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The Effect Of Gaba A Antagonism On Locomotor Activity And Dopamine Release In The Mouse Caudate Putamen Following Acute Toluene Inhalation: An In-Vivo Microdialysis Study

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THE EFFECT OF GABA A ANTAGONISM ON LOCOMOTOR ACTIVITY AND DOPAMINE RELEASE IN THE MOUSE CAUDATE PUTAMEN FOLLOWING ACUTE TOLUENE INHALATION: AN IN-VIVO MICRODIALYSIS STUDY

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DEDICATION

This thesis is dedicated to my parents, who would settle for nothing less from a son, and to my wife Jade, obviously.

ACKNOWLEDGEMENTS

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1.0 Chapter One: Introduction

The deliberate inhalation of volatile substances with the intention of intoxication is one of the most under-appreciated and under-researched substance abuse concerns in the world (Cruz, 2011; Cruz and Bowen, 2008; Howard *et al.*, 2011). This is despite the highly dangerous and volatile nature of solvents, and the risk of serious neurological harm or death that can result from such abuse (Bowen, *et al.*, 1999; Butland *et al.*, 2012). The scientific study of inhalant abuse is complicated by the diverse chemical nature of inhalants, which comprise several distinct chemical groups (see Table 1) with numerous different molecular structures (see Bowen and Cruz, 2014 for a review). The aromatic hydrocarbon toluene (methylbenzene), found in a variety of household and industrial products, is one of the most ubiquitous substances with thousands of industrial and commercial products containing some level of this solvent (Hass, 1999) including airplane glue, gasoline, paint thinner, spray paint, and sealant. Estimates of toluene production per year are in the millions of tons, with upwards of a third of that amount being used in commercial products (IARC, 1989; ATSDR, 2000).

1.1 Inhalants

Inhalants are comprised of an extensive category of abused compounds. This group is comprised of compounds that are volatile at room temperature. It should be noted therefore that drugs which are inhaled but are not volatile at room temperature (e.g., cocaine, tobacco, marihuana, etc.) are not considered to be inhalants. Many substances can be included in this definition of inhalants and, as such, several classification schemes can be used. If we classify inhalants based

on the commercial products that contain them, we can distinguish categories such as adhesives, aerosols, cleaning agents, solvents and odorizers, among others.

Table 1. Commonly misused inhalants. Adapted from Bowen and Cruz (2012).

Group	**Characteristics**	**Effects**	**Commercial Products**
Alkyl-nitrites	A nitrite group	Vasodilation, smooth muscle relaxation	"Poppers" Room Odorizers
Nitrous Oxide	Inorganic anesthetic gas / propellant	CNS depression, hilarity, analgesia, anesthesia	Whipped dairy cream chargers, nitrous oxide tanks
Other Anesthetics	Gases or volatile liquids	Relaxation, numbness analgesia, anesthesia, respiratory depression	Anesthesia bottles (ether, Halothane®, Isoflurane®, Desflurane®)
Propellants-fuels	Flammable gases that are liquefied under pressure	Relaxation, hallucinations, illusions	Cigarette lighters, cooking fuels, hairsprays, central air conditioning units
Solvents	Liquids that dissolve other substances without any change in chemical composition	Initial disinhibition followed by a more prolonged depression. Illusions, hallucinations.	Paints, paint thinners, glues, inks, lacquers, varnish removers, degreaser agents, spot removers, motor fuels

Solvents (e.g., toluene) as a class of chemicals are especially noted for their high level of solubility. The high lipid solubility displayed by solvents results in these compounds easily absorbing into bodily tissues. Toluene, for example, is absorbed efficiently by lung, fat, and brain tissue and in high concentrations is known to produce "intoxication" (Cruz, 2011). Given how widely available and cheap products containing toluene are it is no surprise that toluene-based inhalants are prime targets for recreational misuse.

1.2. Epidemiology of Inhalant Abuse

The abuse of volatile substances, such as toluene, is a worldwide phenomenon of considerable importance (Dell *et al.*, 2011; Medina-Mora and Real, 2008). According to SAMHSA's National Survey on Drug Use and Health, it has been estimated that, among United States residents over the age of 12, 8% admit to deliberately misusing an inhalant at least once, and of those individuals who began using illicit drugs for the first time within the past month, 7.5% chose to abuse inhalants (SAMHSA, 2012). Furthermore, in 2011 inhalants were the fifth most initiated illicit drug amongst United States residents over the age of 12, ahead of both cocaine and heroin (SAMHSA, 2012). Inhalant abuse is very common among adolescents and pre-adolescents with 14.9% of eighth graders admitting to having deliberately inhaled something in order to get high at least once in their lives. This places inhalant abuse as the second most commonly abused substance in this age group behind cannabis inhalation (Bowen 2011).

The problem of inhalant abuse begins early. According to the results of the 2007 Monitoring the Future National Survey, over half of students who admit to misusing an inhalant did so for the first time by the end of their first year in high school (Johnston, *et al.*, 2007). However, evidence from the same survey suggests that, of high school individuals who admit to misusing an inhalant, half had not done so in the past year. Thus, individuals who experiment with inhalant misuse at a young age generally do not continue to regularly abuse the compound. However, it is also possible that abuse continues and the children simply drop out of school. The number of users is estimated to increase at a rate exceeding three-quarters of a million individuals every year (Howard *et al.*, 2011). Evidence also suggests, that among high school students, the perception that inhalant abuse is harmful is decreasing (Williams and Storck, 2007), which is an indicator of the risk for future increases in inhalant abuse amongst high school students.

Initially, research suggested that inhalant misuse (toluene misuse in particular) was more likely among adolescent boys than girls. However, recent survey data has suggested that this gap has closed and there is now almost no sex difference in the prevalence of deliberate solvent abuse (SAMHSA, 2007). The above evidence produces a disheartening picture of inhalant abuse. According to a recent review of the morbidity and mortality of inhalant abuse, 22 % of individuals found dead of an inhalant-related consequence had no history of prior use of the inhalant itself, indicating that almost a quarter of new (or, at least, relatively new) users face death (Williams and Storck, 2007). In addition, Bennett *et al.* (2000) suggests that adolescent or pre-adolescent inhalant abuse doubles the rate of adulthood binge drinking and significantly increases "hard drug" abuse in adult college students, supporting the notion that toluene and other inhalants act as "gateway" drugs and that the life-damaging consequences of inhalant abuse do not end when the individual stops experimenting with the substance.

1.3. Methods of Inhalant Abuse

Inhalation of these chemicals is not complicated and several methods exist for quickly achieving high blood/tissue concentrations following self-administration. Among the many methods of abuse is to spray or pour a judicious amount of the substance into a container, such as a paper/plastic bag or a balloon, and then to breathe deeply from the container (Bowen, 2011). Other methods of abuse include placing objects such as permanent markers, directly under the nose and inhaling the contents ("sniffing"), painting the product onto the fingernails and raising the fingernails to the nose and/or mouth ("nailing"), inhaling the product directly from the container ("sniffing" or "snorting"), soaking cotton balls, cotton swabs or cloth and placing them into the nose or mouth ("soaking"), turning a spray can upside down and spraying the aerosolized compounds directly into the nose and/or mouth ("spraying") and "huffing" which is soaking a

cloth with a solvent, such as toluene, and inhaling the fumes that arise therein (Flanagan *et al.*, 1990; Cruz and Bowen, 2008). With each of these procedures, the individual deeply inhales the high concentrations of the desired solvent. The behavioral effects occur very rapidly, usually within just a few seconds (Cruz and Bowen, 2008). Evidence suggests that nearly 80% of toluene vapor inhaled is absorbed into the bloodstream through the lungs (due to the high lipid solubility noted above) with the intoxicating effects continuing for up to 60 min, making inhalant abuse an extremely effective form of drug abuse (reviewed in Donald *et al.*, 1991).

1.4. Effects of Inhalant Abuse

Like many psychoactive compounds, toluene and other solvents are known to have pronounced behavioral effects in both humans and animals (for review, see Bowen *et al.*, 2006; Bowen and Cruz, 2014; Cruz and Bowen, 2008). Individuals who have abused toluene report that initial or subjectively low-level exposure to this solvent produces ethanol-like disinhibition and euphoria along with the sensation of lightheadedness (Cruz, 2011; Siegel *et al.*, 2009; Cruz and Bowen, 2008; Henretig, 1996). Despite the similarity to ethanol, toluene and other solvents appear to be much more potent than ethanol, and therefore require much lower concentrations to produce behaviorally meaningful effects (Cruz, 2011). As the process of inhalant abuse is so heavily reliant on personal experimentation, and allowing humans to voluntarily administer toluene in a laboratory setting is unethical, it is difficult to estimate an inhaled concentration of a "typical" human abuse episode. As such, most dosimetric information about toluene abuse comes from animal studies. In a further similarity to ethanol, individuals exposed to higher solvent concentrations (in excess of 12,000 ppm) report ataxia, confusion, slurred speech, shallow breathing, tiredness, and slowed reflexes (reviewed by Bowen, 2011). Extremely high concentrations of toluene (> 22,000 ppm) have been observed to cause respiratory suppression

leading to death, similar to what is observed in ethanol overdose (Moser and Balster, 1985). In addition, solvents have been observed in both animal and human studies to have a variety of pseudo-beneficial effects (so-called because the deleterious effects of solvent misuse far outweigh any incidental benefits) including anxiolytic, antidepressant and anesthetic effects, making solvent misuse a popular and readily available method of "self-medicating" for mood and anxiety disorders (Cruz, 2011).

This higher potency is especially problematic with solvents as they do not have pre-standardized doses for effective and/or safe use. An individual set upon "huffing" a particular commercial product generally has to rely on word of mouth as to the strength of product they are about to inhale (e.g., anecdotal rumor suggests that gold spray paint produces a more intense "high" than other colors). This lack of standardization for what dose accomplishes what effect, coupled with the imprecise methods of administration (e.g., a "bag full" of paint thinner is hardly an exact measurement), means that accidental overdoses are alarmingly easy (e.g., "Sudden Sniffing Death;" Bowen, 2011). In fact, acute exposure to solvents is known to cause an increase in the sensitivity of the heart to the effects of the so-called "fight or flight" hormones (i.e., adrenaline and noradrenaline), meaning that even a single bout of toluene abuse can result in heart failure and death (Cruz, 2011).

1.5. Neurochemical mechanisms of action

Toluene, like many psychoactive compounds, has wide action in the brain with c-Fos data suggesting that toluene administration increases activity in a large number of distinct brain regions, including the ventral tegmental area (VTA), nucleus accumbens (NAc), amygdala, caudate putamen (CPu), hypothalamus, and cingulate cortex (Perit *et al.*, 2012; Tomaszycki et al.*et al.*,

2013). Additionally, inhalation of abuse levels of toluene vapor is known to change brain neurotransmitter dynamics, particularly in the dopamine (DA) system. However, the neurochemical mechanisms that underlie toluene intoxication are not yet fully understood (Yavich & Zvartau, 1994; Funada *et al.*, 2002; Bowen *et al.*, 1999). For example, Riegel *et al.* (2007) found that directly administering toluene into the VTA resulted in an increase in DA release in the nucleus NAc, while Stengard *et al.* (1994) found that acutely inhaled toluene increased DA levels in the striatum CPu. This evidence is corroborated by further research showing that sub-chronic injections of toluene (600 mg/kg, i.p.) increased levels of DA present in the CPu and the NAc ex-vivo and that the increased DA found in the CPu was present for several hours after the last toluene treatment (Riegel *et al.*, 2004). The *in-vivo* microdialysis technique has seen limited use in a repeated measures design, and no use in a chronic abuse paradigm. However, Beyer *et al.* (2001) demonstrated that repeated exposure to 8000 ppm toluene increases DA levels in the NAc following a cocaine challenge. The impact of toluene on the DA system is also supported by evidence that toluene may be a D_2 receptor agonist. There is evidence that the effect of toluene on behavioral activity is at least in part mediated by the D_2 receptor, as pretreatment with 5mg/kg (i.p.) remoxipride (a D_2 antagonist) reduces the locomotor stimulating effects of toluene injections (600 -1200 mg/kg i.p.; Riegel and French, 1999).

