CHARACTERIZATION OF THE EFFECTS OF AMPHETAMINES IN THE PLANARIAN SPECIES *Dugesia dorotocephala*

By

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To my mom, dad, and brother, whose encouragement and support have been essential

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Drugs in the amphetamine class, 3,4-methylenedioxymethamphetamine (MDMA), and amphetamine (AMPH) are among the most popular drugs of abuse. Chronic users of amphetamine or analogs can have long-lasting deficits in dopaminergic or serotonergic systems. Planaria, thought to possess the first true central nervous system, exhibit pharmacological and behavioral similarities to vertebrates for many serotonin, dopamine, acetylcholine, GABA, glutamate, and endorphin receptors and drugs, have regenerative capabilities, and exhibit an anti-tropism to light. This study evaluated the toxic effects of AMPH and MDMA on the planarian species *D. dorotocephala*.

The first set of experiments focused on evaluating general toxic effects induced by these drugs. We found that AMPH and MDMA dose-dependently caused decreased locomotor speed and head loss. These effects were not prevented by treatment with drugs shown to be neuroprotective in some other models. Both also caused depletion of monoamine levels and increased indices of dopamine turnover, an effect observed in many vertebrate models and indicative of toxicity. The next set of experiments sought to determine the long-term effects from head loss resulting from AMPH or MDMA exposure. We found that following head loss in

AMPH, but not MDMA, planaria did not recover locomotor speed to control levels. The final set of experiments evaluated the effects of low doses of AMPH and MDMA on regeneration following head removal. We found that low doses of either drug did not inhibit regeneration.

Our studies showed that both AMPH and MDMA are toxic to planaria and appear to be affecting the monoaminergic systems in a way similar to what is observed in other vertebrate models. This model could be useful for studying the dopamine and serotonin related toxicity resulting from abuse of amphetamines.

CHAPTER 1
AMPHETAMINES

Epidemiology

Drug abuse is a major health problem throughout the world. The estimated costs of drug abuse, including loss of productivity, crime, and other health-related expenses for the United States alone was estimated at over $140 billion in 1998 (NIDA, 2001). This was an increase by $40 billion from 1992, and such use is projected to continue increasing according to the Office of National Drug Control Policy. One particular group of abused drugs, the amphetamines, has gained in popularity especially throughout California and the midwest and is spreading to other areas throughout the country as well. According to the Drug Abuse Warning Network (DAWN), stimulants, including all amphetamines, but particularly amphetamine (AMPH) and methamphetamine (METH), were involved in over 100,000 Emergency Department (ED) visits, accounting for about 5% of drug-related ED visits overall in 2004 (SAMHSA, 2006a). Although stimulant abuse is not as high now as it was in mid 1900s, these remain heavily abused among illicit drug users. With accumulating evidence of the lasting effects on the dopaminergic systems of chronic amphetamine abusers in humans, this poses a serious and urgent need for an understanding of the mechanisms involved in AMPH toxicity.

AMPH first came into widespread use during the 1950s and ‘60s when it was frequently prescribed for medical purposes such as: as a bronchodilator in the form of an inhaler, as an anorectic to promote weight loss, and as a treatment for narcolepsy. It was even given to soldiers as a stimulant to combat fatigue during duty. This raised general awareness about the drug and its euphoric effects, which subsequently catalyzed the epidemic of illicit recreational use. Laws were then passed in the U.S. and many other countries, providing sanctions in order to counteract the epidemic and limit the legal production of AMPH. It is still prescribed, in low doses, to treat

attention deficit disorder, narcolepsy, and obesity. Recreational use of AMPH, however, remains popular and the substituted form METH, which has similar effects, has especially gained popularity. AMPH for abuse can be obtained through prescriptions sold illegally or it can be synthesized in clandestine laboratories. It can be ingested orally or injected intravenously (or smoked in the case of METH). The use pattern of smoking represents the most hazardous form for ingesting METH.

Another popular drug, 3,4-methylenedioxymethamphetamine, MDMA (ecstasy), a ring-substituted amphetamine and a "club drug," is popular among young people, especially in the dance club and rave scene in the U.S., the United Kingdom, and Europe. According to the "monitoring the future" survey published by the National Institute on Drug Abuse, lifetime ecstasy use in the U.S. decreased between 2002 and 2005 (from 3.3% to 1.6%) in the 12-17 age group and among 18-25 year olds (15.1% in 2002 to 13.7% in 2005) (SAMHSA, 2006b). However, according to the Office of National Drug Control Policy (ONCDP), the number of new users increased from 2005 to 2006 and the number of past month users also increased in the same time period (ONCDP, 2008). MDMA was initially synthesized in 1912 by Merck, but it was not produced and did not come into use until the 1970s and '80s where it was used to aid in psychotherapy. It was reported to facilitate open communication and an increased sense of well being in patients (Grinspoon and Bakalar, 1986). It then gained popularity with recreational users for its mood-enhancing effects and the food and drug administration (FDA) classified it as a Schedule I drug in 1985, thus eliminating any legal use in this country. There is currently a growing movement to reevaluate this classification and make allowances for its limited use in some psychiatric conditions (such as in the treatment of post-traumatic stress disorder) and also

to aid terminal cancer patients by enhancing their mood and perceived quality of life as they approach their impending death.

Mechanism of Action

AMPH and MDMA exert their effects by facilitating release of catecholamines and serotonin through the transporters and blocking reuptake at presynaptic terminals (Rudnick and Wall, 1992; Sulzer et al., 1995; Mlinar and Corradetti, 2003). AMPH exerts its effects primarily through the dopamine transporter (DAT). It enters the nerve terminal by two mechanisms: passive diffusion and through the transporter at the nerve terminal (Zaczek et al., 1991). MDMA acts by similar mechanisms predominantly at the serotonin transporter (SERT) at least in some but not all mammals (Rudnick and Wall, 1992). It should be noted, however, that in some species MDMA has different effects: in some mice models, MDMA acts primarily at the dopaminergic system (Easton and Marsden, 2006), while in rats and non-human primates it acts primarily at the serotonergic system (Rudnick and Wall, 1992). Both also act at the norepinephrine transporter (NET), though to a lesser extent. Vesicular monoamine transporter 2 (VMAT 2) is also involved in vesicular release which is calcium- and impulse-dependent. In mice lacking the DAT (KO mice), AMPH fails to cause an increase of DA release in the striatum (in the extracellular space), but it does not inhibit its vesicular releasing action (Jones et al., 1998).

Effect on Monoamine Levels

Both AMPH and MDMA induce lasting depletions in monoamine levels in animal models. While METH appears to affect both the dopaminergic and the serotonergic systems, AMPH primarily affects the DA system and MDMA primarily affects the serotonergic system. AMPH, and the substituted form METH, exert actions as indirect agonists of DA receptors, as reuptake blockers through the transporters at the synapse (Heikkila et al., 1975; Han and Gu, 2006) and

also as inhibitors of monoamine oxidase (Kita et al., 2000). Taken together, these actions initially lead to an accumulation of DA in the synapse followed by lasting dopamine depletions (Wagner et al., 1980; Ali et al., 1994). MDMA has similar effects on the serotonin system by releasing serotonin into the synaptic space, causing release from synaptic vesicles within the terminal, binding to the reuptake site and effectively blocking reuptake while facilitating uptake of itself into the nerve terminal (Rudnick and Wall, 1992). One method both AMPH and MDMA use to release monoamines from the transporter is through exchange, in which AMPH or MDMA get taken up by the DAT or SERT, respectively, which then binds the respective neurotransmitter (DA or 5-HT) and subsequently carries it into the extracellular space, in what has been termed the exchange diffusion model (Fischer and Cho, 1979). AMPH further prevents DA reuptake by facilitating movement of the DAT away from the cell surface, lowering the capacity for reuptake (Kahlig et al., 2006). MDMA has also been shown to inhibit synaptosomal and vesicular uptake of both serotonin and dopamine (Bogen et al., 2003).

Neurotoxicity

Administration of multiple high doses of AMPH to mice resulted in decreased DAT binding in the striatum, indicating nerve terminal destruction (Scheffel et al., 1996). Continuous administration of high doses of METH to rats resulted in toxicity to both DA and 5-HT terminals (Ricaurte et al., 1980; Armstrong and Noguchi, 2004). Nerve terminal loss is evidenced by a loss of DAT, SERT, and VMAT 2 on the membrane surface (Guilarte et al., 2003). This reduced presence could also be due to an adaptive down-regulation of the membrane proteins, as some pharmacological agents have been shown to alter DAT and SET levels (Gordon et al., 1996; Zhou et al., 1996). However, VMAT 2 does not appear to be as sensitive (Wilson et al., 1996), indicating that a decrease in levels of VMAT 2 in combination with decreases in DAT or SERT represents terminal damage rather than down-regulation at the membrane surface. Following a

prolonged abstinence period, there was a significant recovery of dopamine function (Friedman et al., 1998; Cass and Manning, 1999). However, these results should be interpreted cautiously as they are based upon the acute toxic dose model rather than the chronic use pattern seen in many METH and AMPH abusers. High doses of amphetamines have also been shown to cause lasting depletions of 5-HT in the striatum, hippocampus, and frontal cortex (Ricuarte et al., 1980; Friedman et al., 1998). There are also lasting depletions in tyrosine hydroxylase (Bowyer et al., 1998) and tryptophan hydroxylase activities (Che et al., 1995). There are numerous theories of how amphetamines cause damage, including formation of ROS and oxidative stress, reactive nitrogen species (RNS), mitochondrial disruption, and glutamate-mediated excitotoxicity (Fleckenstein et al., 2007). One group has gone so far as to postulate that it is the neurotransmitter-releasing action of amphetamines (due to their ability to facilitate monoamine transport outward through the transporter) on DA and 5-HT that is responsible for the neuronal damage and that simply acting as a transport blocker is not only inadequate to cause damage, but can also have protective effects (Kita et al, 2003). DA may partially mediate the 5-HT depletions induced by MDMA since depletion of DA stores with reserpine attenuates damage, but this may be due to thermoregulation since reserpine counteracts the hyperthermic effect observed following MDMA administration (Yuan et al., 2002). This hyperthermic effect has been implicated in the neurotoxicity observed with MDMA in animal models (Malberg and Seiden, 1998). In addition, depletion of DA prior to MDMA administration also attenuates long-term damage (Brodkin et al., 1993). In mice deficient of MAO-B, toxicity to 5-HT neurons was attenuated (Fornai et al., 2001). This is thought to be due to the uptake of MDMA-induced DA release into 5-HT neurons, where DA is subsequently metabolized by MAO-B, a process which was already described to produce reactive products (Sprague et al., 1998).

It was originally thought that amphetamines were only toxic to nerve terminals; that view, however, is currently under fire. Cadet and his colleagues have repeatedly shown evidence of apoptotic cell bodies in mouse brains following administration of METH and AMPH (Deng et al., 2002a; Jayanthi et al., 2004; Krasnova et al., 2005). They and others have provided further evidence that AMPH and METH can cause an up-regulation of pro-apoptotic genes and down-regulation of anti-apoptotic genes, possibly due to the generation of reactive oxygen species (ROS), and induce apoptosis directly (Stumm et al., 1999; Thiriet et al., 2001; Deng et al., 2002b). There is also evidence in animal models showing that acute exposure of rat brain to MDMA selectively destroys neurites of serotonergic neurons in brain regions such as the hippocampus and cerebral cortex (Ricaurte et al., 2000; Meyer and Ali, 2002). MDMA is also known to induce apoptosis in human serotonergic choriocarcinoma (JAR) cells (Simantov and Tauber, 1997) and in rat neocortical neurons in culture. Thus, MDMA might induce neurite degeneration as well as apoptotic death in serotonergic systems. There is also evidence that oxidative stress plays a role in MDMA-induced neurotoxicity. Lipid peroxidation, an indicator of free radical damage, was increased following MDMA administration (Sprague and Nichols, 1995; Colado et al., 1997).

Reactive Oxygen Species

Oxidative stress has been implicated in aging and a number of degenerative diseases including, but not limited to, Alzheimer's (Sompol et al., 2008), where it is thought to play a pivotal role, Parkinson's (Chin et al., 2008), and amyotrophic lateral sclerosis (ALS) (Rakhit et al., 2004). Generation of ROS occurs naturally through cellular metabolism and mitochondrial production of ATP. These basal levels are kept low by endogenous antioxidants and enzymes such as superoxide and glutathione peroxidase. There is strong support for the ROS and oxidative stress hypothesis of amphetamines toxicity with two main theories. One theory is that

following the outward flood of DA into the extracellular space, it auto-oxidizes to 6-hydroxy (OH) DA, producing hydrogen peroxide in the process (Kita et al, 2003). There is also evidence for the involvement of oxygen-based free radicals given that over-expression of the copper-zinc superoxide dismutase gene in transgenic mice provides protection from the toxic effects of METH (Deng and Cadet, 2000). In addition, administration of antioxidants such as ascorbic acid (Wagner et al, 1986), L-carnitine (Virmani et al., 2003), and N-acetyl-L-cysteine (Hashimoto et al., 2004) protect against METH-induced damage. Similarly, N-acetyl-L-cyteine and α-phenyl-N-tert-butyl nitrone, another free radical scavenger, protect against the lipid peroxidation, hydroxyl radical formation, and DA depletion caused by AMPH (Wan et al., 2005).

The second major theory involves the accumulation of oxidizable dopamine through redistribution from synaptic vesicles into cytoplasmic compartments, ultimately resulting in terminal injury. In support of this, METH toxicity was increased in vesicular monoamine transporter 2 (VMAT 2) knockout mice (Fumagalli et al., 1999).

Generally there is a resulting depletion in antioxidants and enzymes necessary for antioxidant reactions after toxic AMPH or MDMA exposure. The thought is that amphetamines increase the level of reactive species beyond the capabilities of the antioxidants present and also through the excess DA released. It is thought that the excess DA released then autooxidizes and produces DA-quinones, hydroxyl radicals, superoxide radicals, and hydrogen peroxide both intra- and extracellularly. Hydrogen peroxide is also formed when MAO deaminates DA and thus, with all the extra DA released, adds to the load for antioxidants.

Reactive Nitrogen Species

Meanwhile other groups have focused on a role for glutamate-mediated excitotoxicity. AMPH and METH administration results in an increase in glutamate release in the ventral

tegmental area (VTA) (Wolf et al., 2000; Mark et al., 2004) and is prevented by both MK-801, an NMDA antagonist, and SCH 23390, a dopamine D1 receptor antagonist administered prior to AMPH (Wolf and Xue, 1999). Additionally, blockade of the metabotropic glutamate receptor mGlu5 protects against METH toxicity (Battaglia et al., 2002a). Inhibition of neuronal nitric oxide synthase, nNOS, or knockout of the gene encoding nNOS has also been shown to protect against toxicity following amphetamine treatment (Imam et al., 2001; Bashkatova et al., 2004). nNOS generates nitric oxide (NO) which can then combine with superoxide radicals (which are increased following AMPH treatment) to form peroxynitrite, which in turn can then induce DNA strand breaks, lipid oxidation, protein oxidation and nitration (Koppenol et al., 1992). The role for glutamate or the nitrergic system in MDMA induced toxicity is less clear. Evidence suggests that the nitrergic system plays a role in DA toxicity, but not for serotonin toxicity (Itzhak et al., 2004).

Mitochondrial Dysfunction

There is some evidence that amphetamines inhibit complexes in the electron transport chain (Burrows et al., 2000). However, it is more likely the combination of both ROS and RNS leads to mitochondrial failure and cell death. This mitochondrial disruption leads to a decrease in ATP production (Wan et al., 1999), while there is generally an increase in energy demands (Virmani et al., 2002). Administration of substrates for the electron transport chain can attenuate toxicity from amphetamines (Stephans et al., 1998), providing support for the involvement of the mitochondria. While there is evidence of apoptotic cell death (Krasnova et al., 2005), characterized by membrane blebbing, activation of apoptotic genes, and compartmentalization, there are more nerve terminals that are affected, indicating that necrosis plays a larger role, possibly due to the lack of ATP available to activate the apoptotic cascade.

Behavioral Effects in Animals

Both AMPH and MDMA have been shown to result in behavioral changes, such as psychosis, impaired memory, aggressiveness, impulsivity, and others in humans. These effects have been tied to nerve terminal damage as well as cell death and plastic restructuring (hyperexcitablility etc). In a recent study involving administration of MDMA to mice at levels seen in human users, oxidative stress in the hippocampal region was reported (Frenzilli et al., 2007). In addition, they observed hyperexcitability and a reduced threshold for seizure in the hippocampus. Animal studies also show impairments in cognitive function. Object recognition memory impairments were shown after both acute (Bisagno et al., 2003) and repeated (Kamei et al., 2006) METH exposure. Neonatal METH exposure causes impaired hippocampus-dependent spatial memory in rodents in the Morris water maze (Vorhees et a., 2000; Acevedo et al., 2007) and the Barnes maze (Williams et al., 2003). In addition, neurodegeneration in the hippocampus has been observed (Schmued and Bowyer, 1997) as well as impairment of long-term potentiation in hippocampal-prefrontal cortex pathway (Ishikawa et al., 2005). Sequential learning on a radial arm maze task along with changes in basal ganglia neurochemistry were observed following a toxic dose of METH in rats (Chapman et al., 2001).

Human Data

A recent report demonstrated lasting depletions in prefrontal grey matter density using magnetic resonance imaging (MRI) and impairment in frontal executive function even after more than 6 months of abstinence from METH abuse (Kim et al., 2006). Another report using positron emission tomography (PET) showed a loss of dopamine transporters (DAT) in the striatum which was associated with impairments of memory and motor function (Volkow et al., 2001a). This same group showed a partial recovery of DAT following at least a year of abstinence, but there was no significant recovery of memory and motor function (Volkow et al., 2001b). An

earlier study showed lasting depletions in striatal DAT following an average of 3 years of abstinence (McCann et al., 1998). This leads to the question of whether the loss of DAT represents an adaptive down-regulation or nerve terminal damage, which may be long-term. Although some evidence suggests irreversible damage, the discrepancies in findings could be explained by length of abstinence compared to duration and extent of use. Using subjects with a range of abstinence from months to years, while at the same time these subjects have use patterns ranging from years to decades, one could reasonably infer that effects of longer abstinence times may be hidden or reduced. Confounding factors in the human populations sampled may make interpretation less than certain. In support of this theory, however, studies in non-human primates indicate a significant recovery in dopamine function following an acute dose of METH only after a prolonged period (Melega et al., 1997; Harvey et al., 2000). These effects are not limited to DAT, a recent study showed significantly decreased serotonin transporter density in currently abstinent METH abusers associated with elevated levels of aggression (Sekine et al., 2006).

There is ample evidence that AMPH is neurotoxic to humans, especially the dopaminergic system, however data for whether MDMA is neurotoxic to humans is controversial. It has been stipulated that thus far there have been no differences found between control subjects and MDMA users in studies using PET, MRI, or EEG (Gouzoulis-Mayfrank and Daumann, 2006). In contrast, in imaging studies, there appears to be lasting deficits in the SERT (Buchert et al., 2003; McCann et al., 2005). Results from McCann were obtained from abstinent human users of MDMA using PET to image the serotonin reuptake transporter (SERT). In this study, levels of SERT were decreased compared to controls with a relatively short abstinence period (the requirement being only 2 weeks, and an average of 4 months). However, this report's results were later critiqued as being due to an effect of reduced binding potential rather than reduced

SERT availability by one of the same groups who co-authored the original report (Buchert et al., 2007). There are reports of differences in metabolic function in some regions such as the hippocampus and amygdala in ecstasy users compared to controls, indicating altered brain activity (Obrocki et al., 1999).

Some of the problems with human drug abuse data in general include: often there is more than one drug being abused, reported usage may not be accurate, and investigators don't know what levels (baseline) were there prior to drug use. Also, purity of the tablet cannot be established, especially in the 1990s, where MDA (3,4-methylenedioxyamphetamine) or MDEA (3,4-methylenedioxyethylamphetamine) were commonly detected in ecstasy pills. A typical tablet of ecstasy is reported to contain 50-150 mg of MDMA (Parrott 2004). Parrott's study also said that tablets were fairly pure in the 1980s, but that purity then decreased in the mid-1990s, and it has since increased. However, in a study by Tanner-Smith (2006), pills were submitted anonymously between 1999-2005, and it appears that the purity has decreased from 1999-2005. In fact, it was just recently reported that MDMA coming out of Canada had high concentrations of METH, which is a known neurotoxicant (ONDCP, 2008). Adding to the controversy over MDMA are the conflicting results obtained from different groups. Some studies show deficits just from polydrug use regardless of whether MDMA was involved. This would suggest that it is the combinations of the effects of different drugs that make it neurotoxic. Finally, in human studies we frequently have the problem of non-random assortment of secondary variables (endogenous or exogenous) that might have contributed to the subject's abuse patters, and/or inclusion in the study.

While there is yet to be direct, conclusive evidence of cellular toxicity/structural damage in humans, there is much evidence of the effects of MDMA on behavior. Regular ecstasy users

demonstrate impaired memory compared to controls (Parrott and Lasky, 1998) and also when compared to other polydrug users who had never used ecstasy (Morgan, 1999). Regular use has also been reported to result in psychosis, depression, anxiety, and impulsivity. Some have psychiatric illness in the family, suggesting that they may have been predisposed (see above). However, in a prospective study, MDMA users displayed more psychiatric symptoms than other drug users or normal controls, indicating a direct role for MDMA (Parrot, 2006). The possibility that users of MDMA already had psychiatric symptoms and had not sought treatment, that they are at an increased risk, or that use of other combinations of drugs are confounding factors cannot be ruled out. Therefore, there is no conclusive evidence for a direct involvement of MDMA. Yet there is, however, evidence that MDMA causes oxidative stress in humans. In a study of 120 MDMA users (who had been using for at least one month), levels of lipoperoxidase were increased and levels of Vitamins C and E, β-carotene, SOD, and catalase were decreased in the MDMA group compared to controls (Zhou et al., 2003).

Acutely, AMPH is a stimulant and results in increased alertness, decreased fatigue, elevated blood pressure, and increased metabolic rate. Low doses are used therapeutically to treat a wide variety of conditions. It is widely used to treat attention deficit hyperactivity disorder (ADHD) due to its ability to increase focus for individuals with this condition (Faraone, 2007). It has also been used to treat obesity due to its effects on metabolic rate (Bray and Greenway, 1999), and narcolepsy (Soldatos et al., 1979). Chronic AMPH can lead to deficits in attention, memory, and decision-making skills in humans. Long-term use can also result in what has been termed amphetamine-induced psychosis (Kokkinidis and Anisman, 1981), typically consisting of paranoia, hallucinations, and behavioral disorganization. Treatment with antipsychotics generally ameliorates this condition (Leelahanaj et al., 2005). Impairment of neuropsychological functions

including frontal executive function, memory recall impairment, and attention are observed in human chronic METH abusers (Volkow et al., 2001a; Sim et al., 2002; Kim et al., 2006).

