mg/kg s.c. methamphetamine injection condition was significantly greater than the vehicle control condition.

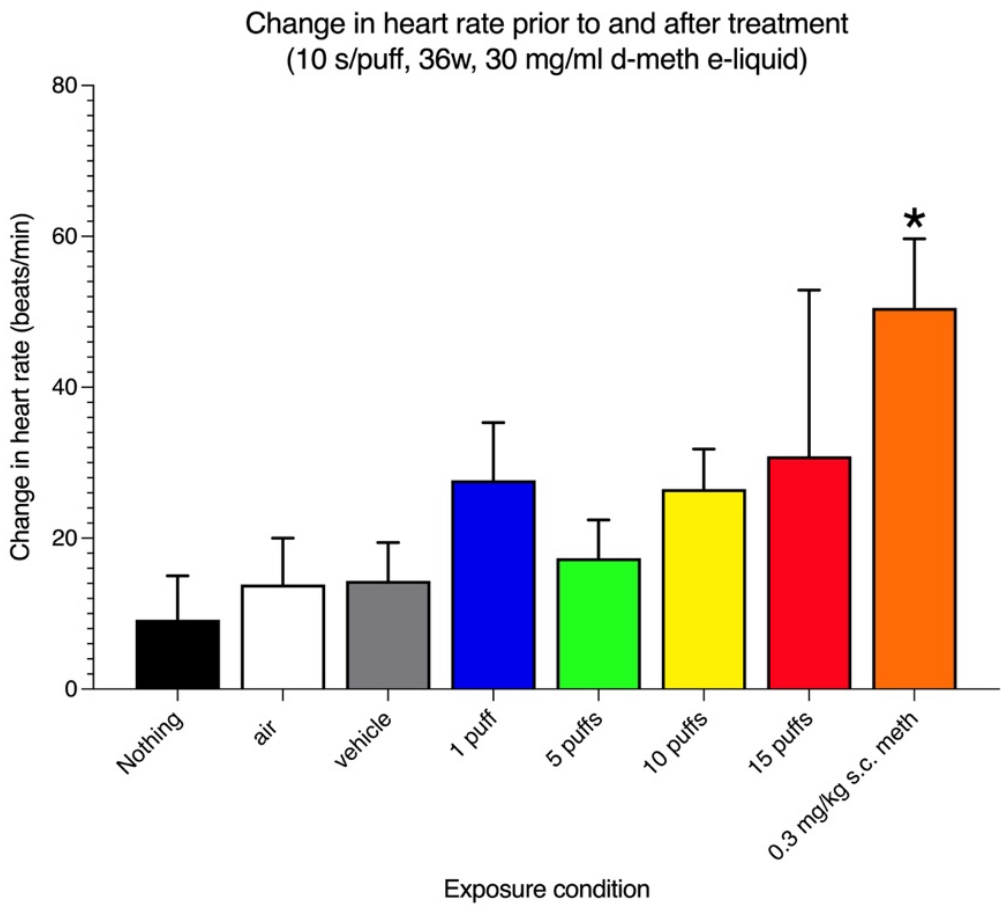

Figure 15: Mean (+/- SEM) change in heart rate of 8 subjects after exposure to 30 mg/ml d-methamphetamine aerosol at an e-cigarette output setting of 36 Watts. Show are 1 (blue), 5 (green), 10 (yellow), and 15 (red) puffs. Methamphetamine 0.3 mg/kg *s.c.* injection (orange) was used as a positive control. The nothing condition (black) represented a baseline measurement with no aerosol exposure. The air condition (white) represented the animal being in the exposure chamber for the duration of 10 puffs with no aerosol. * Indicates statistically significant ($p<0.05$) changes in heart rate compared to 50:50 PG:VG vehicle aerosol (gray).

Aim 3: Correlating behavioral and physiological effects of aerosolized and injected d-methamphetamine with blood drug levels.

As a final aim, we wished to correlate methamphetamine blood levels with results shown prior from behavioral and physiological measurements.

Experiment 4: Examine the blood plasma levels of methamphetamine administered via e-cigarette aerosolization and subcutaneous injection.

Twelve adult male Long-Evans hooded rats were used as subjects. One group of 3 rats was administered 0.3 mg/kg s.c. methamphetamine, the same dose used in the prior studies exploring locomotor activity and physiological effects. Additional groups of 3 rats each were administered 1, 5 or 10 puffs of 30 mg/ml methamphetamine aerosol at an e-cigarette output set at 36 Watts. Pretreatment with methamphetamine aerosol had a puff dependent effect on methamphetamine plasma concentration (Figure 16). There was a significant main effect of puff number [$F(3,8)=10.57$, $P=0.0006$]. Post hoc Fisher's tests showed that the 5, 10 puff, and 0.3 mg/kg methamphetamine *s.c.* injection all resulted in significantly greater methamphetamine plasma concentration than the 1 puff condition. A second Fisher's post-hoc test showed that the 10 puff condition resulted significantly higher plasma concentration of methamphetamine than did the 0.3mg/kg *s.c.* methamphetamine positive control condition.

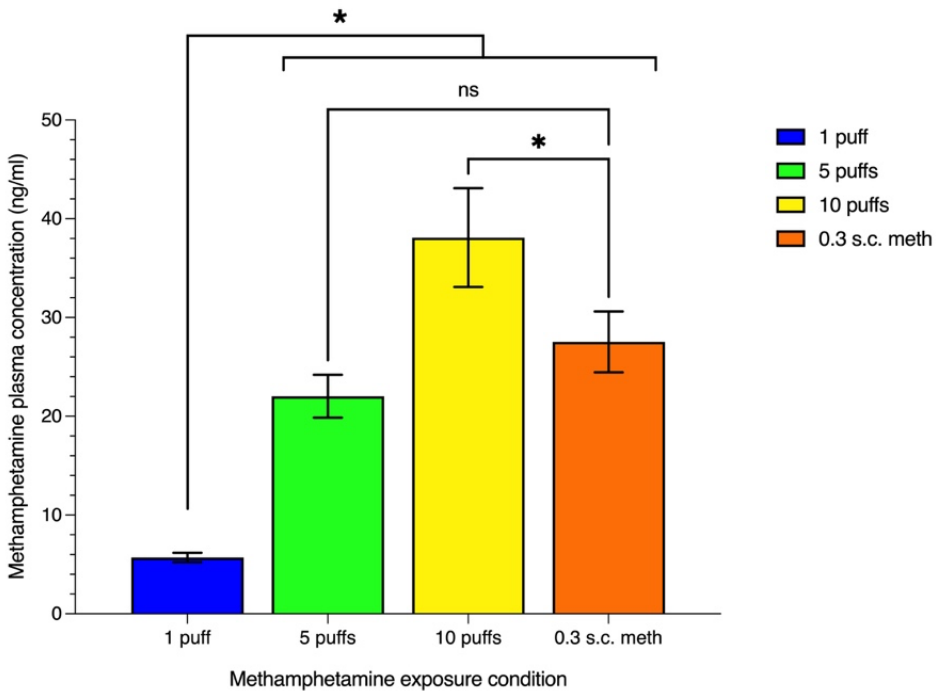

Figure 16: Methamphetamine plasma concentrations in ng/ml (+/- SEM) following exposure to 30 mg/ml methamphetamine aerosol or 0.3 mg/kg s.c. methamphetamine. Puff conditions included 1 (blue), 5 (green) or 10 (yellow) puffs. The 0.3 mg/kg s.c. methamphetamine control condition is shown in orange. * Indicates statistically significant ($p<0.05$) differences compared to the corresponding condition linked with brackets.