However, the evidence for the impact of toluene abuse on the DA system is not necessarily clear-cut. Kondo *et al.* (1995) found no effect of acutely injected toluene on DA levels in the striatum, inferring that toluene does not act upon DA metabolism in the ventral striatum. Likewise, Gerasimov *et al.* (2002) did not find an effect of acute toluene alone on DA levels in the NAc, though they did find a super-additive effect of toluene and cocaine. These conflicting reports are

particularly important, as microdialysis remains a highly utilized technique for categorizing neurochemical effects.

While DA is of primary importance for the abuse potential of inhaled toluene, research has also suggested that toluene exerts its effects on other neurotransmitters. For example, antagonism of $GABA_A$ receptor with bicuculline has been shown to block the hypothermia induced by acutely inhaled toluene (Paez-Martinez *et al.*, 2013). Likewise, *in vitro* research has suggested that toluene administration enhances $GABA_A$ receptor function, reversibly increasing synaptic currents at those sites (Beckstead *et al.*, 2000). Bale *et al.* (2005) found that repeated *in vitro* application of toluene to neurons increased NMDA current amplitude but decreased the amplitude of GABA-activated currents. This pattern of application is similar to what has been observed with ethanol administration. When taken together, these studies implicate $GABA_A$ as a potential target for toluene's action in the brain. However, even here the effect of toluene is far from clear. O'Leary-Moore *et al.* (2007) reported that acutely administered toluene reduced hippocampal GABA levels in juvenile rats. These effects, when taken together, suggest that GABA itself is a promising target for research.

In addition, *in vitro* evidence suggests that toluene also has an inhibitory action upon the glutamate system, specifically NMDA receptors (Bale *et al.*, 2005). The same study demonstrated that repeated *in vitro* exposure to toluene resulted in up-regulation of NMDA sub-receptors N2A and N2B, suggesting that the action of toluene upon NMDA is inhibitory at those binding sites. However, mice lacking the calcium/calmodulin adenylyl cyclases 1 and 8 (important plasticity-related second messenger proteins thought to be associated with intracellular actions of NMDA receptors) still show increased ambulatory activity in response to acutely administered toluene vapor (Conti *et al.*, 2012). This suggests that calcium-related adenylyl cyclases (and by extension,

NMDA) function is not implicated in toluene-induced locomotion. Likewise, acute and repeated administration of toluene appears to have anticonvulsant properties such that mice that have been exposed to toluene are resistant to NMDA-induced seizures (Cruz, *et al.*, 2003). This further suggests that toluene has NMDA antagonist properties.

In vivo microdialysis is a sampling method where extracellular neurotransmitter levels can be examined. Several studies have attempted to simultaneously use *in vivo* microdialysis during their toluene abuse models (e.g., Riegel et al, 2007; Gerasimov et al, 2002; Stengard et al, 1993; Stengard et al, 1994). In addition, an established technique for *in vivo* microdialysis is infusing high concentration of potassium to chemically induce action potentials via depolarization of the cell membrane (e.g., Casanova *et al.*, 2013; Fuentealba *et al.*, 2010). This chemical induction of action potentials can have a super-additive effect when paired with drugs of abuse (as is seen in Casanova *et al.*, 2013). Infusing a specific brain region with a compound through the microdialysis probe is referred to as "reverse-dialysis," and relies upon the principle that the drug goes down its concentration gradient, moving in the direction from high to low concentration from the probe to the brain (e.g., Farrar *et al.*, 2011; Mabrouk *et al.*, 2013). Additionally, reverse dialysis can be used to locally administer a receptor antagonist to a specific brain region without the need for a separate infusion or the cessation of sample collection (Mabrouk *et al.*, 2013).

A review of the current research has identified a need for additional investigation into the neurochemical systems involved with toluene abuse, specifically changes to DA and GABA levels following toluene intoxication. While evidence exists that toluene acts upon both DA and GABA, these findings are conflicted, and therefore require further investigation. Additionally, the effect of toluene inhalation on the DA and GABA relationship has not been examined and doing so would provide further information about the actions of toluene upon the brain as a whole. This is

especially important in the CPu, where DA and GABA comprise two of the primary neurotransmitters. Understanding not only the impact of toluene inhalation on DA in the CPu, but the role of toluene's action on GABA in this region would be highly instructive regarding toluene's effects on both drug-seeking behavior and increased locomotor activity. Based on the above research, it is logical to assume that inhalation of abuse levels of toluene vapor alters brain neurotransmitter dynamics (specifically DA), which in turn has implications both for cross-sensitization and susceptibility to drug abuse after repeated exposure.

Therefore, the purpose of this thesis was threefold. First, I aimed to establish a mouse model for *in vivo* microdialysis following exposure to volatilized solvents. This would allow for the eventual use of techniques and methods specific to mouse models, including the use of genetic models to achieve greater target specificity. Second, I aimed to use this model to examine the relationship between acutely inhaled toluene and DA levels in the CPu. Finally, I sought to explore the relationship between toluene's action on GABA and DA in the mouse CPu.

1.6. Hypotheses

The hypotheses for this study were as follows: First, acutely administered toluene would alter locomotor levels in a dose-dependent manner with 4000 ppm increasing overall activity and 8000 ppm depressing activity. Acutely inhaled toluene would also increase DA levels present in the CPu, indicating the importance of the CPu in the behavioral actions of toluene and identifying the CPu as a target for further neurochemical investigation. Second, the acute administration of toluene would sensitize the neurons of the CPu to further stimulation such that treatment with high-potassium aCSF would increase DA levels, indicating that toluene exposure altered cellular sensitivity to external stimulation. Third, the increase in DA levels and locomotor

activity observed after exposure to 4000 ppm toluene would be attenuated by pretreatment with the $GABA_A$ antagonist bicuculline, suggesting that toluene's action on GABA is linked both to toluene's locomotor-enhancing effects and to toluene's action on extracellular DA.

2.0. Chapter Two: Methods

2.1. Subjects

Adult male Swiss Webster mice (N = 26, approximate postnatal day (PND) = 30), were used for this experiment. The mice were purchased from Harlan Breeding Laboratories (Portage, MI, USA). The Institutional Animal Care and Use Committee at Wayne State University approved all animal procedures and behavioral experiments which were in accordance with the NIH "Guide for the Care and Use of Laboratory Animals: Eighth edition" (National Academy of Sciences 2011, revised 2010). For this study, there were five groups which included three doses of toluene (0 ppm, 4000 ppm, and 8000 ppm) and two toluene-plus-drug conditions (4000 ppm toluene or air control, both with a 30 min pretreatment of 10 μM bicuculline with approximately N = 6 for each group). The selected dose of bicuculline was shown by prior research to be effective at locally antagonizing $GABA_a$ receptors using reverse dialysis (e.g., Farrar *et al.*, 2011 and Mabrouk *et al.*, 2013).

Prior to the microdialysis surgery, mice were housed in groups of 4 - 5 in polypropylene cages (18 cm x 29 cm x 13 cm) with hardwood chip bedding and steel-wire tops within a certified vivarium (Association for Assessment and Accreditation of Laboratory Animal Care; AAALAC) at Wayne State University. Mice were allowed *ad lib* access to food and water (Rodent Lab Diet 5001) in their home cages. The vivarium was temperature controlled (20 – 22 ^{o}C) and kept on a standard 12 hour light dark cycle with lights on at 0600 hrs. While in the vivarium, animal welfare was monitored by trained veterinary staff. The animals were transported from the vivarium in the basement of the Biological Sciences building to the adjacent Chemistry building to undergo

surgery, after which they were singly housed. After surgery recovery, the animals were transported back to Biological Sciences to undergo behavioral/neurochemical testing.

2.2. Surgery

After arrival to the vivarium, the animals were allowed to acclimate for one week before undergoing microdialysis surgery. Prior to surgery, all surfaces of the surgical area were cleaned and disinfected and the surgeon thoroughly scrubbed their hands prior to putting on sterile surgery garments (i.e., gloves, cap, mask, and lab coat). Prior to surgery, all instruments were sterilized using an autoclave or sterilized with a disinfectant (in the case of instruments like the drill that could not be autoclaved).

Animals were anesthetized with Isoflurane® until the animal was judged to have lost reflexive movement in response to a pinch to the webbing between the toes on the hind limb ("toe pinch test"). The animal was then transported from the anesthesia-induction chamber to the stereotaxic frame (David Kopf Instruments, Tujunga, CA) where it was placed upon a microwavable heating pad covered in paper towels. After the animal was affixed to the stereotaxic frame using two non-puncturing ear bars and a mouse palate adapter, tubing was placed on the nose of the animal and Isoflurane® and oxygen administration was continued to ensure proper anesthesia. The "toe pinch test" was re-administered prior to surgery. To protect the eyes of the mouse from dehydration during the surgical procedure, sterile artificial tear lubricant ophthalmic ointment was applied to the eyes. The fur covering the skull was removed with a commercial electric razor and a local analgesic (Lidocaine®) was applied in a circle around the surgery site. The surgical site was disinfected with Betadine and ethanol before the skin was incised to expose the skull. Hydrogen peroxide was then applied to the skull with a Q-tip®. This step was both to

ensure the removal of the membrane covering the skull and to highlight the Bregma and Lambda markings. Using a standard mouse atlas (Paxinos, 2001) the anterior-posterior and medial-lateral coordinates of the ventral portion of the striatum, specifically the CPu were located and marked. Once completed, a hole was drilled through the skull at the marked location. A sterile stainless steel guide cannula (CMA/7, 2mm) was then stereotaxically implanted unilaterally (right side) according to coordinates extrapolated from the mouse brain atlas. The coordinates (in mm) relative to the Bregma suture and the top of the skull were: AP +1, ML -1.6, DV -2.5. A second hole was drilled for a screw support. Dental cement was used to affix the cannula to the skull and hold it in place. The dental cement completely covered the exposed skull region so that no sutures or stitches were necessary. Each guide cannula was fitted with a dummy cannula that extended the length of the guide to keep it clear and free of debris. The animal was then placed in a heated recovery chamber and was monitored until independent locomotion was re-established. The animal was then individually housed until the beginning of microdialysis. The animals were allowed to recover for at least 72 hours before testing.

2.3. *In vivo* microdialysis measurements

Following recovery from surgery, the dummy cannula was removed and replaced with a CMA/7 dialysis probe 12 - 24 hours prior to beginning of the dialysis experiment. The cannula was perfused overnight with artificial cerebral spinal fluid (aCSF) consisting of 0.4 mM ascorbic acid, 126 mM NaCl, 2.5 mM KCl, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.2 mM NaH_2PO_4, 11 mM D-glucose, pH 7.4, according to procedures described previously (see Bosse *et al.*, 2011). During data collection, flow rates for the dialysis pump were set to 1.1 μL/min for the duration of collection. To measure changes in DA levels, eight 15-min baseline dialysis samples were collected before toluene exposure began, two 15-min fractions were collected during toluene

exposure, and four subsequent 15-min samples were collected immediately following toluene inhalation. Following the conclusion of this recovery period, aCSF was switched to an aCSF solution containing 60 mM potassium for one fraction (15 min) before being returned to normal aCSF for four more fractions to monitor the effect of the high-potassium stimulation. During the receptor antagonist study, bicuculline was mixed into aCSF at a 10 μM concentration and was perfused directly into the CPu for the 30 min prior to toluene administration to locally antagonize $GABA_A$ receptors within the CPu. After the 30 min of bicuculline-aCSF administration, regular aCSF was used for toluene administration and recovery. For a schematic of the exposure paradigm, see Fig. 1.

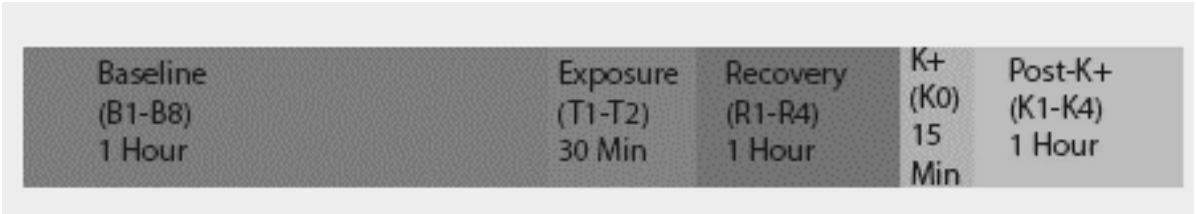

Figure 1: Schematic outline of the exposure paradigm. After collecting 8 baseline fractions, animals were exposed to 30 min of toluene, followed by 1 hour of recovery. Then, animals were exposed to high-potassium aCSF for 15 min before being observed for 1 hour.