In Vitro and In Vivo Models

Most *in vitro* models consist of using cell cultures containing monoaminergic neurons or synaptosome preparations. These cultures provide an easily controlled environment where variables can be manipulated and effects easily observed. Doses used are generally between 1 and 2 mM, although lower doses can be used, generally with limited observable effects (Deng et al., 2002a; Hayat et al., 2006; Warren et al., 2007). These models are typically used to elucidate involvement of a particular biochemical pathway or to evaluate effectiveness of interventions. Slice culture models are also used to evaluate effects on specific brain regions (Miller et al., 2007).

Animal models used depend on the effect being evaluated. For toxicity studies, the two main models used are acute and chronic, though the acute toxic dose model is the most common. Acute models are meant to mimic the (binge) use pattern seen in many recreational users. Generally it is either a single acute toxic dose or multiple high doses given within a period of 8-72 h (McGregor et al., 2003; Itzhak and Achat-Mendes, 2004). Chronic models generally give a lower dose daily for a period of days to weeks (Frederick et al., 1995; Frey et al., 2006). However, there can be substantial differences between the animal models. In mice, MDMA primarily affects the dopaminergic system, while this does not occur in rats and non-human primates. Therefore, it is important to evaluate the advantages and disadvantages of each species employed for experiments.

CHAPTER 2
PLANARIA

General Characteristics

Planaria are a type of free-living flatworm of the phylum platyhelminthes (class turbellaria) that are believed to possess the first true CNS and bilateral symmetry. They may be similar to the ancestors of all other bilaterians. They are found throughout the world in freshwater streams and lakes and are well known for their immense regenerative capabilities; thus their popularity in school biology classes. It takes approximately 10,000 (Sanchez Alvarado, 2004) cells to regenerate an entire worm regardless of whence they come. Studies on evolutionary origin of the brain suggest similar platyhelminthes are the common ancestor for a centralized nervous system in all other vertebrates and invertebrates (Sarnat and Netsky, 2002; Mineta et al., 2003). Thus, they are usually phylogenetically located at the base of the bilateria in the protostome subdivision, although their precise location in such evolution is not completely established.

The worms are triploblastic, meaning they have three layers: an ectoderm, endoderm, and mesoderm. They do not contain a body cavity (acoelomate) and the digestive tract has only one opening for ingestion and elimination. However, they still have complex organ systems. The method of reproduction depends on the species, with some species exhibiting fissionary reproduction and others exhibiting hermaphroditic sexual reproduction, or both, which usually depends on the season. Sexually reproducing animals are hermaphroditic, while asexual animals undergo transverse fission to reproduce. They do not possess a circulatory or respiratory system and therefore must rely on simple diffusion; this may account for their flat shape. The digestive system has a mouth (the opening) which leads to the pharynx and culminates in the branched gastrovascular cavity. One branch extends anteriorly into the head region and two branches

extend posteriorly into the tail section. The excretory system is comprised of flame cells (protonephridia) which have fast-beating cilia that function to promote movement of fluids out of the body through porous openings on the surface (Ishii, 1980). The protonephridial system is a highly connected, branched system located in all parts of the body. Parenchyma (the mesenchyme) fills the space between organ systems.

Planarian movement is largely under cephalic control (Kato et al., 2004), and they demonstrate little movement in the absence of a head. They exhibit two types of movement: via cilia, which cover the ventral surface of the body, and via contractions of the large muscles (more resembling smooth than striated tissue), the latter enabling faster locomotion.

Nervous System

As stated previously, flatworms are believed to possess the first true CNS. They have a rudimentary bilobar brain consisting of cephalic ganglia connected by two commissures. This gives rise to two ventral nerve cords, also connected by multiple commissures, forming a ladder-like structure extending the length of the body. These innervate the muscle fibers and sensory receptors. In early studies of the nervous system, many investigators did not realize the complexity of the nervous system as its fine structure cannot be observed with a light microscope. Later studies with the electron microscope revealed a much more complex system with synaptic connections and other features characteristic of "higher animals". They possess catecholamine (DA and NE), serotonin, glutamate, GABA, and opioid systems, mostly confirmed only by pharmacological analysis. The brain is arranged in an inverse U-shape. Planaria possess monopolar neurons, typical of invertebrates, in addition to bipolar and multipolar neurons (Koopowitz and Chien, 1975). Neurons located in the commissures in the ventral nerve cords tend to possess bipolar neurons, possibly functioning as interneurons (Rieger

et al., 1991). The axons are generally not myelinated (Morita and Best, 1966), although some sheathing has been found in other species of flatworm (Trawicki et al., 1988). Cell bodies contain many neurosecretory granules. Neurons can be characterized morphologically by the presence of dense synaptic vesicles. The nervous system appears to be secretory in nature, with synapses close together (Cebria, 2007).

CNS Systems Present

Planaria possess many mammalian-relevant neurotransmitter systems, most characterized only via pharmacological methods. However, recent advances in molecular biology and the perseverance of a select few laboratories have led to molecular evidence for serotonin and dopamine systems. The dopamine system may also have different receptor types, although this has not been characterized by cloning or sequencing. There are also dopaminergic-cholinergic interactions similar to mammalian systems. Much of the early work investigating planarian nervous system was conducted physiologically, providing little evidence for the types of receptors involved. The availability of selective agonists/antagonists has shed more light on the subject, however, molecular characterizations need to be conducted.

Serotonin

Serotonin is a substance found throughout the animal kingdom, in single celled organisms and even some plants (Feldman and Lee, 1985; Johnson et al., 2007). In humans it is found in large quantities in the gastrointestinal tract, in blood components, and in the central nervous system. Some of its many functions include playing a role in appetite, anxiety, depression, aggression, learning and memory, temperature regulation, muscular contraction, cardiovascular function, and endocrine regulation. In planaria, serotonin is present throughout the central and peripheral nervous system, particularly along the ventral nerve cords (Welsh and Williams, 1970; Reuter et al., 1995), with confirmation by HPLC (Fernandes et al., 2003; Umeda et al., 2005).

Knockdown of the gene encoding tryptophan hydroxylase in planaria, the rate limiting enzyme in serotonin synthesis, resulted in significant decreases in serotonin, indicating that the synthesis in planarians is by a similar pathway (Nishimura et al., 2007a). It is not clear what the function of serotonin has in flatworms, but it appears to have roles in regeneration, movement, and circadian rhythms. When given p-Chlorophenalanine, a serotonin depletor, the ability to glide, a function provided by the cilia on the ventral surface of the worm, decreases significantly (Kimmel and Carlyon, 1990). Instead, these worms rely on the large muscle movement termed "looping" in the aforementioned paper. Immunohistochemical studies have shown high concentrations of serotonin in the peripheral nerve plexus located in close proximity to the ventral cilia (Welsh and Williams, 1970) and administration of serotonin to muscle fibers stimulates muscle contraction (Moneypenny et al., 2001). Studies in parasitic flatworms show a more definitive involvement of serotonin, where it is located in the nerve plexuses innervating muscle cells (Marks et al., 1994), and administration of serotonin induces muscle contraction, though whether this is a direct or indirect effect remains to be determined. To date, two G protein-coupled receptors with sequence homology to the 5-HT1A receptor have been cloned in planaria (Saitoh et al., 1997). This receptor was also characterized with LSD binding (Saitoh et al., 1996) and 8-OH-DPAT, a 5-HT1A agonist, administration stimulates adenylate cyclase activity, providing further evidence for a mammalian-like 5-HT1A receptor (Creti et al., 1992). In mammals, serotonin is involved in synchronization of circadian rhythms (Marchant et al., 1997). Planaria exhibit fluctuations in serotonin levels corresponding with a light-dark cycle, even in the absence of light, although its precise role is not yet determined (Itoh and Igarashi, 2000). Its role in regeneration has been studied and will be discussed later (see below).

Catecholamines

Catacholamines are also present throughout the animal kingdom and have been found in plants. They are involved in a number of behavioral functions. There is also a substantial amount of evidence for a dopaminergic system, however evidence of an adrenergic system is not as strong. The presence of DA and NE has been confirmed by HPLC and LCED (liquid chromatography with electrochemical detection) (Algeri et al., 1983). The presence of two different DA receptors has putatively been determined, but this was only characterized pharmacologically (Algeri et al., 1983; Venturini et al., 1989). According to Venturini (1989), there are two distinct behavioral responses to specific drugs (though at high doses): stimulation of D1 receptors results in screw-like hyperkinesias (SLH), whereas stimulation of D2 receptors results in C-like position (CLP). These effects are blocked by the relevant selective antagonists. Administration of both D1 and D2 agonists also leads to increases in cAMP levels which is also blocked by the antagonists, a characteristic that has been demonstrated for D1 receptors in vertebrates, but is atypical for D2 receptors (Venturini et al., 1989; Palladini et al., 1996). An increase in cAMP production has been observed with simultaneous stimulation of cannabinoid (CB1) and D2 receptors in a culture of hamster striatal cells, but this was only in combination (Glass and Felder, 1997). A compound commonly used to ablate dopamine systems in vertebrates, 6-hydroxydopamine (6-OHDA) also appears to destroy the DA system in planaria in a similar fashion, resulting in an absence in movement and a marked decrease in catecholamines (Caronti et al., 1999). More recently, a gene encoding tyrosine hydroxylase has been cloned in a planarian species and knockdown of this gene attenuates DA synthesis along with movement controlled by the musculature (Nishimura et al., 2007b). Furthermore, 3-Iodo-L-tyrosine, a tyrosine hydroxylase inhibitor, also leads to a decrease in dopamine production. However, it also leads to a decrease in serotonin (Ness et al., 1996), too, indicating that it may not be specific for

tyrosine hydroxylase as it was demonstrated that knockdown of the actual gene led to a decrease in DA, but not 5-HT (Nishimura et al., 2007b). There also appears to be an interaction with the opioid and cholinergic systems in planaria. The cholinergic system may act as an inhibitor to DA-induced movement, as administration of a cholinergic antagonist results in screw-like hyperkinesia (SLH), a behavior typical of D1 agonists (Venturini et al., 1989; Palladini et al., 1996; Buttarelli et al., 2000)

Acetylcholine

Acetylcholine is widely known for its role in the muscular system as well as the autonomic nervous system in mammals. It is also present as interneurons and projection neurons in the CNS, where it is believed to play a role in arousal, sleep, and learning and memory. The two main receptor classes are the ligand-gated nicotinic receptors and the metabotropic G-protein coupled muscarinic receptors. There is evidence for the presence of both receptor types in planaria. Physostigmine, an acetylcholinesterase inhibitor, causes a characteristic movement, or hypokinesia, called bridge-like position (BLP) (Carolei et al., 1975), an effect that is reversed upon addition of atropine, a muscarinic antagonist (Buttarelli et al., 2000). Administration of nicotine produces a different motor behavior called walnut position (Buttarelli et al., 2000). It should be noted, however, these studies are somewhat compromised by the very high concentrations of drugs employed, at which they may not be as specific. Recently, cloning revealed a planarian gene with sequence homology to nicotininc acetylcholine receptor alpha 7-1 subunit with broad expression in the planarian CNS (Cebria et al., 2002).

Other neurotransmitters

Other neuroactive substances such as GABA (Eriksson and Panula, 1994), glutamate (Rawls et al., 2006a), and nitric oxide (Eriksson, 1996) have also been found in planaria. Although GABA is an important inhibitory transmitter in vertebrates, its role in planaria has not

been established. Even in mammals, during early development some GABA elements are excitatory (Cherubini et al., 1991). Nitric oxide synthase activity has been shown in the pharynx region and is speculated to play a role in feeding behavior, as it has that function in other invertebrates (Eriksson, 1996). Administration of a nitric oxide synthase inhibitor did attenuate cocaine withdrawal in planaria, suggesting that it may be involved in the withdrawal pathway (Rawls et al., 2006b). However, its function has not been definitively determined. They also possess a cannabinoid system which has been characterized pharmacologically (Buttarelli et al., 2002).

Regenerative Abilities

The regenerative ability of planaria has fascinated scientists since the 1800s. Planaria are capable of regenerating an entire worm from as few as 10,000 cells (Sanchez Alvarado, 2004). This is due to the presence of totipotent stem cells, called neoblasts, located in the parenchyma. They are the only proliferating cells present in these animals; from them, all others differentiate. These neoblast cells continuously replace aged cells throughout the lifetime of the animal in a homeostatic process, and also form the "regeneration blastema" for wound healing and regeneration of body parts. Planaria appear to use a combination of morphallaxis, characterized by a reorganization or exchange of existing tissues, and epimorphosis, characterized by a significant increase in cellular proliferation during regeneration. They also appear to go through a continuous process of morphallaxis due to their ability to shrink under conditions of food shortage; presumably the tissue is rendered for metabolic substrates. The mechanisms controlling cellular proliferation, migration, and differentiation are under extensive study.

Regeneration is initiated by injury or fission. The first step is for the wound to be closed by attaching the epidermis on the dorsal and ventral surfaces together (Chandebois, 1985). This event is followed by abundant cellular proliferation of the neoblasts which migrate to the wound

surface and form the regeneration blastema (Salo, 2006). This generates the missing parts with correct polarity.

The mechanisms governing pattern formation and tissue polarity for planarians could shed light on why human nervous systems have such limited regeneration potential. Netrin is a chemotropic protein involved in axon guidance. It acts as either a chemoattractant or a chemorepellant depending on the surface receptors getting the signal (Serafini et al., 1996). Planarian homologs of netrin are required for proper pattern formation of the cephalic ganglia as well as the ventral nerve cords (Cebria and Newmark, 2005). New technologies have led to even more information on the genes required for regeneration in planaria. Many of these, such as PIWI, wnt, and glycogen synthase kinase-3 display similar functions to vertebrates (Marsal et al., 2003; Reddien et al., 2005; Adell et al., 2008).

Many other factors are involved in the control of regeneration. Serotonin, dopamine, norepinephrine, acetylcholine, and Substance P have all been implicated. Serotonin is believed to be an important early factor stimulating DNA synthesis (Martelly, 1984). Serotonin has also been implicated in neurogenesis in vertebrates, so this might represent a conserved role. In rats, both destruction of serotonergic axons with the toxin 5,7- dihydroxytryptamine (5,7-DHT) and depletion of serotonin with parachlorophenylalanine (PCPA), a 5-HT synthesis inhibitor, results in a significant reduction in the number of bromodeoxyuridine (BrdU)-labelled neurons in the dentate gyrus (DG) compared to control animals (Brezun and Daszuta, 2000). BrdU, a thymidine analog, specifically labels dividing cells (Gratzner, 1982). In contrast, increasing serotonin levels in the DG results in an increase in neurogenesis in that region (Malberg et al., 2000). Though the exact mechanism by which serotonin regulates neurogenesis remains to be elucidated, it clearly plays a prominent role. Substance P administration enhances cellular proliferation in

regenerating planarians (Salo and Baguna, 1986) and acetylcholine has been shown to have inhibitory effects (Lenicque and Feral, 1976).

Negative Phototaxis

Planarians display a characteristic negative phototaxis to light, providing an easily studied behavioral endpoint (MacRae, 1964; Dasheiff and Dasheiff, 2002). The response is under complex central control, with the brain as the central processing center. Previous studies in *D. dorotocephela* revealed an absence of a light response when eyes were removed, confirming the specificity of the response to sensing by the eyes (Arees, 1986).The sensing organs responsible for detecting light are located in the "eyespots" on the dorsal surface of the body. The cell types that comprise these eyespots are made up of pigment cells and photoreceptor cells. The photoreceptor cells are bipolar neurons which utilize a rhabdomere as the photosensing organelle (Takano et al., 2007). Pigment cells form a cup around the photosensitive elements of the photoreceptor cells. The cell bodies themselves are outside of the pigment cups.

Relative to Vertebrate Systems

Neurons in planarian have many similarities to vertebrates, displaying multipolar shape, dendritic spines with synaptic boutons, a single axon, expression of vertebrate-like neural proteins, and relatively low spontaneously generated electrical activity. In addition, sequence similarity and evolutionary conservation of many genes important in vertebrate systems have been documented in planaria. Some of these include genes important for axon guidance and neural differentiation such as noggin, an FGF signaling molecule, and β-catenin (Mineta et al., 2003). Also, homeobox genes in planaria show high sequence identity with vertebrate orthologs. In addition, they represent distinct brain regions with high correlation to vertebrate genes (Umesono et al., 1999). Expression patterns are complicated by the fact that planarians may be undergoing continuous pattern formation as a result of their ability to shrink and/or grow

depending on the availability of food, water temperature, and other factors. This results in the activity of some anterior-posterior homeobox genes in intact planaria, an event that does not occur in vertebrates (Orii et al., 1999). An understanding of the mechanisms, controls, and signals used in planarian regeneration could add significant insight into the regeneration process for vertebrates and may help elucidate necessary components for therapies using stem cells in vertebrates and humans.

Rationale

The purpose of the present set of experiments was to characterize the effects of AMPH and MDMA in planaria as a potential model for amphetamines toxicity. Cell culture can provide a good system for evaluating specific effects on specific systems and biochemical pathways, however it fails to provide a good model for the microenvironment present *in vivo*. It fails to account for other mitigating factors. Studies *in vivo* using more complicated animals such as rodents or even insects, on the other hand, involve so many complicated interactions that it is hard to separate out what is important for causing the effects versus what is not. Planaria offer an in between- their nervous system is simple enough to be able to evaluate effects, yet still offers the microenvironment.

The first step of this study was to determine whether these drugs were toxic at relevant concentrations. In cell culture, doses used are often in the mM range. We used endpoints of head loss, death, and locomotor speed. The next step was to determine if loss of heads in AMPH or MDMA resulted in long-lasting effects following regeneration by looking at locomotor speed over time, along with morphological characteristics. We also wanted to determine if these drugs would have effects on regeneration of heads when present during the regeneration process as measured morphologically and by effects on locomotor speed. In addition, we tried

pharmacological interventions that have been shown to be protective in some, but not all, animal and cell culture models, along with other drugs as comparisons and active controls.

CHAPTER 3
METHODS

Maintenance of Planarian Colony

Planaria (*Dugesia dorotocephala*) were initially obtained from Carolina Biological Supply (Burlington, NC) and maintained in an aerated aquarium with standardized "artificial spring water" (1 mM $MgSO_4$, 2.7 mM $CaCl_2$, 0.7 mM $NaHCO_3$, 0.08 mM KCl; pH 7.8). The colony was kept under controlled light conditions (lights on at 8AM and off at 5PM). They were fed chicken liver once per week and liver was removed immediately afterwards to prevent bacterial growth. Water in the tank was changed every two weeks. Planaria were harvested on the same day once per week. Once harvested, animals were kept in stainless steel dishes and fed every 5 days. Water was changed daily. Animals to be used in experiments were starved for 3 days and then placed in 60 mm plastic dishes (Fisher Scientific, USA) with appropriate drug or water.

Head Loss and Death

Head loss and death were expressed as a ratio of the number of animals with intact, functional heads or the number of animals alive, respectively, over the number of animals at the beginning of the experiment. Data were expressed as the percentage with intact heads and the percentage alive over time.

Negative Phototaxis to Light

Planaria exhibit an anti-tropism to light, providing an easily measured behavioral endpoint to study the effects of compounds on locomotor speed. Speed was measured as time to move away from a standard light gradient over 5cm. The light source was a 40 watt fluorescent lamp placed above the track. The track used was composed of a white plastic tray with channels (Figure 3-1). Either drug or water was used to fill the channels. The tray was washed in between

drug treatments and rinsed with distilled water. This procedure was used as a measure of motor function, with a decrease in speed indicating decreased motor function. Only planaria with intact heads were used to measure speed. Values were graphed as the time to travel 5cm, in cm/min. A repeated measures two-way ANOVA with Tukey's honest significant differences post-hoc test was used to detect statistically significant ($p<0.05$) differences between groups for all locomotor data.

Drug Treatment

Amphetamine Dose-Response

d-amphetamine was purchased from Sigma Chemical Co. (St Louis, MO). The initial dose-response was carried out by using doses of 10nM, 100nM, 1μM, 10μM, and 100μM in 10 mL of standardized water, for 3 days. In a subsequent dose-response experiment, doses of 10, 20, and 30μM were used for a period of 5 days. Drug and water were changed daily. Head loss, death, and locomotor speed were noted daily. Only animals with intact heads and at least some adverse response to light were used to measure locomotor speed.

MDMA Dose-Response

3,4-methylenedioxymethamphetamine was purchased from Sigma Chemical Co. (St Louis, MO). The dose-response was carried out by using doses of 10nM, 100nM, 1μM, 10μM, and 100μM in 10 mL of standardized water, for 4 days. Drug and water were changed daily. Head loss, death, and locomotor speed were noted daily. Only animals with intact heads and light response were used to measure locomotor speed.

DOI Dose-Response

DOI (2,5-dimethoxy-4-iodoamphetamine), an amphetamine which acts primarily as a 5-HT2 receptor agonist with no effects on dopamine or serotonin transporters in mammals, was

purchased from Sigma Chemical Co. (St Louis, MO). This provided an active control drug. The dose-response was carried out by using doses of 10nM, 100nM, 1μM, 10μM, and 100μM in 10 mL of standardized water, for 4 days. Drug and water were changed daily. Head loss, death, and locomotor speed were noted daily. Only animals with intact heads and light response were used to measure locomotor speed.

Methiothepin

Methiothepin was purchased from Tocris Bioscience (Ellisville, MO). In mammals this affects a number of 5-HT receptors, but not 5-HT reuptake sites. Planaria were treated with water or methiothepin (100nM), for 3 days. Drug and water were changed daily. Head loss, death, and locomotor speed were noted daily. Only animals with intact heads and light response were used to measure locomotor speed.

Regeneration after Amphetamine

Planaria were kept in either artificial water (control) or amphetamine (30μM) for 3 days. On the third day, animals that had lost heads in amphetamine were removed to a new dish and washed 3 times with artificial water for 5 minutes each to remove drug. Animals that had not lost heads were not used. Control animals were placed in another dish where heads were removed as described (see below) and they were subsequently placed in new dishes. Animals were then allowed to regenerate in water for 14 days. Light response (reaction to a pen light) was checked and recorded daily. Once all animals had regained a light response, locomotor speed was measured until Day 14.

Regeneration after MDMA

Planaria were kept in either artificial water (control) or MDMA (100μM) for 5 days. On the fifth day, animals that had lost heads in MDMA were removed to a new dish and washed 3

times with artificial water for 5 minutes each to remove drug. Animals that had not lost heads were not used. Control animals were placed in another dish where heads were removed as described (see below) and they were subsequently placed in new dishes. Animals were then allowed to regenerate in water for 14 days. Light response (reaction to a pen light) was checked and recorded daily. Once all animals had regained a light response, locomotor speed was measured until Day 12.

Figure 3-1. Test apparatus used to determine locomotor speed of anti-tropism to a standard light gradient.

Protein Concentration Determination

Protein concentrations in the pellet were determined using a bicinchoninic acid (BCA) protein assay (BCA protein assay kit, Pierce Inc., USA) with albumin standards (Peirce, USA).

