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3,4-methylenedioxymethamphetamine (MDMA): pharmacology, toxicology, usage patterns, and neurological effects in humans

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Thesis

3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA):
PHARMACOLOGY, TOXICOLOGY, USAGE PATTERNS, AND
NEUROLOGICAL EFFECTS IN HUMANS

by

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3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA):
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NEUROLOGICAL EFFECTS IN HUMANS
ALEXANDER IAN HELFAND

ABSTRACT

3,4-Methylenedioxymethamphetamine (MDMA) is a ring-substituted amphetamine with a potential for abuse. Although originally developed by Merck, MDMA is an illegal drug that is popular recreationally, and is more recently being touted as a therapeutic agent. Unlike some other drugs in the amphetamine class, the mechanism(s) by which MDMA produces its subjective effects are not well understood.

MDMA is a selective serotonin (5-HT) neurotoxin. Exposure to MDMA can lead to lasting reductions in brain 5-HT and 5-HT axonal markers. Somewhat paradoxically, its acute pharmacological effects involve a dramatic acute increase in serotonin (and other monoamine) levels in the brain and the periphery. MDMA is also a direct agonist at several different monoaminergic receptors. Although these pharmacological properties of MDMA are known, they don’t appear to fully explain the subjective effects of MDMA, which include feelings of well-being and euphoria. One unfortunate notion held by many MDMA users is that the drug is safe, or at least safer than many other illegal drugs. This is a notion that is strengthened by MDMA’s current and past use as a psychotherapeutic agent, although definitive safety/efficacy reports have yet to appear in the literature. In recent years, there has been a renewed push to acknowledge the potential utility of MDMA in the treatment of conditions such as post-traumatic stress disorder.
MDMA has been reported to damage a number of organ systems in addition to its properties as a selective 5-HT neurotoxin in the brain. Furthermore, recreational MDMA users develop tolerance, which results in a need to increase the dose to achieve the same subjective effects, thereby also increasing the risk for dose-related adverse effects. A number of research laboratories have demonstrated that abstinent MDMA users develop both a loss of brain 5-HT markers, in addition to potential functional consequences of 5-HT neurotoxicity, including deficits in cognitive function, endocrine modulation, and sleep regulation. Although these effects have been well-described, the mechanism by which MDMA leads to neurotoxicity remains unclear, and multiple theories have been suggested.

There are many unanswered questions when it comes to MDMA. Without knowing more about how MDMA acts in the body and how it produces toxicities, use of the drug constitutes a significant risk. Not only are the acute, systemic and potentially fatal effects of MDMA problematic, but longer term functional consequences secondary to serotonin depletion may pose significant problems for abstinent MDMA users as they age. In light of the drug’s popularity, the need for answers and increased public awareness has never been more pressing.

Although MDMA is classified by the Drug Enforcement Agency (DEA) as schedule I, popular musicians have begun to positively reference MDMA in their lyrics, which has likely contributed to the observed rise in MDMA-related hospital visits and fatalities. Communities, parents, and healthcare professionals must make a more concerted effort to raise public awareness of the potential dangers of MDMA use.
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<tr>
<td>5-HIAA</td>
<td>5-hydroxyindoleacetic acid</td>
</tr>
<tr>
<td>5-HT</td>
<td>Serotonin</td>
</tr>
<tr>
<td>5-NAC-HHMA</td>
<td>5-(N-acetylcystein-S-yl)-3,4-dihydroxymethamphetamine</td>
</tr>
<tr>
<td>ACTH</td>
<td>Adrenocorticotropic hormone</td>
</tr>
<tr>
<td>AMPT</td>
<td>α-methyl-(p)-tyrosine</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>COMT</td>
<td>Catechol-O-methyl transferase</td>
</tr>
<tr>
<td>CP</td>
<td>Plasma concentration</td>
</tr>
<tr>
<td>DA</td>
<td>Dopamine</td>
</tr>
<tr>
<td>DAWN</td>
<td>Drug Abuse and Warning Network</td>
</tr>
<tr>
<td>DAT</td>
<td>Dopamine transporter</td>
</tr>
<tr>
<td>DEA</td>
<td>Drug Enforcement Agency</td>
</tr>
<tr>
<td>DVR</td>
<td>Distribution volume ratio</td>
</tr>
<tr>
<td>GABA</td>
<td>(\gamma)-Aminobutyric acid</td>
</tr>
<tr>
<td>GPCR</td>
<td>G protein-coupled receptor</td>
</tr>
<tr>
<td>HHMA</td>
<td>3,4-dihydroxymethamphetamine</td>
</tr>
<tr>
<td>HMA</td>
<td>4-hydroxy-3-methoxyamphetamine</td>
</tr>
<tr>
<td>HMMA</td>
<td>4-hydroxy-3-methoxymethamphetamine</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
</tr>
<tr>
<td>---------</td>
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</tr>
<tr>
<td>HPA</td>
<td>Hypothalamic-Pituitary-Adrenal Axis</td>
</tr>
<tr>
<td>HPC</td>
<td>Hippocampus</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Median lethal dose</td>
</tr>
<tr>
<td>MAO</td>
<td>Monoamine oxidase</td>
</tr>
<tr>
<td>MAOI</td>
<td>Monoamine oxidase inhibitor</td>
</tr>
<tr>
<td>MAPS</td>
<td>Multidisciplinary Association for Psychedelic Studies</td>
</tr>
<tr>
<td>m-CPP</td>
<td>Meta-chlorophenylpiperazine</td>
</tr>
<tr>
<td>MDA</td>
<td>3,4-methylenedioxyamphetamine</td>
</tr>
<tr>
<td>MDEA</td>
<td>3,4-methylenedioxy-N-ethylamphetamine</td>
</tr>
<tr>
<td>MDMA</td>
<td>3,4-methylenedioxyamphetamine</td>
</tr>
<tr>
<td>NACC</td>
<td>Nucleus accumbens</td>
</tr>
<tr>
<td>NE</td>
<td>Norepinephrine</td>
</tr>
<tr>
<td>NET</td>
<td>Norepinephrine transporter</td>
</tr>
<tr>
<td>NIDA</td>
<td>National Institute on Drug Abuse</td>
</tr>
<tr>
<td>NREM</td>
<td>Nonrapid eye movement</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PFC</td>
<td>Prefrontal cortex</td>
</tr>
<tr>
<td>PVH</td>
<td>Paraventricular hypothalamus</td>
</tr>
<tr>
<td>REM</td>
<td>Rapid eye movement</td>
</tr>
<tr>
<td>SERT</td>
<td>Serotonin transporter</td>
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SPECT  ........................................Single photon emission computed tomography
VMAT  ............................................................Vesicular monoamine transporter
I. Introduction: MDMA Today