Figure 17 shows the average amphetamine plasma concentration as the number of puffs of methamphetamine 30 mg/ml aerosol at an e-cigarette output setting of 36 watts increased. There was a significant main effect of puff number [$F(3,8)=11.85$, $P=0.0026$]. Post hoc Fisher's test showed that the 5, 10 puff, and 0.3 mg/kg methamphetamine *s.c.* injection all resulted in a significant increase in amphetamine plasma concentration compared to the 1 puff condition. There was a trend suggesting that the 10 puff condition may result in higher amphetamine blood levels than either the 5 puff condition or the 0.3 mg/kg *s.c.* methamphetamine positive control. However, a second Fisher's post hoc test comparing all test conditions to the 0.3 mg/kg *s.c.* methamphetamine positive control showed this was not statistically significant.

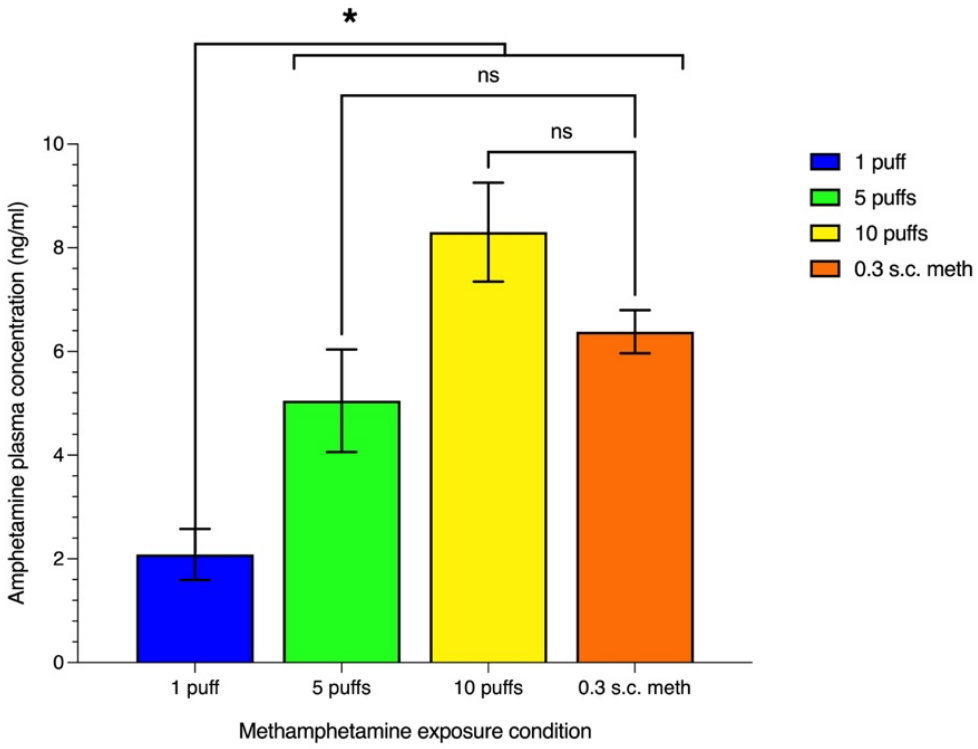

Figure 17: Amphetamine plasma concentrations in ng/ml (+/- SEM) following exposure to 30 mg/ml methamphetamine aerosol or 0.3 mg/kg s.c. methamphetamine. Puff conditions included 1 (blue), 5 (green) or 10 (yellow) puffs. The 0.3 mg/kg s.c. methamphetamine control condition is shown in orange.

Discussion:

Locomotor Activity:

Locomotor activity is a valid and well characterized behavioral procedure that can demonstrate the CNS mediated effects of psychostimulants (Nazari, 2020; Mihalčíková, 2019; Ortman, 2021). Little work has been done on the CNS effects of aerosolized methamphetamine (Nguyen, 2016) and no studies of which we are aware have examined exposure conditions that attempt to model those likely to occur in humans experimenting with methamphetamine use in e-cigarettes. As such, the purpose of the locomotor assay in the present study was to determine if behaviorally relevant doses of methamphetamine were being administered via e-cigarette aerosolization to the rats in order to validate the model. The primary metric that was assessed in the assay was distance traveled by the animal. The positive control of subcutaneously injected methamphetamine produced a dose-dependent statistically significant increase in total distance traveled at all three tested doses: 0.03, 0.1, and 0.3 mg/kg. This was consistent with previous studies done using injected methamphetamine (Mihalčíková 2019; Kelly, 2008). Mihalčíková et. al focused on locomotor activity of adult male rats after subcutaneous methamphetamine injected at a dosage of 1 mg/kg. That group also used a 60 min time session for assessment of locomotor activity. They found that total distance traveled was increased in the methamphetamine injection group, similar to that which was seen in our study. However, the Mihalčíková group did not conduct a dose-response analysis as was done in the present experiment. Given that our highest test dose was 0.3 mg/kg and other experiments have utilized higher methamphetamine doses it is likely that our dose range did

not test a sufficiently high dose to produce maximal increases in locomotor activity, although a very substantial 200% increase in distance traveled occurred at our highest 0.3 mg/kg s.c. dose. While assessing a full dose-effect curve of s.c. administered drug would have allowed us to determine if the maximum efficacy of aerosolized methamphetamine was greater than that of s.c. injected drug, exploring maximal effects was not our primary goal. Instead, our goal was to assess the ascending portion of the methamphetamine dose-effect curve in order to determine the minimum s.c. dose which would produce a statistically significant increase in distance travelled. Our lowest dose of 0.03 mg/kg s.c. methamphetamine was likely near that threshold in that it produced a statistically significant increase in locomotor activity but the change in distance traveled over the saline control was relatively small. It was our hypothesis that this lower range would be more relevant as a comparison to what we believed might be relatively modest effects of methamphetamine aerosol.

In humans experimenting with aerosolized drugs of abuse including methamphetamine, there are several variables which might be manipulated. Users report discussing how much methamphetamine to mix with the e-liquid. Other easily manipulated variables include the power output setting of the e-cigarette as well as how many puffs a user might inhale before they determined that using methamphetamine in an e-cigarette didn't produce the desired response. We hypothesized that the amount of drug dissolved in e-liquid would likely be highly variable among users both due to the haphazard nature of estimating drug weights outside a laboratory as well as the relative purity of illicitly acquired drug. In contrast, manipulation of total number of puffs as well as e-cigarette power setting could be much more easily and

precisely controlled by the user. As such we believed that exploring these factors would be valuable to determine if aerosolized methamphetamine might have abuse liability.

In our study, methamphetamine aerosol resulted in a puff-dependent and a wattage-dependent increase in locomotor activity. The vehicle vapor condition confirmed that the 50% VG/50% PG vehicle alone did not alter locomotor activity compared to the no treatment condition. A previously mentioned, a relevant prior study done by Nguyen et al. (Nguyen, 2016), examined the effects of aerosolized methamphetamine, mephedrone, and 3,4-methylenedioxypyrovalerone (MDPV). In the published study the rats were implanted with radiotelemetry devices to measure behavioral effects and methamphetamine also increased locomotor activity as occurred in our experiment utilizing a photobeam-based test system. As such our experiments provide confirmation across two different procedures that methamphetamine aerosol has CNS-mediate behavioral effects.

However, it was our hypothesis that the exposure conditions utilized in the prior study were not well suited to assessing a likely scenario in a human user engaging in experimentation. Specifically, the Nguyen group utilized only one condition in which they exposed rats to aerosolized drug in four 10 second aerosol puffs every 5 min for 40 min. This exposure procedure provides useful information about the consequence of a lengthy exposure to methamphetamine aerosol rather that that resulting from a more realistic experimentation situation. In contrast our puff condition was defined as a 10 second aerosol emitted into the chamber, a 10 second hold in the chamber, a 10 second exhaust out of the chamber, and a 10 second interval between one puff and the subsequent one. Importantly we manipulated the number of puffs to determine the minimum number required to elicit a statistically significant

increase in locomotion based on our hypothesis that users that do not experience the desired effects fairly quickly would likely not continue to engage in the behavior over an extended period of time.