High Performance Liquid Chromatography

Planaria were treated with either 10 or 100μM of amphetamine or MDMA. Samples were collected at 1 h, 24 h, and 72 h and placed in 2.0mL plastic tubes. Only animals with intact heads were collected. Excess water was removed with a pipet. Samples were then immediately frozen

on dry ice and stored in the dark at -80 C until they were ready to be used. In preparation for high performance liquid chromatography (HPLC), 1.0mL of a 0.1% perchloric acid solution containing 100 uM EDTA was added. The tubes were then vortexed and sonicated for 10 seconds. A 100μL aliquot of the mixture was placed in a separate tube to be used for protein analysis. Samples were then centrifuged at 15000 G for 10 minutes at 4°C. Next, supernatant was removed and filtered with a 0.2 μm filter and collected in a separate tube. Filtered supernatant was then injected into an ESA Coulochem II HPLC-ECD (ESA, Chelmsford, MA) using an RP-C18 microdialysis column with a 20-ul injection loop to determine the concentrations of neurotransmitters and their metabolites. The mobile phase consisted of an acetonitrile mixture (MD-TM, ESA). Flow rate was set at 0.6 ml/min. Potential settings on the HPLC were E1 at -175 mV, E2 at 250 mV, GC at 350 mV. The peaks were displayed, integrated and stored by means of an ESA 501 Chromatography Data System (ESA) Standards for DA, 5-HT, and DOPAC (Sigma) run the same day were used to identify and quantitate peaks.

Values for picograms of compound per sample were calculated by plotting area under the peak against concentration of compound for standard solutions. Values were then normalized to protein concentration for each respective sample. The values for control animals were averaged and values for treatment groups are expressed as %control. A two-way ANOVA with Tukey's post-hoc test was used to detect statistically significant ($p<0.05$) differences between groups for all HPLC data.

Head Removal for Regeneration

For head removal, animals were placed in a large glass Petri dish (100cm wide) and most of the water was removed to minimize movement. Heads were removed with a razor blade just behind the auricles (Figure 3-2), which effected the removal of the entire cephalic ganglia.

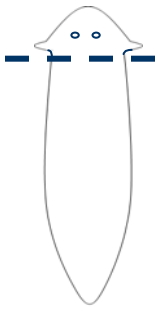

Figure 3-2. Location of head removal. Dotted lines represent the point at which cuts were made.

Drug Combinations

In order to ascertain possible mechanisms and whether putative protective agents in mammals would also work in this model, different combinations of drugs were used. In order to evaluate whether drug interventions that have been shown to be protective in cell culture or animal models, we used combinations of some of these drugs with AMPH. Lithium was purchased from Tocris Bioscience (Ellisville, MO). Nomifensine maleate and 7-nitroindazole were purchased from Sigma Chemical Co. (St Louis, MO). The first combination used was AMPH and Lithium. In this experiment, planaria were treated with either water, AMPH (27μM), Lithium (1mM, 2mM), or a combination of Lithium and AMPH. Lithium has antioxidant effects (Shao et al., 2005) and is believed to be protective against oxidative stress caused by AMPH (Frey et al., 2006); it also has stabilizing effects on second messenger systems. The next combination was nomifensine (Nom), a DA reuptake inhibitor, and AMPH. AMPH doses of 10

and 100 μM were used in combination with a dose of 1 μM for Nom. The last combination used was 7-NI, a neuronal Nitric oxide synthase inhibitor. Doses of 20 μM AMPH and 50 μM 7-NI were used. Doses were chosen based upon both reports in the literature (Shao et al., 1995; Itzhak and Ali, 1996) and from separate dose-response experiments conducted with the planaria.

Receptor Binding Assays

Samples for the assay were prepared by adding whole animals to 5 mL of assay buffer (50 mM Tris-HCl, 120 mM NaCl, 5 mM KCl) and vortexing for 2 minutes. Homogenates were then centrifuged at 10000 G for 10 minutes at 4ºC. Supernatant was removed and the procedure was repeated for a total of 3 times. The resulting pellet was resuspended in the total assay volume plus 10% assay buffer. In each well 25 uL each of nonradioactive ligand, radioactive ligand, and sample were combined with 175 uL of assay buffer and incubated for 90 minutes. The filter was washed in 0.9% saline to stop the reaction and the plate was filtered through a GF/B filter strip. The filter was allowed to dry overnight, and scintillation fluid was added to get DPM . Binding assays were conducted to determine receptor interactions and to estimate doses and are reported as dissociation constants (Table 4-2).

CHAPTER 4
RESULTS

Toxicity of Amphetamine and MDMA

Head Loss and Death

The first step in evaluating toxicity of amphetamines in this model was to establish a general dose-response. The endpoints used were head loss and death.

Treatment with both AMPH and MDMA resulted in head loss. However, AMPH has a steep dose-response curve with head loss occurring only at the high dose of 100μM by Day 2, in which there is 100% head loss and no head loss at 10μM or any lower doses until Day 3 (Figure 4-1). Amphetamine at 100 μM resulted in 100% death by Day 3, with lower doses having no effect (Figure 4-2). In a subsequent experiment to determine effects of intermediate doses, a range of 10μM, 20μM, and 30μM was used. This range produced a greater distribution, with the most head loss occurring at 30μM (100% by Day 4) followed by 20μM (79% by Day 5) and then 10μM (29% by Day 5) (Figure 4-4). Only the highest dose (30mM) resulted in any death (20%) (Figure 4-5). Following treatment with MDMA, maximal head loss of 65% occurs at 100 μM, with 25% occurring at 10μM and 10% at 1μM (Figure 4-7). Lower doses resulted in no head loss. MDMA did not cause death by Day 4 at any dose (data not shown).

Locomotor Speed

As mentioned above, dopamine is known to influence locomotor movement in planaria (Palladini et al., 1996). Amphetamines are also known to have effects on the dopamine system, therefore locomotor speed was incorporated as an endpoint for measuring toxicity. Control levels were typically between 12 and 14 cm/min. Amphetamine caused decreased locomotor speed at 20 and 30 μM for the observed period ($p<0.001$) (Figure 4-6), and at 100μM, where most animals did not move (Figure 4-3). Locomotor speed was only measured for 3 days with 30μM

AMPH since all worms had lost heads by Day 4. At 20μM, the largest decreases were observed on Days 4 and 5. Locomotor speed was also significantly decreased during administration of 100μM MDMA ($p<0.001$), but not for any other dose (Figure 4-8). As observed in earlier experiments with AMPH, control values remained between 12 and 14 cm/min. MDMA (100μM) resulted in observable decreases starting on Day 1 and speed remained decreased throughout the experiment. The slight increase observed on Day 4 was most likely due to animals that were excluded due to head loss.

Morphology

Head loss was examined using gross morphological effects as the metric. Planaria were treated with either water or drug. Concentrations of AMPH and MDMA causing maximal numbers of animal with complete head loss without death were used. Following treatment with both (independently) AMPH (30μM) and MDMA (100μM), the tissue on the head appears to be degrading over time (Figure 4-9). Initially, the auricles, where the chemoreceptors are located, disappear (Figure 4-9B). Following this event, the anterior portion of the head begins to disappear and has the appearance of lesions on the ventral surface (Figure 4-9B). Lastly, the head appears to be hollow and disintegrates (Figure 4-9C), leaving a ragged wound surface posterior to where the head used to be (Figure 4-9D). Only the head tissue is affected by the drugs; the bodies remain unaffected. This same pattern of events is observed after treatment with MDMA (100μM) (Figure 4-10A-C).

Effects on Monoamines

In cell culture and animal models, MDMA and AMPH initially cause an acute increase in serotonin and dopamine release, which is followed by substantial depletions. In order to determine if these effects would be obtained in planaria, monoamine levels were measured

following drug administration. AMPH caused significant decreases in serotonin levels at 24h with both the 10 and 100μM dose (46% and 62% below control, respectively) (Figure 4-11). MDMA (100μM) resulted in decreased serotonin levels at 24h (51% below control) and 72 h (33% below control) (Figure 4-11). While not significant, MDMA at both doses appears to cause an increase in serotonin levels at 1h. MDMA (10μM and 100μM) also resulted in significant increases in dopamine at 1h (279% and 214% over control, respectively), with levels returning to normal by 24h and remaining stable at 72h (Figure 4-12). Amph (100μM) caused decreases in DA (39% below control) at 24h (figure 4-12), while 10μM resulted in no significant changes at any timepoint (Figure 4-12). DA turnover, as measured by calculating the ratio of DOPAC/DA, was decreased at 1h following administration of both 10 and 100μM MDMA (33% and 51% below control, respectively, $p<0.05$) (Figure 4-13). For 100μM MDMA, the levels remain depressed at 24h (51% below control, not significant) before jumping up at 72h (261% over control, $p<0.05$). AMPH at 100μM also induced increases in DA turnover at 1 and 24 h (140% and 210% over control, respectively, $p<0.05$) (Figure 4-13).

AMPH Has Long-Term Effects after Regeneration

Following head loss due to AMPH, it was thought that there might be persistent effects once the animals regenerated. This was measured by evaluating recovery of locomotor speed. Indeed, planaria that had been treated with AMPH (30μM) were significantly ($p<0.05$) slower than control animals, lasting 2 weeks after head loss (Figure 4-14). In contrast, when heads were mechanically removed prior to disintegration in AMPH (30μM), the effects were not significant (Figure 4-17). Worms that lost heads were slower than controls by approximately 4 cm/min.

Morphology of Heads Regenerated after AMPH

Gross morphology of heads was evaluated to determine if there were differences after regeneration. Representative photographs depict the appearance of heads on Day 14 after they have regenerated (Figure 4-15). Morphology of the heads of some animals that lost heads in AMPH (Figure 4-15B) and subsequently regenerated them display slightly different gross morphology. The anterior portion of the head region in these animals appears blunted. The auricles and eyespots appear normal, however. Not all animals display such gross differences in appearance, however; some appear morphologically normal. Animals that had heads mechanically removed do not appear to be different from controls morphologically once heads are regenerated (Figure 4-15C).

Loss of Heads in MDMA Does Not Effect Head Regeneration or Subsequent Locomotor Speed

Since loss of heads in AMPH had drastic effects on regeneration, as evaluated with locomotor speed, the effect of losing heads in MDMA was subsequently evaluated. It was found that loss of heads in 100μM MDMA did not effect locomotor speed at any timepoint tested (Figure 4-16). Further, if heads were cut rather than allowed to disintegrate, there were still no effects on locomotor speed as compared to controls (data not shown).

Other Drugs

DOI Toxicity

DOI, another amphetamine, which acts primarily as a 5-HT2 receptor agonist is a more potent toxin than either MDMA or AMPH and results in 100% head loss after only one day at 100μM and 60% head loss after 4 days at 10 μM (Figure 4-18). A dose of 100μM also results in 100% death after only two days (Figure 4-19). Head loss prevented measuring locomotor speed for the high dose of DOI, but a dose of 10μM resulted in a significant decrease in locomotor

speed as compared to controls (Figure 4-20). Control levels were slightly lower than what had been previously observed and were between 10 and 12 cm/min. However, locomotor speed of animals that had been treated with DOI (10μM) began to decline on Day 1 and stayed depressed until Day 4, where the average speed was around 6 cm/min, approximately 5cm/min slower than controls.

Methiothepin Potently Inhibits Movement

To further compare results, methiothepin, a general serotonin antagonist was administered to planaria. It resulted in drastic decreases in locomotor speed starting on Day 1 and continuing to Day 2 (Figure 4-21) Control animals started in the range typically observed (between 12 and 14 cm/min) and did decrease slightly to 11 cm/min. Animals treated with methiothepin on the other hand began at control values and decreased to 5 and less than 3 cm/min on Days 1 and 2, respectively. While the effects on speed were quite pronounced, there was no effect on head loss or death (data not shown).

Nomifensine, Lithium, and 7-Nitroindazole Are Not Protective of AMPH Effects

In cell culture and animal models, nomifensine, lithium, amd 7-nitroindazole have all been shown to be protective. We wanted to determine if these same agents would be protective in this model. None of the drug combinations used protected against amphetamine-induced toxicity as defined by head loss and death (Table 4-1). The higher dose of Lithium (2mM) appeared to exacerbate the effects, while the lower dose of lithium (1mM), nomifensine, and 7-nitroindazole had no effect on the endpoints measured.

Low Doses of AMPH and MDMA Do Not Affect Regeneration after Mechanical Removal

Serotonin, dopamine, and norepinephrine have been shown to be important players in regeneration (Lenique, 1973). Since AMPH and MDMA both affect these neurotransmitter systems, we wanted to ascertain the effects of these compounds on planarian regeneration. Since

high doses proved to be toxic as demonstrated inn earlier experiments, doses in the lower range were chosen. Administration of low doses of AMPH (5μM) (Figure 4-22) and MDMA (10nM, 100nM, 1μM) (Figure 4-23) do not inhibit regeneration as measured by an index of locomotor speed. Although not significant, planaria treated with MDMA (1μM) perform slightly better than controls. Neither AMPH nor MDMA caused head loss, death, or locomotor speed at these dosage levels (Figures 4-1 to 4-3 and 4-7 to 4-9, respectively).

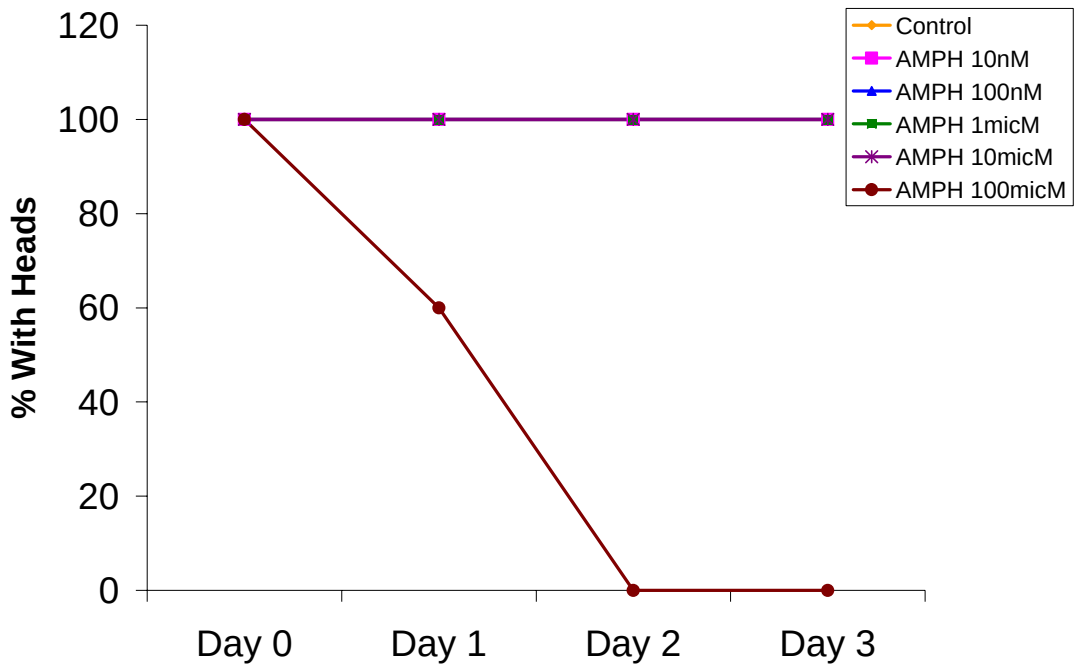

Figure 4-1. Head loss resulting from amphetamine exposure. Planaria were treated with increasing doses of amphetamine and head loss was recorded. Maximal head loss occurred at the highest dose (N=10/group). Data are represented as the percent of animals with intact heads.

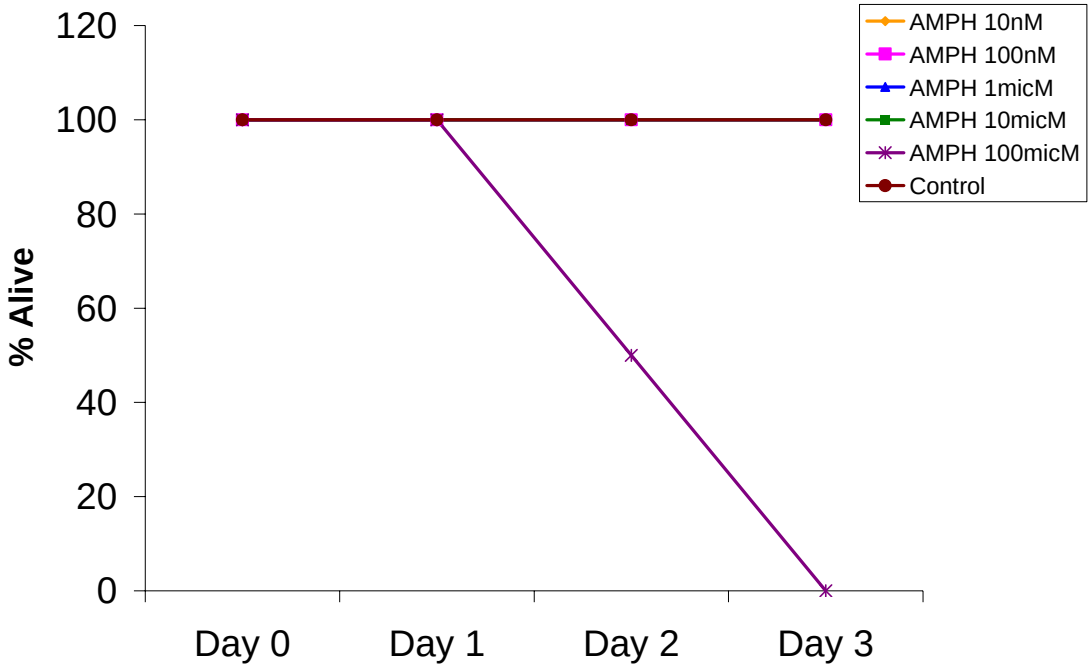

Figure 4-2. Death resulting from amphetamine exposure. Planaria were treated with increasing doses of amphetamine and death was recorded. Only the highest dose used caused death, resulting in 100% death (N=10/group).

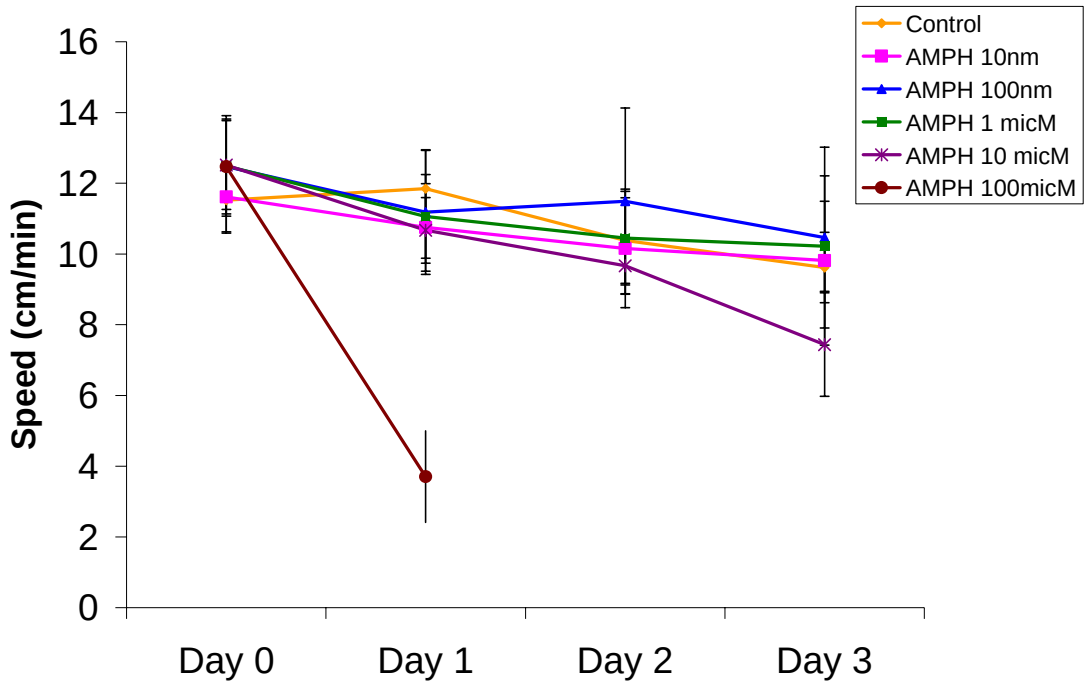

Figure 4-3. Locomotor speed following treatment with water (control) or amphetamine (AMPH) at specified concentrations over time. Data are expressed as the mean ± S.D. of the time to travel 5cm in cm/min. Only the highest dose affected locomotor speed, with most animals failing to move (N=10/group).

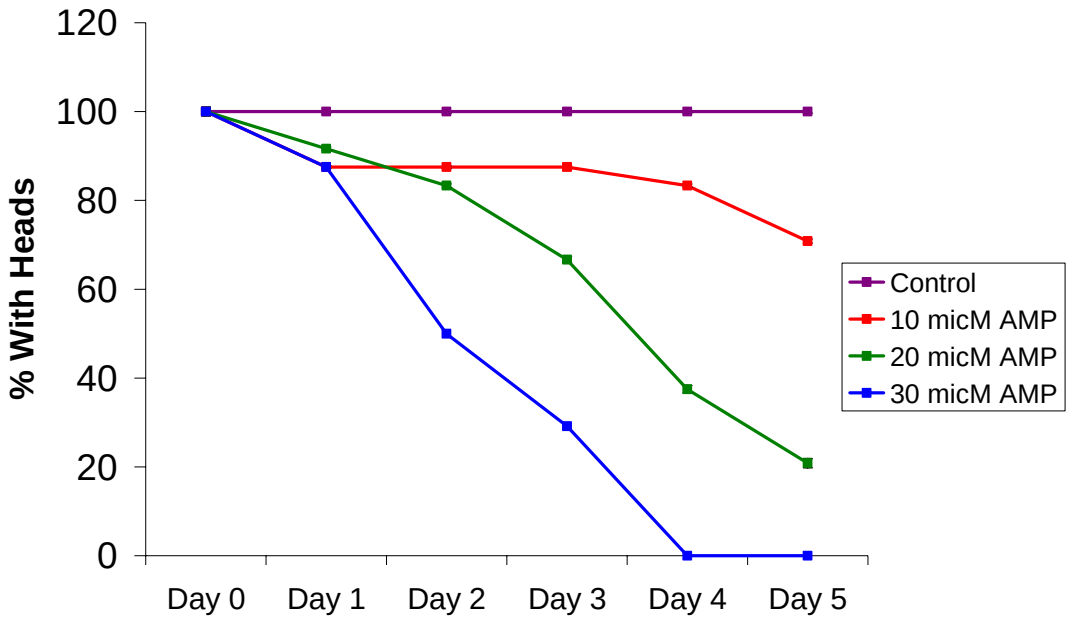

Figure 4-4. Dose-dependent head loss resulting from amphetamine exposure. Amphetamine dose-dependently results in head loss, with maximal head loss occurring at the highest dose. Data are represented as the percent of animals with intact heads (N=24/group).