3,4-Methylenedioxymethamphetamine

3,4-Methylenedioxymethamphetamine, or MDMA, is a ring-substituted amphetamine with a potential for abuse. MDMA has been in existence since at least 1914.\(^1\) MDMA-related hospitalizations have been rising over the past 15 years, as the drug increased in popularity\(^2\). MDMA has been described as an entactogen\(^3\), meaning that it heightens feelings of empathy, trust, and belonging. Recreational MDMA users have historically been individuals who frequent “raves” and nightclubs.\(^4\) This context is especially problematic for MDMA use, because the drug produces a rise in body temperature, and drug consumption in the warm setting of an active nightclub or similar situation may lead to more severe, sometimes life-threatening hyperthermia. Animal studies show that the effects of MDMA are compounded by the temperature of the ambient environment.\(^5\) MDMA is a selective serotonin (5-HT) neurotoxin, while other amphetamines may act as a dopamine (DA) neurotoxin or as both a DA and 5-HT neurotoxin.\(^6\) For reasons that are unclear, MDMA is a selective 5-HT neurotoxin in all animal species tested to date (as well as humans) with one exception; in mice, MDMA is a selective DA neurotoxin.\(^7\) MDMA-mediated 5-HT neurotoxicity is defined by long-lasting depletion of brain indole markers after the drug has left the body, including 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), and serotonin reuptake transporters (SERTs). MDMA is neurotoxic only to neuron terminals and axons, largely sparing the cell body or soma. In addition to its selective 5-HT effects, MDMA exerts acute toxicity on many different organ systems and possesses a diverse set of pharmacological properties,
although the present focus will be mainly on the neural effects and the mechanisms by which MDMA might produce long-lasting neurotoxicity, as well as on relevant pharmacology and drug metabolism. Both the long-term and acute effects of MDMA will be discussed.

**Why Take MDMA?**

As one user explains, “All I wanted to do was smile, I was so wide awake, and I felt in love for everything and everyone.” MDMA has been described as an “entactogen”, enhancing feelings of love, trust, and emotional closeness, in addition to these “entactogen” properties, MDMA is an amphetamine and, as such, has stimulant properties typical of this drug class. Individuals may choose to take a drug for many different reasons, but MDMA has historically been classified a “club drug”, a drug for partying, having a good time, and staying awake. When taking the drug at a concert, the purpose may be to change the subjective perception of the sensory experience, as users report experiencing altered perception in both light and sound. These effects usually peak within two hours of administration. MDMA is often sold as a tablet, which allows users to take the drug discretely. In the setting of a loud concert or nightclub, behavioral effects like euphoria and a heightened energy level do not draw any unwanted attention. In recent years, MDMA use has become especially common in private homes, and a 2009 review of MDMA-related fatalities found that the majority of the MDMA use occurred in private homes.
Variations in Drug Composition

MDMA has many names on the street, including “ecstasy” and, more recently “molly”. It is worth noting that not all “ecstasy” tablets contain pure MDMA. In 1997, ecstasy tablets from Holland were tested and only 34% were found to contain MDMA. However, by 1998, over 75% of Dutch ecstasy contained MDMA. This finding has prompted the World Health Organization to treat the term “ecstasy” as an umbrella term referring to several different compounds. Ecstasy may contain 3,4-methylenedioxy-N-ethylamphetamine, also referred to as MDEA or MDE. MDE has been called “Eve”, indicative of the similarities between the effects produced by MDE and those produced by MDMA, which has also been called “Adam”. Ecstasy may also contain 3,4-methylenedioxyamphetamine, better known as MDA, or the “love drug”. While MDA is a drug of abuse in its own right, it is also a metabolic product of MDMA and also a serotonin neurotoxin. The three compounds mentioned above (MDMA, MDA, and MDEA) are chemically related, and produce similar effects pharmacologically.

Reference will be made to ecstasy with the assumption that the tablets in question contain MDMA, but this is not always the case. Still, relatively pure MDMA is present and available for drug users; anecdotal reports of seizures by police in Liverpool, UK describe the MDMA seized as “pure” and “almost crystalline”.

History of MDMA

Merck first applied for a patent on MDMA in 1912, and the patent application was granted in 1914. The patent was granted in Germany, and suggests that MDMA was only to be an intermediate compound used in the production of other therapeutics.
The drug was relatively unknown until 1953, when the U.S. Army conducted a study exploring MDMA’s toxic potential. The research was declassified in 1969, and published in 1973. Several years later, in 1976, Dr. Leo Zeff, Ph.D., began to use the substance as a supplement to psychiatric therapy. A comprehensive review detailing the use of MDMA in psychotherapy was not published until 1983. At that time, the drug’s popularity in the psychotherapeutic community was on the rise, although initially, results of MDMA-assisted sessions were not made public for fear of attracting the DEA’s and public media’s attention to this new drug. The drug picked up the name “ecstasy” in 1981, and recreational use of MDMA expanded thanks to mass production by organized groups of chemists and shameless promotion as a “fun dance drug” The rapid explosion of MDMA’s recreational use finally alerted law enforcement and the therapeutic communities. As a result, in 1984, the DEA recommended that MDMA be classified Schedule I, a status shared with drugs such as heroin, which would make the drug illegal. Psychotherapists fought back, to the surprise of the DEA, which was unaware of the drug’s purported therapeutic value. Meanwhile, mass production of the drug continued. The DEA responded by scheduling the drug emergently, classifying it Schedule I for a period of one year while hearings took place. For a drug to be classified Schedule I, there must be a high potential for abuse, no accepted medical use, and an inability to make medical use of the drug safe. The courts recommended the substance be classified Schedule III. The DEA rejected this recommendation, and with the exception of a brief (three month) removal of Schedule I status due to an appeal made by a medical doctor, the drug has remained classified Schedule I.
Therapeutic Uses for MDMA