We found that both puff number and e-cigarette wattage interacted in their ability to produce locomotor activation. At a power setting of 18 watts, our minimum exposure condition of 10 puffs resulted in a significant increase of locomotion from 17 meters to over 35 meters, whereas 10 puffs at 36 watts resulted in a mean of 66 meters travelled. This approximately doubling of distance traveled at twice the wattage setting was unlikely to have been the result of a change in particle size at the higher wattage (Mulder 2019). Instead, we hypothesize that the greater increase in activity at the 36-watt condition was a consequence of a greater volume of vapor being generated by each puff. As we were introducing the aerosol into a semi-enclosed space, a greater amount of aerosol would be expected to produce a higher drug concentration within that space and an effectively higher inhaled dose by each rat. It is also interesting to note that mechanically it appears that, at least in the wattage range we tested that the internal circuitry of the e-cigarette utilized produces power outputs that are consistent with our behavioral data in that the locomotor activation produced at 36 watts was roughly double that produced by 18 watts. Although we did not directly measure drug aerosolized at each wattage these data are suggestive that a doubled wattage may result in a doubling of aerosolized drug.

Another important factor relevant to the abuse liability of a drug is the rapidity of effects following administration (Allain, 2015). Our minimum exposure duration was quite short relative to the prior published study with only a 3 min and 20 second time between the

start of exposure and the beginning of locomotor testing in the shortest test in which we showed significant locomotor increases (5 puffs/36 watts). The present data confirms the hypothesis that effects of aerosolized methamphetamine are rapid in that locomotor activating effects were increased significantly within the first 15 min of vapor exposure. It is possible that it might have been possible to detect even more rapid effects had we set a shorter minimum time increment. Future studies will be necessary to answer this question.

Unlike the prior study, we also utilized a subcutaneous injection of methamphetamine as a positive control in order to compare efficacies across routes of administration. Although the maximal locomotor stimulatory dose was probably was not achieved, total distance traveled in each bin at a methamphetamine concentration of 30 mg/ml for 15 puffs and 20 puffs at 18 watts and 30 mg/ml for 10 puffs at 36 watts were all higher than the highest 0.3 mg/kg dose of methamphetamine administered subcutaneously. This would suggest that the efficacy of aerosolized methamphetamine for producing increases in locomotor activity was at least as high and very likely higher than a moderately high dose of injected methamphetamine. In addition to efficacy, aerosolized methamphetamine also had longer lasting effects in distance traveled when compared with the 0.3 mg/kg dose of methamphetamine used as a positive control. This could be explained by a dose being achieved through the numerous puffs administered to the animal which is actually greater than the 0.3 mg/kg dose. This was made evident by our methamphetamine blood level results, showing that our 10-puff condition had achieved a higher methamphetamine concentration than the 0.3 mg/kg dose which would explain both efficacy as well as greater duration of effect seen at the higher puff conditions.

Another possible explanation would be that methamphetamine vapor has slower absorption than injected methamphetamine. Unlike humans, rodents are obligate nose-breathers and have a more extensive ability to filter particles and prevent lung absorption, which might have resulted in much of the methamphetamine aerosol not reaching deep lung tissue which would promote rapid absorption (Fröhlich, 2021). However, aerosols particles are known to deposit in the nasopharyngeal and tracheobronchial airways, which are mucosilated. This results in transportation of drugs into the gastrointestinal tract and enabling GI tract absorption resulting in greater systemic level of drug due to multiple site absorption (Sakagami, 2003. As previously mentioned, time dependent effects of methamphetamine on locomotion were examined across four bins. It was noticed that the significant effects were observed 0-15 minutes post aerosol exposure suggesting that some absorption occurred quite rapidly but the peak in locomotor activity in the highest puff conditions at both 18 and 36w occurred at the 15-30 min time point which may be indicative of more gradual absorption through the nasal mucosa.

It must also be noted that all of our testing with methamphetamine aerosol and injections was done in the same group of 8 rats. Methamphetamine and other psychomotor stimulants have been well documented to produce locomotor sensitization as exhibited by escalating locomotion following repeated administration (Buchanan, 2009; Hutchinson, 2013; Wearne, 2015). Habituation sessions that were conducted prior to methamphetamine testing as well as interposed between each methamphetamine treatment session were conducted to reduce the likelihood of this possibility. Between the 1st and 2nd methamphetamine exposure

locomotor activity during habituation sessions did increase over that prior to any exposure. After that point no additional trends in locomotion on habituation sessions were apparent which could be interpreted as some degree of rapid sensitization which did not develop further despite many repeated tests. One limitation is that, although the doses and puff conditions were presented in pseudorandom order, we did not replicate any of our early exposure conditions after the completion of the remainder of the tests. Without this datapoint it is impossible to know with certainty if the animals became sensitized and if so the degree to which it had occurred.

Physiological Effects:

Following the examination of behavioral effects of methamphetamine via locomotor activity we examined the physiological effects of aerosolized methamphetamine. There have been a number of studies in rodents examining the sympathomimetic effects of methamphetamine after injection (Yoshida, 1993; Clark, 1986). However, we are aware of only one study examining the sympathomimetic effects of aerosolized methamphetamine (Nguyen, 2016). Given that the sympathomimetic effects methamphetamine can lead to significant adverse health consequences such as strokes and heart attacks, understanding the degree to which aerosolized methamphetamine produces these effects after brief exposures likely to occur in human users is important (Winslow, 2007). The three physiological conditions measured were heart rate, body temperature, and blood pressure (systolic and diastolic). In this study, the vehicle aerosol condition was utilized as the control and an air condition was also

utilized as a means to confirm that the exposure chamber was not causing any physiological agitation in the animal.

In most prior studies of the physiological effects of methamphetamine the rats were either anesthetized during testing or a surgically-implanted radiotelemetry receiver was utilized (Nguyen, 2016, Yoshida, 1993). For our studies we wished to avoid the confounds associated with anesthesia as well as the difficulty inherent to a significant surgical manipulation. To accomplish this goal, we utilized an automated blood pressure device (the CODA monitor) attached to the tail of the animals while they were briefly immobilized within a rodent restrain tube. Because brief restraint was necessary to utilize this equipment there is the possibility that our physiological measures may have been confounded by restraint stress. We attempted to minimize this possibility by habituation of the subjects to the restraint tubes prior to testing but it is a compromise associated with the method which must be noted.

The positive control, 0.3 mg/kg s*,c* methamphetamine injection, identified in the locomotor activity assay as producing a robust increase in activity was used as the positive control for our physiological studies. For the entirety of our physiological testing, the e-cigarette output was set at 36 watts as we found more profound change in locomotor activity at that wattage as compared to the lower 18-watt test condition. As the results from our locomotor activity assay confirmed our hypothesis that effects of aerosolized methamphetamine were rapid, we used the 0-15 min time frame to measure the physiological effects of methamphetamine.

The primary metric used in blood pressure measurements (systolic and diastolic) was the change between the animal's pre-treatment blood pressure to their post-treatment blood

pressure. The individual change score was chosen because it controlled for individual variability between subjects, providing greater sensitivity and statistical power over simply comparing mean blood pressure in each test condition. The vehicle aerosol condition confirmed that the 50%/50% PG:VG vehicle alone did not alter neither systolic nor diastolic blood pressure compared to control condition in which no aerosol exposure occurred. This might suggest that exposure to vehicle aerosol itself was not a stressor. To confirm this hypothesis, it would have been necessary to assess if increases in blood pressure can be detected utilizing our procedure.