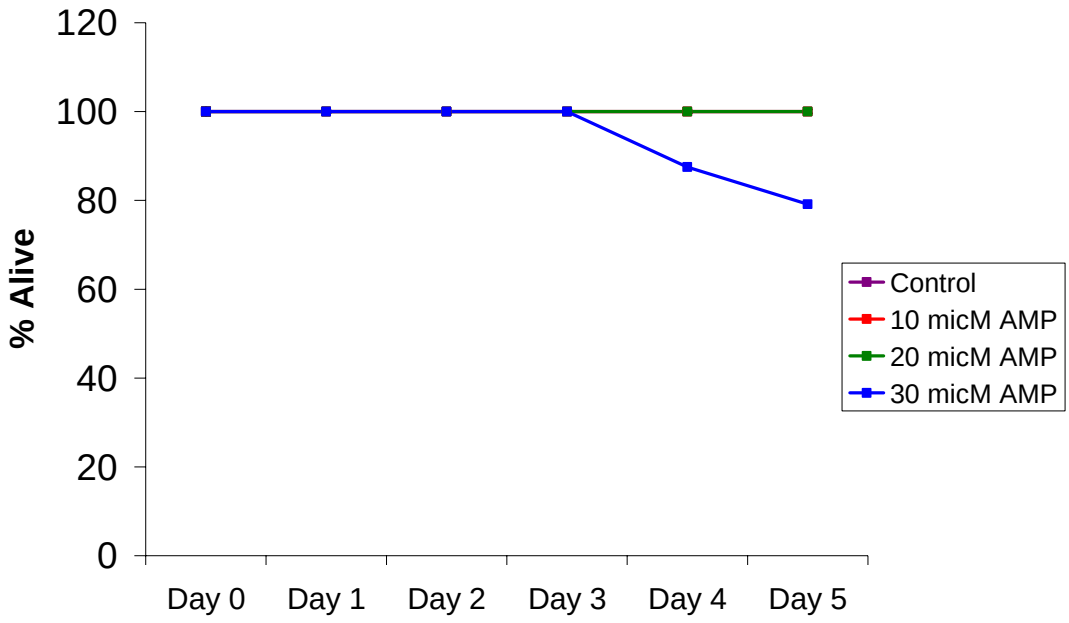

Figure 4-5. Intermediate doses of amphetamine result in minimal death. Planaria were treated with increasing doses of amphetamine and death was recorded. Only the highest dose used caused death, resulting in 20% death (N=24/group).

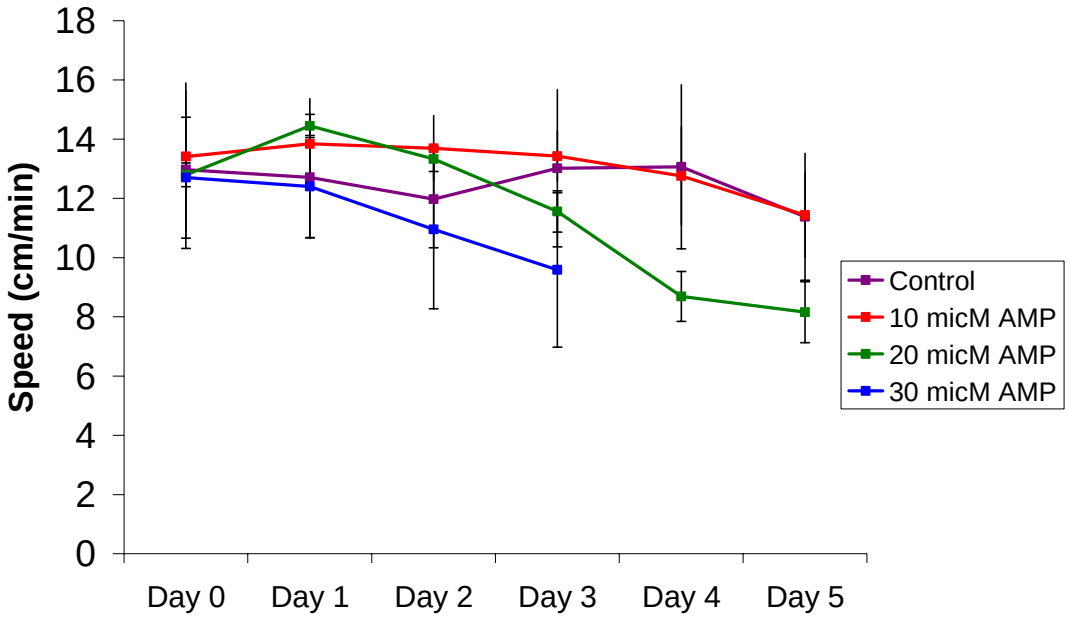

Figure 4-6. Locomotor speed following treatment with water (control) or amphetamine (AMP) at specified concentrations over time. Data are expressed as the mean ± S.D. of the time to travel 5cm in cm/min. AMPH at doses of 20 and 30mM significantly reduced locomotor speed ($p<0.01$) (N=24/group).

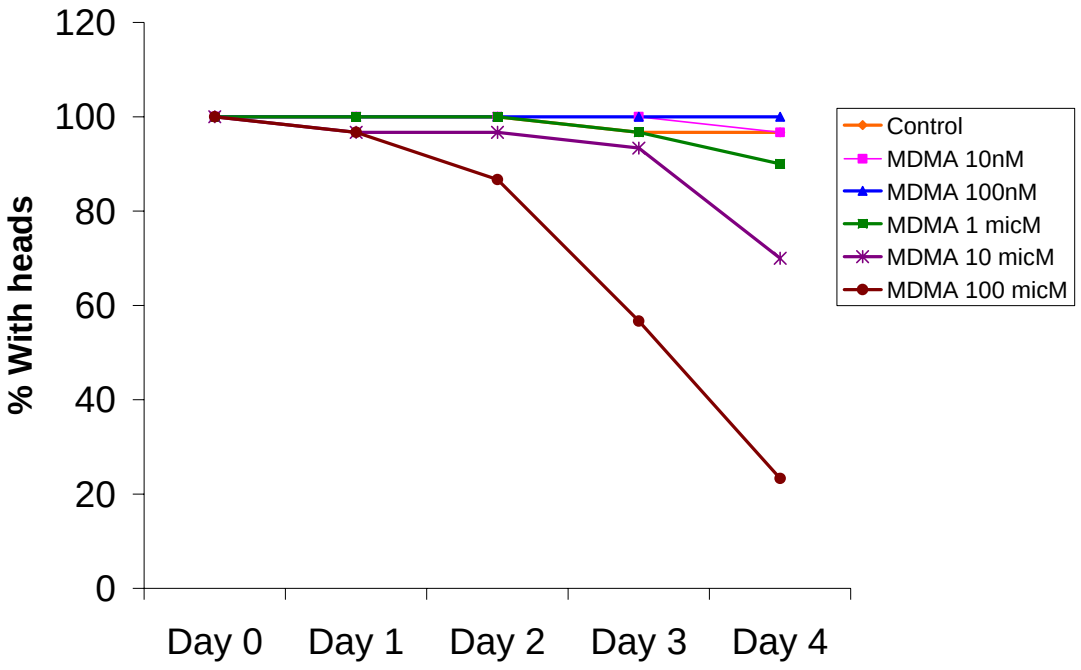

Figure 4-7. Head loss following MDMA administration. MDMA dose-dependently results in head loss, with maximal head loss occurring at the highest dose (N=30). Data are represented as the percent of animals with intact heads.

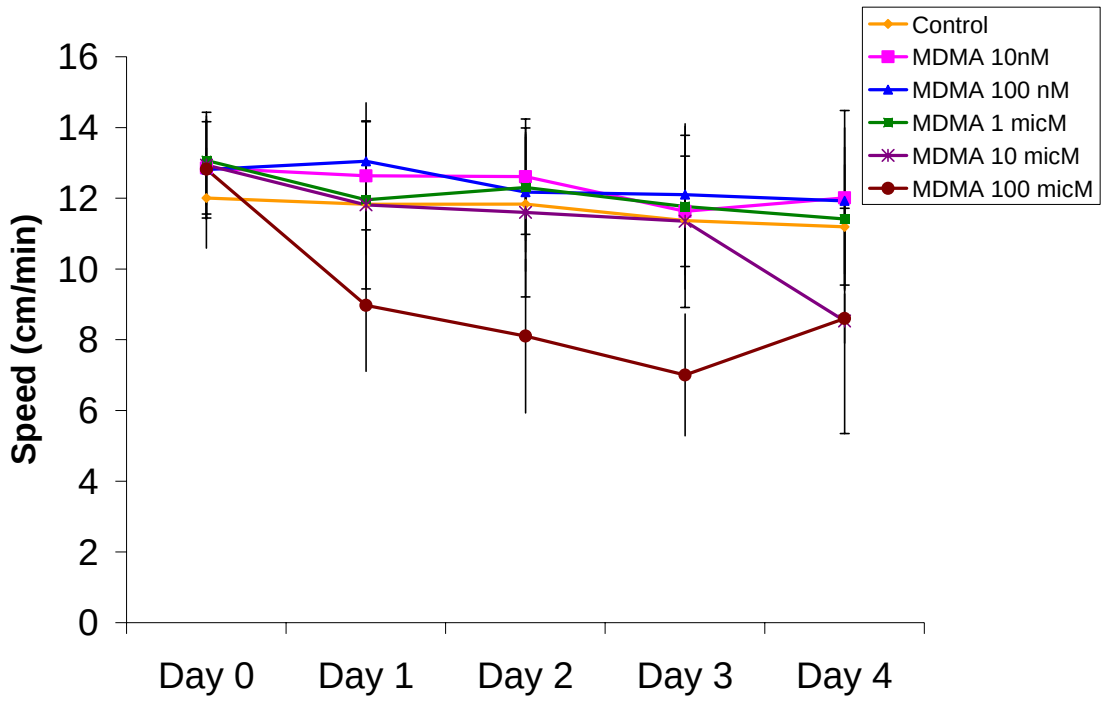

Figure 4-8. Locomotor speed following MDMA administration. Data are expressed as the mean ± S.D. of the time to travel 5cm in cm/min. The highest dose of MDMA results in locomotor slowing ($p<0.05$, N=30).

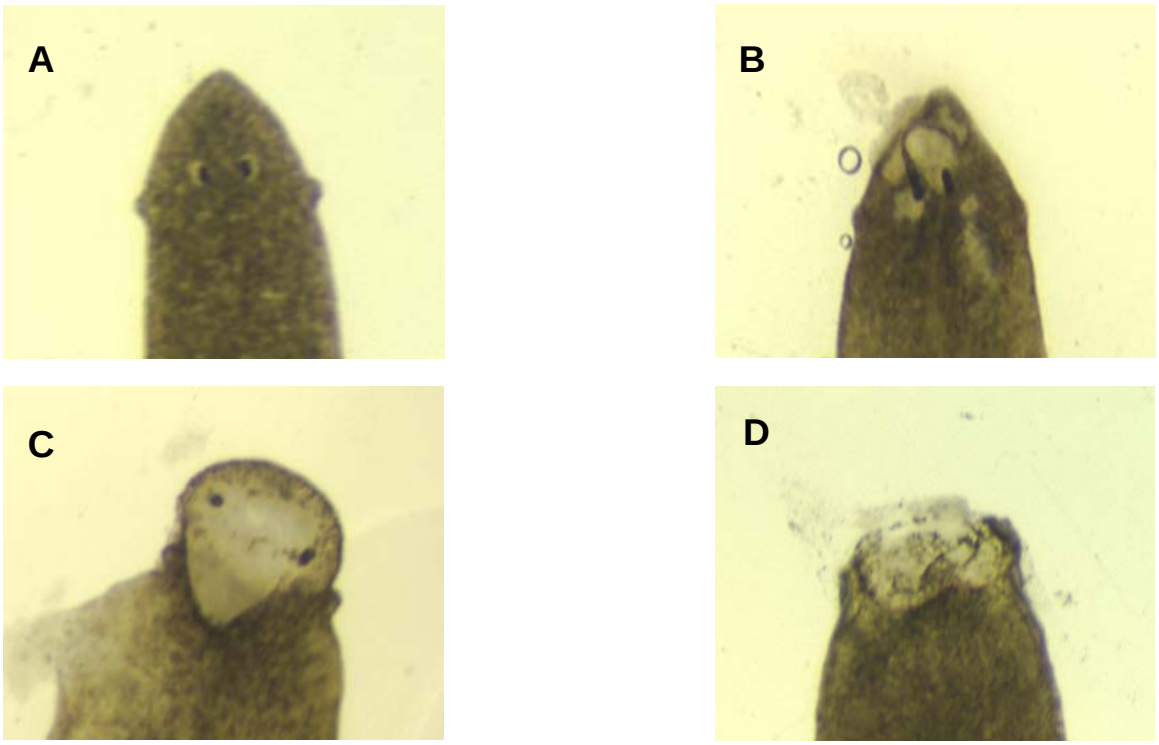

Figure 4-9. Representative photograph of degeneration in head region following exposure to AMPH. In AMPH treated animals (B-D), the auricles begin to disappear and lesions on the head become apparent (B). Lastly, the head appears to be hollow (C) and disintegrates (D), leaving a ragged wound surface posterior to where the head used to be. Normal worms have a triangular head and intact auricles (A).

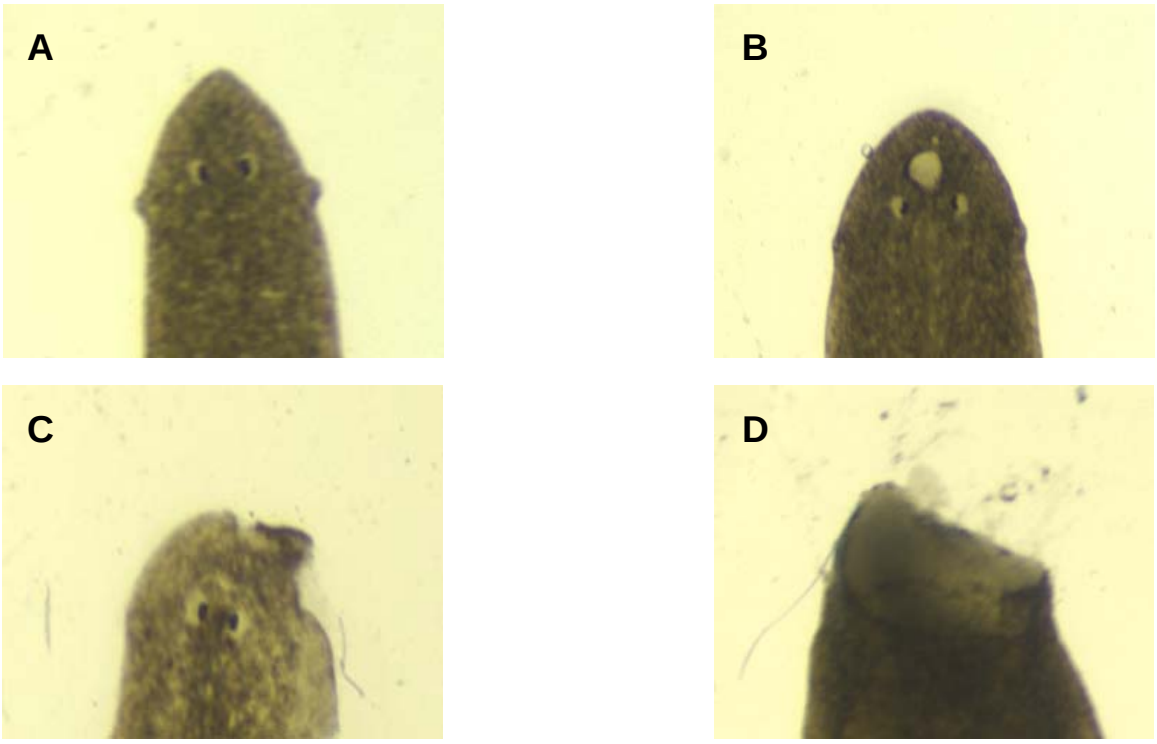

Figure 4-10. Representative photograph of degeneration in head region following exposure to MDMA. In MDMA treated animals (B-D), the auricles begin to disappear and lesions on the head become apparent (B). Lastly, the head appears to be hollow (C) and disintegrates (D), leaving a ragged wound surface posterior to where the head used to be. Normal worms have a triangular head and intact auricles (A).

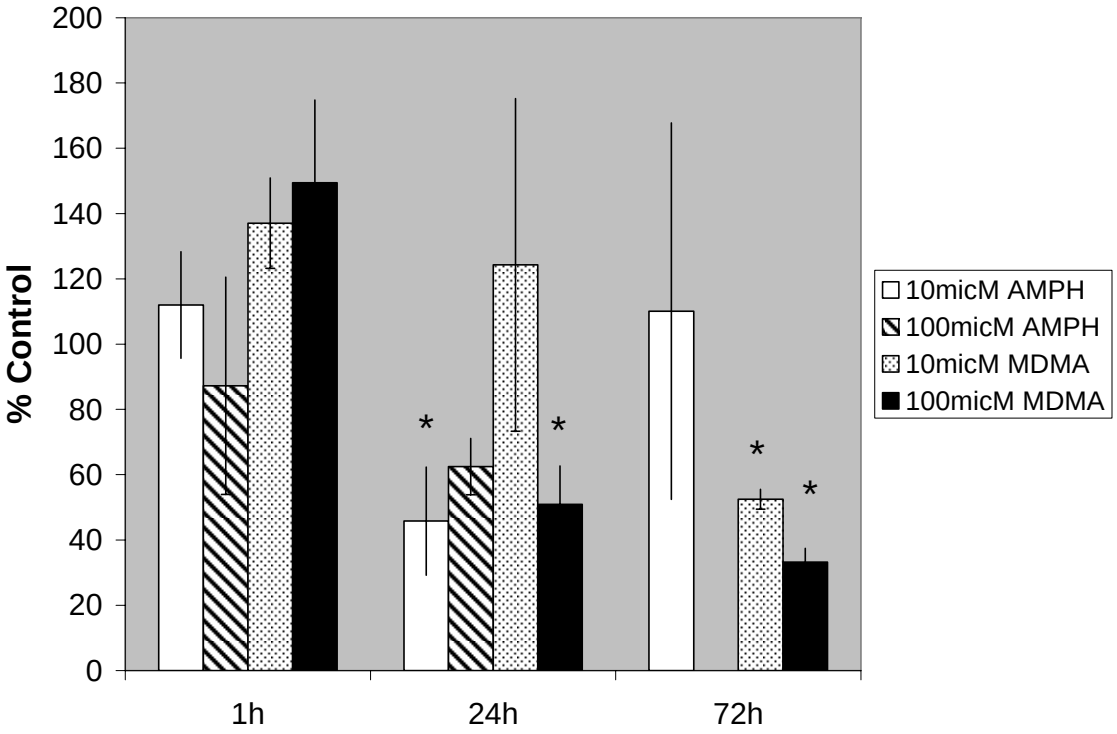

Figure 4-11. Changes in levels of 5-HT following treatment with AMPH and MDMA. Levels are reduced at 24 h following AMPH and return to normal at 72 h for the low dose (the high dose resulted in complete head loss by 72 h and therefore could not be measured). MDMA induced a decrease at 24 and 72 h with the high dose and at 72 h with the low dose. Values are expressed as % Control, N=6 samples/group, 3 animals/sample. *$p<0.05$.

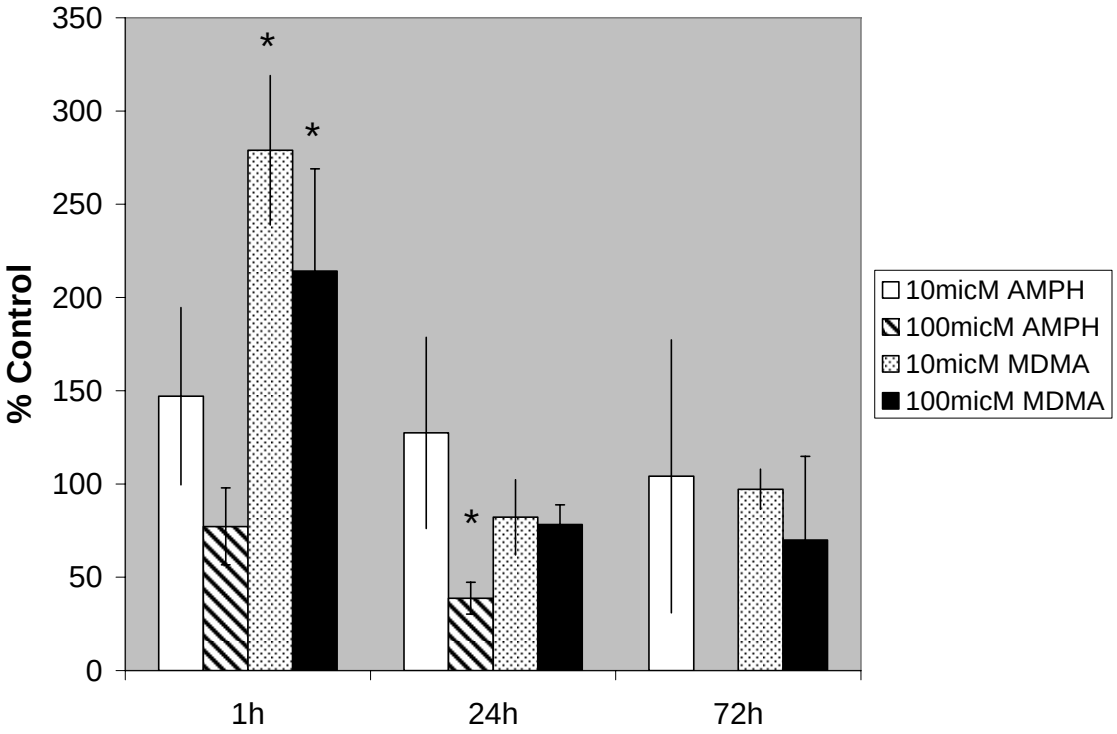

Figure 4-12. Changes in levels of DA following treatment with AMPH and MDMA. Levels are reduced at 24 h following the high dose of AMPH (the high dose resulted in complete head loss by 72 h and therefore could not be measured). MDMA induced an increase at 1 h for both doses with levels returning to normal at 24 and 72 h. N=6 samples/group, 3 animals/sample. *$p<0.05$.