As mentioned above, some maintain that MDMA has a role in psychotherapy, despite the significant body of literature that describes the damage MDMA can potentially inflict on the brain and bodily organs. Such arguments often claim that the negative outcomes associated with MDMA have been overstated. MDMA’s therapeutic potential has been proposed on account of its ability to promote “relaxation”, “facilitate a loosening of the ego”, and “encourage an increased thoughtfulness and contemplativeness”.24 Together, these qualities are said to promote enhanced insight and an increased ability to explore “painful repressed memories”.25,26 Proponents have cited the use of MDMA in couples therapy, where it was used in place of LSD.27 Others suggest that it might be useful in treating chronic pain, another utility with previously documented success.25 In this case, a patient suffering from multiple myeloma received 4 MDMA treatments over the course of 9 months. The patient reported complete alleviation of his pain during the treatments. Although the pain returned during the interim between sessions, the patient claimed that he was able to manage the pain by recalling his experiences with MDMA. The treatments ceased once MDMA was made illegal. The pain eventually returned, and the patient died of his illness.25 Still others have recently demonstrated the success with which MDMA can be used to treat post-traumatic stress disorder (PTSD).28 Currently, the Multidisciplinary Association for Psychedelic Studies (MAPS) is funding clinical trials to investigate the efficacy of “MDMA-assisted psychotherapy” in the treatment of PTSD, with the goal of an FDA-approved form of MDMA on the market by the year 2021.29
Many who advocate for the therapeutic effects of MDMA refer to a study by Halpern et al. in which the authors were unable to find any lasting residual cognitive effects of MDMA use. Halpern et al. suggest that MDMA use alone might “not generally produce lasting residual neurotoxicity.” Halpern et al.’s results and conclusion have prompted several responses, including reevaluations of the data. Responding to the author’s conclusion that MDMA use might not cause toxicity, A.C. Parrott offers “a rather different interpretation of their findings”, and suggests that Halpern et al. have only demonstrated the dramatic variability of the effects produced by MDMA. Currently, groups like MAPS are spending large amounts of money on clinical trials in order to test the efficacy of MDMA as an adjunct to psychotherapy. Such reports are likely to contribute to the mistaken notion that MDMA is “safe”. As previously noted, the repeated use of MDMA may be problematic, because tolerance to its subjective effects develops quickly, requiring higher dosages to achieve the same subjective response. If such tolerance occurred, the potential therapeutic benefit of MDMA may become limited. Tolerance to MDMA will be discussed subsequently. In addition to the problem of tolerance, 25% of users report the occurrence of at least one adverse reaction to the drug. In some individuals, especially those with a genetic predisposition, MDMA can induce a panic attack, and, in some individuals, such an attack can bring about the development of panic disorder. When MDMA-induced panic attacks do occur, they are generally within the first hour after administration, and they occur more frequently in women.
Public Health Concerns

MDMA is widely used, and population surveys indicate that it is among the most widely used illegal substances in the world. Popular musicians such as Jay-Z, Madonna, and Miley Cyrus, promote “molly”, slang for molecular MDMA, in their lyrics and performances. The inclusion of the drug in popular culture can only add to the drug’s appeal, especially to younger audiences, and may contribute to the notion widely held by many users of MDMA that the drug is “safe”. Between 1997 and 1999, the prevalence of MDMA use among nearly all types of college students rose from 2.8% to 4.7%, an increase of 69%. Recent data shows that MDMA use may be rising. Figures published by The Drug Abuse and Warning Network (DAWN) estimated that there were 10,227 emergency department visits related to MDMA in 2004. In 2008, this number increased to 17,888. In 2011, they estimated over 22,498 emergency department visits related to MDMA. Unfortunately, these figures are likely underestimates of what is actually occurring; not every health care provider reports to DAWN, and not every drug-related emergency department visit is accurately reported, as drug users may be reluctant to share information concerning drug use with health care providers. In addition to its popularity in the United States, MDMA is also widely abused in Europe. As recently as 2013, a raid coordinated by European police in three countries seized 60 million ecstasy tablets estimated to be worth €1.3 billion. The public health problem associated with MDMA may be worsened by reports of healthcare professionals who advocate for the use of MDMA in the treatment of various psychological disorders. One website, “theDEA.org”, which the webmaster states is an acronym for “Drug Enjoying
Americans”, 39 states prominently at the top of its homepage that “MAPS has officially gotten approval from the FDA (US government) to begin testing MDMA (‘ecstasy’) on military veterans with post-traumatic stress disorder (PSTD)!"40 Elsewhere, on the same website’s page covering neurotoxicity, the webmaster states that “science has proven (at least in my opinion) that moderate MDMA use does not cause any lasting harm...”41

Addiction and Behaviors Associated with MDMA

The National Institute on Drug Abuse (NIDA) classifies MDMA as a “club drug”, commonly used by individuals who frequent nightclubs and raves.9 Within that group, ecstasy use can be commonplace. A study in 2001 suggested that as many as 96% of club-goers have used the drug4,42, indicating that the present increase in MDMA use may be part of a long lasting pattern. MDMA users now include a wide range of groups and ethnicities.9

While the drug has an addictive potential, physical dependence is unlike that observed with other types of drugs, such as opioids.43 Bruno et al. suggest that the “underlying structure of dependence symptoms differs for ecstasy compared to other drug classes”44. MDMA is not a drug that engenders a state of physical dependence such that regular administration of the drug is required in order for the user to feel “normal”, as may be observed in chronic heroin users.45 However, MDMA users have self-reported withdrawal symptoms and tolerance, both of which are hallmarks of physical dependence.43 Degenhardt suggests “the biological basis for a dependence syndrome similar to other drugs is present, but that other issues, for example, behavioral reinforcement or learning, may additionally play a role for some.”43 In humans, one
pattern of MDMA usage is similar to that observed with hallucinogens such as LSD; drug users commonly undergo a “two-factor structure” consisting of compulsive use followed by escalating use. Frequent users experience profound tolerance, and self-administer increasingly large quantities of MDMA as the subjectively experienced “positive” effects begin to decline. Although uncommon, there are accounts of intravenous MDMA use; one study by Topp et al. found that 16% of a large sample of Australian recreational MDMA users had injected the drug. Additionally, some individuals may consume the drug by insufflation. Users now report taking the drug in a diverse array of environments: MDMA use is no longer restricted to nightclubs, and its classification as a “club drug” has become misleading, especially in light of reports of individuals dying after taking MDMA in private homes. Regardless of the expansion of the drug into wider audiences, club-goers were fourteen times more likely to have used the drug, and about 90% of club-going individuals in the UK have reported use.
II. Chemical Properties

Chemical Structure and Properties

Figure 1: Chemical Structure of MDMA

As shown in Figure 1, MDMA is a ring-substituted amphetamine, and a chiral compound. Its enantiomers may exhibit slight differences in their properties. Current research indicates differences in the metabolism and activities of the enantiomers\textsuperscript{49}, although the functional effect of this finding is not clear. MDMA is highly lipophilic, and fairly small in size (193.24 amu\textsuperscript{49}) which allows the drug to readily cross the blood-brain barrier. It is these same properties that may allow MDMA to diffuse across a cell membrane. Although usually administered as a racemic mixture\textsuperscript{50}, both enantiomers are potent in generating the desired subjective effects. The \textit{S}(+) enantiomer is more potent,\textsuperscript{51} and more easily induces “euphoria, energy and a desire to socialize.”\textsuperscript{50} The \textit{S}(+) configuration is also eliminated more rapidly.\textsuperscript{51}
Similarities to Other Compounds

Figure 2: Chemical Structures of MDMA, Mescaline, and Related Compounds.