2.4. Test Chemicals and Exposures

Testing consisted of a single 30 min exposure to toluene vapor, a duration chosen to be consistent with prior research by this lab (Tomaszycki *et al.*, 2013; Bowen and Hannigan, 2013). The two doses chosen for this experiment, 4000 ppm and 8000 ppm, constitute moderate to high doses of toluene that have been shown in previous studies to be behaviorally active doses (Conti *et al.*, 2012). The 10 μM dose of bicuculline used may appear high, but the diffusion of bicuculline

out of the semi-permeable membrane is very low (< 20% of the concentration administered), suggesting that the administered dose must be high to ensure a meaningful concentration reaches the brain. The dose used was based on prior published work with bicuculline administered via reverse dialysis (e.g. Zackheim and Abercrombie, 2005; Whitehead, *et al.*, 2001).

2.5. Solvent Exposure and Behavioral Testing

A dynamic exposure system similar to that described by Bowen and Balster (1996) was used for toluene exposure (see Fig. 2). The chamber consists of a 37.8 L glass rectangular tank (51cm x 28cm x 12cm, 1428cm^2 floor area) fitted with a Plexiglas® lid into which toluene was delivered in the airflow. The lid was equipped with 1.2 cm access ports located at opposite ends which allowed for delivery of toluene vapor as well as the collection of dialysis samples. The mouse was restricted to a 30.4 cm x 20.32 cm section of the chamber through the insertion of two Plexiglas® walls. The stand for the microdialysis swivel was fixed to one of the Plexiglas walls (affixed approximately 2.5 cm from the back wall of the chamber). This allowed the injection and collection tubes to exit the chamber through one of the portholes. The second Plexiglas® wall (affixed approximately 15.2 cm from the back wall) was perforated with 12 equally spaced 2.5 cm diameter holes to allow airflow to pass unobstructed throughout the chamber. The porthole through which toluene vapor was administered was located 7.6 cm from this second wall. Vapor generation occurred by initially directing air flow through a bubbler that was immersed in a 500-ml solvent bath contained in a 1-L round-bottom flask. Air saturated with vapor exited the bath and was mixed with filtered laboratory (fresh) air that was then delivered to the exposure chamber for 30 min (see Fig. 1). Flow rates through the exposure chambers were maintained at 10 L/min and toluene concentration was determined on line using a single wavelength monitoring infrared (IR) spectrometer (Miran 1A, Foxboro Analytical, North Haven, CT). At the end of the exposure

period, toluene exposure was discontinued and the toluene tubing was replaced with a suction tube to facilitate evacuation of toluene vapor from the chamber. The dynamic exposure system was housed under a fume hood, which also served to provide white background noise and isolation from the laboratory environment.

During the exposure period, animals were allowed to freely move around the interior of the chamber. Locomotor activity was measured within the dynamic exposure chamber via 3 sets of 16-beam infrared (I/R) emitter–detector arrays (Med Associates, St. Albans, VT) mounted on Plexiglas bases around the sides of the exposure chambers. Interruptions of I/R beams resulted in

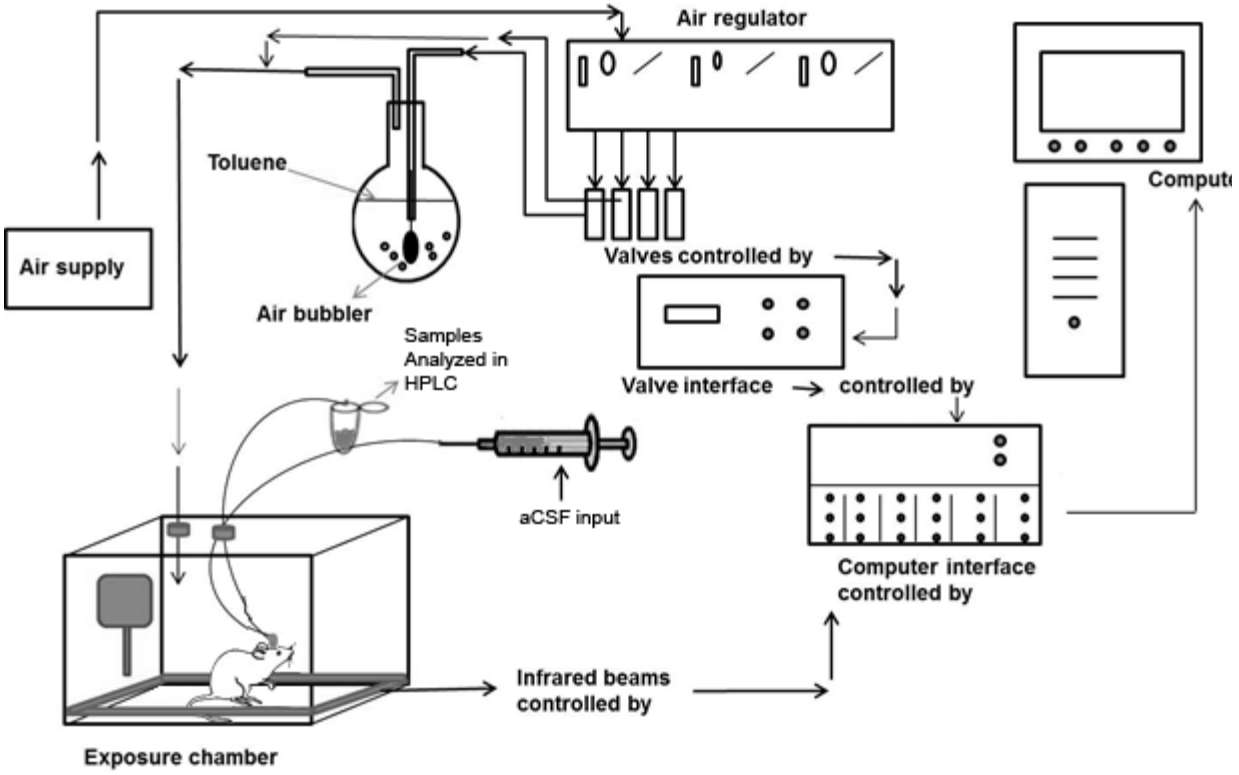

Figure 2: Schematic representation of the dynamic exposure system. Airflow from the air regulator is passed through the air bubbler filled with liquid toluene. The resulting toluene vapor is carried to the exposure chamber and mixed with clean air to control concentration and flow rate. The infrared beam grid is controlled by a computer interface that allows for real-time recording of locomotion.

an analog signal being recorded by automated activity software (Open Field Activity Software [SOF-811], Med Associates, St. Albans, VT). This system quantified total beam breaks in both the vertical and horizontal planes, specifically encoding measures of distance traveled (cm; calculated from number of breaks of adjacent beams) and number of rears (see Fig. 2). This automated measure of activity was transformed into 15-min blocks over the duration of the session.

2.6. Liquid Chromatography

All collected dialysate samples were stored in a - 80 ^{o} C freezer and analyzed within two weeks of collection. High performance liquid chromatograph (HPLC; LC-20AD pump; Shimadzu, Columbia, MD) was used to analyze samples by manually injecting 10 µL of dialysate samples into a 10 µL injection loop. The mobile phase that was used to help separate the analytes was the ESA MDTM mobile phase (which consisted of: 75 mM NaH_2PO_4, 3 mM 1-octanesulfonic acid, 0.125 mM EDTA, 9 % acetonitrile, and 0.2 - 0.5 % triethylamine; pH = 3.0) and was operated at a flow rate of 0.4 mL/min. The analytes were further separated using a C_{18} column (Luna 100 x 3 mm, C_{18}, 2.6 µm column; Phenomenex, Torrance, CA). DA was electrochemically detected using ESA 5014B microdialysis cell (E1 = -150 mV; E2 = +220 mV; ESA Coulochem III (Thermo-Fisher, Chelmsford, MA) with an in-line ESA 5020 guard cell (potential of guard cell: + 350 mv) positioned before the injection loop. Separation and quantification of the analytes on the HPLC system was controlled by LC Solutions Software (Shimadzu, Columbia, MD). The approximate retention time of DA was at approximately 7 - 8 min. Integration and quantification of DA peak area were performed against known concentrations of DA standards (0, 2.5, 5, and 10 nM).

2.7. Histological verification of microdialysis probe placement

After the conclusion of microdialysis testing, the mice were sacrificed using CO_2 narcosis. Their brains were then removed and stored in 3.7% formaldehyde solution until histology. During histology, brains were transferred to a solution containing 2% Cresyl violent dye and allowed to sit for approximately 18 hours. Brains were then removed and rinsed three times with 95% ethanol before being placed in a solution of 70% ethanol and water and set on a plate shaker for 3-4 hours to wash off excess dye. The solution was changed and replaced and the brains were left in 70% ethanol and allowed to shake overnight. The next day, the brains were rinsed again by letting them sit in 95% ethanol in water for 30 min followed by 45 min sitting in water. After this, brains were fixed in 2% agarose solution to aid in cutting. After the agarose had set, brains were sectioned into 150 μm coronal slices using a Vibratome (Vibratome, St. Louis, MO) until slices of the striatum containing the CPu were obtained. These slices were then examined under a microscope (Olympus SZX7) and photomicrographs of the track marks were taken.

2.8. Data analysis

Spontaneous locomotor data was converted to a percentage of baseline activity to control for increased variability due to the small sample size. Without conversion to percent of baseline, individual differences in basal ambulatory distance would have obscured any locomotor trends. Additionally, the use of percent of control for the locomotor data allowed for easy comparison with the dialysis data. Locomotor data was analyzed with SPSS using a mixed-factorial ANOVA for both exposure and recovery, with time bins serving as the repeated measures variable (2 bins for exposure and 4 bins for recovery) and dose-condition and bicuculline condition serving as the independent variables in both analyses. An alpha level of $p<0.05$ determined statistical significance. Tukey's HSD post-hoc contrasts were used to determine the locus of significant

main effects and interactions. Individual fractions were assessed as single univariate ANOVAs with dose and bicuculline condition serving as between-subjects variables.

Statistical analyses of all neurochemical measurements were also done using SPSS. Baseline DA levels were calculated by averaging DA levels found in the 8 baseline fractions. Outlier baseline fractions were determined using the criteria $Z > 2.5$. DA levels during the experimental phase were represented as a percent of that baseline average as previously reported in Bosse and Mathews (2011) and Mathews *et al.* (2006). The toluene administration study was analyzed with a 2 x 3 x 2 Mixed Design ANOVA for exposure and a 2 x 3 x 4 Mixed Design ANOVA for recovery, with dose and bicuculline condition serving as the between subjects variables, and the two fractions of toluene exposure or four fractions of recovery serving as the within subjects factors. Post-hoc tests were performed using Tukey's HSD. Extracellular DA levels was further analyzed for the two toluene administration fractions with two 2 x 2 ANOVAs with dose and bicuculline condition serving as the between-subjects variables. The high potassium experiments were analyzed with two separate univariate ANOVAs (both 3 x 2 ANOVAs), one each for the 15 min of potassium exposure and the 15 min immediately following exposure. For the Mixed ANOVAs analyses, in cases where sphericity was violated, the Huynh-Feldt degrees of freedom correction was utilized.

3.0. Results

3.1. Dynamic Exposure System

For the 4000 ppm condition, the average time until concentration asymptote and concentration elimination was approximately 7.5 min (see Fig. 3), while the 8000 ppm condition took approximately 7.5 min to asymptote and 9.5 min for elimination. The concentration

remained stable for the entire exposure duration, and no meaningful traces of the solvent remained in the chamber after testing.