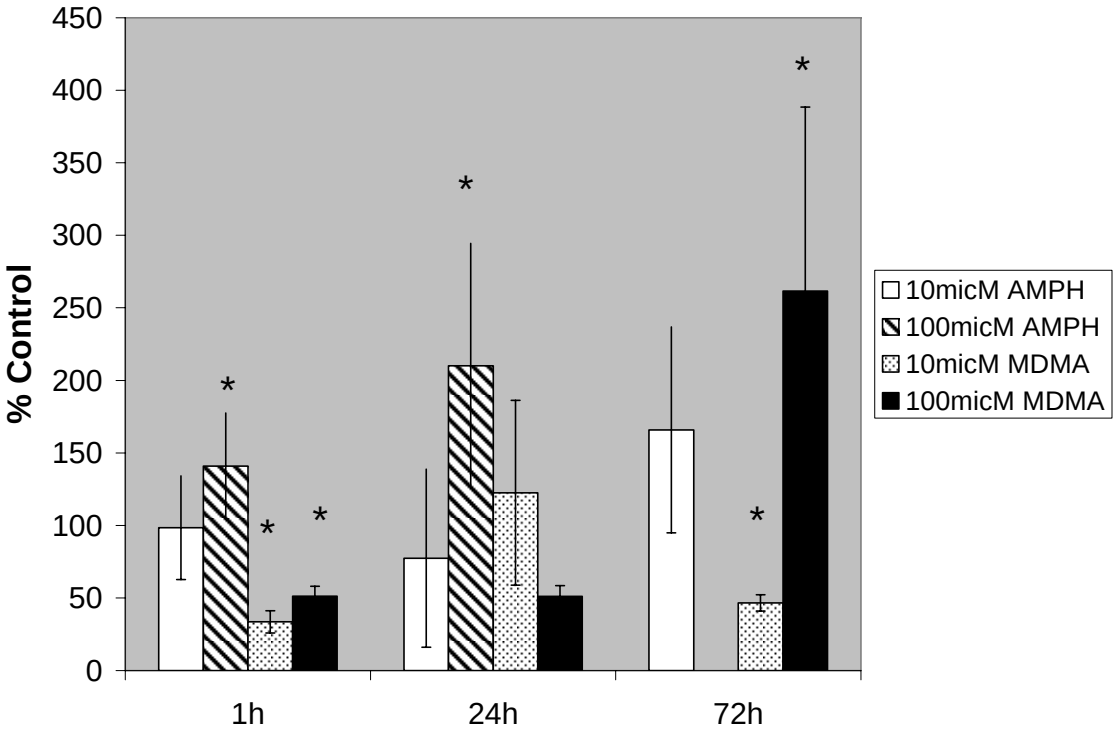

Figure 4-13. Changes in levels of DOPAC/DA following treatment with AMPH and MDMA. . Data are presented as mean ± S.D. of the % of control values. Levels are reduced at 24 h following the high dose of AMPH (the high dose resulted in complete head loss by 72 h and therefore could not be measured). MDMA induced an increase at 1 h for both doses with levels returning to normal at 24 and 72 h. N=6 samples/group, 3 animals/sample. *$p<0.05$

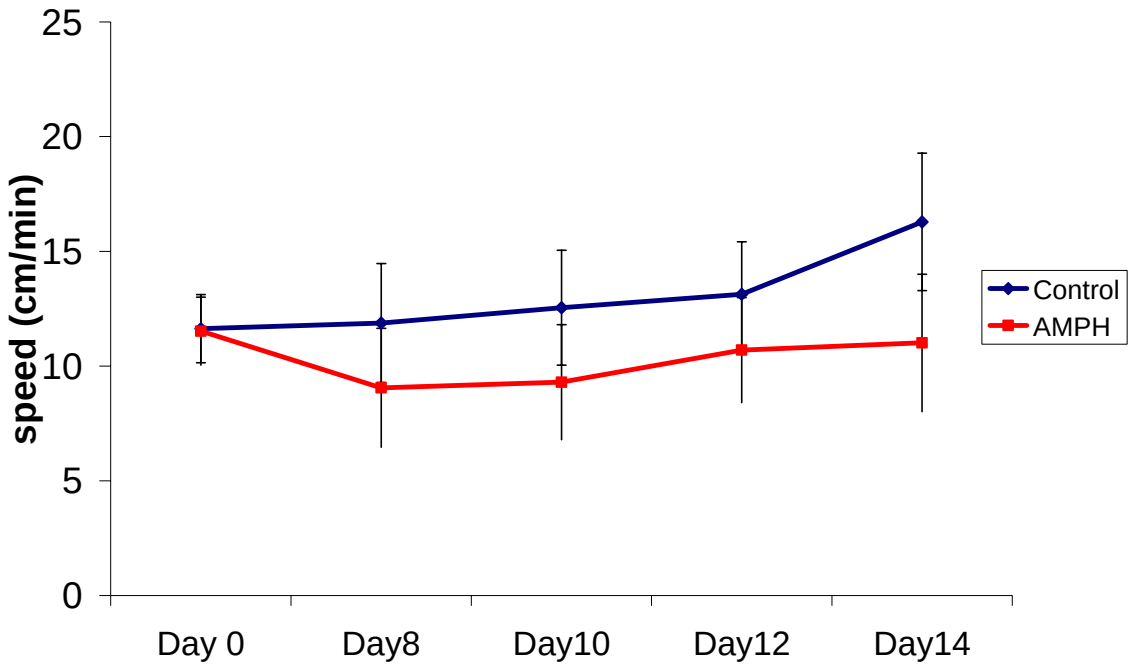

Figure 4-14. Head loss following amphetamine exposure results in long-term locomotor deficits. Planaria that lost heads following amphetamine exposure and allowed to recover in water fail to return to control levels after 14 days. Data are expressed as the mean ± S.D. of the time to travel 5cm in cm/min. (N=24).

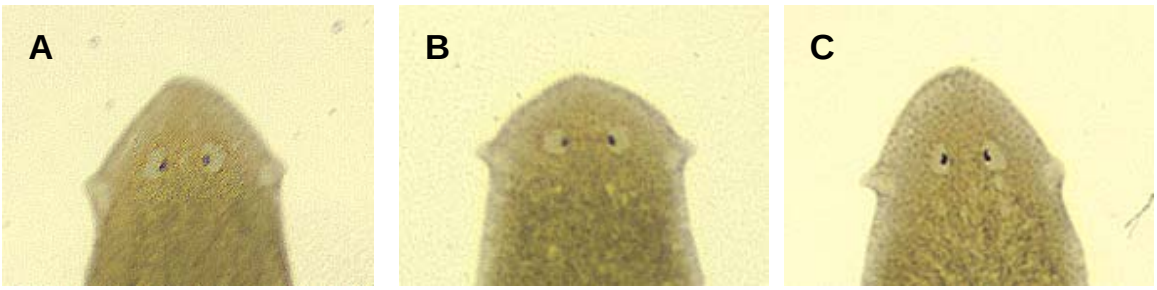

Figure 4-15. Representative photographs of head morphology after AMPH. Worms treated with AMPH (30μM) and had heads mechanically removed (C) did not appear different than normal controls (A) after regeneration. Worms that had lost heads in AMPH and regenerated appear slightly blunted at the anterior portion of the head(B).

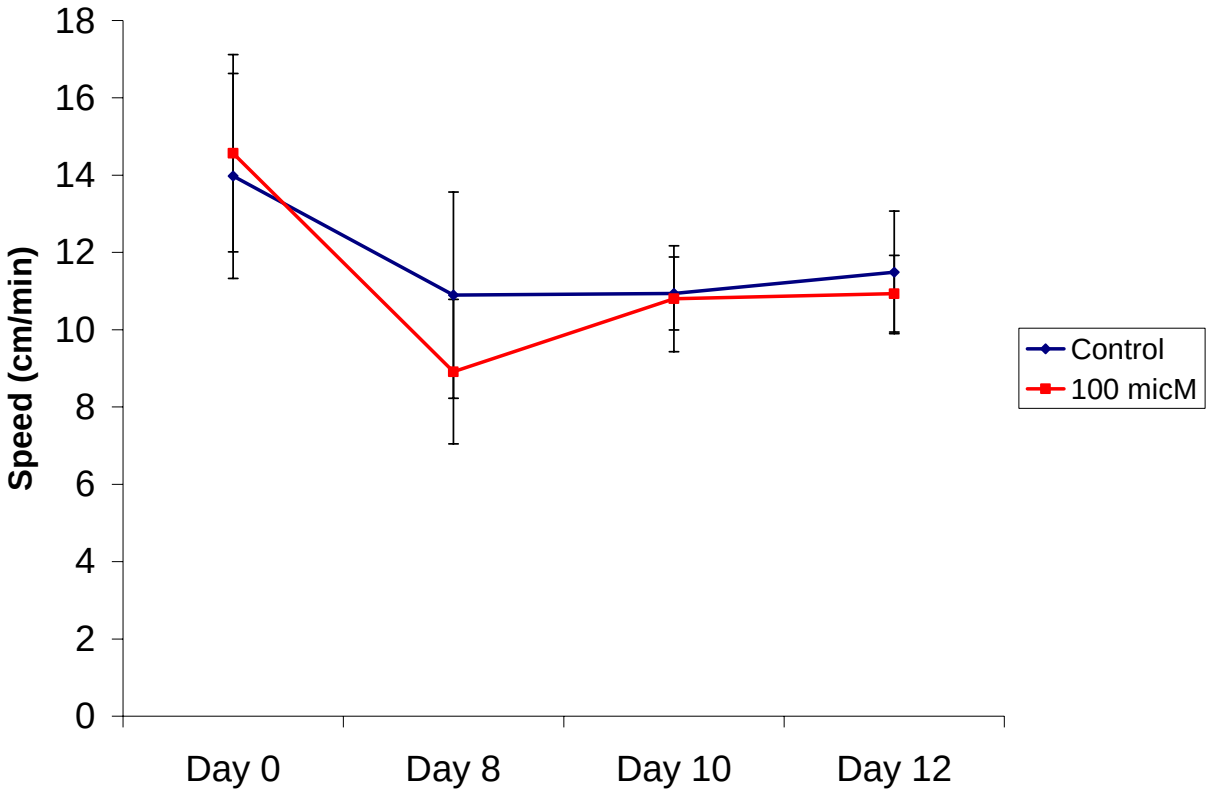

Figure 4-16. Head loss following MDMA exposure does not result in long-term locomotor deficits. Planaria that lost heads following MDMA exposure and allowed to recover in water for 12 days were not significantly different from controls. Data are expressed as the mean ± S.D. of the time to travel 5cm in cm/min. (N=24).

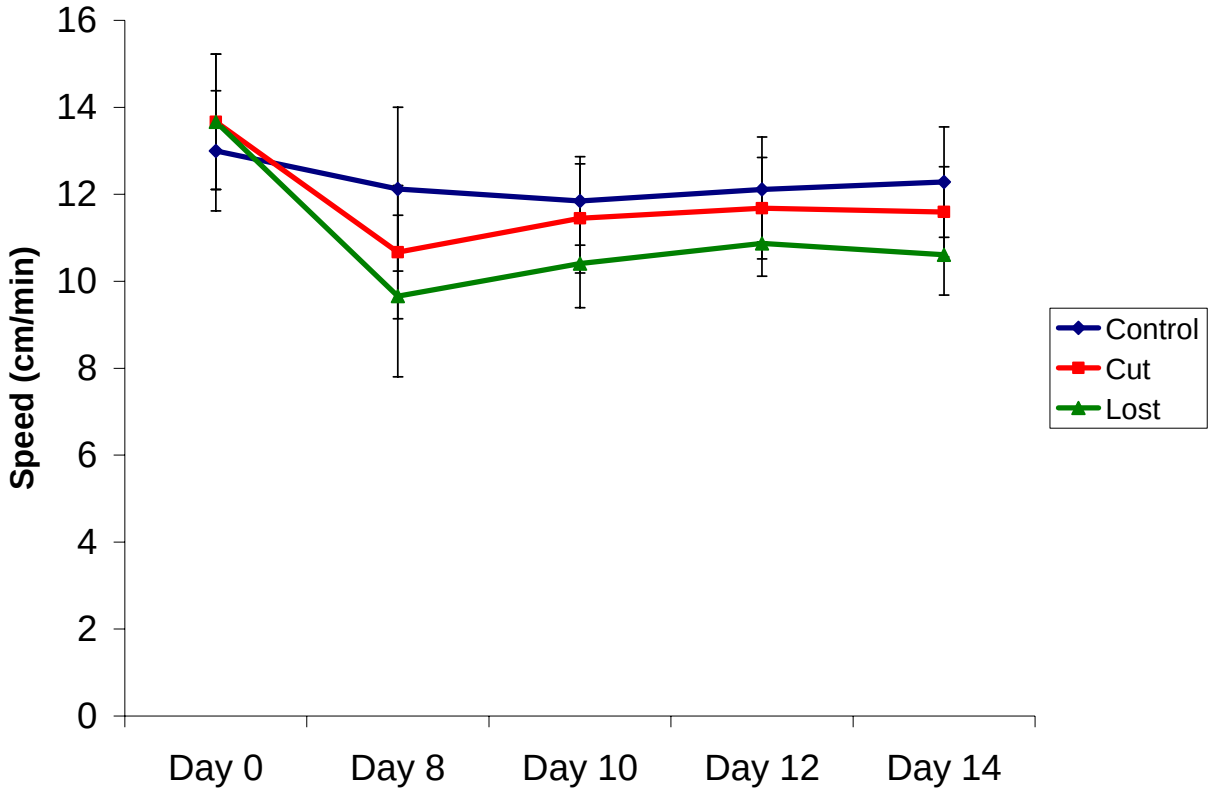

Figure 4-17. Differential effects of losing heads in AMPH vs. being mechanically removed. Consistent with previous observations, animals that lost heads (Lost) following AMPH treatment (30μM) were significantly slower than control animals ($p<0.05$). Removal of heads (Cut) before head loss occurs does not result in a significant difference from control. Data are expressed as the mean ± S.D. of the time to travel 5cm in cm/min. (N=24).

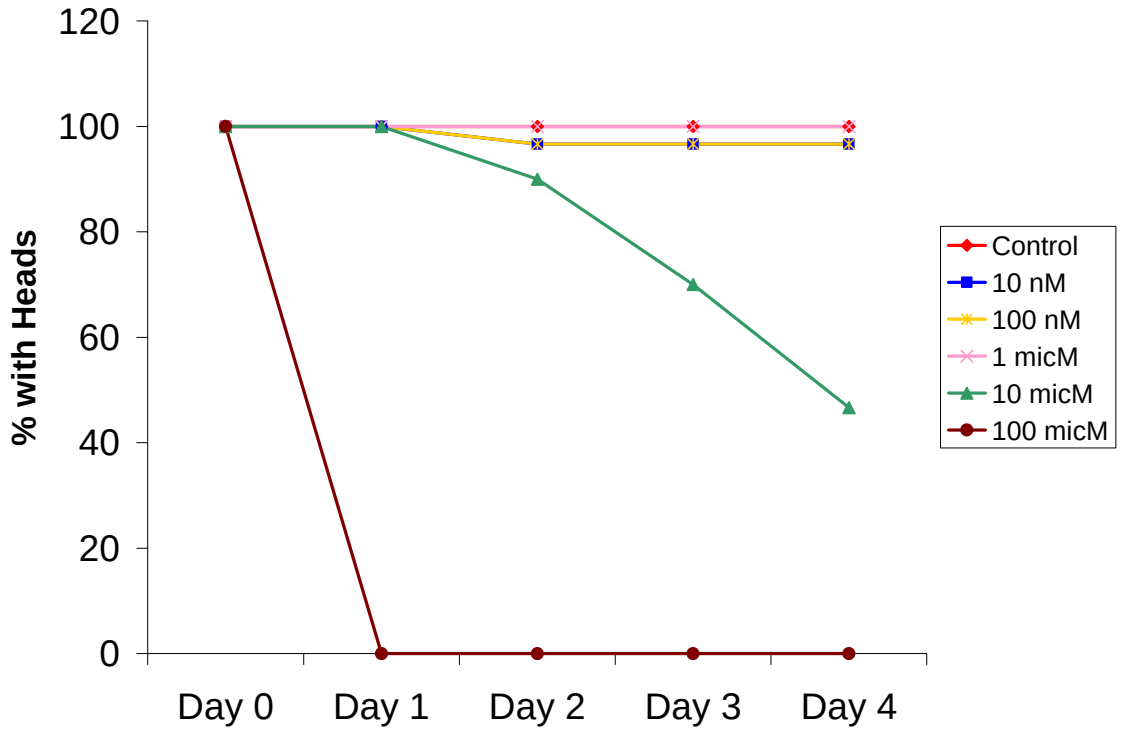

Figure 4-18. Dose-dependent head loss resulting from DOI exposure. DOI dose-dependently results in head loss, with maximal head loss occurring at the highest dose. Data are represented as the percent of animals with intact heads (N=30/group).

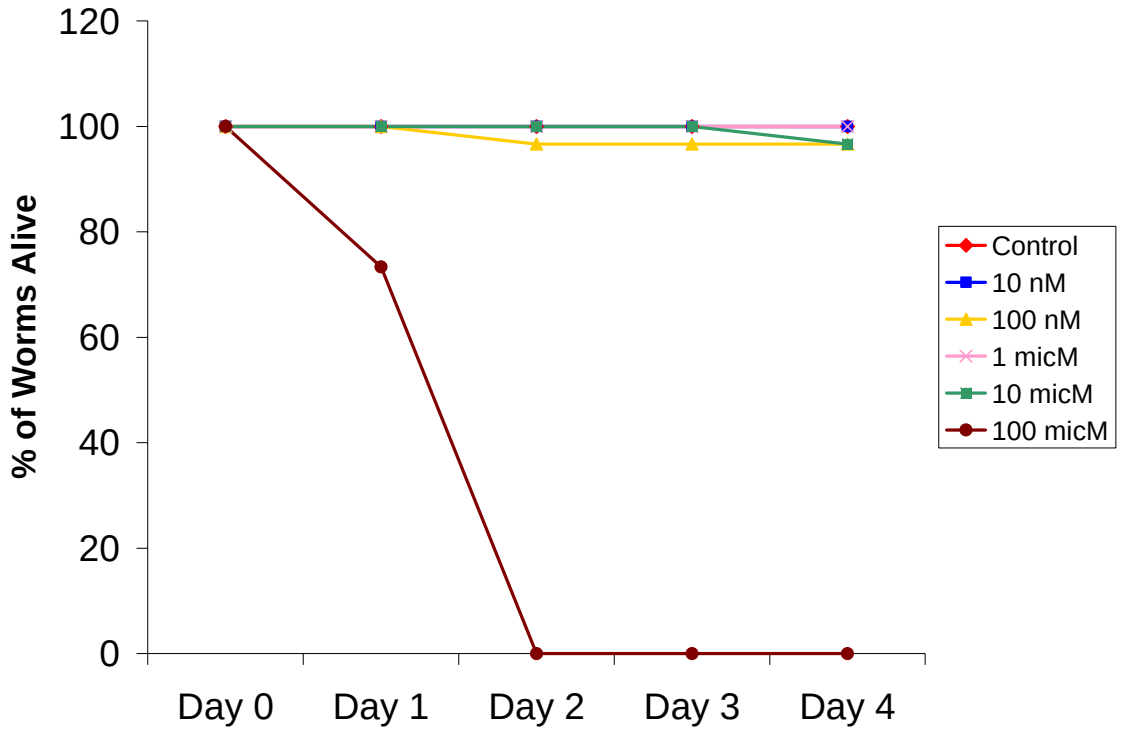

Figure 4-19. Death resulting from DOI. Planaria were treated with increasing doses of DOI and death was recorded. Only the highest dose used caused death, resulting in 100% death (N=30/group). Data are presented as % of animals alive.

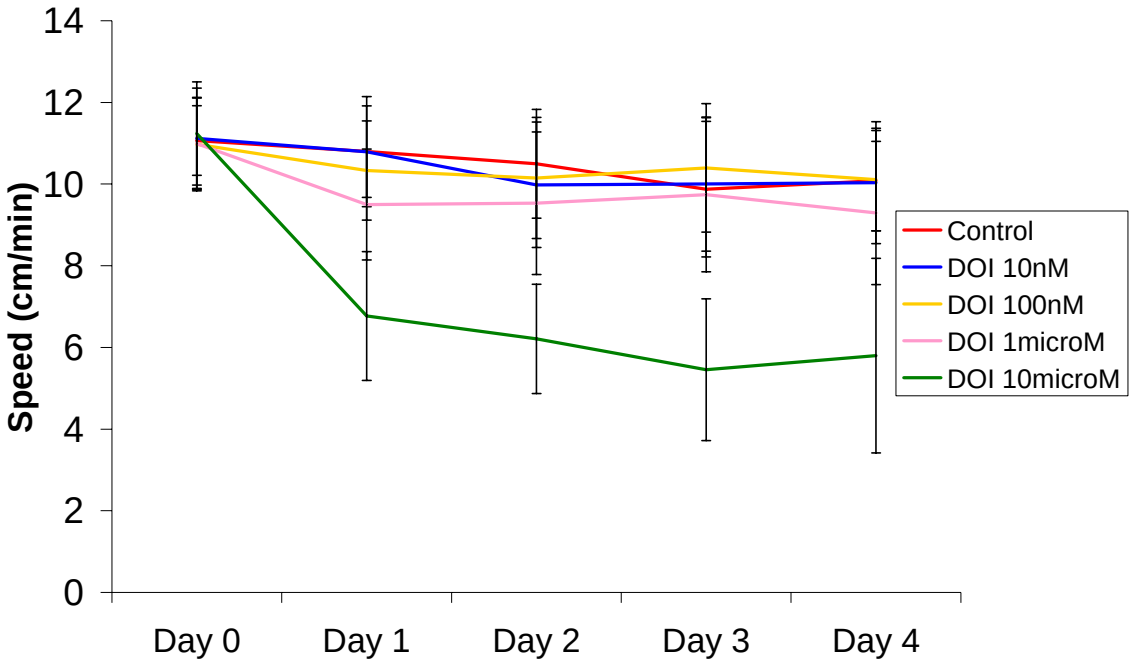

Figure 4-20. DOI dose-dependently results in a decrease in locomotor speed. Data are expressed as the mean ± S.D. of the time to travel 5cm in cm/min. (N=30/group).DOI (10mM) resulted in a significant decrease in locomotor speed ($p<0.01$).

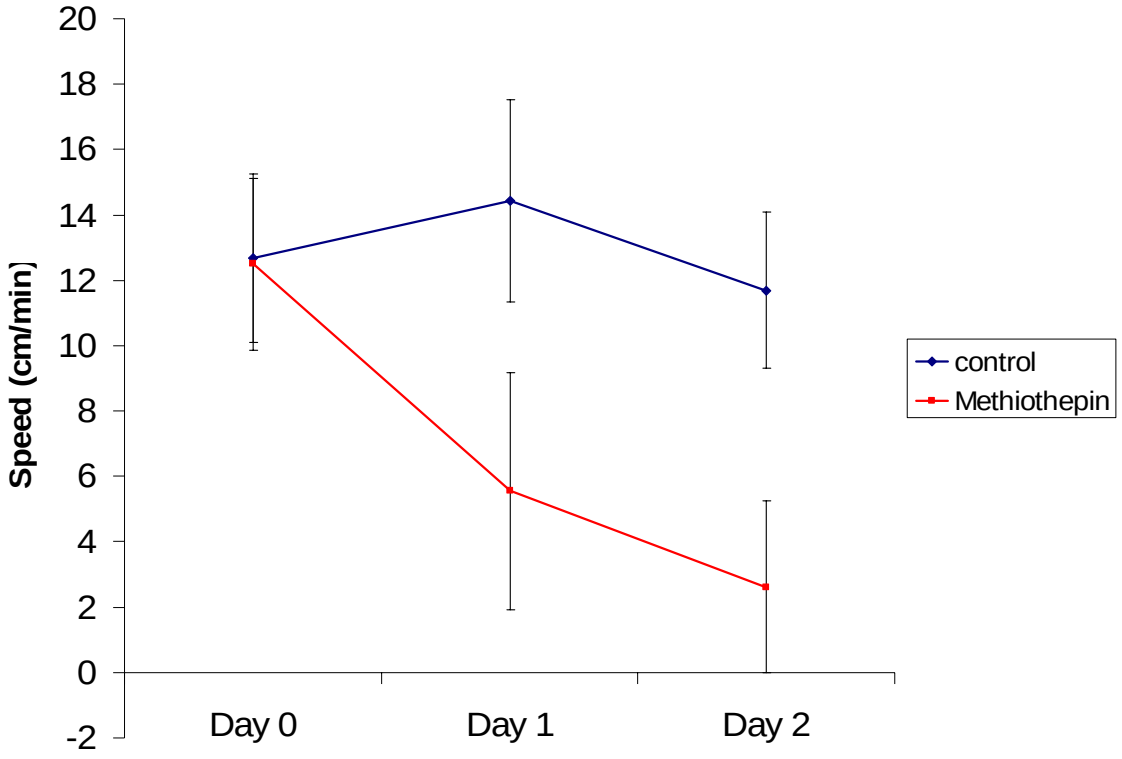

Figure 4-21. Methiothepin potently effects locomotor speed. Data are expressed as the mean ± S.D. of the time to travel 5cm in cm/min. Methiothepin (100nM) causes a significant reduction in locomotor speed ($p<0.001$). (N=30/group)

Table 4-1. Effect of co-exposure with different drugs on AMPH toxicity.