We found a methamphetamine puff number-dependent increase in both systolic and diastolic blood pressure. Interestingly it was observed that at the highest puff number administered (15 puff), resulted in a downward effect in both systolic and diastolic blood pressure over the 10 puff condition. This result is inconsistent with that from locomotor activity in which 15 puffs produced a larger effect than 10 puffs. It is possible that this reduction in blood pressure could result in potential cardiotoxic effects of methamphetamine at high doses. Our methamphetamine blood level studies examined a maximum of 10 puffs of methamphetamine administered at 36 watts, which resulted in significantly higher methamphetamine concentration than that produced by fewer puffs. Thus, it could be assumed that 15 puffs would have even greater methamphetamine blood levels which might have resulted in a cardiotoxic effect and concomitant decrease in blood pressure. Hassan et. al (Hassan, 2016) reported observing cardiotoxic effects in methamphetamine at a very high dosage of 10 mg/kg. Blood methamphetamine levels after 10 puffs of methamphetamine were significantly higher than our s.c. comparison dose of 0.3 mg/kg, however, given the trajectory of increasing blood concentrations as a function of puff number it would be unlikely that an

additional 5 puffs would yield the same blood levels as a 10 mg/kg dose. However, further investigation should be done to examine methamphetamine blood levels when an animal is exposed to 15 puffs of methamphetamine aerosol at an e-cigarette output of 36 watts to address the possibility.

A study done by Yoshida et. al (Yoshida, 1993) specifically examined methamphetamine effects on mean arterial blood pressure. A 1 mg/kg methamphetamine injected dose resulted in greatest peak in blood pressure increases in the first 15 min. Although our study tested a lower 0.3 mg/kg dose, we also noted a significant increase in blood pressure. In the published study, an implanted radio telemetry device was used to assess mean arterial blood pressure whereas out study utilized a tail blood pressure test system. Our results indicate that non-invasive blood pressure testing is probably as sensitive or potentially more sensitive than testing by implanted transceiver making this method an attractive alternative to more expensive and invasive techniques. Unlike the published study, we examined the effects of aerosolized methamphetamine within the first 5 min after aerosol exposure. Although it is not possible to determine if absorption of methamphetamine was within the lung, the nasal mucosa or both, the rapidity of the response confirms our hypothesis that effects of aerosolized methamphetamine in rats is quite rapid. This rapidity of onset of effect is an important factor in leading to high abuse liability of the aerosolized drug. If absorption of aerosolized methamphetamine is even more rapid humans than in rats due to greater relative lung absorption it would lead us to speculate that the abuse liability of aerosolized methamphetamine is likely quite high.

Body temperature of animals pre and post methamphetamine administration was also assessed using a handheld infrared thermometer. As with blood pressure, we found that as puff number increased body temperature also increased. The study done by Nguyen et. al (Nguyen, 2016) also examined the effect of methamphetamine aerosol on body temperature using implanted radiotelemetry devices similar to prior experiments on injected methamphetamine (Yoshida, 1993; Schindler, 2013). The authors observed that body temperature increased at the 20 min post methamphetamine aerosol exposure; consistent with the results of our study utilizing infrared thermometer, despite significant differences in methamphetamine aerosol exposure conditions. Specifically study done by Nguyen et. al (Nguyen, 2016) utilized a paired aerosol exposure method for a duration of 40 min with four 10 second aerosol puffs every 5 min. These exposure parameters were useful for understanding the effect of lengthy exposure of methamphetamine aerosol. In contrast our study was designed to examine more realistic conditions of brief exposures which might occur in humans experimenting with aerosolized methamphetamine. Specifically, our study used puff parameters defined as 10 second aerosol emitted into the chamber, a 10 second hold in the chamber, a 10 second exhaust out of the chamber, and a 10 second interval between one puff and the subsequent one. Our effects were also seen in a faster timeframe than the published study. Thus, further confirming our hypothesis that methamphetamine aerosol has not only rapid locomotor activating effects but also rapid onset sympathomimetic effects. It was also observed that under certain puff conditions both body temperature and blood pressure effects are of similar magnitude of our injected methamphetamine dose. This would confirm our

hypothesis that these comparable effects between both aerosolization and injection may also have similar abuse liability.

Our final physiological test was examining the effects of methamphetamine on heart rate. Unlike body temperature and blood pressure, methamphetamine aerosol did not have significant effects on heart rate. This is possibly due to the robust effect on blood pressure causing a lack of effect on heart rate or that the methamphetamine dose was too low to impact heart rate. However, we did see a significant heart rate increasing effect of our 0.3 mg/kg s.c. methamphetamine test condition. The greater effect on heart rate in the injection condition relative to the aerosol conditions is the inverse of that noted in locomotor activity. It could be possible that our testing was conducted too soon after removal from the exposure chamber and that the absorption of methamphetamine aerosol via mucosal absorption over increased time might have had a more pronounced effect whereas the s.c. methamphetamine might have produced a more rapid increase in blood levels. However, further experimentation would need to be done looking further than 5 min post aerosol exposure to address this possibility.

Methamphetamine Plasma Levels

After confirmation of effects physiological and behaviorally, we set out to assess blood levels of methamphetamine resulting from methamphetamine aerosol exposure. Methamphetamine plasma levels were measured after 1, 5, and 10 puffs with the e-cigarette output set at 36 watts along with a 0.3 mg/kg *s.c.* injection, consistently with our positive

control in all other studies. It was observed that methamphetamine levels of the 5, 10 puff conditions were greater than the 1 puff condition. When compared with the 0.3 mg/kg methamphetamine injection, the 10-puff condition produce methamphetamine blood levels that were significantly higher. These data are consistent with our locomotor data in which the 10-puff condition generally resulted in greater locomotor activation than did the 0.3 mg/kg injected dose. In contrast they are less consistent with the effects of aerosolized and injected methamphetamine on blood pressure, heart rate and temperature. This may be the result of the fact that locomotor activity, even when broken down into 15 min intervals over a 1 hr session, provides a longer window of assessment than did our single point in time used with the physiology assays. Had additional later time points been examined in these procedures the results may have been more consistent.

As mentioned earlier, there are three main biotransformation pathways involved in methamphetamine clearance: 1) demethylation of methamphetamine to produce amphetamine 2) aromatic hydroxylation producing pholedrine and 3) beta-hydroxylation to produce norephedrine (Torre, 2012). It was observed that there was not a significant increase in levels of amphetamine when compared with the *s.c.* injected condition although both the 5, 10 and 0.3 mg/kg injected drug dose were significantly higher than the 1 puff conditions. It could be the case that differences would have emerged at longer time points following administration as little time had elapsed after exposure and as such the amount of methamphetamine metabolized was likely small. Additional studies at longer time points post exposure would be necessary to address this question. Overall, the results on blood levels confirm that more puffs result in greater the methamphetamine blood concentrations. The

general trend for puff number and methamphetamine blood plasma levels to be proportional suggests that overdose by methamphetamine aerosol exposure is certainly a possibility in a user that self-administers repeated puffs.

Theoretical Calculations:

In order to approximate how much drug our test subjects might have been inhaling during an exposure session, theoretical calculations were performed to approximate this. Initially, our e-liquid tank was filled and weighed to get a baseline weight of the tank. The tank was attached to the aerosol exposure chamber and a 5 puff program was activated 5 times for 10 seconds each at an e-cigarette output wattage of 36 Watts. It was observed that the average weight that was lost with each puff was calculated to be 0.28 g. This number was divided by the total number of seconds in which the atomizer was active, at the program set it was 50 seconds, in order to determine total weight of vehicle vaporized per second. This value was found to be 0.0056 g/s. This value was divided by the molecular weight of d-methamphetamine, which is 149.23 g/mol, to express the value in moles delivered per second of e-cigarette activation, which was calculated to be 0.000038 mol/s. The total volume of the aerosol exposure chamber used was 14.0 liters. The amount of d-methamphetamine aerosolized per session was divided by the chamber volume to determine the amount of drug present per 14.0 liters with the assumption being made that aerosol would distribute evenly throughout the chamber. This was calculated to 0.0027 μmol/cm^3. Finally, the tidal volume and respiratory rate of the rat was factored into the calculation in order to determine how much d-methamphetamine the rat is possibly inhaling. Multiplying tidal volume, 7 cm^3 for an adult Sprague Dawley rat, by 85 breathes/min for a typical adult rat male was found to be 595

cm^3/min (Frank, 2001). This was divided by 60 seconds to determine the volume inhaled per second and then was multiple by the number of seconds the rat was exposed to aerosol, 50 seconds. This was then multiplied by the methamphetamine distribution in the chamber, which should yield an approximation of the amount of methamphetamine inhaled per puff of aerosol. Under these conditions for 5 individual 10 second puffs, a rat would theoretically inhale 1.33875 µmol of methamphetamine per puff. This was converted to moles and multiplied by the molecular weight of methamphetamine, 149.23 g. This was found to be 0.20 mg per puff. Theoretically, this would provide a far greater dosage with multiple puffs administered than the 0.3 mg/kg methamphetamine *s.c.* injection. These values are a theoretical calculations it would be as a limiting factor in this is the volume of the chamber, which is not a vacuum chamber and possess the ability to lose aerosol being generated.