MDMA is structurally related to amphetamines and the hallucinogen mescaline. In fact, the only chemical difference between MDMA and methamphetamine is the addition of a substituted ring on the other side of the molecule. In addition to its effects on the brain, MDMA affects several other organ systems, and those effects may be similar to those produced by related compounds. For example, in rats, MDMA produces cardiovascular effects similar to those elicited by d-amphetamine.

III. Pharmacodynamics

Neurochemistry Overview

MDMA, like many amphetamines, is a strong central nervous system (CNS) stimulant. MDMA is a so-called indirect agent because it exerts its effects primarily through release of 5-HT and other monoamines rather than by direct actions on
monoaminergic receptors. MDMA exhibits a high affinity on presynaptic serotonin transporters (SERTs) located in the nerve terminals. One hypothesis posits that once MDMA reaches the brain, it is co-transported with Na\(^+\) into the terminal via SERT, competitively inhibiting 5-HT uptake.\(^{54}\) Once inside the cell, MDMA is carried into the storage vesicles by the vesicular monoamine transporter (VMAT). Concentrations of 5-HT may increase in the vesicle, and 5-HT will be pumped out into the cytosol by the VMAT. As a result, cytosolic 5-HT and Na\(^+\) levels rise. Cellular 5-HT is further increased by MDMA-mediated inhibition of the monoamine oxidase (MAO), which normally metabolizes 5-HT. 5-HT binds to the now inward facing SERT and, together with Na\(^+\), is transported out of the terminal into the synaptic cleft where it activates postsynaptic receptors.\(^{54}\) Transporting MDMA has effectively ‘reversed’ the flow of SERT.

**Normal 5-HT Activity**

A brief overview of normal serotonergic transmission is necessary to better understand why the effects of MDMA on the 5-HT systems are so damaging. Ordinarily, 5-HT is stored within the neuron in vesicles, having been packaged by the vesicular monoamine transporter (VMAT)\(^{55(p141)}\). This keeps concentrations of free 5-HT within the neuron at acceptably low levels. In a serotonergic neuron, many different signals can cause a 5-HT containing vesicle to merge with the cellular membrane, allowing vesicular release of the 5-HT into the synaptic cleft, where an effect is produced due to the binding of 5-HT on the post-synaptic (and pre-synaptic) receptors. There are many different types of 5-HT receptors, and at least three different types of 5-HT\(_2\) receptors\(^{56(chap13)}\), but all
researched 5-HT2 receptors are classified as G protein coupled receptors (GPCRs). This means that after 5-HT (or a 5-HT agonist) binds to the receptor, pharmacologic effects are produced by way of second messengers; a cascade of such second messengers amplifies the signal produced. When 5-HT interacts with a receptor, a variety of effects may be produced, depending on the type of receptor and its location and function. Activation of many types of 5-HT receptors may lead to neuronal activation or “depolarization”, but 5-HT1 receptors do not perform this function. 5-HT1 receptors may be called “autoreceptors” because they are located on the presynaptic neuron, the neuron which is releasing the 5-HT. 5-HT1 receptors are also GPCRs. In general, the function of an autoreceptor is to modulate the amount of neurotransmitter released by the neuron. The family of 5-HT1 receptors is the only known 5-HT receptor family which modulates the release of 5-HT. From the synaptic cleft, 5-HT can be taken back up into the axon where it is metabolized by monoamine oxidase (MAO).

**Cellular Consequences of MDMA Activity**

MDMA binds to all three monoaminergic transporters (i.e., serotonergic, dopaminergic and noradrenergic) and is an indirect monoamine agonist. Although MDMA binds to all three monoaminergic transporter, it largely targets the 5-HT system, and up to 80% of presynaptic 5-HT may be dumped into the synaptic cleft upon administration. In addition to the previously described effect on the SERT, elevated synaptic levels of 5-HT are caused by increased vesicular release, reduced pre-synaptic reuptake, and reduced MAO activity. In addition, MDMA exhibits comparably high affinities for several receptors including the 5-HT2 receptor, as well as the α2 adrenergic,
H₁ histamine, and M₁ muscarinic receptors. MDMA exhibits somewhat lower affinity binding at the 5-HT₁, α₁ adrenergic, M₂ muscarinic, and β adrenergic receptor. MDMA additionally exhibits similarly low binding affinities for the D₁ and D₂ DA receptors. Although early experimentation initially suggested that MDMA exhibited little selectivity for the 5-HT₁ receptor compared to the 5-HT₂ receptor, subsequent research has demonstrated otherwise. Additionally, MDMA has been demonstrated to act on VMAT. VMAT is a key player in the storage of neurotransmitter within discrete vesicles, and MDMA’s previously described activity at VMAT may lead to elevated cytosolic levels of 5-HT. Free cytosolic 5-HT may increase the oxidative burden on the neuron, and would also contribute to elevated extracellular 5-HT due to MDMA’s activity on SERT. In addition, MDMA has also been shown to cause release of NE due to inhibition and/or reversal of the NE transporter (NET). MDMA has also been demonstrated to inhibit and reverse the direction of the DA transporter (DAT), increasing extracellular DA levels. The affinity of MDMA for DAT is lower than the affinity MDMA possesses for SERT, such that “MDMA releases 5-HT from striatal slices at concentrations that are ~10-fold lower than concentrations required for stimulating DA release.”