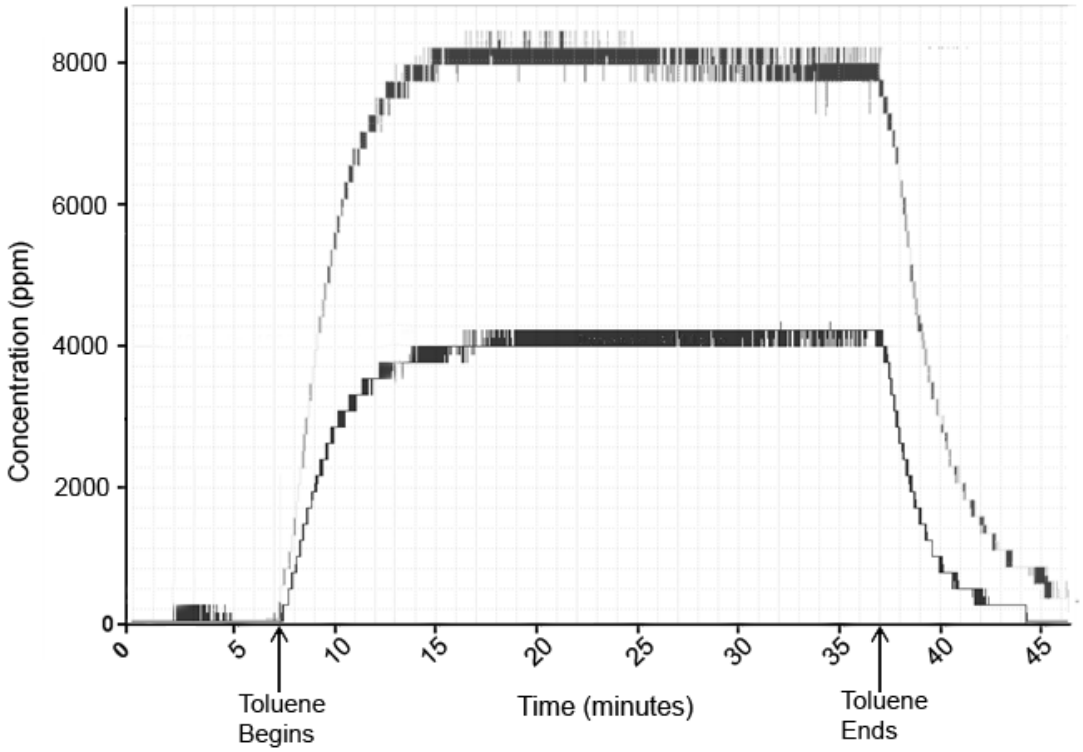

Figure 3: Infrared spectroscopy plot of increasing and decreasing toluene concentrations within the dynamic exposure chamber. Each vertical line represents one min. Toluene concentration reached the target 4000 ppm or 8000 ppm in approximately 7.5 min and remained constant until exposure was discontinued at thirty min. Evacuation of toluene from the chamber took between 7.5 and 9.5 min, depending on the dose utilized.

3.2. Locomotor activity (or distance travelled) during acute toluene administration

During toluene exposure, there was a significant effect of time, F (1, 22) = 8.44, $p < 0.05$, such that as time increased, locomotor activity decreased, irrespective of treatment condition (see Fig. 4). While there were no significant time x dose (F = 1.8, p = 0.18), time x bicuculline (F =

0.045, $p = 0.83$), or time x dose x bicuculline interactions ($F = 0.087$, $p = 0.77$), there was a significant between-subjects effect of toluene dose, F (2, 22) = 3.90, $p < 0.05$, with animals in the 4000 ppm group showing significantly elevated locomotor distances as compared to air controls. There was no effect of bicuculline, F (1, 22) = 1.03, $p = 0.32$, and no dose x bicuculline interaction ($F = 0.72$, $p = 0.39$).

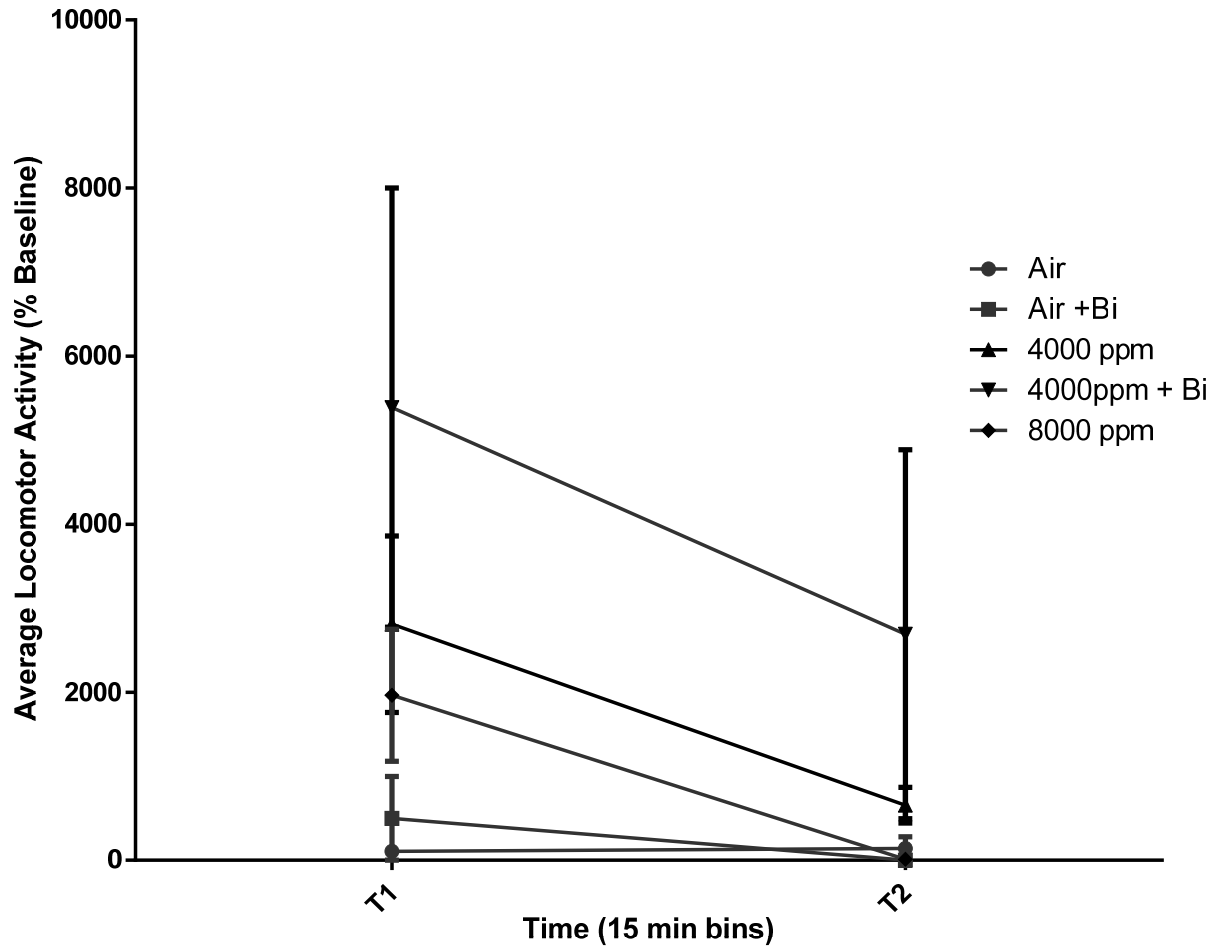

Figure 4. Locomotor distance traveled (mean ± SEM) during toluene exposure in fifteen min bins. T1 denotes the first fifteen min of exposure while T2 denotes the second fifteen min. Data is represented as percent of average locomotor distance traveled during the second hour of baseline.

3.3. Locomotor activity (or distance travelled) during acute toluene recovery

During recovery from toluene exposure, there was a significant main effect of time, F (1.44, 31.75) = 7.09, $p < 0.001$, such that as time increased, locomotor activity decreased irrespective of treatment condition (see Fig. 5). There was no significant time x dose (F = 1.14, p = 0.34), time x bicuculline (F = 1.93, p = 0.13), or time x dose x bicuculline interactions (F = 1.08, p = 0.32). There was no between-subjects effect of dose, F (1, 22) = 1.58, p = 0.22, or of bicuculline, F (1,22) = 2.78, p = 0.109. There was a trending dose x bicuculline interaction, F(1, 22) = 3.23, p = 0.086, suggesting that the animals in the toluene x bicuculline condition were more active during the recovery phase, and remained more active than animals in the toluene, air, or air x bicuculline conditions.

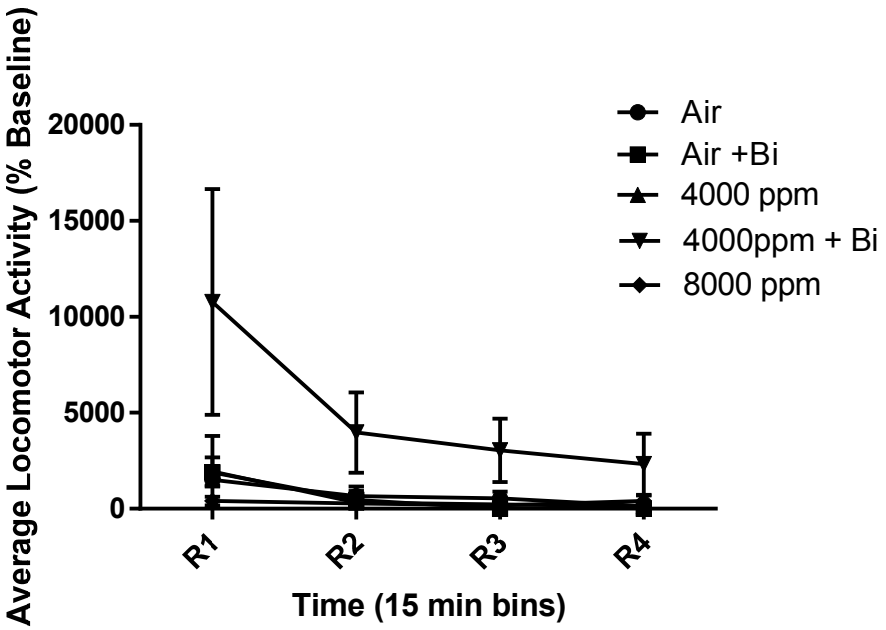

Figure 5. Locomotor distance traveled (mean ± SEM) after toluene exposure in fifteen min bins (R1, R2, etc.). Data is represented as percent of average locomotor distance traveled during the second hour of baseline.

3.4. Neurochemistry

3.4.1. Extracellular DA levels during acute toluene administration

During toluene administration, no significant main effect of time was observed, F (1, 21) = 0.069, p = 0.795. However, a between-subjects effect of dose was found, F (2, 21) = 7.67, p < 0.01. *Post-hoc* analysis revealed that the 8000 ppm dose of toluene significantly elevated extracellular DA levels as compared to both air and 4000 ppm doses (p < 0.05; see Fig. 6). Finally, there was a trending non-significant time x dose interaction, F (2, 21) = 2.78, p = 0.084, with the 8000 ppm condition displaying an upward trend across time, while all other conditions remained stable or slightly decreased over time.

3.4.2. *Extracellular DA levels during acute toluene administration following bicuculline pretreatment*

As seen in Fig. 6, there was no effect of bicuculline treatment (F = 0.041, p = 0.67). No significant interactions were found for time x bicuculline, or time x bicuculline x dose effects (p's = 0.18 and 0.18, respectively). Analysis of the two fractions during toluene exposure revealed no effect of toluene (p = 0.40) or bicuculline (p = 0.41) during the first 15 min. However, during the second fifteen min of exposure there was a significant effect of toluene dose, F (1, 17) = 5.79, p < 0.05, with the 4000 ppm conditions having higher extracellular DA, as well as a significant effect of bicuculline, F (1, 17) = 22.95, p < 0.001, with the bicuculline conditions having higher extracellular DA. There was also a significant toluene dose x bicuculline interaction, F (1, 17) = 17.09, p < 0.01, indicating that DA levels for the 4000 ppm x bicuculline condition increased during the second 15 min of exposure, while the other three conditions remained at baseline levels for all other conditions (see Fig. 6).