Future Directions

Further studies in several directions might be carried out in order to better understand the abuse potential of methamphetamine aerosol. First, analyzing the vapor that is emitted from the e-cigarette to help establish the dosage each animal is being exposed to per puff relative to the amount of drug utilized would be important as it might shed some light on the likelihood of aerosol drug abuse in price sensitive human users. The solubility of the drug as well as the vehicle components must be taken into consideration to assess aerosol and aerosol capture. Furthermore, our dose used throughout the majority of experimentation was 30 mg/ml. Both locomotor activity and the testing of physiological effects should be assessed with the use of both higher and lower drug concentration. There have been studies done showing

that high methamphetamine doses can result in stereotypical behavior (Mueller 1989, Milesi-Hallé, 2007) which can possibly reduce locomotor activity but blood pressure, heart rate, and body temperature can still be assessed. Additional studies assessing other aspects of locomotor activity (Mihalčíková, 2019; Matsumoto, 1990) such as time spent immobile time spent rearing, time spent grooming, average speed might also be used to follow changes in behavior from habituation sessions and test sessions. Further studies assessing the effects of aerosol over increase in time could also provide further insight into both duration of effect of methamphetamine aerosol as well as possible increase in effect as time increases. Other experiments that better assess anxiogenic-like effects in rodents should be carried out to assess methamphetamine aerosol as these types of responses might be predicted to be use limiting as opposed to use promoting. Two possible experiment that could be carried out are the elevated plus maze procedure which is a reliable assessor of anxiety-like effects in rodents in pharmacological experiments (Komada, 2008; Kraeuter, 2019) and the elevated T maze, which has been used to study anxiety-like behavior and can assess panic (Jardim, 1999; Deacon, 2006).

Conclusion

Overall, the current data show that aerosolized methamphetamine produces behavioral and physiological effects. Locomotor data indicated that behaviorally relevant does of methamphetamine were being achieved under our test conditions and exposure system producing effects similar to *s.c.* injection and under certain conditions even greater effects.

Aerosolized methamphetamine also produces effects on blood pressure and body temperature similar effects produced by the more traditional route of injection. Lastly, methamphetamine blood levels were also similar to those resulting from injection. These data are consistent with our hypothesis that methamphetamine aerosol is likely to have comparable abuse liability to that produced by other routes. Given this data, surveillance aimed at capturing increases in anecdotal reports of methamphetamine use in e-cigarettes should be undertaken and appropriate interventions instituted to prevent widespread adoption of aerosolized methamphetamine use and abuse.

References

Abuse, N. I. on D. (--). *What is the scope of methamphetamine use in the United States?* National Institute on Drug Abuse. https://nida.nih.gov/publications/research-reports/methamphetamine/what-scope-methamphetamine-misuse-in-united-states

Allain, F., Minogianis, E.-A., Roberts, D. C. S., & Samaha, A.-N. (2015). How fast and how often: The pharmacokinetics of drug use are decisive in addiction. *Neuroscience and Biobehavioral Reviews*, *56*, 166–179. https://doi.org/10.1016/j.neubiorev.2015.06.012

Alqahtani, M. M., Pavela, G., Lein, D. H., Vilcassim, R., & Hendricks, P. S. (2022). The Influence of Mental Health and Respiratory Symptoms on the Association Between Chronic Lung Disease and E-Cigarette Use in Adults in the United States. *Respiratory Care*, *67*(7), 814–822. https://doi.org/10.4187/respcare.09579

Alzahrani, T., Pena, I., Temesgen, N., & Glantz, S. A. (2018). Association Between Electronic Cigarette Use and Myocardial Infarction. *American Journal of Preventive Medicine*, *55*(4), 455–461. https://doi.org/10.1016/j.amepre.2018.05.004

Balster, R. L., & Schuster, C. R. (1973). Fixed-interval schedule of cocaine reinforcement: Effect of dose and infusion duration. *Journal of the Experimental Analysis of Behavior*, *20*(1), 119–129. https://doi.org/10.1901/jeab.1973.20-119

Bexis, S., & Docherty, J. R. (2006). Effects of MDMA, MDA and MDEA on blood pressure, heart rate, locomotor activity and body temperature in the rat involve alpha-adrenoceptors. *British Journal of Pharmacology*, *147*(8), 926–934. https://doi.org/10.1038/sj.bjp.0706688

Bracken-Clarke, D., Kapoor, D., Baird, A. M., Buchanan, P. J., Gately, K., Cuffe, S., & Finn, S. P. (2021a). Vaping and lung cancer – A review of current data and recommendations. *Lung Cancer*, *153*, 11–20. https://doi.org/10.1016/j.lungcan.2020.12.030

Bracken-Clarke, D., Kapoor, D., Baird, A. M., Buchanan, P. J., Gately, K., Cuffe, S., & Finn, S. P. (2021b). Vaping and lung cancer—A review of current data and recommendations. *Lung Cancer (Amsterdam, Netherlands)*, *153*, 11–20. https://doi.org/10.1016/j.lungcan.2020.12.030

Breland, A., Soule, E., Lopez, A., Ramôa, C., El-Hellani, A., & Eissenberg, T. (2017). Electronic cigarettes: What are they and what do they do? *Annals of the New York Academy of Sciences*, *1394*(1), 5–30. https://doi.org/10.1111/nyas.12977

Buchanan, J. B., Sparkman, N. L., & Johnson, R. W. (2010). Methamphetamine sensitization attenuates the febrile and neuroinflammatory response to a subsequent peripheral immune stimulus. *Brain, Behavior, and Immunity*, *24*(3), 502–511. https://doi.org/10.1016/j.bbi.2009.12.008

Cheng, T. (2014). Chemical evaluation of electronic cigarettes. *Tobacco Control*, *23 Suppl 2*, ii11-17. https://doi.org/10.1136/tobaccocontrol-2013-051482

Clark, W. G., & Lipton, J. M. (1986). Changes in body temperature after administration of adrenergic and serotonergic agents and related drugs including antidepressants: II. *Neuroscience and Biobehavioral Reviews*, *10*(2), 153–220. https://doi.org/10.1016/0149-7634(86)90025-4

Courtney, K. E., & Ray, L. A. (2014). Methamphetamine: An update on epidemiology, pharmacology, clinical phenomenology, and treatment literature. *Drug and Alcohol Dependence*, *143*, 11–21. https://doi.org/10.1016/j.drugalcdep.2014.08.003

Creamer, M., Case, K., Loukas, A., Cooper, M., & Perry, C. L. (2019). Patterns of sustained e-cigarette use in a sample of young adults. *Addictive Behaviors*, *92*, 28–31. https://doi.org/10.1016/j.addbeh.2018.12.011

de la Torre, R., Yubero-Lahoz, S., Pardo-Lozano, R., & Farré, M. (2012). MDMA, methamphetamine, and CYP2D6 pharmacogenetics: What is clinically relevant? *Frontiers in Genetics*, *3*, 235. https://doi.org/10.3389/fgene.2012.00235