With an understanding of where MDMA is active, it is possible to conceptualize what MDMA is doing to 5-HT and other neurotransmitter systems. Once in the brain, MDMA may enter a serotonergic neuron in at least two different ways. It can either diffuse across the cell membrane, or it can bind to an outward facing SERT. Once inside the neuron, MDMA can cause release of 5-HT as described previously. Binding of
synaptic 5-HT to post-synaptic 5-HT$_2$ receptors causes the post-synaptic neurons to depolarize, pushing them closer to activation. The net effect of MDMA is to increase 5-HT neurotransmission, with huge amounts of 5-HT free in the synapse available to depolarize post-synaptic neurons. Neurons may become depleted of 5-HT, and this problem is compounded by an inability to make more; MDMA inhibits the rate-limiting enzyme in the biosynthesis of 5-HT, tryptophan-5-hydroxylase. This effect occurs very quickly, and such inhibition can last for days.$^{10,55(p141)}$

**Acute and Subacute Pharmacodynamic Effects of MDMA Administration**

As reviewed in the previous section, the acute pharmacological effects of MDMA include increases in 5-HT, DA, and norepinephrine (NE) neurotransmission in the brain and the periphery, in addition to direct actions at a variety of 5-HT and non-5-HT receptor types. Its effects at 5-HT neurons are the most prominent,$^{52,63,64}$ but 5-HT release may not alone produce the subjective effects of the drug, as other drugs causing 5-HT release, such as the diet drug fenfluramine$^{65}$, do not produce this subjective response. In addition to actions at neuronal targets, MDMA leads to the release of the hormones oxytocin and vasopressin, which have also been hypothesized to contribute to MDMA’s subjective effects of love, trust, arousal, and belonging.$^{9,66,67}$ Elevated heart rate, blood pressure, and energy level occur following acute administration, and these effects are likely due to the action MDMA-induced increases in NE.$^{11}$ While MDMA can produce a wide array of symptoms, one particularly dangerous feature is the tremendous variability found in different individuals’ reactions to the drug. Importantly, the effects produced as well as an individual’s ability to tolerate them are extremely variable; a dose of the same
size can do very different things to different people. Not every MDMA experience is positive; in addition to adverse reactions, recent studies have shown that MDMA may amplify negative emotions as well. Considering undesired effects, the surge of 5-HT can cause adverse physical effects if the increase in 5-HT reaches especially high levels, a state known as “serotonin syndrome”. In more mild states of serotonin syndrome, hyperthermia, hyperkinesia, and confusion are observed in the user. In more serious cases, seizures, and loss of consciousness may occur. In the most severe cases, rhabdomyolysis, disseminated intravascular coagulation, multi-organ failure, and death may occur. High temperature environments exacerbate the situation and increase the likelihood of unfavorable outcomes. Serotonin syndrome will be further discussed subsequently. While MDMA often causes hyperthermia, experimentation with rats has demonstrated that administration in a cool environment can lead to large drops in body temperature; indeed, the homeostatic control of body temperature is lost as a result of MDMA administration. In humans, a similar effect has also been observed. In 2009, Greene et al. described 332 cases of patients receiving treatment at a London hospital for reactions to MDMA. While hyperthermia was common, other individuals presented with low body temperatures or even hypothermia.

In addition to its acute and subacute subjective and physiological effects, MDMA may weaken the immune system. MDMA has been shown to suppress the innate and adaptive branches of the immune system. MDMA may impair a neutrophil’s ability to phagocytose, and also interferes with the production of a number of macrophage-derived pro-inflammatory cytokines. These cytokines include Tumor Necrosis Factor α,
Interleukin (IL) 1-β, IL-15, and IL-12, to name a few. Subacutely, in the 24 hours following administration of neurotoxic dosages of MDMA, the brain is depleted of 5-HT, and there is also a marked depletion of 5-hydroxyindoleacetic acid (5-HIAA), which is the major metabolite of 5-HT. Cerebrospinal fluid (CSF) levels of 5-HIAA can be used to indirectly assess serotonergic injury (including that caused by MDMA) in both humans and non-human primates. With respect to rats, about 24 hours following this period of depletion, 5-HIAA levels return to “normal”. This return is short-lived; within 3 days, levels of 5-HT and 5-HIAA drop once more; CSF levels of 5-HT and 5-HIAA remain depressed for at least 12 months. The subacute depletion of 5-HT and 5-HIAA may cause the user to feel fatigued, depressed, or anxious. These feelings are especially pronounced in the days that immediately follow drug administration, a phenomenon which has been nicknamed the ‘Tuesday blues’ or the ‘midweek blues’. Some research has suggested an increase in irritability and anger which is worst about four days after taking a neurotoxic dose of MDMA. In addition to the effects described, MDMA also increases levels of the hormone cortisol; some studies have found reported increases in cortisol levels during and immediately after MDMA use. Elevated cortisol levels and associated dysregulation of the hypothalamic-pituitary-adrenal axis (HPA axis) have been conclusively linked to major depression, but it is unclear what role cortisol plays with respect to the ‘midweek blues’. Long-term alterations in cortisol or HPA axis functioning may result in cognitive deficits.

In a review of 12 studies describing human reactions to MDMA administration, Dumont and Verkes note that 11 of the studies found a significant increase in cortisol
levels following MDMA administration. In another separate report, Harris et al. found a 100% increase in cortisol levels following a dose of 1.0 mg/kg, and a 150% increase following a dose of 1.5 mg/kg. One study found that cortisol levels may be elevated by as much as 800% while under the effects of MDMA. This finding has been contested, as the reported 800% increase is somewhat incredible. In rebuttal, Wolff and Aitchison have pointed out the conclusion is somewhat misleading, as the cortisol levels were measured differently (total cortisol quantity as compared to salivary cortisol concentration). Furthermore, Wolff and Aitchison measured cortisol levels immediately after having taken MDMA, while the 800% increase was observed in a dance club. The authors do not dispute the finding that cortisol levels are elevated, but instead clear up the apparent incongruency. In another study using healthy MDMA-naïve volunteers, a 125mg dose of MDMA was found to significantly increase plasma prolactin levels. The acute effects of MDMA include a complex pattern of endocrine changes.

**The Serotonin Syndrome**

Symptoms of serotonin syndrome include “restlessness, confusion, shivering, tachycardia, diarrhea, muscle twitches/rigidity, fever, seizures, loss of consciousness, and death.” Serotonin syndrome is caused by elevated 5-HT levels, and produces symptoms according to the extent of 5-HT toxicity. Mild instances of serotonin syndrome generally do not require treatment. One especially dangerous component is the potentially rapid progression of serotonin syndrome, such that a mild case can become quite serious in less than one hour. While a mild case can be treated with rest and a cool environment, a more serious case may require active cooling of the body, physical
restraint, and administration of drugs with 5-HT antagonistic effects in order to prevent death. Occasionally, the symptoms (but not the cause) of serotonin syndrome may be treated with a benzodiazepine. The cause of serotonin syndrome is not just limited to MDMA, but may also occur as a reaction to a combination of drugs administered with therapeutic intent, such as drugs of the MAOI and SSRI class. Most MDMA users exhibit some degree of serotonin syndrome, and individuals may actually use the presentation of serotonin syndrome as a benchmark for establishing the quality and MDMA content of the drug which has been taken.