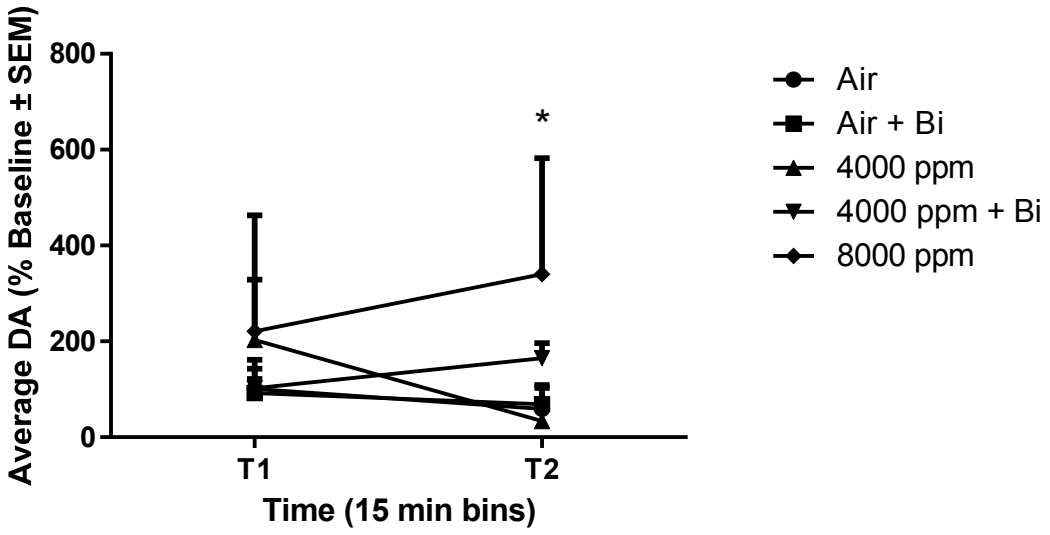

Figure 6. The effect of acute toluene on extracellular DA (mean ± SEM) during toluene exposure in fifteen min bins. Data is represented as percent of baseline DA levels.

3.4.3. Extracellular DA during acute toluene recovery

During recovery from toluene exposure, there was no main effect of time, $p = 0.645$ (see Fig. 6). There were no time x dose, time x bicuculline, or time x dose x bicuculline interaction effects (p's = 0.30, 0.26, and 0.20, respectively). However, there was a significant between-subjects effect of toluene dose, F (2, 21) = 8.39, $p < 0.01$. *Post-hoc* analysis revealed that the 8000 ppm dose significantly elevated DA levels as compared to both air control and 4000 ppm groups ($p < 0.05$). There was no effect of bicuculline or drug x bicuculline interaction (p's = 0.55 and 0.22, respectively).

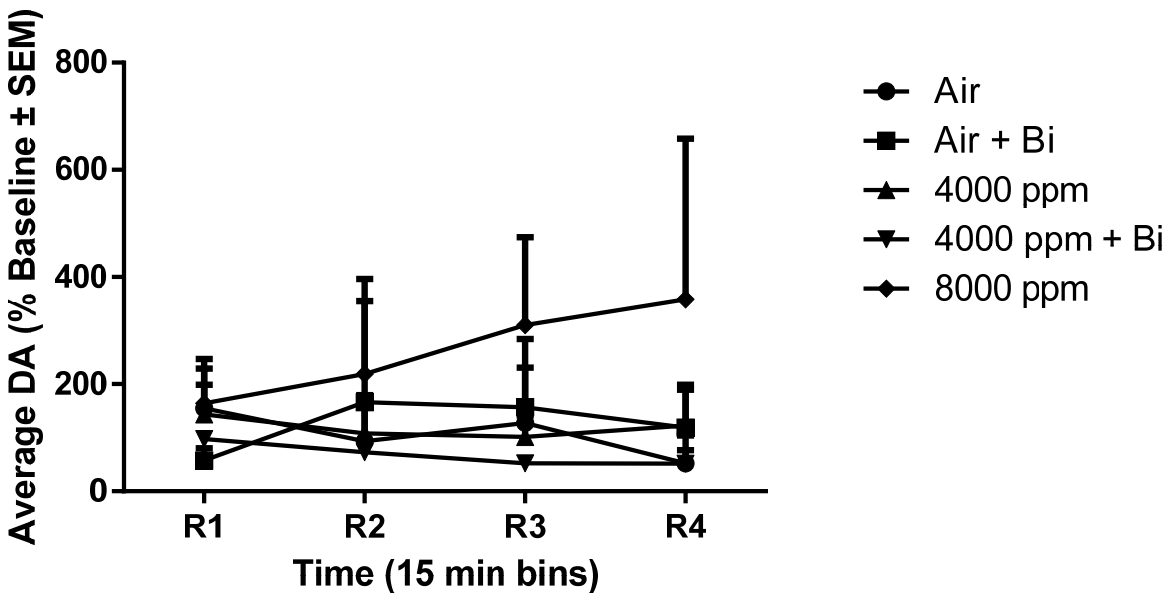

Figure 7. Extracellular DA levels in the mouse CPu during recovery from toluene exposure (mean ± SEM). Data are represented as a percent of average baseline DA levels in fifteen min bins (R1, R2, etc.).

3.4.4. High potassium stimulation following acute toluene administration

During the 15 min of high potassium stimulation, there was no main effect of dose ($1, 21$) = 1.91, $p = 0.17$, or bicuculline, F (1, 21) = 1.12, $p = 0.30$. However, there was a significant dose x bicuculline interaction, F (1, 21) = 7.59, $p < 0.05$, with animals in the 4000 ppm and 8000 ppm groups showing elevated DA levels as compared to animals in the air control group or the two bicuculline groups (see Fig. 8).

During the fifteen min immediately following the high potassium infusion, there was no main effect of dose, F (2, 21) = 2.18, $p = 0.14$, or bicuculline, F (1, 21) = 0.28, $p = 0.60$, nor was there a dose x bicuculline interaction, F (1, 21) = 2.08, $p = .164$.

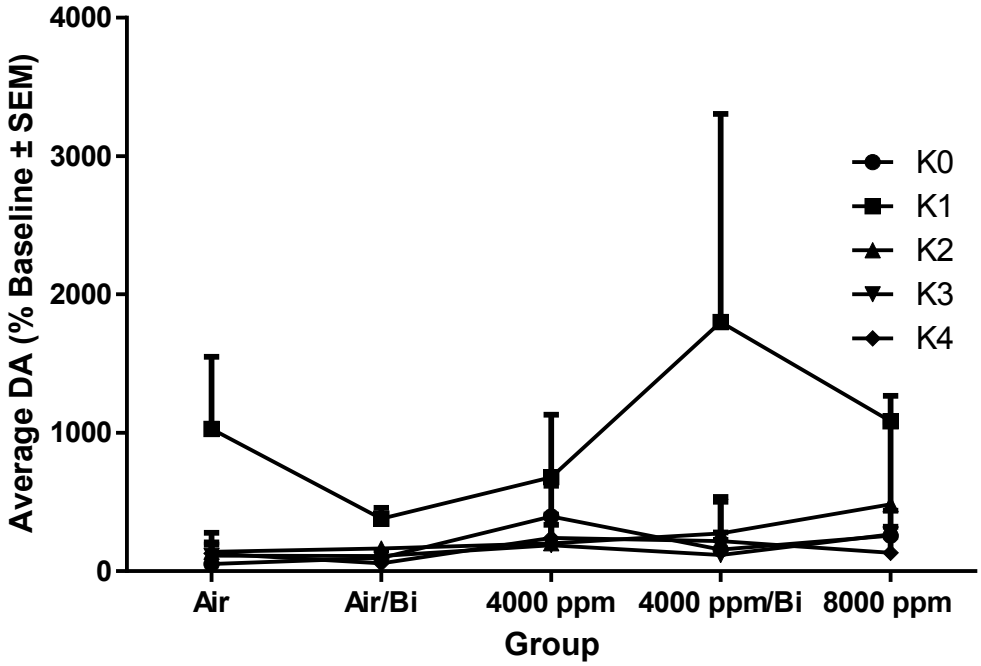

Figure 8: The effect of high potassium stimulation on DA levels following toluene exposure. Data are represented as percent of extracellular DA levels during baseline in fifteen min bins. Bicuculline conditions are checkered for ease of comparison.

4.0. Discussion

The primary purpose of the present research was to develop a novel method for characterizing *in vivo* neurochemical and behavioral characteristics of freely moving mice while being exposed to the volatilized solvent toluene. In this study, a unique dynamic exposure system custom-built to enable the concurrent use of *in vivo* microdialysis and infrared locomotor measurement techniques during solvent exposure was employed. I demonstrated toluene dose-dependent alterations in locomotor activity and extracellular DA levels in free-moving, acutely exposed mice. To my knowledge, this is the first such report to examine these variables concurrently during active solvent exposure, and the first to examine toluene-induced alterations in extracellular DA in mice at all. Furthermore, my work here is the first to utilize reverse-dialysis infusion as a brain-region specific pretreatment in free-moving mice exposed to toluene. Finally, I utilized the $GABA_A$ antagonist, bicuculline, to investigate whether or not the actions of toluene on the striatal DA system are related to toluene's action on GABA.

4.1. Dynamic Exposure System

This dynamic exposure system is the first free-moving microdialysis system developed for solvent abuse models that does not utilize some sort of restraint on animal movement. Additionally, this system is the first apparatus of any kind designed to enable simultaneous toluene exposure and dialysate collection in a mouse model. The chamber achieved the requisite dose quickly and maintained the dose throughout testing (see Fig 2). Additionally, this system represents the first use by this research group of a dynamic chamber for solvent abuse research.

Compared to the static exposure system utilized by this research group, the dynamic exposure system took considerably longer to reach asymptotic concentration. The static system

utilized by this group reached asymptotic concentration between 1 and 3 min after initiation of exposure (Warren et al, 2000), while the present dynamic system took approximately 7.5 min to reach stable levels**.** Consistent with the findings of Balster *et al.* (1982), who reported that 4000 ppm and 8000 ppm in their dynamic exposure system took approximately the same amount of time to reach asymptote and to empty after exposure, our dynamic exposure system was very similar in the amount of time it took for 4000 ppm and 8000 ppm of toluene to fill the chamber (7.5 min for both) with only a slightly longer time to void the chamber (8000 ppm took 2 min longer to void than 4000 ppm). The toluene concentration for both conditions was very stable, varying by approximately ±5% for both conditions (±200 ppm for 4000 ppm, and ±400 ppm for 8000 ppm). It is doubtful that the difference in elimination time would impact behavioral results, and the difference is an unavoidable product of the higher concentration present in our dynamic chamber. In addition, the higher toluene concentration of 8000 ppm appears to have a deleterious effect on the rubber tubing used for carrying the vapor to the test chamber. There was evidence of liquid toluene accumulation inside the tubing during exposure, and the tubing had to be changed regularly because of signs of discoloration in the tubing, suggesting toluene accumulation was damaging the tubes. For these reasons, it appears that 8000 ppm toluene might be an upper limit on the concentration curve achievable by this system.

4.2. Toluene administration dose-dependently altered locomotor behavior in naïve animals

A key finding in the present experiment was that acute toluene administration resulted in qualitatively similar acute behavioral effects in our mice (see Fig. 3) as compared to previous reports (Bowen *et al.*, 2010; Conti *et al.*, 2012). That is, in the present experiment, the results clearly demonstrated that the new dynamic toluene inhalation exposure system resulted in dose-dependent biphasic effects on spontaneous locomotor activity in mice. At the lowest

concentration (4000 ppm), toluene increased locomotor activity during the first fifteen min of exposure (see Fig. 4). However, unlike previous reports (Conti *et al.,* 2012; Bowen et al, 2010), this activity was not persistent over time, but instead returned to baseline levels during the next fifteen min of exposure. In these prior reports, activity for locomotor stimulating doses (2000 ppm for both studies) increased activity, which remained elevated for the duration of toluene exposure (Conti *et al.,* 2012; Bowen et al., 2010).

At the highest concentration (8000 ppm), locomotor activity was suppressed with animals displaying little to no motor activity following the first fifteen min of exposure (T1) for the remainder of the exposure and recovery periods (see Fig. 4 and 5), although functional observational battery assessments were not performed. Even after recovery, most animals appeared sedated and did not engage in voluntary locomotor activity. During the 8000 ppm toluene exposure, there was evidence of myoclonic tail twitching ("straub tail") in all of the animals. While toluene is classically reported to have anticonvulsant properties (Wood *et al.*, 1984), the dose utilized here was significantly higher than those used in previous reports and may be suggestive of toluene's convulsant properties at higher concentrations. This twitching resulted in locomotor beam breaks that accounted for the small amount of activity seen following the T1 time block. The initial increase in locomotor activity during T1 is most likely the result of the relatively slow rise to asymptote of the dynamic system. As noted above, it took approximately half of the first exposure dialysate collection period for the concentration to become stable (7.5 min). During that time, the toluene concentration in the chamber was still rising. The rate of rise was asymptotic, such that the chamber concentrations spent more time at doses approaching 8000 ppm than at lower concentrations. However, there was still a period of

time when the toluene concentrations within the chamber were below 8000 ppm and at a locomotor-stimulating level (< 6000 ppm; Bowen and Balster, 1998).