Deacon, R. M. J., & Rawlins, J. N. P. (2006). T-maze alternation in the rodent. *Nature Protocols*, *1*(1), 7–12. https://doi.org/10.1038/nprot.2006.2

DeVito, E. E., & Krishnan-Sarin, S. (2018a). E-cigarettes: Impact of E-Liquid Components and Device Characteristics on Nicotine Exposure. *Current Neuropharmacology*, *16*(4), 438–459. https://doi.org/10.2174/1570159X15666171016164430

DeVito, E. E., & Krishnan-Sarin, S. (2018b). E-cigarettes: Impact of E-liquid Components and Device Characteristics on Nicotine Exposure. *Current Neuropharmacology*, *16*(4), 438–459. https://doi.org/10.2174/1570159X15666171016164430

Effects of nicotine and THC vapor inhalation administered by an electronic nicotine delivery system (ENDS) in male rats | Elsevier Enhanced Reader. (n.d.). https://doi.org/10.1016/j.drugalcdep.2019.01.027

Fadus, M. C., Smith, T. T., & Squeglia, L. M. (2019). The rise of e-cigarettes, pod mod devices, and JUUL among youth: Factors influencing use, health implications, and downstream effects. *Drug and Alcohol Dependence*, *201*, 85–93. https://doi.org/10.1016/j.drugalcdep.2019.04.011

Frank, J. A., Gutierrez, J. A., Jones, K. D., Allen, L., Dobbs, L., & Matthay, M. A. (2002). Low tidal volume reduces epithelial and endothelial injury in acid-injured rat lungs. *American Journal of*

Respiratory and Critical Care Medicine, *165*(2), 242–249. https://doi.org/10.1164/ajrccm.165.2.2108087

Fröhlich, E. (2021). Replacement Strategies for Animal Studies in Inhalation Testing. *Sci*, *3*(4), 45. https://doi.org/10.3390/sci3040045

Galvalisi, M., Prieto, J. P., Martínez, M., Abin-Carriquiry, J. A., & Scorza, C. (2017). Caffeine Induces a Stimulant Effect and Increases Dopamine Release in the Nucleus Accumbens Shell Through the Pulmonary Inhalation Route of Administration in Rats. *Neurotoxicity Research*, *31*(1), 90–98. https://doi.org/10.1007/s12640-016-9667-8

Garnier, L. M., Arria, A. M., Caldeira, K. M., Vincent, K. B., O'Grady, K. E., & Wish, E. D. (2010). Sharing and selling of prescription medications in a college student sample. *The Journal of Clinical Psychiatry*, *71*(3), 262–269. https://doi.org/10.4088/JCP.09m05189ecr

Gutierrez, A., Nguyen, J. D., Creehan, K. M., Javadi-Paydar, M., Grant, Y., & Taffe, M. A. (2022). Effects of combined THC and heroin vapor inhalation in rats. *Psychopharmacology*, *239*(5), 1321–1335. https://doi.org/10.1007/s00213-021-05904-w

H, Ü. S. (2019). A Schematic Overview of Addiction: Molecular Effects of Cocaine, Methamphetamine and Morphine on Limbic Neurons. *Forensic Science & Addiction Research*, *4*(4), 1–11.

Hart, J. L., Walker, K. L., Sears, C. G., Lee, A. S., Ridner, S. L., & Keith, R. J. (2018a). E-cigarette use and perceived health change: Better health through vaping? *Tobacco Induced Diseases*, *16*, 48. https://doi.org/10.18332/tid/95218

Hart, J. L., Walker, K. L., Sears, C. G., Lee, A. S., Ridner, S. L., & Keith, R. J. (2018b). E-cigarette use and perceived health change: Better health through vaping? *Tobacco Induced Diseases*, *16*, 48. https://doi.org/10.18332/tid/95218

Hartmann-Boyce, J., Begh, R., & Aveyard, P. (2018). Electronic cigarettes for smoking cessation. *BMJ (Clinical Research Ed.)*, *360*, j5543. https://doi.org/10.1136/bmj.j5543

Hassan, S. F., Wearne, T. A., Cornish, J. L., & Goodchild, A. K. (2016a). Effects of acute and chronic systemic methamphetamine on respiratory, cardiovascular and metabolic function, and cardiorespiratory reflexes. *The Journal of Physiology*, *594*(3), 763–780. https://doi.org/10.1113/JP271257

Hassan, S. F., Wearne, T. A., Cornish, J. L., & Goodchild, A. K. (2016b). Effects of acute and chronic systemic methamphetamine on respiratory, cardiovascular and metabolic function, and cardiorespiratory reflexes. *The Journal of Physiology*, *594*(3), 763–780. https://doi.org/10.1113/JP271257

Herman, T. F., & Santos, C. (2022). First Pass Effect. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK551679/

How do I electronically vape meth? (n.d.). Quora. Retrieved July 19, 2022, from https://www.quora.com/How-do-I-electronically-vape-meth

Hutchinson, A. J., Ma, J., Liu, J., Hudson, R. L., & Dubocovich, M. L. (2014). Role of MT1 melatonin receptors in methamphetamine-induced locomotor sensitization in C57BL/6 mice. *Psychopharmacology*, *231*(1), 257–267. https://doi.org/10.1007/s00213-013-3228-0

Jaffe, A. B., Sharpe, L. G., & Jaffe, J. H. (1989). Rats self-administer sufentanil in aerosol form. *Psychopharmacology*, *99*(3), 289–293. https://doi.org/10.1007/BF00445545

Jardim, M. C., Nogueira, R. L., Graeff, F. G., & Nunes-de-Souza, R. L. (1999). Evaluation of the elevated T-maze as an animal model of anxiety in the mouse. *Brain Research Bulletin*, *48*(4), 407–411. https://doi.org/10.1016/s0361-9230(99)00018-0

Javadi-Paydar, M., Kerr, T. M., Harvey, E. L., Cole, M., & Taffe, M. A. (2019). Effects of nicotine and THC vapor inhalation administered by an electronic nicotine delivery system (ENDS) in male rats. *Drug and Alcohol Dependence*, *198*, 54–62. https://doi.org/10.1016/j.drugalcdep.2019.01.027

Jerzyński, T., Stimson, G. V., Shapiro, H., & Król, G. (2021a). Estimation of the global number of e-cigarette users in 2020. *Harm Reduction Journal*, *18*(1), 109. https://doi.org/10.1186/s12954-021-00556-7

Jerzyński, T., Stimson, G. V., Shapiro, H., & Król, G. (2021b). Estimation of the global number of e-cigarette users in 2020. *Harm Reduction Journal*, *18*(1), 109. https://doi.org/10.1186/s12954-021-00556-7

Juarez-Portilla, C., Kim, R. D., Robotham, M., Tariq, M., Pitter, M., LeSauter, J., & Silver, R. (2017). Voluntary inhalation of methamphetamine: A novel strategy for studying intake non-invasively. *Psychopharmacology*, *234*(5), 739–747. https://doi.org/10.1007/s00213-016-4510-8

Kelly, M. A., Low, M. J., Rubinstein, M., & Phillips, T. J. (2008). Role of dopamine D1-like receptors in methamphetamine locomotor responses of D2 receptor knockout mice. *Genes, Brain, and Behavior*, *7*(5), 568–577. https://doi.org/10.1111/j.1601-183X.2008.00392.x

Kimmel, D. B. (2007). Mechanism of action, pharmacokinetic and pharmacodynamic profile, and clinical applications of nitrogen-containing bisphosphonates. *Journal of Dental Research*, *86*(11), 1022–1033. https://doi.org/10.1177/154405910708601102