**Fatal Cases**

Despite the potential confound created by polysubstance use in MDMA users, there should be no doubt about the role MDMA plays in the development of potentially lethal scenarios. Considering data from UK coroners between 1997 and 2007, Schifano et al. found that fatalities subsequent to MDMA use were significantly more frequent than fatalities subsequent to methamphetamine use. That being said, MDMA, while clearly dangerous and potentially lethal, may be less dangerous than other drugs of abuse. Emergency department and mortality data suggests that the occurrence of life-threatening complications resulting from MDMA ingestion may be less common than those associated with other drugs of abuse, such as methamphetamine or opioids. As previously discussed, the incidence of complications and fatalities related to MDMA use is rising; while MDMA-related complications are currently uncommon, they are increasing in frequency. This trend demands future research leading to a better understanding of MDMA and how it causes damage. MDMA-induced fatalities vary in
their proximate cause of death. Though MDMA causes hyperthermia, Smith et al. describe MDMA-induced fatalities resulting from acute liver failure; MDMA is known to cause cultured rat liver cells to undergo apoptosis. In other fatal cases involving MDMA usage, the causes of death have included seizures, rhabdomyolysis, cardiac arrest, multiple types of organ failure (including renal failure and liver failure), and even disseminated intravascular coagulopathy. Many of these pathologies can be caused by hyperthermia itself. Due to the many different organ systems affected by MDMA, MDMA-related fatalities are not a homogenous group of cases, and many involve preexisting conditions or other illegal substances. Kaye et al. considered 82 MDMA related fatalities in Australia which spanned 5 years. They observed that 59% of the fatalities were attributed to a mixture of other drugs in addition to MDMA. We have primarily focused on the pharmacology and neurological activities of MDMA, but cerebrovascular pathologies were found in only 12% of cases considered by Kaye et al. Instead, cardiovascular pathologies were found in 58% of the fatalities examined. 5-HT performs a wide variety of functions both within the brain and the periphery, and perhaps the most salient of these functions relate to blood pressure. In the brain, 5-HT activation causes constriction of cranial blood vessels. In smooth muscle, such as is found surrounding most blood vessels, 5-HT activation causes vasoconstriction, raising blood pressure. Vasoconstriction of capillaries prevents the efficient loss of heat through radiation, potentially exacerbating a state of hyperthermia. When vasoconstriction is coupled with tachycardia, resulting from MDMA or anything else, the result is typically an acute increase in blood pressure. When the individual finds himself dancing in a hot
and crowded environment, the effect can only be to further increase blood pressure, and so increase the risk for life-threatening complications. Indeed, hypertension is just one of the acute effects of MDMA ingestion. It is therefore not surprising that cardiovascular pathologies were found by Kaye et al. in the majority of the cases they considered. Although uncommon, there are cases of MDMA-induced aortic dissection\textsuperscript{91} as well as MDMA-induced myocardial infarction.\textsuperscript{92,93} The role of unknown pre-existing conditions cannot be understated: aneurisms and arteriovenous malformations significantly increase the likelihood for vascular complications of MDMA use. While we have discussed several ways that MDMA seems to be more dangerous in women, Kaye et al. found that 83\% of the deceased were male.\textsuperscript{14} Whether this is due to behavioral differences (e.g., males may take higher dosages or behave differently when under the influence of MDMA) or to biological differences between males and females remains unclear. The incidence of various pathologies and causes of death bears further consideration. Of the 82 cases considered, MDMA was the proximate or antecedent cause of death in 67 cases. Of these 67, drug-induced toxicity was observed in 91\% of cases.
Table 1\textsuperscript{a}: Incidence of Various Findings in 67 MDMA-Related Deaths\textsuperscript{14}

<table>
<thead>
<tr>
<th>Cause of Death</th>
<th>Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular</td>
<td>10%</td>
</tr>
<tr>
<td>Cerebrovascular</td>
<td>7%</td>
</tr>
<tr>
<td>Aspiration</td>
<td>4%</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>3%</td>
</tr>
<tr>
<td>Hyperthermia</td>
<td>1%</td>
</tr>
</tbody>
</table>

Of the cardiovascular complications, atherosclerosis was the most common pathology observed, having been identified in 6 cases.\textsuperscript{14} Atherosclerosis reduces the diameter of affected vasculature, contributing to the previously described hypertension. Kaye \textit{et al.} reviewed other cases from a variety of sources, including autopsy findings. The authors found atherosclerosis to be a common pathology in many of the fatalities reviewed. Of the cases previously described in Table 1, it is interesting that hyperthermia was the proximate cause of death in only 1\% of the 67 cases, although it is possible that hyperthermia acted as an antecedent cause, initiating or worsening other life-threatening conditions. In two cases considered by De Letter \textit{et al.}, the proximate cause of death was found to be hyperthermia\textsuperscript{68} wherein the body loses the ability to regulate temperature due to “altered hypothalamic control”\textsuperscript{8}. Many MDMA users are aware of the potential for MDMA to produce hyperthermia and dehydration, and attempt to compensate by drinking large amounts of water, and perhaps by taking breaks from physical exertion. However, drinking large amounts of water poses a new risk; some MDMA users actually consume too much water, which can lead to hyponatraemia, another potentially fatal condition.\textsuperscript{8} Hyponatraemia, like 5-HT syndrome can induce an altered mental state and

\textsuperscript{a} Adapted from Kaye \textit{et al.}, 2009. Used with permission.
confusion, and may also cause cerebral edema.\textsuperscript{14,87,94,95} The study by Kaye \textit{et al.} reported an unexpected finding related to fatalities following MDMA use; their data demonstrated that the majority of the fatalities considered did not occur in nightclubs, but instead occurred in private homes\textsuperscript{14}. This finding demonstrates the danger that MDMA poses even outside of a hot and crowded environment, and demands a reevaluation of the contexts in which MDMA is being used. Furthermore, the large proportion\textsuperscript{b} of deaths directly caused by combined drug toxicity reflects the fact that polydrug use is the norm among MDMA users".\textsuperscript{14}

\textbf{SERT-Blocking Compounds}

Fluoxetine, better known to consumers as Prozac, is a selective serotonin reuptake inhibitor (SSRI), which interacts with MDMA in interesting ways. In animal models, the co-administration of fluoxetine (or any other SSRI) in conjunction with MDMA has been shown to prevent MDMA-induced neurotoxicity.\textsuperscript{96} That being said, fluoxetine is not a universally effective way to prevent the effects of MDMA; hyperthermia still occurs.\textsuperscript{96} The ability of SSRIs to provide neuroprotection can be measured in a variety of ways, including a method called autoradiography. In particular, by radioactively labeling a drug or marker that is known to bind to the serotonin terminal (e.g., \textsuperscript{3}H-paroxetine, which binds to the SERT), it is possible to measure the density of 5-HT terminals in animals previously treated with MDMA.\textsuperscript{4,97,98} For example, in rats, after giving a single dose of MDMA (15 mg/kg), there was a marked reduction of 5-HT and 5-HIAA in the striatum, cortex, and HPC. Seven days later, the rats demonstrated significantly reduced \textsuperscript{3}H-