	[AMPH]	[Drug]	Number With Heads (Day)				Number Alive (Day)			
			1	2	3	4	1	2	3	4
Control	---	---	10	10	10	10	10	10	10	10
AMPH	27μM	---	8	1	0	0	10	10	10	5
Li	---	1mM	10	10	9	6	10	10	10	6
Li	---	2mM	10	10	3	0	10	10	5	4
AMPH + Li	27μM	1mM	10	2	1	1	10	6	4	3
AMPH + Li	27μM	2mM	10	0	0	0	10	2	2	2
Control	---	---	10	10	10	10	10	10	10	10
AMPH	100μM	---	10	6	0	0	10	10	5	0
Nom	---	1μM	10	10	10	10	10	10	10	10
AMPH + Nom	100μM	1μM	10	5	0	0	10	10	5	0
Control	---	---	10	10	10	10	10	10	10	10
DMSO	---	1%	10	10	10	10	10	10	10	10
7-NI	---	50μM	10	10	7	5	10	10	10	8
AMPH + 7-NI	20μM	50μM	10	10	7	4	10	10	10	8

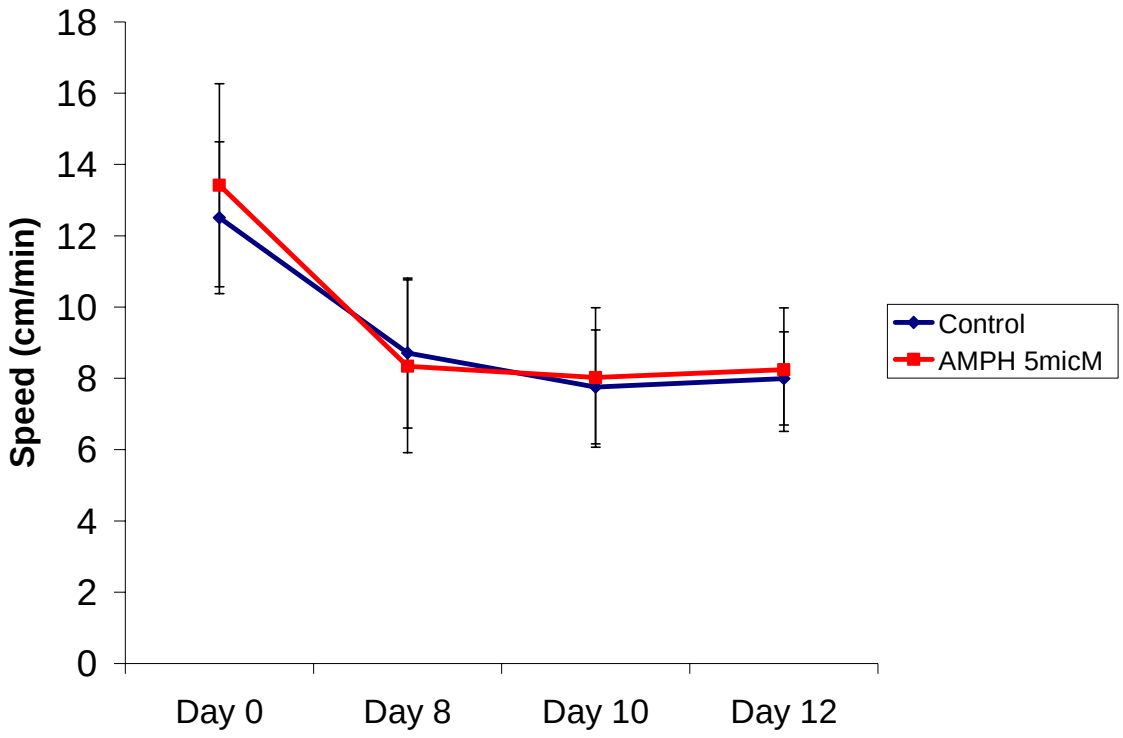

Figure 4-22. Amphetamine did not inhibit head regeneration. Head regeneration was evaluated by measuring locomotor speed which was not affected by the presence of amphetamine at any point during regeneration. Data are expressed as the mean ± S.D. of the time to travel 5cm in cm/min. (N=10/group)

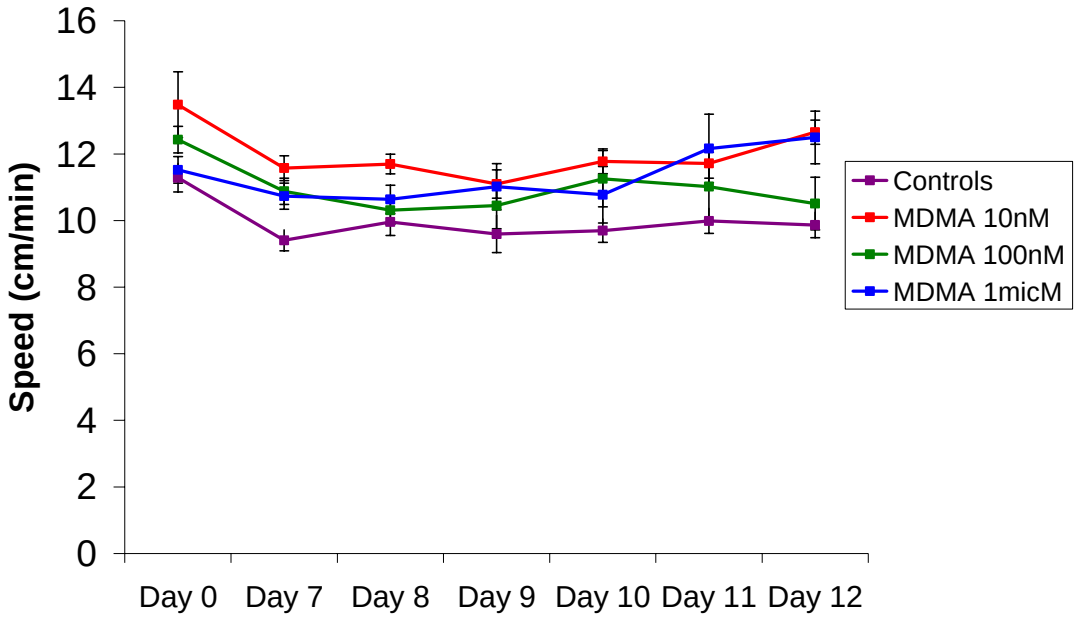

Figure 4-23. MDMA did not inhibit head regeneration. Head regeneration was evaluated by measuring locomotor speed which was not affected by the presence of MDMA at any point during regeneration at all doses. Data are expressed as the mean ± S.D. of the time to travel 5cm in cm/min. (N=10/group)

Table 4-2. Radio-ligand displacement kinetics.

Ligand and Displacers	**K_i = __**	**X $10^{\underline{X}}$ M**
[H^3]-5-HT		
5-HT	5	-8
methiothepin	2	-8
d-amphetamine	3	-8
MDMA	2	-6
DA	1	-6
[H^3]-DA		
DA	2	-8
d-amphetamine	4	-8
MDMA	7	-8
5-HT	1	-6

CHAPTER 5
DISCUSSION

This study sought to characterize the effects of amphetamines in a model using the free-living freshwater planarian species *Dugesia dorotocephala*. Animal models are time-consuming and costly. Cell culture models allow for investigation of specific effects and are more easily manipulated, but fail to represent the complexities found in the CNS. The goal of these studies was to characterize the effects of AMPH and MDMA in a new model employing planaria in order to determine the potential of this invertebrate for use in drug abuse neurotoxicity studies.

Planarians are useful for this type of study because of the simplicity of their nervous system and the existing literature on the behavioral effects of dopaminergic and serotonergic drugs, as well as others (Venturini et al., 1989; Buttarelli et al., 2000). In addition, colonies are easy to maintain and they are very inexpensive.

Planarians are already being used as a model system for Parkinson's disease (Kitamura et al., 1998). In this system, MPTP and rotenone treatment cause whole body autolysis which is inhibited by typical anti-Parkinsonian drugs such as talipexole and pramipexole (Kitamira et al., 2003). Planarians have also been suggested as a model system for tumorigenesis studies as well as for environmental toxins (Hansen et al., 1993; Schaeffer, 1993). In addition, planarians are being used as a model for drug withdrawal and combinatorial effects of drugs of abuse (Raffa et al., 2006). This same group has also shown a withdrawal effect from AMPH, using a measure of locomotor velocity, which is inhibited by an opioid antagonist (Raffa et al., 2008). In contrast to the measure of locomotor speed we used, this group used a measure of the number of gridlines crossed in a particular time period under static light conditions. Although AMPH has been purported to inhibit regeneration in planaria (Liotti, 1961), there have been no studies to date of which our group is aware investigating the neurotoxicity of amphetamines in planaria.

Initial Toxicity Studies

We initially found that AMPH and MDMA induce head loss in a dose x time-dependent manner, with the highest doses exhibiting maximal head loss. The ability of drugs to cause head loss in planaria has not been reported previously and as such is a novel finding. AMPH appears to have a steep dose-response curve, with the window of effect being between 30 and 100μM. At 30μM significant head loss is induced, but does not result in as much death as at 100μM. The fact that AMPH and MDMA do this in a dose-dependent manner suggests that it is a direct effect of the drug. As only heads are affected, it also suggests a unique vulnerability in the cephalic ganglia. Osmotic disregulation is not likely because the excretory system responsible for maintainence of osmotic balance, the protonephridial system, is located throughout the body of the worm (Ishii, 1980). If AMPH and MDMA were simply disrupting the equilibrium, then it would be expected to affect the whole body.

As the highest dosage levels were also shown to cause reductions in serotonin, which is involved in regeneration, it could be interfering with general tissue homeostasis and cellular repair. However, these are speculative and the exact mechanism by which this occurs remains to be elucidated. Morphological examination of heads that were treated with AMPH and MDMA revealed a gradual autolysis. This could be due to a number of factors, including apoptosis or non-specific necrosis. In worms treated with MPTP and rotenone, whole body autolysis was exhibited which could be inhibited with caspase inhibitors (Kitamura et al., 2003). Proteinases, such as caspase as well as aspartic proteinases, are present in planaria and are active in remodeling and regeneration (Zamora-Veyl et al., 2002; Hwang et al., 2004). Apoptosis has been observed in cell culture and in rodent brain after AMPH and METH administration (Cubells et

al., 1994; Cunha-Oliveira et at., 2006; Zhu et al., 2006). Biochemical analysis of caspase levels or levels of apoptosis could help elucidate these effects.

Additionally, AMPH and MDMA caused a dose dependent decrease in locomotor speed which also correlated with doses causing head loss and death. Earlier studies indicated that amphetamine behaviorally acts as an indirect agonist at both the D1 and D2-like receptor types in planaria, resulting in both C-like position (CLP) and screw-like hyperkinesia (SLH) (Venturini et al., 1989). Both DA and serotonin have been implicated in movement control. DA appears to modulate the large muscular contractions (Nishimura et al., 2007b) whereas serotonin is thought to modulate ciliary movement (Kimmel and Carlyon, 1990). The decreased speed could be due to decreased availability of one or both of these neurotransmitters, although other effects such as decreased energy availability might also be possible.

The decrease in locomotor speed observed at 24 h following administration of AMPH (100μM) (Figure 4-3) appears to correlate with the decrease in both 5-HT and DA at 24 h, as measured by HPLC (Figures 4-9 and 4-10, respectively). Acutely, MDMA increases both 5-HT and DA, although this effect is only significant for DA (Figures 4-9 and 4-10, respectively). MDMA at a dose of 100μM resulted in significant decreases in levels of 5-HT at 24 h that remained depressed at 72 h. The decreases in levels of serotonin following MDMA treatment are consistent with data in the literature since MDMA acts primarily at the serotonergic system. The increased rate of DA turnover observed with AMPH and MDMA appears to correlate with the timepoint at which maximal head loss occurs for each drug. DA turnover is indicative of an increase in neurotransmitter release which could lead to an increase in oxidative stress. This is an interesting effect since AMPH does not increase levels of DA at 1h, but it appears to inhibit the metabolism since the turnover is much higher than in controls. This is in agreement with current

data that AMPH increases DA turnover while decreasing the levels of DA, possibly due to inhibition of monoamine oxidase (Pereira et al., 2006).

Another amphetamine, DOI, which acts primarily as a 5-HT2 receptor agonist was used as a comparison. This compound proved to be the most potent toxin examined here, with the highest dose of 100μM inducing head loss and death in 100% of animals by Day 1 and Day 2, respectively. A lower dose of 10μM also resulted in 60% head loss by Day 4 and significant decreases in locomotor speed as compared to controls.

In humans, DOI is a hallucinogen and exerts complex actions, disrupting the balance between inhibitory and excitatory influences (Martin-Ruiz et al., 2001). The toxicity caused by DOI in planarians does not occur in rodents. This difference in toxicity could be partially due to disruption of the serotonin system by DOI, which plays an important role in regeneration and tissue homeostasis in planarians (Franquinet, 1979).

Methiothepin, a general serotonin antagonist, was also used as a comparison to further elucidate results obtained from AMPH and MDMA. It proved to be a potent inhibitor of locomotor movement at a comparatively low dose (100nM). The results obtained with methiothepin were not surprising since it has previously been shown to be a potent inhibitor of regeneration, although the concentration used in that experiment was higher than the concentration used here (500nM and 100nM, respectively) (Saitoh et al., 1996).

It has previously been shown that reuptake blockers are protective against neurotoxicity induced by amphetamines (Seiden and Ricuarte, 1987; Malberg et al., 1996). Nomifensine, a DA reuptake blocker, was investigated to determine if it would be protective against the toxic effects of amphetamine in this model, as measured by indices of head loss and death. Although it did not have any effect on these parameters, this could be due to a number of issues. Both AMPH and

nomifensine cause C-like position (CLP) and screw-like hyperkinesias (SLH) when given acutely, indicating that they are acting by similar mechanisms (Venturini et al., 1989). However, it is not known how these two compounds interact with the transporter or if they would inhibit each other. Another possibility is that the doses used were not sufficient to protect against AMPH toxicity in this model. Evaluating the affinity of nomifensine with the DAT in planaria using radioligand binding experiments could help determine not only the best dose range but also could help elucidate the pharmacological activity of this drug in planaria. Further work needs to be done to indicate whether the effect of head loss is strictly neuronal. The decreased locomotor speed indicates that there are effects on the DA system, possibly due to oxidative stress or impaired regenerative ability. The possibility that support cells are also being affected cannot be ruled out.

Lithium has been shown to have antioxidant effects in mammals (Shao et al., 2005) and is believed to protect against oxidative stress caused by AMPH (Frey et al., 2006). In this model, it exacerbates the effects of AMPH. However, Lithium has also been shown to inhibit regeneration in planaria (Lenicque, 1974) and the result could be due to loss of ability to maintain normal tissue homeostasis, combined with toxicity exerted by AMPH.

There is also evidence that reactive nitrogen species play a role in toxicity of amphetamines, such as increases in nitrotyrosine (Darvesh et al., 2005). A neuronal nitric oxide synthase (nNOS) inhibitor, 7-nitroindazole (7-NI), was shown to be protective against METH-induced toxicity in the striatum of mice (Itzhak and Ali, 1996). There is also evidence that nitric oxide plays an inhibitory role in muscle movement (Gustaffson et al., 2001). The inability of 7-NI to protect against AMPH-induced toxicity, by measures of head loss and death, could be due to several factors. 7-NI is a lipophilic compound and requires dimethylsulfoxide (DMSO) to

dissolve it. DMSO has been shown to be toxic, though at levels much higher than what the animals in this study were exposed to (Pagan et al., 2006). Although vehicle controls (in DMSO only) did not display toxicity, it is possible that the amount present facilitated uptake of AMPH, or that the two compounds acted in a synergistic way, with AMPH lowering the threshold of resistance to the toxicity of DMSO. Also, since very little is known about the nitric oxide system in planaria, it may not be interacting in the same way it does in mammals. As stated previously, these were conducted as pilot studies and therefore an exhaustive evaluation of dosages of the putative neuroprotective agents was not conducted. It is possible that these agents might be protective at different dose levels.

This model has also been shown to have dopaminergic-cholinergic interactions. The cholinergic system may act as an inhibitor to DA-induced movement, as administration of a cholinergic antagonist results in SLH, a behavior typical of D1 agonists (Venturini et al., 1989; Palladini et al., 1996; Buttarelli et al., 2000). In a rat model, stimulation of the muscarinic acetylcholine receptors inhibited the amphetamine-induced increase in DA release in the nucleus accumbens (Ichikawa et al., 2002). Administration of an alpha-7 nicotinic acetylcholine receptor (nAChR) antagonist also protected against oxidative stress and toxicity of MDMA in vitro and in mice brains (Chipana et al., 2008). It would be interesting to examine the effects of cholinergic agonists on the effects of amphetamines in this invertebrate model.

Lasting Effects after Head Loss

Another interesting finding was that once planaria lose their heads in AMPH, they fail to recover, as measured by locomotor speed, to control levels (Figure 4-13). This would indicate a lasting change. This could be due to lasting depletions in monoamine levels which subsequently prevent proper regeneration, as they are implicated in playing a large role in regeneration (Franquinet, 1979). More specifically, serotonin is important in the early stages of regeneration

and the rapid increase in DNA synthesis (Martelly, 1984). In support of the theory that monoamine depletion causes lasting changes, when planaria are treated with the serotonin depletor para-chlorophenylalanine, sliced, and allowed to regenerate, they fail to recover normal function (Kimmel and Carlyon, 1990). Thus, it is possible that AMPH induces a plastic change in the nervous system which gets perpetuated. However, the possibility that effects on other components such as tyrosine hydroxylase, which is depleted in mammalian models (Bowyer et al., 1998), cannot be excluded. In this model, AMPH also causes reductions in serotonin levels and therefore could also be affecting tryptophan hydroxylase. In addition, since serotonin is thought to be an important modulator of ciliary movement, while DA is thought to be important for gross muscle movement, it is unclear whether there was abnormal development in the musculature or if levels of serotonin and dopamine simply remain depressed in the regenerates. We did not measure serotonin or dopamine levels in regenerates and therefore do not know what levels were present. The decreased locomotor speed appears, in part, to be dependent on AMPH as the causative agent for head loss since heads that were mechanically removed were not affected to the same level. These animals did not remain at levels that were significantly below control levels. One explanation for this differential effect could be that animals that did not lose heads also did not have as much monoamine depletion, or toxic effect of AMPH in general. In contrast, animals that lost heads in MDMA and were allowed to regenerate were not different from controls. This differential effect is intriguing and it could be that toxicity from MDMA is less severe than what occurs with AMPH. The data presented here do show that MDMA is less toxic than AMPH on head loss, death, and locomotor speed. It is possible that MDMA is not causing enough of a disruption, or depletion of serotonin and dopamine, to have lasting effects that are perpetuated after regeneration. Effects on tryptophan hydroxylase could also participate.

Since AMPH does cause depletions in serotonin in this model, it could also be acting as an inhibitor of tryptophan hydroxylase. Given the belief that serotonin is important early in regeneration, a depletion in its levels combined with inhibition of synthesis could lead to the continued effects. Histological studies evaluating the effects on the serotonin and dopamine systems could help elucidate these differential effects.

Effect on Regeneration

The importance of the monoamines in regeneration led to the hypothesis that AMPH and MDMA, by altering levels of the monoamines, might impede or inhibit regeneration. In a gerbil model, a single dose of METH temporarily inhibited granule cell proliferation in the hippocampus (Teuchert-Noodt et al., 2000). Similar observations were made following a high binge dose of MDMA in rat brain (Hernández-Rabaza et al., 2006). In mammals, there are two main sources of neural stem cells in adults. These arise from the sub-granular layer of the hippocampus and the sub-ventricular zone of the lateral ventricles (Zhao et al., 2008) and are speculated to play a role in depression (Elder et al., 2006) and neurodegenerative diseases (Steiner et al., 2006). In addition, it had previously been reported that AMPH inhibited regeneration in planaria (Liotti, 1961). However, this was not the case as evidenced here (Figures 4-17 and 4-18). This disparity is most likely due to a difference in dosages and one possibility is that at low doses these drugs are not producing a level of monoamine depletion that would be inhibitory. However, since higher doses resulted in toxicity in intact worms, we wanted to test doses that were not toxic by themselves. These lower doses could actually have been helpful by increasing availability of dopamine and serotonin for regeneration. Although the endpoint used may not be sensitive enough to detect subtle changes, there was a slight, though not significant, improvement in worms treated with 1μM MDMA. Another, though less likely, possibility is that

intact heads might be required for the two drugs to exert their effects and that in the absence of a CNS they fail to modulate monoamine activity. This would further indicate that the toxicity of these two drugs is mediated by the CNS in planarians.

Summary and Future Directions

In summary, we found that AMPH and MDMA were differentially toxic in the planarian, with AMPH exerting more toxic effects. AMPH also displaced 5-HT with high affinity whereas MDMA only bound at micromolar concentrations. Both drugs exerted effects on the serotonin and dopamine systems as evidenced by their effects on the transmitter levels. We also found that AMPH induces lasting effects on regenerated worms, in contrast to MDMA. Additionally, we found that a 5HT-2 agonist, DOI, was more potent than either AMPH or MDMA. A general serotonin antagonist, methiothepin, did not cause toxicity in the form of head loss or death, but had very potent effects on locomotor speed. Thus, the drugs that acted as 5-HT agonists, AMPH and DOI, appeared to exert the most toxicity in terms of head loss and death. Drug intervention used in combination with AMPH did not attenuate effects in this model. It may be that AMPH does not act by the same mechanisms in this system, or that the drugs used as protectants are not interacting in the same way, or that different doses were needed. We also found that neither MDMA nor AMPH had effects on regeneration at low doses.