Because this report represents the first use of this exposure apparatus, the locomotor activity results cannot be directly compared to those previously achieved with the static exposure chambers used previously (e.g. Bowen *et al.*, 2010). However, the present results are similar to previous reports which have demonstrated that acute inhalation of toluene in Swiss Webster mice produces a profile of effects that progress from motor excitation at lower concentrations (i.e., 500–4000 ppm) to motor impairment, sedation, and anesthesia at concentrations above 6000 ppm (Bowen and Balster, 1998). Similar increases in motor activity have been found when toluene was administered in rats via inhalation (Himnan, 1984) and systemically (Riegel *et al.*, 2003; Riegel and French, 1999). It is also important to note that the animals in these experiments were placed in the exposure chamber the night before toluene exposure to allow for physical habituation to the microdialysis probe. An unintended side-effect of this step could be that the animals were able to habituate to the testing chamber itself, resulting in much lower baseline locomotor rates. To our knowledge, these findings are the first to report toluene effects on mouse locomotor activity in a dynamic system, and the first to explore the administration of toluene vapor to animals that have spent the previous night in the exposure chamber. It is possible that the different behavioral pattern observed in the 4000 ppm condition is an artifact of habituation or a unique feature of this dynamic exposure protocol. If the observed pattern of locomotor activity is due to habituation, this might suggest that toluene exposure might be better viewed as a locomotion-moderator rather than a locomotion-inducer. Direct experimental investigation is necessary to determine the cause of this discrepant result.

4.3. The GABA antagonist, bicuculline, enhances the locomotor increasing effects of toluene

The locomotor effects of the $GABA_A$ antagonist, bicuculline, on CPu DA levels are particularly interesting. To our knowledge, this investigation represents the first use of bicuculline pretreatment in a toluene locomotor experiment. As would be expected of a GABA antagonist, the bicuculline dose of 10 μM had no effect on locomotor behavior during air control exposures (see Figs. 4 and 5). However, when mice were pretreated with bicuculline and then exposed to inhaled toluene, an increase in locomotor activity was observed which persisted for the duration of both the toluene exposure and recovery phases. The present locomotor results argue that toluene's ability to induce dose-dependent, biphasic alterations in spontaneous locomotor activity is likely related to decreased GABA neurotransmission in the CPu, as this effect was significantly enhanced following blockade of CPu GABA receptors.

These results are contrary to the prior work of Beckstead *et al.* (2000), who found that *in vitro* toluene administration increased $GABA_A$ receptor function in rat hippocampal slices. However, the current work differs from Beckstead *et al.* (2000) in several ways. The present study was *in vivo* and examined extracellular DA, instead of receptor functioning. Extracellular levels of DA in the CPu may be moderated by additional mechanisms, including internal cellular actions, afferent pathway signaling, and neurotransmission from neurons within the CPu (reviewed by Kreitzer and Malenka, 2008). It is distinctly possible that receptor functioning in response to drug administration may vary from brain region to brain region due to the influences of these outside factors. Next, the present study used mice instead of rats, and there may be species-specific differences in CPu DA-GABA dynamics. Finally, in the present study, the region of interest was the CPu instead of the hippocampus. The effect of toluene exposure on the CPu GABA receptors may well differ from the hippocampus due to the action of presynaptic

modulation by other neurons. This current finding is also contrary to Stengard and O'Connor (1994), who found that administration of 2000 ppm toluene did not decrease striatal GABA in rats. However, it is possible that 2000 ppm toluene, even when given for 2 hours, is not sufficient to depress striatal GABA levels. Indeed, the use of quantitative pharmacokinetic modeling indicates that 2000 ppm toluene, if inhaled for two hours, resulted in an estimated 31.19 ppm blood toluene concentration, which is less than the estimated 50.88 ppm obtained with a half hour of 4000 ppm exposure (estimated peak blood concentrations based on formula developed in Callan *et al.*, 2014, in revision). This suggests that a higher dose of toluene, even if given for a shorter time, would result in a higher concentration of toluene present in the body. It is possible that the effect of toluene and GABA receptor function in the CPu is biphasic: excitatory at low doses, but inhibitory at high doses. Additionally, the present study used a unilateral administration of bicuculline which may have attributed to our result, as total CPu antagonism was impossible in the current design. Future studies should investigate the effect of bilateral CPu infusion of bicuculline prior to toluene exposure on locomotor behavior.

4.4. Acute Toluene exposure increases extracellular DA in the mouse CPu

Acute toluene administration had a dose-dependent effect of CPu DA levels. While 4000 ppm of toluene did not significantly elevate DA levels, the highest exposure of 8000 ppm toluene significantly increased extracellular DA levels and this persisted throughout exposure and recovery. The increase in extracellular DA in the CPu is consistent with findings from other toluene doses in rats (Riegel *et al.*, 2004; Stengard *et al.*, 1994). Stengard *et al.* (1994) demonstrated that in rats, two hour exposures of 2000 ppm of toluene increased extracellular DA within the striatum. Gospe and Al-Bayati (1994) have also demonstrated that the blood concentration of toluene will continue to rise for up to two hours before becoming asymptotic.

As such, it is entirely plausible that the difference between the current findings with 4000 ppm and those of Stengard *et al.* (1994) with 2000 ppm is a function of duration of exposure. Additionally, although their work was done *ex vivo,* Riegel *et al.* (2004) demonstrated that repeated injections of toluene increased CPu DA which persisted for several hours. These results are consistent with our current findings at 8000 ppm, in which the increase in extracellular DA persisted for at least an hour after exposure. These findings suggest that CPu DA alone is not sufficient to alter locomotor activity, as locomotor activity increased in the 4000 ppm condition despite DA levels remaining unchanged. Indeed, this effect is similar to Rose *et al.* (2013), who reported that ethanol-induced locomotor activity was not related to differences in striatal DA dynamics in two strains of mice. This suggests that increases in locomotor activity during toluene exposure may be related to an interaction between DA and GABA providing further evidence for the hypothesis that toluene acts as a moderator of basal locomotor activity, rather than as an inducer of novel activity.

Despite the discrepancies between this report and other published microdialysis studies, these findings are noteworthy in several ways. First, the results obtained here represent the first time toluene-induced DA effects have been observed in a mouse model. Secondly, these results obtained here represents the first time that freely-moving animals have been exposed to toluene during microdialysis. The present study indicates that toluene appears to have a dose-dependent effect upon CPu DA dynamics in the mouse brain, suggesting that toluene's effect on the DA system may not be uniform between different brain regions, and that the action of toluene at neurotransmitter receptors may be dose dependent. Additionally, the CPu is classically thought to be important for aspects of planning and executive functioning (Kreitzer and Malenka, 2008), and alterations to neurotransmitters within this region provide insight into the impact of acute

toluene misuse on these processes. Indeed, the apparent dose-dependent effect of toluene on CPu DA might provide an explanation for seemingly transient nature of solvent misuse, as this critical region for the planning of drug seeking behavior does not appear to be as strongly impacted by toluene inhalation as other brain regions (e.g. the NAc; Riegel *et al.*, 2007).

4.5. GABA Antagonism increases DA levels during acute toluene exposure

This paper represents the first use of bicuculline in a toluene microdialysis study. Prior *ex-vivo* work via fast-scan cyclic voltammetry has suggested that $GABA_A$ agonism increases striatal DA levels (Avshalumov *et al.*, 2003). These authors proposed a mechanism through which GABAergic neurons act upon glutamatergic neurons (specifically, AMPA neurons), which in turn have a negative modulatory effect on dopaminergic neurons. However, in the current report, $GABA_A$ antagonism in conjunction with toluene administration led to a small increase in extracellular DA. It is possible that the action of toluene on DA is also mediated by glutamatergic neurons, and in the absence of GABAergic stimulation, the excitatory effect of glutamate was increased. It is also possible that not all GABAergic neurons in the striatum are acting to inhibit AMPA receptor-containing neurons. There may alternative inhibitory pathways acting directly on DA (see Fig. 9). Inhibition of these pathways by bicuculline may have enhanced DA levels. Likewise, it is possible that there are $GABA_B$ or $GABA_C$ containing neurons that are acting on the pathway explored by Avshalumov *et al.* (2003), and it is possible that toluene's actions at these sub-receptors was sufficient to increase DA levels in the CPu. Indeed, there is evidence that suggests that the theory put forward by Avshalumov *et al.* (2003) may not be accurate. In their review on the function of the CPu, Kreitzer and Malenka (2008) note that the CPu is almost entirely absent of glutamatergic signaling, which would make the mediated mechanism suggested by Avshalumov impossible. This theoretical framework suggests

that $GABA_A$ receptors within the CPU may have a primarily inhibitory action on DA neurons, and antagonism of $GABA_A$ would be expected to produce the obtained increase in extracellular DA (see Fig. 9). Indeed, the mechanism for this pathway may occur outside the CPu itself. Toluene's action on CPu GABA could have an inhibitory effect on the dis-inhibitory efferent GABA pathway from the CPu to the substantia nigra (SNr). This, in turn, would decrease efferent DA pathway from the SNr the CPU. Pretreatment with bicuculline blocked internal CPu GABA signaling, which stopped toluene's inhibitory action on the CPu-SNr GABA pathway. This resulted in an increase in efferent GABA to the SNr, which acted to dis-inhibit DA release in the SNr, which resulted in increased DA release from the SNr to the CPu. In this manner, toluene's mechanism of action upon DA within the CPu, at least at high concentrations of toluene, can be viewed as dis-dis-inhibitory. Thus, the current results provide evidence for the hypothesis that, while not critical, DA does play a role in toluene-related locomotor activity.

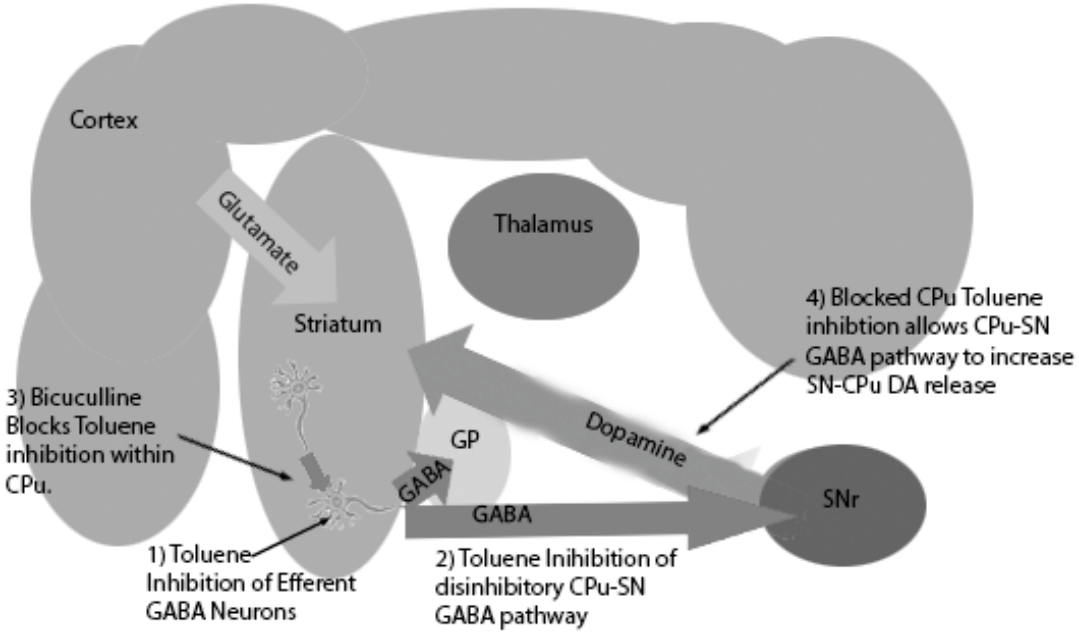

Figure 9: Schematic representation of toluene's action within the CPu. Toluene inhibits CPu GABAergic neurons, which in turn increases CPu DA release within the CPu.