\textsuperscript{b} 44 of the 67 deaths described in Table 1 were due to polydrug toxicity.
paroxetine density in the cortex.\textsuperscript{96} In a different paradigm, 10 mg/kg of fluoxetine was given 5 minutes before and 55 minutes after the 15 mg/kg MDMA dose. Consequently, indole content was not lost, and the reduction in SERTs was attenuated.\textsuperscript{96} In still another paradigm, fluoxetine (2x 10 mg/kg doses, separated by one hour) was given either 2 or 4 days before administration of MDMA. In these cases, the result was complete neuroprotection.\textsuperscript{96} Fluoxetine had no effect on the amount and concentration of MDMA in the brain,\textsuperscript{96} but other studies suggest that fluoxetine may increase the concentrations of MDMA in the blood.\textsuperscript{60} Fluoxetine may inhibit CYP2D6\textsuperscript{c}, and MDMA $C_{\text{MAX}}$ was found to increase by 30\% in rats pre-treated with fluoxetine.\textsuperscript{60} In humans, pretreatment with fluoxetine was found to cause an attenuation of the physiological and subjective effects of MDMA administration in some studies,\textsuperscript{99} but not in others.\textsuperscript{100}

\textsuperscript{c} CYP is a prefix used for naming cytochromes of the P450 family of cytochromes, enzymes usually found in the liver.
IV. Pharmacokinetics

MDMA Metabolism in Humans

Figure 3: MDMA Metabolic Pathways

MDMA is metabolized through two different phase I pathways which operate in unison but at different rates in different species; $O$-demethylation predominates in humans and in nonhuman primates. The goal of the metabolic process is the conjugation of the substrate with a sulfate or glucuronic acid moiety in order to render the product more prone to renal clearance. Hartman *et al.* note that $N$-demethylation is mediated by CYP1A2 and by CYP2B6, while $O$-demethylation is mediated by CYP2D6.\(^{11}\)

\(^{a}\) Adapted from Mueller *et al.*, 2013. Used with permission.
Considering the stereoselectivity of the cytochrome P450s, Meyer et al. found CYP2B6 to be a very large contributor to \( N \)-demethylation activity.\(^{51}\) It should be noted that multiple P450 cytochromes are capable of performing demethylation and demethylenation reactions, but with varying efficiency. In addition to the reactions shown above, HHMA and HMMA are susceptible to \( N \)-demethylation, generating HHA and 4-hydroxy-3-methoxyamphetamine. It is important to note that the \( O \)-demethylenation reaction, which is mainly catalyzed by CYP2D6 in humans, is subject to inhibition by MDMA. Consequently, the progression of MDMA metabolism in humans is not linear, because plasma concentrations of MDMA as low as 125-200 ng/ml can inhibit CYP2D6, such that a single dose is sufficient to block one of the metabolic pathways for MDMA breakdown.\(^{19}\) In human liver microsomes, such inhibition was found to decrease the production of the metabolite HHMA by as much as 75\%.\(^{60}\) A single recreational dose of MDMA may be enough to inactivate most hepatic CYP2D6; a dose of 1.5 mg/kg is capable of causing rapid inhibition of CYP2D6\(^{60}\). Although recovery of CYP2D6 activity is slow, possibly taking 10 days for complete recovery,\(^{60}\) the recovery half-life for CYP2D6 is about 2 days. As a result, a second dose of MDMA taken within a few days of the first is much more likely to produce toxicity due to elevated concentrations of MDMA in the blood exacerbated by diminished metabolic capabilities.\(^{60}\) Rietjens et al. have suggested that frequent MDMA users may have permanently reduced CYP2D6 activity.\(^{60}\) Another result of CYP2D6 inhibition is an increased amount of MDMA available for \( N \)-demethylation, which results in increased amounts of the metabolite 3,4-Methylenedioxyamphetamine (MDA). MDA’s metabolism is slowed dramatically, as \( O\-
demethylenation is now inhibited, causing an even larger build up of MDA. MDA is biologically active, and acts on many of the same substrates as MDMA, and is also a serotonin neurotoxin. The inhibition of CYP2D6 has consequences for further downstream metabolic products as well; plasma concentrations of HHMA and HMMA remain the same despite larger doses of MDMA. Furthermore, this inhibition causes disproportionately large increases in plasma and brain MDMA concentrations following small increases in dose, an example of the non-linear nature of MDMA metabolism.

Most if not all of the metabolic products of MDMA, as well as the parent drug itself, are eliminated in the urine. A full urinalysis was performed on ten individuals to further elucidate the major methods of MDMA elimination. In this study, subjects received either 1.0 mg/kg or 1.6 mg/kg of oral MDMA. The major metabolite was conjugated HMMA-sulfate, which was present in 98% of the urine analyzed.
Table 2: Proportions of MDMA Metabolites in Human Urine\textsuperscript{101}

<table>
<thead>
<tr>
<th>Compound</th>
<th>Proportion of positive urine samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA</td>
<td>.63</td>
</tr>
<tr>
<td>HHMA</td>
<td>.32</td>
</tr>
<tr>
<td>HHMA 3-glucuronide</td>
<td>.51</td>
</tr>
<tr>
<td>HHMA 4-glucuronide</td>
<td>.40</td>
</tr>
<tr>
<td>HHMA 3-sulfate</td>
<td>.69</td>
</tr>
<tr>
<td>HHMA 4-sulfate</td>
<td>.59</td>
</tr>
<tr>
<td>HMMA</td>
<td>.38</td>
</tr>
<tr>
<td>HMMA glucuronide</td>
<td>.84</td>
</tr>
<tr>
<td>HMMA sulfate</td>
<td>.98</td>
</tr>
<tr>
<td>MDA</td>
<td>.42</td>
</tr>
<tr>
<td>HHA</td>
<td>.03</td>
</tr>
<tr>
<td>HHA glucuronides</td>
<td>.13</td>
</tr>
<tr>
<td>HHA sulfates</td>
<td>.32</td>
</tr>
<tr>
<td>HMA</td>
<td>.23</td>
</tr>
<tr>
<td>HMA glucuronide</td>
<td>.51</td>
</tr>
<tr>
<td>HMA sulfate</td>
<td>.50</td>
</tr>
</tbody>
</table>

Urine was collected for 7 days following MDMA administration, although a negligible amount of the original dose was excreted in the urine after day 5. Overall, the amount of the original MDMA dose recovered in urine ranged from 24% to 52\%\textsuperscript{101}.