Kimmel, H. L., O'Connor, J. A., Carroll, F. I., & Howell, L. L. (2007). Faster onset and dopamine transporter selectivity predict stimulant and reinforcing effects of cocaine analogs in squirrel

monkeys. *Pharmacology, Biochemistry, and Behavior*, *86*(1), 45–54. https://doi.org/10.1016/j.pbb.2006.12.006

Komada, M., Takao, K., & Miyakawa, T. (2008). Elevated plus maze for mice. *Journal of Visualized Experiments: JoVE*, *22*, 1088. https://doi.org/10.3791/1088

Kraeuter, A.-K., Guest, P. C., & Sarnyai, Z. (2019). The Elevated Plus Maze Test for Measuring Anxiety-Like Behavior in Rodents. *Methods in Molecular Biology (Clifton, N.J.)*, *1916*, 69–74. https://doi.org/10.1007/978-1-4939-8994-2_4

Krakowiak, R. I., Poklis, J. L., & Peace, M. R. (2019). The Analysis of Aerosolized Methamphetamine From E-cigarettes Using High Resolution Mass Spectrometry and Gas Chromatography Mass Spectrometry. *Journal of Analytical Toxicology*, *43*(8), 592–599. https://doi.org/10.1093/jat/bkz067

Lallai, V., Chen, Y.-C., Roybal, M. M., Kotha, E. R., Fowler, J. P., Staben, A., Cortez, A., & Fowler, C. D. (2021). Nicotine e-cigarette vapor inhalation and self-administration in a rodent model: Sex- and nicotine delivery-specific effects on metabolism and behavior. *Addiction Biology*, *26*(6), e13024. https://doi.org/10.1111/adb.13024

Li, Y., Burns, A. E., Tran, L. N., Abellar, K. A., Poindexter, M., Li, X., Madl, A. K., Pinkerton, K. E., & Nguyen, T. B. (2021). Impact of e-Liquid Composition, Coil Temperature, and Puff Topography on the Aerosol Chemistry of Electronic Cigarettes. *Chemical Research in Toxicology*, *34*(6), 1640–1654. https://doi.org/10.1021/acs.chemrestox.1c00070

Lin, L. Y., Di Stefano, E. W., Schmitz, D. A., Hsu, L., Ellis, S. W., Lennard, M. S., Tucker, G. T., & Cho, A. K. (1997). Oxidation of methamphetamine and methylenedioxymethamphetamine by CYP2D6. *Drug Metabolism and Disposition: The Biological Fate of Chemicals*, *25*(9), 1059–1064.

Marusich, J. A., Lefever, T. W., Blough, B. E., Thomas, B. F., & Wiley, J. L. (2016). Pharmacological effects of methamphetamine and alpha-PVP vapor and injection. *Neurotoxicology*, *55*, 83–91. https://doi.org/10.1016/j.neuro.2016.05.015

Meng, Y., Dukat, M., Bridgen, D. T., Martin, B. R., & Lichtman, A. H. (1999a). Pharmacological effects of methamphetamine and other stimulants via inhalation exposure. *Drug and Alcohol Dependence*, *53*(2), 111–120. https://doi.org/10.1016/s0376-8716(98)00120-3

Meng, Y., Dukat, M., Bridgen, D. T., Martin, B. R., & Lichtman, A. H. (1999b). Pharmacological effects of methamphetamine and other stimulants via inhalation exposure. *Drug and Alcohol Dependence*, *53*(2), 111–120. https://doi.org/10.1016/s0376-8716(98)00120-3

Mihalčíková, L., Ochozková, A., & Šlamberová, R. (2019). Effect of methamphetamine exposure on sexual behavior and locomotor activity of adult male rats. *Physiological Research*, *68*(Suppl 3), S339–S346. https://doi.org/10.33549/physiolres.934357

Milesi-Hallé, A., Hambuchen, M. D., McMillan, D. E., & Michael Owens, S. (2015). The pharmacokinetics of methamphetamine self-administration in male and female rats. *Drug and Alcohol Dependence*, *150*, 164–169. https://doi.org/10.1016/j.drugalcdep.2015.02.032

Milesi-Hallé, A., Hambuchen, M. D., McMillan, D. E., & Owens, S. M. (2015). The Pharmacokinetics of Methamphetamine Self-Administration in Male and Female Rats. *Drug and Alcohol Dependence*, *150*, 164–169. https://doi.org/10.1016/j.drugalcdep.2015.02.032

Mueller, K., Kunko, P. M., Whiteside, D., & Haskett, C. (1989). Time course of amphetamine-induced locomotor stereotypy in an open field. *Psychopharmacology*, *99*(4), 501–507. https://doi.org/10.1007/BF00589899

Mulder, H. A., Patterson, J. L., Halquist, M. S., Kosmider, L., Turner, J. B. M., Poklis, J. L., Poklis, A., & Peace, M. R. (2019a). The Effect of Electronic Cigarette User Modifications and E-liquid Adulteration on the Particle Size Profile of an Aerosolized Product. *Scientific Reports*, *9*(1), 10221. https://doi.org/10.1038/s41598-019-46387-2

Mulder, H. A., Patterson, J. L., Halquist, M. S., Kosmider, L., Turner, J. B. M., Poklis, J. L., Poklis, A., & Peace, M. R. (2019b). The Effect of Electronic Cigarette User Modifications and E-liquid Adulteration on the Particle Size Profile of an Aerosolized Product. *Scientific Reports*, *9*, 10221. https://doi.org/10.1038/s41598-019-46387-2

Mulder, H. A., Stewart, J. B., Blue, I. P., Krakowiak, R. I., Patterson, J. L., Karin, K. N., Royals, J. M., DuPont, A. C., Forsythe, K. E., Poklis, J. L., Poklis, A., Butler, S. N., Turner, J. B. M., & Peace, M. R. (2020). Characterization of E-cigarette coil temperature and toxic metal analysis by infrared temperature sensing and scanning electron microscopy—Energy-dispersive X-ray. *Inhalation Toxicology*, *32*(13–14), 447–455. https://doi.org/10.1080/08958378.2020.1840678

National Youth Tobacco Survey. 12th grade use in the past 30 days from 1999 to 2019. (n.d.). https://tobacco21.org/the-juul-epidemic/

Nazari, A., Perez-Fernandez, C., Flores, P., Moreno, M., & Sánchez-Santed, F. (2020). Age-dependent effects of repeated methamphetamine exposure on locomotor activity and attentional function in rats. *Pharmacology, Biochemistry, and Behavior*, *191*, 172879. https://doi.org/10.1016/j.pbb.2020.172879

Nguyen, J. D., Aarde, S. M., Cole, M., Vandewater, S. A., Grant, Y., & Taffe, M. A. (2016a). Locomotor Stimulant and Rewarding Effects of Inhaling Methamphetamine, MDPV, and Mephedrone via Electronic Cigarette-Type Technology. *Neuropsychopharmacology: Official Publication of the*

American College of Neuropsychopharmacology, *41*(11), 2759–2771. https://doi.org/10.1038/npp.2016.88

Nguyen, J. D., Aarde, S. M., Cole, M., Vandewater, S. A., Grant, Y., & Taffe, M. A. (2016b). Locomotor Stimulant and Rewarding Effects of Inhaling Methamphetamine, MDPV, and Mephedrone via Electronic Cigarette-Type Technology. *Neuropsychopharmacology*, *41*(11), 2759–2771. https://doi.org/10.1038/npp.2016.88

Nguyen, J. D., Aarde, S. M., Vandewater, S. A., Grant, Y., Stouffer, D. G., Parsons, L. H., Cole, M., & Taffe, M. A. (2016). Inhaled delivery of Δ9-tetrahydrocannabinol (THC) to rats by e-cigarette vapor technology. *Neuropharmacology*, *109*, 112–120. https://doi.org/10.1016/j.neuropharm.2016.05.021