4.6. Acute Toluene Inhalation Increases Sensitivity to High Potassium Stimulation

Following acute toluene exposure, high potassium was directly infused into the mouse's CPu. While the overall magnitude of the increase in DA levels following high potassium stimulation didn't differ between groups, the 4000 ppm and 8000 ppm toluene groups had elevated DA levels one fraction sooner (15 min) than air controls. Indeed, the 4000 ppm toluene exposure group, which had no effect of toluene administration, demonstrated a robust response to potassium during the fifteen min of administration, while the animals in the air condition did not respond until fifteen min later. This finding differs from what has been observed with amphetamine (Casanova *et al.*, 2013) and opioid agonists (Fuentealba *et al.*, 2010) which increased the amplitude of the high potassium stimulated DA release in the medial prefrontal cortex, but not the speed at which DA release occurred. Our high potassium extracellular DA levels suggest cellular sensitization, though the mechanism of this action is unclear and requires further investigation. This hypothesis of cellular sensitization fits with the locomotor findings of this paper, suggesting that toluene's actions (both cellular and behavioral) are at least partially modulatory in nature. In this experiment, 4000 ppm toluene was not sufficient to raise extracellular DA levels. However, 4000 ppm of toluene exposure was sufficient to increase the sensitivity of DA neurons to other forms of stimulation. This sensitizing effect is consistent with Gerasimov *et al.* (2002), who found that toluene increased DA levels in the NAc only when paired with cocaine stimulation. This sensitization suggests that toluene is simultaneously a modest releaser of DA and a positive modulator for cellular sensitivity, though whether or not that modulation is mediation or moderation is presently unknown. The effects of bicuculline on

toluene-induced locomotor effects and changes to extracellular DA levels suggests that the sensitizing actions of toluene appear to be dependent on normal GABAergic functioning. It is possible that the cellular sensitization that underlies the increase in stimulated DA release is occurring in GABA receptor-containing neurons and antagonism of GABA inhibits the as yet-unknown mechanism that causes this effect. It is worth noting the seeming discrepancy of the toluene + bicuculline group during high potassium stimulation. While the toluene + bicuculline group's stimulated DA levels seem low, the effect is not significant and as such should not be over-interpreted.

4.7. Future Directions

This report has created a number of opportunities for future research, both in terms of the findings of the project and the avenues of research opened by the creation of this new combined method of simultaneously measuring activity and performing microdialysis in freely moving mice during solvent inhalation. First, the findings of this study should be validated by replication to confirm the validity of this method. Secondly, the exposure window should be varied experimentally to determine that the effect of 4000 ppm toluene on DA levels is indeed an artifact of exposure timing and not a species-specific difference in toluene sensitivity. Third, our findings suggest that the action of toluene on locomotor activity is, in part, mediated by GABA activity, and future studies should investigate this by measuring extracellular GABA during toluene exposure with and without pretreatment with bicuculline. The effect of bicuculline discovered by our present results strongly suggests that a change in extracellular GABA levels will occur in the CPu during toluene administration. Fourth, the action of other GABA receptors should be explored to provide further insight into the relationship between toluene, GABA, and DA. Fifth, the effects of repeated toluene exposure on DA levels in the CPu should be explored.

Sixth, this exposure chamber allows for the unique opportunity to selectively apply pharmacological mediation to specific brain regions before, during, and after toluene exposure. Indeed, future studies should concentrate on the effects of applying sub-receptor specific antagonists and agonists to CPu and NAc tissue before toluene administration to observe the effects of various receptors on toluene-induced activity. Seventh, as this study represents the first freely moving microdialysis model of inhalant abuse in mice, this project sets the stage for future work only possible in mouse models. Indeed, future studies should make use of genetically altered mouse models, or site-specific genetic knockdowns, to provide further mechanistic control of their inhalant studies. Finally, this system can easily be adapted to allow for other behavioral assays, including open field, conditioned place preference, marble burying, pain sensitivity, and a variety of operant tasks.

4.8. Conclusions

I hypothesized (Hypothesis 1) that acutely inhaled toluene would increase DA levels present in the CPu. This hypothesis was supported. Acutely administered toluene increased DA levels 3 fold in the mouse CPu at the highest toluene dose, and we are the first to demonstrate this in mice. The prediction that the above effect would be exacerbated by high-potassium aCSF post-toluene exposure (Hypothesis 2) was also supported, albeit in a surprising manner. Instead of having elevated DA levels compared to controls, the toluene-treated animals displayed a sensitization effect with DA levels increasing more quickly than controls. Taken together, these results suggest that toluene both increases extracellular DA and acutely alters cellular dynamics to increase sensitivity to future stimulation, though the mechanism for this second effect is as yet unknown.

The final hypothesis, proposing that the increase in DA levels and locomotor activity observed would be attenuated by pretreatment with the $GABA_A$ antagonist bicuculline, was not supported. Instead, pretreatment with bicuculline increased the effect of toluene on both locomotor behavior and extracellular DA levels in the CPu, suggesting that the mechanism of toluene's action on the brain may be more complicated than hypothesized. This result is contrary to prior published work suggesting that acute toluene either increases or does not affect GABA signaling in the CPu, though this is the first such model to measure DA as an outcome of $GABA_A$ antagonism in conjunction with toluene exposure, and the first *in vivo* microdialysis model of toluene abuse developed in the mouse. As such these discrepant results are in need of further validation and examination.

In conclusion, I have developed a novel approach to inhalant abuse research: a method by which freely moving mice can be exposed to inhalants during microdialysis collection. Additionally, this is the first attempt to examine the DA dynamics of toluene in a living mouse model, which opens the field for numerous studies that take advantage of the unique capabilities of mouse models. Furthermore, toluene may be best viewed as a modulator of behavioral activity, rather than as an activity inducer. Finally, the DA dynamics of acute toluene inhalation are related to GABAergic functioning, suggesting that CPu DA alone does not explain toluene-related locomotor behavior. In conclusion, we have made novel contributions to the inhalant literature on empirical and methodological grounds, and have raised a substantial number of important questions in need of thorough examination.

References

Anderson, CE and Loomis, GA. (2003). Recognition and Prevention of Inhalant Abuse. *American Family Physician*, 68(5), pp. 869-874.

ASTDR. (2000). Toxicological profile for toluene in US Dept. of Health and Human Services. Atlanta, U.S. Public Health Service: 1-357.

Avshalumov, MV; Chen, BT; Marshall, SP; Pena, DM, Rice, ME. (2003). Glutamate-dependent inhibition of dopamine release in striatum is mediated by a new diffusible messenger, H2O2. *Journal of Neuroscience*, 23(7), pp. 2744-2750.

Bale, AS; Tu, Y; Carpenter-Hyland, EP; Chandler, LJ; Woodward, JJ. (2005). Alterations in glutaminergic and GABAergic ion channel activity in hippocampal neurons following exposure to the abused inhalant toluene. *Neuroscience*, 130, pp. 197-206.

Balster, RL; Moser, VC; Woolverton, WL. (1982). Concurrent measurement of solvent vapor concentrations and effects on operant behavior using a dynamic exposure system. *Journal of Pharmacology Methods,* 8(4), pp. 299-309.

Beckstead, MJ; Weiner, JL; Eger II, EI; Gong, DH; Mihic, SJ. (2000). Glycine and γ-Aminobutyric Acid$_A$ Receptor Function Is Enhanced by Inhaled Drugs of Abuse. *Molecular Pharmacology*, 57, pp. 2299-1205.

Bennett, ME; Walters, ST; Miller, JH; Woodall, WG. (2000). Relationship of early inhalant use to substance use in college students. *Journal of Substance Abuse*, 12, pp. 227-240.

Beyer, CE; Stafford, D; LeSage, MG; Glowa, JR; Steketee, JD. (2001). Repeated exposure to inhaled toluene induces behavioral and neurochemical cross-sensitization to cocaine in rats. *Psychopharmacology (Berlin),* 198-204.

Bosse, KE and Mathews, TA. (2011). Ethanol-induced increases in extracellular dopamine are blunted in brain-derived neurotrophic factor heterozygous mice. *Neuroscience letters*, 489(3), pp. 172-176.

Bowen, SE and Balster, RL. (1996). Effects of inhaled 1,1,1-trichloroethane on locomotor activity in mice. *Neurotoxicology and Teratology*, 18(1), pp. 77-81.

Bowen, SE and Cruz, SL (2014). Inhalants: Addiction and toxic effects in the human. In: B. Madras & M. Kuhar (Eds.), The Effects of Drug Abuse on the Human Nervous System (pp 553-569). Oxford, UK: Academic Press. https://www.elsevier.com/books/the-effects-of-drug-abuse-on-the-human-nervous-system/madras/978-0-12-418679-8.

Bowen, SE; Daniel, J; Balster, RL. (1999). Deaths Associated with inhalant abuse in Virginia from 1987 to 1996. *Drug and Alcohol Dependence*, 53, pp. 239-245.

Bowen, SE and Hannigan, JH. (2013). Binge toluene exposure in pregnancy and pre-weaning developmental consequences in rats. *Neurotoxicology and Teratology*, 38, pp. 29-35.

Bowen, SE. (2011). Two serious and challenging medical complications associated with volatile substance misuse: sudden sniffing death and Fetal Solvent Syndrome. *Substance Use & Misuse*, 46, pp 68-72.

Bowen, SE; Batis, JC; Paez-Martinez, N; Cruz, SL. (2006). The last decade of solvent research in animal models of abuse: Mechanistic and behavioral studies. *Neurotoxicology and Teratology*, 28, pp. 636-647.

Bowen, SE; Kimar, S; Irtenkauf, S. (2010). Comparison of toluene-induced locomotor activity in four mouse strains. *Pharmacology, Biochemistry, and Behavior*, 95, pp. 249-257.

Butland, BK; Field-Smith, ME; Ramsey, JD; Anderson, HR. (2012). Twenty-five years of volatile substance abuse mortality: a national mortality surveillance programme. *Addiction*, 108(2), pp. 385-393.

Callan, SP; Kott, JM; Cleary, JP; McCarthy, M; Baltes, BB; Bowen, SE. (2014, in revision). Changes in Developmental Body Weight as a Function of Toluene Exposure: A Meta-Analysis of the Preclinical Literature. *Neurotoxicology and Teratology*, in revision.

Casanova, JP; Velis, GP; Fuentealba, JA. (2013). Amphetamine locomotor sensitization is accompanied with an enhanced high K+-stimulated Dopamine release in the rat medial prefrontal cortex. *Behavioral Brain Research*, 237, pp. 313-317.

Conti, AC; Lowing, JL; Susick, LL; Bowen, SE. (2012). Investigation of calcium-stimulated adenylyl cyclases 1 and 8 on toluene and ethanol neurobehavioral actions. *Neurotoxicology and Teratology*, 34, pp. 481-488.

Cruz, SL. (2011). The Latest Evidence of Solvent Misuse: An Article Written for Service Providers. *Substance Use and Misuse*, 46, 62-67.

Cruz, SL and Bowen, SE. (2008). Inhalant abuse. In Ubach, MM and Mondragon-Ceballos, R (Eds.), *Neural Mechanisms of Action of Drugs of Abuse and Natural Reinforcers* (61-87). Kerala, India: Research Signpost.

Cruz, SL; Gauthereau, MY; Camacho-Munoz, C; Lopez-Rubalcava, C; Balster, RL. (2003). Effects of inhaled toluene and 1,1,1 –trichloroethane on seizures and death produced by N-methyl-D-aspartic acid in mice. *Behavioral Brain Research*, 140(1-2), pp. 195-202.

Dell, CA; Gust, SW; MacLean, S. (2011). Global Issues in Volatile Substance Misuse. *Substance Use and Misuse*, 46 (suppl 1), pp. 1-7.

Donald, JM; Hooper, K; Hopenhayn-Rich, C. (1991) Reproductive and Developmental toxicity of toluene: a review. *Environmental Health Perspectives*, 94, pp. 237-244.

Elfenbein, DS and Felice, ME. (2003). Adolescent pregnancy. *The Pediatric Clinics of North America*, 50, pp. 781-800.