The popularity of AMPH and MDMA and the accumulating evidence for lasting neurotoxicity underscores the need for new models to parse out effects. The preliminary evidence of AMPH and MDMA actions in this model suggest it has potential as a model for measuring toxicity of amphetamines and other drugs. The simplicity of the nervous system and its similarities to mammalian systems would make planaria an ideal model to segue between *in vitro* and *in vivo* systems. However, further studies need to be conducted before it can truly be defined as a legitimate model. The effects need to be confirmed histologically to conclusively

prove that the toxic effects are a direct response to administration of the amphetamines. The recent advances in planarian and molecular biology should help provide the necessary tools to achieve this goal. Specifically, the cloning of the serotonin receptor, tryptophan hydroxylase, and tyrosine hydroxylase genes should help provide for *in situ* examinations. This is an exciting and potentially great model for quickly evaluating drug toxicity. Additionally, the low costs involved could benefit young investigators in a time of financial constraints or for use in smaller side projects that do not have funding.

LIST OF REFERENCES

Acevedo SF, de Esch IJ, Raber J (2007) Sex- and Histamine-Dependent Long-Term Cognitive Effects of Methamphetamine Exposure. Neuropsychopharmacology 32:665-672.

Adell T, Marsal M, Saló E (2008) Planarian GSK3s are involved in neural regeneration. Dev Genes Evol 218:89-103.

Algeri S, Carolei A, Ferretti P, Gallone C, Palladini G, Venturini G (1983) Effects of dopaminergic agents on monoamine levels and motor behaviour in planaria. Comp Biochem Physiol C 74:27-29.

Ali SF, Newport GD, Holson RR, Slikker W Jr, Bowyer JF (1994) Low environmental temperatures or pharmacologic agents that produce hypothermia decrease methamphetamine neurotoxicity in mice. Brain Res 658:33-38.

Arees EA (1986) Absence of light response in eyeless planaria. Physiol Behav 36:445-449.

Armstrong BD, Noguchi KK (2004) The neurotoxic effects of 3,4-methylenedioxymethamphetamine (MDMA) and methamphetamine on serotonin, dopamine, and GABA-ergic terminals: an in-vitro autoradiographic study in rats. Neurotoxicology 25:905-914.

Bashkatova V, Kraus MM, Vanin A, Hornick A, Prast H (2004) Comparative effects of NO-synthase inhibitor and NMDA antagonist on generation of nitric oxide and release of amino acids and acetylcholine in the rat brain elicited by amphetamine neurotoxicity. Ann N Y Acad Sci 1025:221-230.

Battaglia G, Gesi M, Lenzi P, Busceti CL, Soldani P, Orzi F, Rampello L, Nicoletti F, Ruggieri S, Fornai F (2002a) Morphological and biochemical evidence that apomorphine rescues striatal dopamine terminals and prevents methamphetamine toxicity. Ann N Y Acad Sci 965:254-266.

Battaglia G, Fornai F, Busceti CL, Aloisi G, Cerrito F, De Blasi A, Melchiorri D, Nicoletti F (2002b) Selective blockade of mGlu5 metabotropic glutamate receptors is protective against methamphetamine neurotoxicity. J Neurosci 22:2135-2141.

Bisagno V, Ferguson D, Luine VN (2002) Short toxic methamphetamine schedule impairs object recognition task in male rats. Brain Res 940:95-101.

Bogen IL, Haug KH, Myhre O, Fonnum F (2003) Short- and long-term effects of MDMA ("ecstasy") on synaptosomal and vesicular uptake of neurotransmitters in vitro and ex vivo. Neurochem Int 43:393-400.

Booij J, de Bruin K, Gunning WB (2006) Repeated administration of D-amphetamine induces loss of [123I]FP-CIT binding to striatal dopamine transporters in rat brain: a validation study. Nucl Med Biol 33:409-411.

Bowyer JF, Frame LT, Clausing P, Nagamoto-Combs K, Osterhout CA, Sterling CR, Tank AW (1998) Long-term effects of amphetamine neurotoxicity on tyrosine hydroxylase mRNA and protein in aged rats. J Pharmacol Exp Ther 286:1074-1085.

Bray GA, Greenway FL (1999) Current and potential drugs for treatment of obesity. Endocr Rev 20:805-875.

Brezun JM, Daszuta A (2000) Serotonin may stimulate granule cell proliferation in the adult hippocampus, as observed in rats grafted with foetal raphe neurons. Eur J Neurosci. 12:391-396.

Brodkin J, Malyala A, Nash JF (1993) Effect of acute monoamine depletion on 3,4-methylenedioxymethamphetamine-induced neurotoxicity. Pharmacol Biochem Behav 45:647-653.

Buchert R, Thomasius R, Nebeling B, Petersen K, Obrocki J, Jenicke L, Wilke F, Wartberg L, Zapletalova P, Clausen M. (2003) Long-term effects of "ecstasy" use on serotonin transporters of the brain investigated by PET. J Nucl Med. 44:375-384.

Buchert R, Thiele F, Thomasius R, Wilke F, Petersen K, Brenner W, Mester J, Spies L, Clausen M (2007) Ecstasy-induced reduction of the availability of the brain serotonin transporter as revealed by [11C](+)McN5652-PET and the multi-linear reference tissue model: loss of transporters or artifact of tracer kinetic modelling? J Psychopharmacol 21:628-634.

Burrows KB, Gudelsky G, Yamamoto BK (2000) Rapid and transient inhibition of mitochondrial function following methamphetamine or 3,4-methylenedioxymethamphetamine administration. Eur J Pharmacol 398:11-18.

Buttarelli FR, Pontieri FE, Margotta V, Palladini G (2000) Acetylcholine/dopamine interaction in planaria. Comp Biochem Physiol C Toxicol Pharmacol 125:225-231.

Buttarelli FR, Pontieri FE, Margotta V, Palladini G (2002) Cannabinoid-induced stimulation of motor activity in planaria through an opioid receptor-mediated mechanism. Prog Neuropsychopharmacol Biol Psychiatry 26:65-68.

Carolei A, Margotta V, Palladini G (1975) Proposal of a new model with dopaminergic-cholinergic interactions for neuropharmacological investigations. Neuropsychobiology 1:355-364.

Caronti B, Margotta V, Merante A, Pontieri FE, Palladini G (1999) Treatment with 6-hydroxydopamine in planaria (Dugesia gonocephala s.l.): morphological and behavioral study. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 123:201-207.

Cass WA, Manning MW (1999) Recovery of presynaptic dopaminergic functioning in rats treated with neurotoxic doses of methamphetamine. J Neurosci 9:7653-7660.

Cebrià F, Kudome T, Nakazawa M, Mineta K, Ikeo K, Gojobori T, Agata K (2002) The expression of neural-specific genes reveals the structural and molecular complexity of the planarian central nervous system. Mech Dev 116:199-204.

Cebrià F, Newmark PA (2005) Planarian homologs of netrin and netrin receptor are required for proper regeneration of the central nervous system and the maintenance of nervous system architecture. Development 132:3691-3703.

Cebrià F (2007) Regenerating the central nervous system: how easy for planarians! Dev Genes Evol 217:733-748.

Chandebois R (1985) Differentiated epidermal outgrowths in the planarian Dugesia gonocephala: a model for studying cell renewal and patterning in single-layered epithelial tissue. Exp Cell Biol 53:46-58.

Chapman DE, Hanson GR, Kesner RP, Keefe KA (2001) Long-term changes in basal ganglia function after a neurotoxic regimen of methamphetamine. J Pharmacol Exp Ther 296:520-527.

Che S, Johnson M, Hanson GR, Gibb JW (1995) Body temperature effect on methylenedioxymethamphetamine-induced acute decrease in tryptophan hydroxylase activity. Eur J Pharmacol 293:447-453.

Cherubini E, Gaiarsa JL, Ben-Ari Y (1991) GABA: an excitatory transmitter in early postnatal life. Trends Neurosci 14:515-519.

Chin MH, Qian WJ, Wang H, Petyuk VA, Bloom JS, Sforza DM, Laćan G, Liu D, Khan AH, Cantor RM, Bigelow DJ, Melega WP, Camp DG 2nd, Smith RD, Smith DJ (2008) Mitochondrial dysfunction, oxidative stress, and apoptosis revealed by proteomic and transcriptomic analyses of the striata in two mouse models of Parkinson's disease. J Proteome Res 7:666-677.

Chipana C, Camarasa J, Pubill D, Escubedo E (2008) Memantine prevents MDMA-induced neurotoxicity. Neurotoxicology 29:179-183.

Colado MI, O'Shea E, Granados R, Murray TK, Green AR (1997) In vivo evidence for free radical involvement in the degeneration of rat brain 5-HT following administration of MDMA ('ecstasy') and p-chloroamphetamine but not the degeneration following fenfluramine. Br J Pharmacol 121:889-900.

Cretì P, Capasso A, Grasso M, Parisi E (1992) Identification of a 5-HT1A receptor positively coupled to planarian adenylate cyclase. Cell Biol Int Rep 16:427-432.

Cubells JF, Rayport S, Rajendran G, Sulzer D (1994) Methamphetamine neurotoxicity involves vacuolation of endocytic organelles and dopamine-dependent intracellular oxidative stress. J Neurosci 14:2260-2271.

Cunha-Oliveira T, Rego AC, Cardoso SM, Borges F, Swerdlow RH, Macedo T, de Oliveira CR (2006) Mitochondrial dysfunction and caspase activation in rat cortical neurons treated with cocaine or amphetamine. Brain Res 1089:44-54.

Darvesh AS, Yamamoto BK, Gudelsky GA (2005) Evidence for the involvement of nitric oxide in 3,4-methylenedioxymethamphetamine-induced serotonin depletion in the rat brain. J Pharmacol Exp Ther 312:694-701.

Dasheiff BD, Dasheiff RM (2002) Photonegative response in brown planaria (Dugesia tigrina) following regeneration. Ecotoxicol Environ Saf 53:196-199.

Deng X, Cadet JL (2000) Methamphetamine-induced apoptosis is attenuated in the striata of copper-zinc superoxide dismutase transgenic mice. Brain Res Mol Brain Res 83:121-124

Deng X, Cai NS, McCoy MT, Chen W, Trush MA, Cadet JL (2002a) Methamphetamine induces apoptosis in an immortalized rat striatal cell line by activating the mitochondrial cell death pathway. Neuropharmacology 42(6):837-845.

Deng X, Jayanthi S, Ladenheim B, Krasnova IN, Cadet JL (2002b) Mice with partial deficiency of c-Jun show attenuation of methamphetamine-induced neuronal apoptosis. Mol Pharmacol 62:993-1000.

Easton N, Marsden CA (2006) Ecstasy: are animal data consistent between species and can they translate to humans? J Psychopharmacol 20:194-210.

Elder GA, De Gasperi R, Gama Sosa MA (2006) Research update: neurogenesis in adult brain and neuropsychiatric disorders. Mt Sinai J Med 73:931-940.

Eriksson KS, Panula P (1994) gamma-Aminobutyric acid in the nervous system of a planarian. J Comp Neurol 345:528-536.

Eriksson KS (1996) Nitric oxide synthase in the pharynx of the planarian Dugesia tigrina. Cell Tissue Res 286:407-410.

Faraone SV (2007) Stimulant therapy in the management of ADHD: mixed amphetamine salts (extended release). Expert Opin Pharmacother 8:2127-2134.

Feldman JM, Lee EM (1985) Serotonin content of foods: effect on urinary excretion of 5-hydroxyindoleacetic acid. Am J Clin Nutr 42:639-643.

Fernandes MC, Alvares EP, Gama P, Silveira M (2003) Serotonin in the nervous system of the head region of the land planarian Bipalium kewense. Tissue Cell 35:479-486.

Fischer JF, Cho AK (1979) Chemical release of dopamine from striatal homogenates: evidence for an exchange diffusion model. J Pharmacol Exp Ther 208:203-209.

Fleckenstein AE, Volz TJ, Riddle EL, Gibb JW, Hanson GR (2007) New insights into the mechanism of action of amphetamines. Annu Rev Pharmacol Toxicol 47:681-698.

Fornai F, Giorgi FS, Gesi M, Chen K, Alessrì MG, Shih JC (2001) Biochemical effects of the monoamine neurotoxins DSP-4 and MDMA in specific brain regions of MAO-B-deficient mice. Synapse 39:213-221.

Franquinet R (1979) The role of serotonin and catecholamines in the regeneration of the Planaria Polycelis tenvis J Embryol Exp Morphol 51:85-95.

Frederick DL, Ali SF, Slikker W Jr, Gillam MP, Allen RR, Paule MG (1995) Behavioral and neurochemical effects of chronic methylenedioxymethamphetamine (MDMA) treatment in rhesus monkeys. Neurotoxicol Teratol 17:531-543.

Frenzilli G, Ferrucci M, Giorgi FS, Blandini F, Nigro M, Ruggieri S, Murri L, Paparelli A, Fornai F (2007) DNA fragmentation and oxidative stress in the hippocampal formation: a bridge between 3,4-methylenedioxymethamphetamine (ecstasy) intake and long-lasting behavioral alterations. Behav Pharmacol 18:471-481.

Frey BN, Valvassori SS, Réus GZ, Martins MR, Petronilho FC, Bardini K, Dal-Pizzol F, Kapczinski F, Quevedo J (2006) Effects of lithium and valproate on amphetamine-induced oxidative stress generation in an animal model of mania. J Psychiatry Neurosci 31:326-332.

Friedman SD, Castaneda E, Hodge GK (1998) Long-term monoamine depletion, differential recovery, and subtle behavioral impairment following methamphetamine-induced neurotoxicity. Pharmacology Biochemistry and Behavior 61:35-44.

Fumagalli F, Gainetdinov RR, Wang YM, Valenzano KJ, Miller GW, Caron MG (1999) Increased methamphetamine neurotoxicity in heterozygous vesicular monoamine transporter 2 knock-out mice. J Neurosci 19:2424-2431.

Glass M, Felder CC (1997) Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. J Neurosci 17:5327-5333.

Gouzoulis-Mayfrank E, Daumann J (2006) Neurotoxicity of methylenedioxyamphetamines (MDMA; ecstasy) in humans: how strong is the evidence for persistent brain damage? Addiction 101:348-361.

Gratzner HG (1982) Monoclonal antibody to 5-bromo- and 5-iododeoxyuridine: a new reagent for detection of DNA replication. Science 218: 474-475.

Grinspoon L, Bakalar JB (1986) Can drugs be used to enhance the psychotherapeutic process? Am J Psychother 40:393-404.

Guilarte TR, Nihei MK, McGlothan JL, Howard AS (2003) Methamphetamine-induced deficits of brain monoaminergic neuronal markers: distal axotomy or neuronal plasticity. Neuroscience 122:499-513.

Gustafsson MK, Terenina NB, Kreshchenko ND, Reuter M, Maule AG, Halton DW (2001) Comparative study of the spatial relationship between nicotinamide adenine dinucleotide phosphate-diaphorase activity, serotonin immunoreactivity, and GYIRFamide immunoreactivity and the musculature of the adult liver fluke, Fasciola hepatica (Digenea, fasciolidae). J Comp Neurol 429:71-79.

Han DD, Gu HH (2006) Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. BMC Pharmacol 6:6 .

Hansen LG, Tehseen WM, Foley GL, Schaeffer DJ (1993) Modification by polychlorinated biphenyls (PCBs) of cadmium induced lesions in the planarian model, Dugesia dorotocephala. Biomed Environ Sci 6:367-384.

Harvey DC, Lacan G, Tanious SP, Melega WP (2000) Recovery from methamphetamine induced long-term nigrostriatal dopaminergic deficits without substantia nigra cell loss. Brain Research 871:259-270.

Hashimoto K, Tsukada H, Nishiyama S, Fukumoto D, Kakiuchi T, Shimizu E, Iyo M (2004) Protective effects of N-acetyl-L-cysteine on the reduction of dopamine transporters in the striatum of monkeys treated with methamphetamine. Neuropsychopharmacology 29:2018-2023.

Hayat S, Williams RJ, Rattray M (2006) Serotonin transporter expression is not sufficient to confer cytotoxicity to 3,4-methylenedioxymethamphetamine (MDMA) in vitro. J Psychopharmacol 20:257-263.

Heikkila RE, Orlansky H, Mytilineou C, Cohen G (1975) Amphetamine: evaluation of d- and l-isomers as releasing agents and uptake inhibitors for 3H-dopamine and 3H-norepinephrine in slices of rat neostriatum and cerebral cortex. J Pharmacol Exp Ther 194:47-56.

Hernández-Rabaza V, Domínguez-Escribà L, Barcia JA, Rosel JF, Romero FJ, García-Verdugo JM, Canales JJ (2006) Binge administration of 3,4-methylenedioxymethamphetamine ("ecstasy") impairs the survival of neural precursors in adult rat dentate gyrus. Neuropharmacology 51:967-973.

Hwang JS, Kobayashi C, Agata K, Ikeo K, Gojobori T (2004) Detection of apoptosis during planarian regeneration by the expression of apoptosis-related genes and TUNEL assay. Gene 333:15-25.

Ichikawa J, Chung YC, Li Z, Dai J, Meltzer HY (2002) Cholinergic modulation of basal and amphetamine-induced dopamine release in rat medial prefrontal cortex and nucleus accumbens. Brain Res 958:176-184.

Imam SZ, Newport GD, Itzhak Y, Cadet JL, Islam F, Slikker W Jr, Ali SF (2001) Peroxynitrite plays a role in methamphetamine-induced dopaminergic neurotoxicity: evidence from mice lacking neuronal nitric oxide synthase gene or overexpressing copper-zinc superoxide dismutase. J Neurochem 76:745-749.

Ishii S (1980) The ultrastructure of the protonephridial flame cell of the freshwater planarian Bdellocephala brunnea. Cell Tissue Res 206:441-449.

Ishikawa A, Kadota T, Kadota K, Matsumura H, Nakamura S (2005) Essential role of D1 but not D2 receptors in methamphetamine-induced impairment of long-term potentiation in hippocampal-prefrontal cortex pathway. Eur J Neurosci 22:1713-1719.

Itoh MT, Igarashi J (2000) Circadian rhythm of serotonin levels in planarians. Neuroreport 11:473-476.

Itzhak Y, Ali SF (1996) The neuronal nitric oxide synthase inhibitor, 7-nitroindazole, protects against methamphetamine-induced neurotoxicity in vivo. J Neurochem 67:1770-1773.

Itzhak Y, Achat-Mendes C (2004) Methamphetamine and MDMA (ecstasy) neurotoxicity: 'of mice and men'. IUBMB Life 56:249-255.

Itzhak Y, Anderson KL, Ali SF (2004) Differential response of nNOS knockout mice to MDMA ("ecstasy")- and methamphetamine-induced psychomotor sensitization and neurotoxicity. Ann N Y Acad Sci 1025:119-128.

Jayanthi S, Deng X, Noailles PA, Ladenheim B, Cadet JL (2004) Methamphetamine induces neuronal apoptosis via cross-talks between endoplasmic reticulum and mitochondria-dependent death cascades. FASEB J 18:238-251.

Johnson DJ, Sanderson H, Brain RA, Wilson CJ, Solomon KR (2007) Toxicity and hazard of selective serotonin reuptake inhibitor antidepressants fluoxetine, fluvoxamine, and sertraline to algae. Ecotoxicol Environ Saf 67:128-139.

Jones SR, Gainetdinov RR, Wightman RM, Caron MG (1998) Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. J Neurosci 18:1979-1986.

Kahlig KM, Lute BJ, Wei Y, Loland CJ, Gether U, Javitch JA, Galli A (2006) Regulation of dopamine transporter trafficking by intracellular amphetamine. Mol Pharmacol 70:542-548

Kamei H, Nagai T, Nakano H, Togan Y, Takayanagi M, Takahashi K, Kobayashi K, Yoshida S, Maeda K, Takuma K, Nabeshima T, Yamada K (2006) Repeated methamphetamine treatment impairs recognition memory through a failure of novelty-induced ERK1/2 activation in the prefrontal cortex of mice. Biol Psychiatry 59:75-84.

Kato C, Mihashi K, Ishida S (2004) Motility recovery during the process of regeneration in freshwater planarians. Behav Brain Res 150:9-14.

Kim SJ, Lyoo IK, Hwang J, Chung A, Sung YH, Kim J, Kwon D, Chang, KH, Renshaw PF (2006) Prefrontal Grey Matter Changes in Short Term and Long Term Abstinent Methamphetamine Abusers. International Journal of Neurospychopharmacology 9: 221-228.

Kimmel HD, Carlyon WD (1990) Persistent effects of a serotonin depletor (p-chlorophenylalanine) in regenerated planaria (Dugesia dorotocephala). Behav Neurosci 104:127-134.

Kita T, Matsunari Y, Saraya T, Shimada K, O'Hara K, Kubo K, Wagner GC, Nakashima T (2000) Methamphetamine-induced striatal dopamine release, behavior changes and neurotoxicity in BALB/c mice. Int J Dev Neurosci 18:521-530.

Kita T, Wagner GC, Nakashima T (2003) Current research on methamphetamine-induced neurotoxicity: animal models of monoamine disruption. J Pharmacol Sci 92:178-195.

Kitamura Y, Kakimura J, Taniguchi T (1998) Protective effect of talipexole on MPTP-treated planarian, a unique parkinsonian worm model. Jpn J Pharmacol 78:23-29.

Kitamura Y, Inden M, Sanada H, Takata K, Taniguchi T, Shimohama S, Orii H, Mochii M, Agata K, Watanabe K (2003) Inhibitory effects of antiparkinsonian drugs and caspase inhibitors in a parkinsonian flatworm model. J Pharmacol Sci 92:137-142.

Klongpanichapak S, Phansuwan-Pujito P, Ebadi M, Govitrapong P (2007) Melatonin protects SK-N-SH neuroblastoma cells from amphetamine-induced neurotoxicity. J Pineal Res 43(1):65-73.

Kokkinidis L, Anisman H (1981) Amphetamine psychosis and schizophrenia: a dual model. Neurosci Biobehav Rev 5:449-461.

Koopowitz H, Chien P (1975) Ultrastructure of nerve plexus in flatworms. II. Sites of synaptic interactions. Cell Tissue Res 157:207-216.

Koppenol WH, Moreno JJ, Pryor WA, Ischiropoulos H, Beckman JS (1992) Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. Chem Res Toxicol 5:834-842.

Krasnova IN, Ladenheim B, Cadet JL (2005) Amphetamine induces apoptosis of medium spiny striatal projection neurons via the mitochondria-dependent pathway. FASEB J 19:851-853.

Leelahanaj T, Kongsakon R, Netrakom P (2005) A 4-week, double-blind comparison of olanzapine with haloperidol in the treatment of amphetamine psychosis. J Med Assoc Thai 88 Suppl 3:S43-52.

Lenicque PM (1974) Protective effect of 5-hydroxytryptophan and a-Methyl DOPA against the inhibitive effect of lithium on the cicastration and the regeneration of the planarian worm Dugesia tigrina and the hydroid Clava squamata. Acta Zoologica 55:169-171.