Nicotine e-cigarette vapor inhalation and self-administration in a rodent model: Sex- and nicotine delivery-specific effects on metabolism and behavior—Lallai—2021—Addiction Biology—Wiley Online Library. (n.d.). Retrieved July 19, 2022, from https://onlinelibrary-wiley-com.proxy.library.vcu.edu/doi/10.1111/adb.13024

Ortman, H. A., Newby, M. L., Acevedo, J., & Siegel, J. A. (2021). The acute effects of multiple doses of methamphetamine on locomotor activity and anxiety-like behavior in adolescent and adult mice. *Behavioural Brain Research*, *405*, 113186. https://doi.org/10.1016/j.bbr.2021.113186

Poklis, J. L., Wolf, C. E., & Peace, M. R. (2017a). Ethanol concentration in 56 refillable electronic cigarettes liquid formulations determined by headspace gas chromatography with flame ionization detector (HS-GC-FID). *Drug Testing and Analysis*, *9*(10), 1637–1640. https://doi.org/10.1002/dta.2193

Poklis, J. L., Wolf, C. E., & Peace, M. R. (2017b). Ethanol Concentration in 56 Refillable Electronic Cigarettes Liquid Formulations Determined by Headspace Gas Chromatography with Flame Ionization Detector (HS-GC-FID). *Drug Testing and Analysis*, *9*(10), 1637–1640. https://doi.org/10.1002/dta.2193

Prakash, M. D., Tangalakis, K., Antonipillai, J., Stojanovska, L., Nurgali, K., & Apostolopoulos, V. (2017). Methamphetamine: Effects on the brain, gut and immune system. *Pharmacological Research*, *120*, 60–67. https://doi.org/10.1016/j.phrs.2017.03.009

PubChem. (n.d.-a). *Methamphetamine*. Retrieved August 5, 2022, from https://pubchem.ncbi.nlm.nih.gov/compound/10836

PubChem. (n.d.-b). *Nicotine*. Retrieved August 5, 2022, from https://pubchem.ncbi.nlm.nih.gov/compound/89594

Pulvers, K., Nollen, N. L., Rice, M., Schmid, C. H., Qu, K., Benowitz, N. L., & Ahluwalia, J. S. (2020). Effect of Pod e-Cigarettes vs Cigarettes on Carcinogen Exposure Among African American and Latinx Smokers: A Randomized Clinical Trial. *JAMA Network Open*, *3*(11), e2026324. https://doi.org/10.1001/jamanetworkopen.2020.26324

Rau, J. L. (2005). The inhalation of drugs: Advantages and problems. *Respiratory Care*, *50*(3), 367–382.

Rau, T., Ziemniak, J., & Poulsen, D. (2016). The neuroprotective potential of low-dose methamphetamine in preclinical models of stroke and traumatic brain injury. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *64*, 231–236. https://doi.org/10.1016/j.pnpbp.2015.02.013

Rom, O., Pecorelli, A., Valacchi, G., & Reznick, A. Z. (2015). Are E-cigarettes a safe and good alternative to cigarette smoking? *Annals of the New York Academy of Sciences*, *1340*(1), 65–74. https://doi.org/10.1111/nyas.12609

Sakagami, M., Kinoshita, W., Sakon, K., & Makino, Y. (2003). Fractional contribution of lung, nasal and gastrointestinal absorption to the systemic level following nose-only aerosol exposure in rats: A case study of 3.7-µm fluorescein aerosols. *Archives of Toxicology*, *77*(6), 321–329. https://doi.org/10.1007/s00204-003-0450-2

Schindler, C. W., Thorndike, E. B., Blough, B. E., Tella, S. R., Goldberg, S. R., & Baumann, M. H. (2014). Effects of 3,4-methylenedioxymethamphetamine (MDMA) and its main metabolites on cardiovascular function in conscious rats. *British Journal of Pharmacology*, *171*(1), 83–91. https://doi.org/10.1111/bph.12423

Shelton, K. L., & Nicholson, K. L. (2022). Reinforcing effects of fentanyl and sufentanil aerosol puffs in rats. *Psychopharmacology*. https://doi.org/10.1007/s00213-022-06129-1

Son, Y., Wackowski, O., Weisel, C., Schwander, S., Mainelis, G., Delnevo, C., & Meng, Q. (2018). Evaluation of E-Vapor Nicotine and Nicotyrine Concentrations under Various E-Liquid Compositions, Device Settings, and Vaping Topographies. *Chemical Research in Toxicology*, *31*(9), 861–868. https://doi.org/10.1021/acs.chemrestox.8b00063

Species Specific Information: Rat. (n.d.). Retrieved August 6, 2022, from https://web.jhu.edu/animalcare/procedures/rat.html

Taffe, M. A., Nguyen, J. D., Vandewater, S. A., Grant, Y., & Dickerson, T. J. (2021a). Effects of α-pyrrolidino-phenone cathinone stimulants on locomotor behavior in female rats. *Drug and Alcohol Dependence*, *227*, 108910. https://doi.org/10.1016/j.drugalcdep.2021.108910

Taffe, M. A., Nguyen, J. D., Vandewater, S. A., Grant, Y., & Dickerson, T. J. (2021b). Effects of α-pyrrolidino-phenone cathinone stimulants on locomotor behavior in female rats. *Drug and Alcohol Dependence*, *227*, 108910. https://doi.org/10.1016/j.drugalcdep.2021.108910

The neuroprotective potential of low-dose methamphetamine in preclinical models of stroke and traumatic brain injury. (2016). *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *64*, 231–236. https://doi.org/10.1016/j.pnpbp.2015.02.013

Trends in U.S. methamphetamine use and associated deaths. (2021, October 4). National Institutes of Health (NIH). https://www.nih.gov/news-events/nih-research-matters/trends-us-methamphetamine-use-associated-deaths

Ueno, H., Takahashi, Y., Suemitsu, S., Murakami, S., Kitamura, N., Wani, K., Matsumoto, Y., Okamoto, M., & Ishihara, T. (2020). Caffeine inhalation effects on locomotor activity in mice. *Drug Development and Industrial Pharmacy*, *46*(5), 788–794. https://doi.org/10.1080/03639045.2020.1753064

UPENDS. (n.d.). *Meth Vape Pen. A 2021 Review of the Meth Vaporizer*. UPENDS. Retrieved July 19, 2022, from https://www.upends.com/blogs/articles/meth-vape-pen-a-2021-review-of-the-meth-vaporizer

Vaporizing—How to use a vaporizer to smoke meth! (n.d.). Drugs-Forum. Retrieved July 19, 2022, from https://drugs-forum.com/threads/how-to-use-a-vaporizer-to-smoke-meth.280002/

Wearne, T. A., Mirzaei, M., Franklin, J. L., Goodchild, A. K., Haynes, P. A., & Cornish, J. L. (2015). Methamphetamine-induced sensitization is associated with alterations to the proteome of the prefrontal cortex: Implications for the maintenance of psychotic disorders. *Journal of Proteome Research*, *14*(1), 397–410. https://doi.org/10.1021/pr500719f

Why Some Meth and DMT Users Are Using Vapes. (n.d.). Retrieved July 19, 2022, from https://www.vice.com/en/article/a3qbyj/why-some-meth-and-dmt-users-are-using-vapes)

Yasaei, R., & Saadabadi, A. (2022). Methamphetamine. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK535356/

Yoshida, K., Morimoto, A., Makisumi, T., & Murakami, N. (1993). Cardiovascular, thermal and behavioral sensitization to methamphetamine in freely moving rats. *The Journal of Pharmacology and Experimental Therapeutics*, *267*(3), 1538–1543.

Zervas, E., Litsiou, E., Konstantopoulos, K., Poulopoulos, S., & Katsaounou, P. (2018). Physical characterization of the aerosol of an electronic cigarette: Impact of refill liquids. *Inhalation Toxicology*, *30*(6), 218–223. https://doi.org/10.1080/08958378.2018.1500662

Vitae

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