Farrar, AM; Callahan, JW; Abercrombie, ED. (2011). Reduced striatal acetylcholine efflux in the R6/2 mouse model of Huntington's disease: An examination of the role of altered inhibitory and excitatory mechanisms. *Experimental Neurology*, 232, pp. 119-125.

Flanagan, RJ; Ruprah, M; Meredith, TJ; Ramsey, JD. (1990). An Introduction to the Clinical Toxicology of Volatile Substances. *Drug Safety*, 5(5), pp. 359-383.

Fuentealba, JA, Gysling, K; Andres, ME. (2010). Repeated treatment with the k-Opioid agonist U-69593 increases K+-stimulated dopamine release in the rat medial prefrontal cortex. *Synapse*, 60(12), pp. 896-904.

Gerasimov, MR; Schiffer, WK; Marstellar, D; Ferrieri, R; Alexoff, D; Dewey, SL. (2002). Toluene inhalation produces regionally specific changes in extracellular dopamine. *Drug and Alcohol Dependence*, 65(3), pp. 243-251.

Gospe, SM; Al-Bayati, MAS. (1994). Comparison of Oral and Inhalation Exposures to Toluene. *Journal of the American College of Toxicology*, 13(1), pp. 21-32.

Hannigan, JH & Bowen, SE. (2010). Reproductive Toxicology and Teratology of Abused Toluene. *Systems Biology in Reproductive Medicine*, 56, pp, 184-200.

Hass, U; Lund, SP; Hougaard, KS; Simonsen, L. (1999). Developmental Neurotoxicity after toluene inhalation exposure in rats. *Neurotoxicology and Teratology,* 21(4), pp. 349-357.

Henretig, F.(1996). Inhalant abuse in children and adolescents. *Pediatric Annals*, 25(1), pp. 47-52.

Himnan, DJ. (1987). Biphasic Dose-Response relationship for effects of Toluene inhalation on locomotor activity. *Pharmacology Biochemistry and behavior*, 26, pp. 65-69.

Himnan, DJ. (1984). Tolerance and Reverse Tolerance to Toluene Inhalation: Effects on Open-Field Behavior. *Pharmacology, Biochemistry, and Behavior*, 21 (4), pp. 625-631.

Howard, MO; Bowen, SE; Garland, EL; Perron, BE; Vaughn, MG. (2011). Inhalant use and inhalant use disorders in the United States. *Addictive Sciences and Clinical Practice,* 6(1), pp. 18-31.

IARC. (1989). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: Some Organic Solvents, Resin Monomers and Related Compounds, Pigment and Occupational Exposures in Paint Manufacturing and Painting. Lyon, France, World Health Organization.

Johnston, L.D., *et al.*, 2007. *Monitoring the Future National Survey Results on Drug Use, 1975-2006.* Vol. I: Secondary school students. NIH Publication Number 07-6205. Bethesda, MD: National Institute on Drug Abuse.

Kondo, H; Huang, J; Ichihara, G; Kamijima, M; Saito, I; Shibata, E; Ono, Y; Hisanaga, N; Takeuchi, Y; Nakahara, D. (1995). Toluene induces behavioral activation without affecting striatal dopamine metabolism in the rat: behavioral and microdialysis studies. *Pharmacology, Biochemistry, and Behavior*, 51(1), pp. 97-101.

Kreitzer, AC and Malenka, RC. (2008). Striatal Plasticity and Basal Ganglia Circuit Function. *Neuron Review*, 60, pp. 543-554

Mathews, TA; John, CE; Lapa, GB; Budygin, EA; Jones, SR. (2006). No Role of the Dopamine Transporter in Acute Ethanol Effects on Striatal Dopamine Dynamics. *Synapse*, 60, pp. 288-294.

Mabrouk, OS; Semaan, DZ; Mikelman, S; Gnegy, ME; Kennedy, RT. (2013). Amphetamine stimulates movement through thalamocortical glutamate release. *Journal of Neurochemistry*, 128 (1), pp. 152-161.

Medina-Mora, ME and Real, T. (2008). Epidemiology of inhalant use. *Current Opinions in Psychiatry*, 21(3), pp.247-251.

O'Leary-Moore, SK; Galloway, MP; McMechan, AP; Hannigan, JH; Bowen, SE. (2007). Region-dependent alterations in glutamate and GABA measured by high-resolution magnetic resonance spectroscopy following acute binge inhalation of toluene in juvenile rats. *Neurotoxicology and Teratology*, 29(4), pp. 466-475.

Paez-Martinez, N; Aldrete-Audiffred, J; Gallardo-Tenorio, A; Castro-Garcia, M; Estrada-Camarena, E; Lopez-Rubalcava, C. (2013). Participation of $GABA_a$, $GABA_b$ receptors and neurosteroids in toluene-induced hypothermia: Evidence of concentration dependent differences in the mechanism of action. *European Journal of Pharmacology*, 698, pp. 178-185.

Paxinos G, F. K. J. B. (2001) *The Mouse Brain: In Stereotaxic Coordinates*, Second edition ed., Academic Press, San Diego, CA.

Perron, BE; Howard, MO; Maitra, S; Vaugh, MG. (2009). Prevalence, timing, and predictors of transitions from inhalant use to Inhalant Use Disorders. *Drug and Alcohol Dependence*, 100, pp. 277-284.

Plenge-Bonig, A and Karmaus, W. (1999). Exposure to toluene in the printing industry is associated with subfecundity in women but not in men. *Occupational Environmental Medicine*, 56, pp. 443-448.

Perit, KE; Gmaz, JM; Browne, JDC; Matthews, BA; Dunn, MBF; Yang, L; Raaphorst, T; Mallet, PE; McKay, BE. (2012). Distribution of c-Fos immunoreactivity in the rat brain following abuse-like toluene vapor inhalation. *Neurotoxicology and Teratology*, 34, pp. 37-46.

Raman, L; Tkac, I; Ennis, K; Georgieff, MK; Gruetter, R; Rao, R. (2005) In vivo effect of chronic hypoxia on the neurochemical profile of the developing rat hippocampus. *Developmental Brain Research*, 156, pp. 202-209.

Riegel, AC and French, ED. (1999). Acute toluene induces biphasic changes in rat spontaneous locomotor activity which are blocked by remoxipride. *Pharmacology, Biochemistry, and Behavior*, 62(3), pp. 399-402.

Riegel, AC; Zapata, A; Shippenberg, TS; French, ED. (2007). The abused inhalant toluene increases dopamine release in the nucleus accumbens by directly stimulating ventral tegmental area neurons. *Neuropsychopharmacology*, 32(7), pp. 1558-1569.

Riegel, AC; Ali, SF; Torinese, S; French, ED. (2004). Repeated exposure to the abused inhalant toluene alters levels of neurotransmitters and generates peroxynitrite in nigrostriatal and mesolimbic nuclei in rat. Annals of the New York Academy of Science, 1025, pp. 543-551.

Robinson, TE and Berridge, KC. (2003). Addiction. *Annual Review of Psychology*, 54, pp. 25-53.

Rose, JH; Calipari, ES; Mathews, TA; Jones, SR. (2013). Greater ethanol-induced locomotor activation in DBA/2J versus C57BL/6J mice is not predicted by presynaptic striatal dopamine dynamics. *PLoS One*, 8(12), e83852.

Scheeres, JJ and Chudley, AE. (2001) Solvent Abuse in Pregnancy: A Perinatal Perspective. *Journal of obstetrics and Gynecology (Canada)*, 24(1) pp. 22-26.

Siegel, JT; Alvaro, EM; Patel, N; Crano, WD. (2009). "…you would probably want to do it. Cause that's what made them popular": Exploring Perceptions of Inhalant Utility Among Young Adolescent Nonusers and Occasional Users. *Substance Use & Misuse*, 44(5), pp. 597-615.

Solomon, RL. (1980). The Opponent-Process Theory of Acquired Motivation: The Costs of Pleasure and the Benefits of Pain. *American Psychologist*, 35(8), pp. 691-712.

Stengard, K; Hoglund, G; Ungerstedt, U. (1994). Extracellular dopamine levels within the striatum increase during inhalation exposure to toluene: a microdialysis study in awake, freely moving rats. *Toxicology Letters*, 71(3), pp. 245-255.

Stengard, K; Tham, R; O'Connor, WT; Hoglund, G; Ungerstedt, U. (1993). Acute toluene exposure increases extracellular GABA in the cerebellum of rat: a microdialysis study. *Pharmacology and Toxicology*, 73(6), pp. 315-318. , 154(2), pp. 315-318.

Stollery, BT. (1996). Long-Term Cognitive Sequelae of Solvent Intoxication. *Neurotoxicology and Teratology*, 18(4), pp. 471-476.

SAMHSA. (2012). Results from the 2011 National Survey on Drug use and Health: Summary of National Findings.

SAMHSA. (2007). The NSDUH report: Patterns and trends in inhalant use by adolescent males and females, 2002–2005.

Tomaszycki, ML; Aulerich, KE; Bowen, SE. (2013). Repeated toluene exposure increases c-Fos in catecholaminergic cells of the nucleus accumbens shell. *Neurotoxicology and Teratology*, 40, pp. 28-34.

Warren, DA; Bowen, SE; Jennings, WB; Dallas, CE; Balster, RL. (2000). Biphasic Effects of 1,1,1-Trichloroethane on the Locomotor Activity of Mice: Relationship to Blood and Brain Solvent Concentrations. *Toxicological Sciences*, 56, pp. 365-373

Whitehead, KJ; Rose, S; Jenner, P. (2001). Involvement of intrinsic cholinergic and GABAergic innervation in the effect of NMDA on striatal dopamine efflux and metabolism as assessed by microdialysis studies in freely moving rats. *European Journal of Neuroscience*, 14(5), pp. 851-860.

Williams, JF and Storck, M. (2007) Inhalant Abuse. *Pediatrics*, 119, pp. 1009-1017.Yavich, L and Zvartau, E. (1994). A Comparison of the Effects of Individual Organic Solvents and Their Mixture on Brain Stimulation Reward. *Pharmacology, Biochemistry, and Behavior*, 48(3), pp. 661-664.

Wood, RW; Coleman, JB; Schuler, R; Christopher C. (1984). Anticonvulsant and Antipunishment Effects of Toluene. *The Journal of Pharmacology and Experimental Therapeutics*, 230(2), pp. 407-412

Zackheim, J and Abercrombie, E.D. (2005) Thalamic regulation of striatal acetylcholine efflux is both direct and indirect and qualitatively altered in the dopamine-depleted striatum. *Neuroscience*, 131 (2), pp. 423-436.

Zacny, JP; Janiszewski, D; Sadeghi, P; Black, ML. (1999). Reinforcing, subjective, and psychomotor effects of sevoflurane and nitrous oxide in moderate-drinking healthy volunteers. *Addiction*, 94(12), pp. 1817-1828.

ABSTRACT

THE EFFECT OF NMDA AND GABA ANTAGONISM ON DOPAMINE RELEASE IN THE MOUSE CAUDATE PUTAMEN FOLLOWING ACUTE TOLUENE INHALATION: AN IN-VIVO MICRODIALYSIS STUDY

by

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Major: Psychology (Behavioral and Cognitive Neuroscience)

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Toluene is ubiquitous solvent commonly inhaled recreationally. Despite its frequency of misuse, there is little understanding of how toluene acts within the brain. To examine this, this master's thesis examined the impact of acutely inhaled toluene on dopamine (DA) release in the mouse CPu *in vivo* using microdialysis techniques. Toluene inhalation produced dose-dependent increases in DA levels as well as changes in locomotor activity. These effects were potentiated by pre-treatment with the $GABA_A$ antagonist bicuculline via reverse microdialysis delivery. These results suggest that the DA dynamics of toluene abuse are related to toluene's previously explored effects on the GABA system. It is possible that the action of toluene on GABA in the caudate actually inhibits toluene's action on DA. Another theory is that toluene activates multiple GABA receptor sites, and blocking $GABA_A$ blocked a primarily inhibitory pathway.

AUTOBIOGRAPHICAL STATEMENT

Sean Callan is currently a doctoral student in Wayne State University's Psychology department, with a major in Behavioral and Cognitive Neuroscience, and a minor in Statistics. He graduated from Oakland University in 2011 with a Bachelor of Arts in Psychology with a Minor in English, receiving both departmental and university honors. He is currently a member of the Behavioral Pharmacology and Toxicology lab.