Lenicque PM, Feral JP (1976) A mechanism of the action of neurotransmitters on the regeneration of the planarian worm, *Dugesia tigrina:* Role of acetylcholine as a negative feedback. Acta Zool 57: 1-5

Liotti FS (1961) Studies and investigations of regeneration in Planaria. Inhibiting action of amphetamine and other sympathicominetic amines on the regeneration of the head in Dugesia lugubris. Riv Biol 54:415-455.

MacRae EK (1964) Observation on the fine structure of photoreceptor cells in the planarian Dugesia tigrina. J Ultrastruc Res 10:334-349

Malberg JE, Sabol KE, Seiden LS (1996) Co-administration of MDMA with drugs that protect against MDMA neurotoxicity produces different effects on body temperature in the rat. J Pharmacol Exp Ther 278:258–267.

Malberg JE, Seiden LS (1998) Small changes in ambient temperature cause large changes in 3,4-methylenedioxymethamphetamine (MDMA)-induced serotonin neurotoxicity and core body temperature in the rat. J Neurosci 18:5086-5094.

Malberg JE, Eisch AJ, Nestler EJ, Duman RS (2000) Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 20:9104-9110.

Marchant EG, Watson NV, Mistlberger RE (1997) Both neuropeptide Y and serotonin are necessary for entrainment of circadian rhythms in mice by daily treadmill running schedules. J Neurosci 17:7974-7987.

Mark KA, Soghomonian JJ, Yamamoto BK (2004) High-dose methamphetamine acutely activates the striatonigral pathway to increase striatal glutamate and mediate long-term dopamine toxicity. J Neurosci 24:11449-11456.

Marks NJ, Halton DW, Kearn GC, Shaw C, Johnston CF (1994) 5-hydroxytryptamine-immunoreactivity in the monogenean parasite, Entobdella soleae. Int J Parasitol 24:1011-1018.

Marsal M, Pineda D, Saló E (2003) Gtwnt-5 a member of the wnt family expressed in a subpopulation of the nervous system of the planarian Girardia tigrina. Gene Expr Patterns 3:489-489.

Martelly I (1984) Calcium thresholds in the activation of DNA and RNA synthesis in cultured planarian cells: relationship with hormonal and DB cAMP effects. Cell Differ 15:25-36.

Martín-Ruiz R, Puig MV, Celada P, Shapiro DA, Roth BL, Mengod G, Artigas F (2001) Control of serotonergic function in medial prefrontal cortex by serotonin-2A receptors through a glutamate-dependent mechanism. J Neurosci 21:9856-9866.

McCann UD, Wong DF, Yokoi F, Villemagne V, Dannals RF, Ricaurte GA (1998) Reduced striatal dopamine transporter density in abstinent methamphetamine and methcathinone users: evidence from positron emission tomography studies with [11C]WIN-35,428. J Neurosci 18:8417-8422.

McCann UD, Szabo Z, Seckin E, Rosenblatt P, Mathews WB, Ravert HT, Dannals RF, Ricaurte GA. (2005) Quantitative PET studies of the serotonin transporter in MDMA users and

controls using [11C]McN5652 and [11C]DASB. Neuropsychopharmacology. 30(9):1741-1750.

McGregor IS, Gurtman CG, Morley KC, Clemens KJ, Blokland A, Li KM, Cornish JL, Hunt GE (2003) Increased anxiety and "depressive" symptoms months after MDMA ("ecstasy") in rats: drug-induced hyperthermia does not predict long-term outcomes. Psychopharmacology 168:465-474.

Melega WP, Raleigh MJ, Stout DB, Lacan G, Huang SC, Phelps ME (1997) Recovery of striatal dopamine function after acute amphetamine- and methamphetamine-induced neurotoxicity in the vervet monkey. Brain Res 766:113-120.

Meyer JS, Ali SF (2002) Serotonergic neurotoxicity of MDMA (ecstasy) in the developing rat brain. Ann N Y Acad Sci 965:373-380.

Miller DK, Rodvelt KR, Constales C, Putnam WC (2007) Analogs of SR-141716A (Rimonabant) alter d-amphetamine-evoked [3H] dopamine overflow from preloaded striatal slices and amphetamine-induced hyperactivity. Life Sci 81:63-71.

Mineta K, Nakazawa M, Cebria F, Ikeo K, Agata K, Gojobori T (2003) Origin and evolutionary process of the CNS elucidated by comparative genomics analysis of planarian ESTs. Proc Natl Acad Sci U S A 100:7666-7671.

Mlinar B, Corradetti R. (2003) Endogenous 5-HT, released by MDMA through serotonin transporter- and secretory vesicle-dependent mechanisms, reduces hippocampal excitatory synaptic transmission by preferential activation of 5-HT1B receptors located on CA1 pyramidal neurons. Eur J Neurosci. 18(6):1559-1571.

Moneypenny CG, Kreshchenko N, Moffett CL, Halton DW, Day TA, Maule AG (2001) Physiological effects of FMRFamide-related peptides and classical transmitters on dispersed muscle fibres of the turbellarian, Procerodes littoralis. Parasitology 122:447-455.

Morgan MJ (1998) Memory deficits associated with recreational use of "ecstasy" (MDMA). Psychopharmacology (Berl) 141:30-36.

National Institute on Drug Abuse (2001) The economic costs of drug abuse in the united states 1992-1998. NIH Publication No. 190636.

Ness DK, Foley GL, Villar D, Hansen LG (1996) Effects of 3-iodo-L-tyrosine, a tyrosine hydroxylase inhibitor, on eye pigmentation and biogenic amines in the planarian, Dugesia dorotocephala. Fundam Appl Toxicol 30:153-161.

Nishimura K, Kitamura Y, Inoue T, Umesono Y, Yoshimoto K, Takeuchi K, Taniguchi T, Agata K (2007a) Identification and distribution of tryptophan hydroxylase (TPH)-positive neurons in the planarian Dugesia japonica. Neurosci Res 59:101-106.

Nishimura K, Kitamura Y, Inoue T, Umesono Y, Sano S, Yoshimoto K, Inden M, Takata K, Taniguchi T, Shimohama S, Agata K (2007b) Reconstruction of dopaminergic neural network and locomotion function in planarian regenerates. Dev Neurobiol 67:1059-1078.

Obrocki J, Buchert R, Väterlein O, Thomasius R, Beyer W, Schiemann T (1999) Ecstasy--long-term effects on the human central nervous system revealed by positron emission tomography. Br J Psychiatry 175:186-188.

Office of National Drug Control Policy (2008) Canadian-made, METH-laced ecstasy being dumped into U.S. illegal drug markets. Press Release January 3.

Orii H, Kato K, Umesono Y, Sakurai T, Agata K, Watanabe K (1999) The planarian HOM/HOX homeobox genes (Plox) expressed along the anteroposterior axis. Dev Biol 210:456-468.

Pagán OR, Rowlands AL, Urban KR (2006) Toxicity and behavioral effects of dimethylsulfoxide in planaria. Neurosci Lett 407:274-278.

Palladini G, Ruggeri S, Stocchi F, De Pandis MF, Venturini G, Margotta V (1996) A pharmacological study of cocaine activity in planaria. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 115:41-45.

Parrott AC (2004) Is ecstasy MDMA? A review of the proportion of ecstasy tablets containing MDMA, their dosage levels, and the changing perceptions of purity. Psychopharmacology 173:234-241.

Parrott AC, Lasky J (1998) Ecstasy (MDMA) effects upon mood and cognition: before, during and after a Saturday night dance. Psychopharmacology (Berl) 139:261-268.

Pereira FC, Lourenço ES, Borges F, Morgadinho T, Ribeiro CF, Macedo TR, Ali SF (2006) Single or multiple injections of methamphetamine increased dopamine turnover but did not decrease tyrosine hydroxylase levels or cleave caspase-3 in caudate-putamen. Synapse 60:185-193.

Raffa RB, Stagliano GW, Tallarida RJ (2006) Subadditive withdrawal from cocaine/kappa-opioid agonist combinations in Planaria. Brain Res 1114:31-35.

Raffa RB, Stagliano GW, Ross G, Powell JA, Phillips AG, Ding Z, Rawls SM (2008) The kappa-opioid receptor antagonist nor-BNI inhibits cocaine and amphetamine, but not cannabinoid (WIN 52212-2), abstinence-induced withdrawal in planarians: An instance of 'pharmacologic congruence'. Brain Res 1193:51-56.

Rakhit R, Crow JP, Lepock JR, Kondejewski LH, Cashman NR, Chakrabartty A (2004) Monomeric Cu,Zn-superoxide dismutase is a common misfolding intermediate in the oxidation models of sporadic and familial amyotrophic lateral sclerosis. J Biol Chem 279:15499-15504.

Rawls SM, Gomez T, Stagliano GW, Raffa RB (2006a) Measurement of glutamate and aspartate in Planaria. J Pharmacol Toxicol Methods 53:291-295.

Rawls SM, Rodriguez T, Baron DA, Raffa RB (2006b) A nitric oxide synthase inhibitor (L-NAME) attenuates abstinence-induced withdrawal from both cocaine and a cannabinoid agonist (WIN 55212-2) in Planaria. Brain Res 1099:82-87.

Reddien PW, Oviedo NJ, Jennings JR, Jenkin JC, Sánchez Alvarado A (2005) SMEDWI-2 is a PIWI-like protein that regulates planarian stem cells. Science 310:1327-1330.

Reuter M, Gustafsson MK, Sheiman IM, Terenina N, Halton DW, Maule AG, Shaw C (1995) The nervous system of Tricladida. II. Neuroanatomy of Dugesia tigrina (Paludicola, Dugesiidae): an immunocytochemical study. Invert Neurosci 1:133-143.

Ricaurte GA, Schuster CR, Seiden LS (1980) Long-term effects of repeated methylamphetamine administration on dopamine and serotonin neurons in the rat brain: a regional study. Brain Res 193:153-163.

Ricaurte GA, Yuan J, McCann UD (2000) (+/-)3,4-Methylenedioxymethamphetamine ('Ecstasy')-induced serotonin neurotoxicity: studies in animals. Neuropsychobiology 42:5-10.

Rieger RM, Tyler S, Smith JPS III, Rieger GE (1991) Platyhelminthes: turbellaria. In: Harrison FW, Bogitsh BJ (eds) Microscopic anatomy of invertebrates, vol. 3. Wiley–Liss, New York, pp 7–140.

Rudnick G, Wall SC (1992) The molecular mechanism of "ecstasy" [3,4-methylenedioxy-methamphetamine (MDMA)]: serotonin transporters are targets for MDMA-induced serotonin release. Proc Natl Acad Sci U S A 89:1817-1821.

Saitoh O, Yuruzume E, Nakata H (1996) Identification of planarian serotonin receptor by ligand binding and PCR studies. Neuroreport 8:173-178.

Saitoh O, Yuruzume E, Watanabe K, Nakata H (1997) Molecular identification of a G protein-coupled receptor family which is expressed in planarians. Gene 195:55-56.

Saló E, Baguñà J (1986) Stimulation of cellular proliferation and differentiation in the intact and regenerating planarian Dugesia(G) tigrina by the neuropeptide substance P. J Exp Zool 237:129-135.

Saló E (2006) The power of regeneration and the stem-cell kingdom: freshwater planarians (Platyhelminthes). Bioessays 28:546-559.

Sánchez Alvarado A (2004) Planarians. Curr Biol 14:R737-738.

Sarnat HB, Netsky MG (2002) When does a ganglion become a brain? Evolutionary origin of the central nervous system. Semin Pediatr Neurol 9:240-253.

Schaeffer DJ (1993) Planarians as a model system for in vivo tumorigenesis studies. Ecotoxicol Environ Saf 25:1-18.

Scheffel U, Steinert C, Kim SE, Ehlers MD, Boja JW, Kuhar MJ. (1996) Effect of dopaminergic drugs on the in vivo binding of [3H]WIN 35,428 to central dopamine transporters. Synapse. 23:61-69.

Schmued LC, Bowyer JF (1997) Methamphetamine exposure can produce neuronal degeneration in mouse hippocampal remnants. Brain Res 759:135-140.

Seiden LS, Ricaurte GA (1987) Neurotoxicity of methamphetamine and related drugs. In: Meltzer HY, Editor, Psychopharmacology—a generation of progress, Raven Press, New York, pp 359–366.

Sekine Y, Ouchi Y, Takei N, Yoshikawa E, Nakamura K, Futatsubashi M, Okada H, Minabe Y, Suzuki K, Iwata Y, Tsuchiya KJ, Tsukada H, Iyo M, Mori N (2006) Brain serotonin transporter density and aggression in abstinent methamphetamine abusers. Arch Gen Psychiatry 63:90-100.

Serafini T, Colamarino SA, Leonardo ED, Wang H, Beddington R, Skarnes WC, Tessier-Lavigne M (1996) Netrin-1 is required for commissural axon guidance in the developing vertebrate nervous system. Cell 87:1001-1014.

Shao L, Young LT, Wang JF (2005) Chronic treatment with mood stabilizers lithium and valproate prevents excitotoxicity by inhibiting oxidative stress in rat cerebral cortical cells. Biol Psychiatry 58:879-884.

Sim T, Simon SL, Domier CP, Richardson K, Rawson RA, Ling W (2002) Cognitive deficits among methamphetamine users with attention deficit hyperactivity disorder symptomatology. J Addict Dis 21:75-89.

Simantov R, Tauber M (1997) The abused drug MDMA (Ecstasy) induces programmed death of human serotonergic cells. FASEB J 11:141-146.

Soldatos CR, Kales A, Kales JD (1979) Management of insomnia. Annu Rev Med 30:301-312

Sompol P, Ittarat W, Tangpong J, Chen Y, Doubinskaia I, Batinic-Haberle I, Abdul HM, Butterfield DA, St Clair DK (2008) A neuronal model of Alzheimer's disease: An insight into the mechanisms of oxidative stress-mediated mitochondrial injury. Neuroscience. Feb 7

Sprague JE, Nichols DE (1995) The monoamine oxidase-B inhibitor L-deprenyl protects against 3,4-methylenedioxymethamphetamine-induced lipid peroxidation and long-term serotonergic deficits. J Pharmacol Exp Ther 273:667-673.

Sprague JE, Everman SL, Nichols DE (1998) An integrated hypothesis for the serotonergic axonal loss induced by 3,4-methylenedioxymethamphetamine. Neurotoxicology 19:427-441Steiner B, Wolf S, Kempermann G (2006) Adult neurogenesis and neurodegenerative disease. Regen Med 1:15-28.

Steiner B, Wolf S, Kempermann G (2006) Adult neurogenesis and neurodegenerative disease. Regen Med 1:15-28.

Stephans SE, Whittingham TS, Douglas AJ, Lust WD, Yamamoto BK (1998) Substrates of energy metabolism attenuate methamphetamine-induced neurotoxicity in striatum. J Neurochem 71:613-621.

Stumm G, Schlegel J, Schäfer T, Würz C, Mennel HD, Krieg JC, Vedder H. (1999) Amphetamines induce apoptosis and regulation of bcl-x splice variants in neocortical neurons. FASEB J. 13:1065-1072.

Substance Abuse and Mental Health Services Administration. (2006a) National Estimates of Drug-Related Emergency Department Visits in 2004 (Office of Applied Studies), DAWN Series D-28, DHHS Publication No. SMA 06-4143.

Substance Abuse and Mental Health Services Administration. (2006b) Results from the 2005 National Survey on Drug Use and Health (Office of Applied Studies), NSDUH Series H-30, DHHS: Publication No. SMA 06-4194.

Sulzer D, Chen TK, Lau YY, Kristensen H, Rayport S, Ewing A (1995) Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. J Neurosci 15:4102-4108.

Takano T, Pulvers JN, Inoue T, Tarui H, Sakamoto H, Agata K, Umesono Y (2007) Regeneration-dependent conditional gene knockdown (Readyknock) in planarian: demonstration of requirement for Djsnap-25 expression in the brain for negative phototactic behavior. Dev Growth Differ 49:383-394.

Tanner-Smith EE (2006) Pharmacological content of tablets sold as "ecstasy": results from an online testing service. Drug Alcohol Depend 83:247-254.

Teuchert-Noodt G, Dawirs RR, Hildebrandt K (2000) Adult treatment with methamphetamine transiently decreases dentate granule cell proliferation in the gerbil hippocampus. J Neural Transm 107:133-143.

Thiriet N, Jayanthi S, McCoy M, Ladenheim B, Lud Cadet J (2001) Methamphetamine increases expression of the apoptotic c-myc and L-myc genes in the mouse brain. Brain Res Mol Brain Res 90:202-204.

Trawicki W, Czubaj A, Moraczewski J (1988) The brain ultrastructure of Dendrocoelum lacteum (O.F. Muller). Fortschr Zool 36:195-200.

Umeda S, Stagliano GW, Borenstein MR, Raffa RB (2005) A reverse-phase HPLC and fluorescence detection method for measurement of 5-hydroxytryptamine (serotonin) in Planaria. J Pharmacol Toxicol Methods 51:73-76.

Umesono Y, Watanabe K, Agata K (1999) Distinct structural domains in the planarian brain defined by the expression of evolutionarily conserved homeobox genes. Dev Genes Evol 209:31-39

Venturini G, Stocchi F, Margotta V, Ruggieri S, Bravi D, Bellantuono P, Palladini G (1989) A pharmacological study of dopaminergic receptors in planaria. Neuropharmacology 28:1377-1382.

Virmani A, Gaetani F, Imam S, Binienda Z, Ali S (2002) The protective role of L-carnitine against neurotoxicity evoked by drug of abuse, methamphetamine, could be related to mitochondrial dysfunction. Ann N Y Acad Sci 965:225-232.

Virmani A, Gaetani F, Imam S, Binienda Z, Ali S (2003) Possible mechanism for the neuroprotective effects of L-carnitine on methamphetamine-evoked neurotoxicity. Ann N Y Acad Sci 993:197-207.

Volkow ND, Chang L, Wang GJ, Fowler JS, Leonido-Yee M, Franceschi D, Sedler MJ, Gatley SJ, Hitzemann R, Ding YS, Logan J, Wong C, Miller EN (2001a) Association of dopamine transporter reduction with psychomotor impairment in methamphetamine abusers. American Journal of Psychiatry 158:377-382.

Volkow ND, Chang L, Wang GJ, Fowler JS, Franceschi D, Sedler M, Gatley SJ, Miller E, Hitzemann R, Ding YS, Logan J (2001b) Loss of dopamine transporters in methamphetamine abusers recovers with protracted abstinence. J Neurosci 21:9414-9418.

Vorhees CV, Inman-Wood SL, Morford LL, Broening HW, Fukumura M, Moran MS (2000) Adult learning deficits after neonatal exposure to D-methamphetamine: selective effects on spatial navigation and memory. J Neurosci 20:4732-4739.

Wagner GC, Ricaurte GA, Seiden LS, Schuster CR, Miller RJ, Westley J (1980) Long-lasting depletions of striatal dopamine and loss of dopamine uptake sites following repeated administration of methamphetamine. Brain Res 181:151-160.

Wagner GC, Carelli RM, Jarvis MF (1986) Ascorbic acid reduces the dopamine depletion induced by methamphetamine and the 1-methyl-4-phenyl pyridinium ion. Neuropharmacology 25:559-561.

Wan FJ, Lin HC, Kang BH, Tseng CJ, Tung CS (1999) D-amphetamine-induced depletion of energy and dopamine in the rat striatum is attenuated by nicotinamide pretreatment. Brain Res Bull 50:167-171.

Wan FJ, Tung CS, Shiah IS, Lin HC (2006) Effects of alpha-phenyl-N-tert-butyl nitrone and N-acetylcysteine on hydroxyl radical formation and dopamine depletion in the rat striatum produced by d-amphetamine. Eur Neuropsychopharmacol 16:147-153.

Warren MW, Zheng W, Kobeissy FH, Cheng Liu M, Hayes RL, Gold MS, Larner SF, Wang KK (2007) Calpain- and caspase-mediated alphaII-spectrin and tau proteolysis in rat

cerebrocortical neuronal cultures after ecstasy or methamphetamine exposure. Int J Neuropsychopharmacol 10:479-489.

Welsh JH, Williams LD (1970) Monoamine-containing neurons in planaria. J Comp Neurol 138:103-115.

Williams MT, Blankenmeyer TL, Schaefer TL, Brown CA, Gudelsky GA, Vorhees CV (2003) Long-term effects of neonatal methamphetamine exposure in rats on spatial learning in the Barnes maze and on cliff avoidance, corticosterone release, and neurotoxicity in adulthood. Brain Res Dev Brain Res 147:163-175.

Wolf ME, Xue CJ (1999) Amphetamine-induced glutamate efflux in the rat ventral tegmental area is prevented by MK-801, SCH 23390, and ibotenic acid lesions of the prefrontal cortex. J Neurochem. 73:1529-1538.

Wolf ME, Xue CJ, Li Y, Wavak D (2000) Amphetamine increases glutamate efflux in the rat ventral tegmental area by a mechanism involving glutamate transporters and reactive oxygen species. J Neurochem. 75:1634-1644.

Xu W, Zhu JP, Angulo JA (2005) Induction of striatal pre- and postsynaptic damage by methamphetamine requires the dopamine receptors. Synapse 58:110-121.

Yuan J, Cord BJ, McCann UD, Callahan BT, Ricaurte GA (2002) Effect of depleting vesicular and cytoplasmic dopamine on methylenedioxymethamphetamine neurotoxicity. J Neurochem. 80:960-969

Zaczek R, Culp S, De Souza EB (1991) Interactions of [3H]amphetamine with rat brain synaptosomes. II. Active transport. J Pharmacol Exp Ther 257:830-835.

Zamora-Veyl FB, Guedes HL, Giovanni-De-Simone S (2002) Aspartic proteinase in Dugesia tigrina (Girard) planaria. Z Naturforsch [C] 57:541-547.

Zhao C, Deng W, Gage FH (2008) Mechanisms and functional implications of adult neurogenesis. Cell 132:645-660.

Zhou JF, Chen P, Zhou YH, Zhang L, Chen HH (2003) 3,4-Methylenedioxymethamphetamine (MDMA) abuse may cause oxidative stress and potential free radical damage. Free Radic Res 37:491-497.

Zhu JP, Xu W, Angulo JA (2006) Distinct mechanisms mediating methamphetamine-induced neuronal apoptosis and dopamine terminal damage share the neuropeptide substance p in the striatum of mice. Ann N Y Acad Sci 1074:135-148.

BIOGRAPHICAL SKETCH

Shannon Lee Janssen was born in St. Petersburg, FL in 1978. She attended the Center for Advanced Technologies program at Lakewood High School, graduating in 1996. She then went on to attend the University of Florida obtaining a B.S. degree in chemistry. She had originally intended to go to veterinary school to pursue her love of horses, however she decided this was not the lifestyle she wanted. She then decided to pursue research in the field of drug abuse and addiction and applied to the University of Florida Interdisciplinary Program in Biomedical Sciences (IDP). She started working with Mark Gold in 1997 and has focused on the neurotoxicity associated with abuse of both legal and illicit drug.