**Kinetic Profile: \( C_{\text{MAX}}, T_{\text{MAX}}, T_{1/2}, \text{ and } AUC_\infty \)**

\( T_{1/2} \), also called the half-life, is the time required for the body to remove one half of the administered compound from the body, either by excretion or metabolism. The following half-lives were determined by Kolbrich et al. for MDMA and its metabolites in humans after controlled MDMA administration.
Table 3: MDMA Metabolite $T_{1/2}$

<table>
<thead>
<tr>
<th>Compound</th>
<th>$T_{1/2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMA</td>
<td>7-8 hrs</td>
</tr>
<tr>
<td>MDA</td>
<td>10.5-12.5 hrs</td>
</tr>
<tr>
<td>HMMA</td>
<td>11.5-13.5 hrs</td>
</tr>
<tr>
<td>HMA</td>
<td>Highly variable</td>
</tr>
</tbody>
</table>

It may take as many as five to six half-lives for 95% of administered MDMA to be cleared from the body.\(^{102}\) The time required to reach maximum plasma concentration, or $T_{\text{MAX}}$, is dependent upon a number of circumstances, including the chemical structure and molecular weight of the drug, its pH, the pH of the environment, the lipophilicity of the drug, and other factors. In the case of MDMA, $T_{\text{MAX}}$ was about 2.4 hours, regardless of the dose administered.\(^{57}\) The numerical value of $C_{\text{MAX}}$ varies depending on the size of the dose and the size of the subject, but those who receive a “high dose” achieve a larger $C_{\text{MAX}}$ than those receiving a “low dose”.\(^{57}\) The $\text{AUC}_{\infty}$, or area under the plasma concentration-over-time curve, is related to the bioavailability of the drug. AUC is an especially important variable, because it measures how much of the administered drug is actually available and absorbed by the body, a necessary step for the action of most drugs. In the case of the study performed by Kolbrich et al., the “high dose” was 1.6 times larger than the low dose, however the MDMA $\text{AUC}_{\infty}$ for the “high dose” was 1.9 times larger than that found with the “low dose”. This finding indicates a non-linear relationship between dose and plasma concentration.\(^{57}\) Such a non-linear relationship is most likely caused by the inhibition or saturation of the metabolism of MDMA.

Interestingly, such a non-linear relationship was not found by Hartman et al., although this may be because $\text{AUC}_{0-3h}$ was considered, rather than $\text{AUC}_{\infty}$.\(^{11}\)
**MDMA and Metabolite Detection Windows**

One parameter of interest was the amount of time until MDMA taken orally appeared in the plasma.

Figure 4: Time Until Metabolite Appearance in Blood\(^5\)

![Graph showing time until metabolite appearance in plasma](image)

Following administration, it is apparent that MDMA is quickly being broken down by \(O\)-demethylation prior to \(O\)-methylation, resulting in the formation of HMMA.

Inhibition/saturation of CYP2D6 by MDMA may cause a relative increase in the amount of \(N\)-demethylation occurring due to a greater availability of substrate. This results in increased MDA formation. Also of interest, Kolbrich *et al.* report that the detection windows for MDMA, HMMA, and MDA are much larger than have been previously
documented. HMMA had the largest detection window, and was found in the plasma of all but one research subject 47 hours after MDMA administration. Depending on whether the subjects received the high dose (1.6 mg/kg) or low dose (1.0 mg/kg) group, HMMA was still present in the plasma 71 hours after administration in 67% and 14% of subjects, respectively. Concerning MDMA, the drug was found in every subject’s plasma for at least 23 hours after administration. After 47 hours, 82% of subjects in the “high dose” group were found to have MDMA in their plasma, while 23.5% of subjects in the “low dose” group were still found to have MDMA in their plasma. MDA plasma concentrations resemble MDMA concentrations, and at 47 hours, 24% of those receiving the “low dose” of MDMA still had MDA in their plasma, while 82% of those receiving the “high dose” had MDA in their plasma. While metabolite concentrations vary from subject to subject, HHMA concentrations were found to be especially variable. In sum, it is apparent that MDMA remains in the body for several days after administration, and its metabolites may take even longer to be cleared. Due to the ways in which MDMA is metabolized, it is likely that MDMA elimination is also biphasic. It is worth noting that the inhibition of CYP2D6 affects the metabolism of a single dose of MDMA, but also has longer lasting effects which become apparent on repeat administration. In a study by Farré et al., two doses of MDMA were given to human subjects, with 24 hours in between doses. Compared to the first dose, MDMA $C_{\text{MAX}}$ increased by 30%, and AUC increased by 75%. These increases were larger than can be explained by a buildup of MDMA from the first and second doses, which suggests that autoinhibition of CYP2D6 is to blame for the increase.
Pharmacokinetic Parameters of MDMA Metabolites

Concerning the metabolite HMMA, $C_{\text{MAX}}$ was nearly identical in both the “high dose” and “low dose” conditions.\(^{57}\) HMMA is a product of $O$-demethylation followed by $O$-methylation. Saturation/inhibition of the $O$-demethylation reaction is likely to explain the similar $C_{\text{MAX}}$ found for HMMA observed in both administered dosing conditions.\(^{53}\) Furthermore, HMMA $T_{\text{MAX}}$ was reached significantly more quickly than MDMA $T_{\text{MAX}}$,\(^{57}\) indicating saturation/inhibition of the $O$-demethylation reaction (which creates HMMA) even before complete absorption of MDMA from the alimentary canal took place. Due to inhibited formation of HHMA, the ratio of HMMA:MDMA was higher in the “low dose” condition than in the “high dose” condition.\(^{57}\) Concerning the metabolite MDA, it then follows that the MDA:MDMA ratio would increase over time in both dosing conditions as MDMA is broken down by $N$-demethylation. Kolbrich et al. found that “MDA/MDMA ratios increased linearly and similarly from 0.75 to approximately 23 hours…”\(^{57}\) MDA $C_{\text{MAX}}$ was attained approximately 7.5 hours after MDMA administration, and $T_{\text{MAX}}$ was not found to significantly vary depending on the size of the dose.\(^{57}\) Such a finding is demonstrative of zero order kinetics. Conversely, both MDA $C_{\text{MAX}}$ and AUC were significantly different between the “high dose” condition and the “low dose” condition, with larger $C_{\text{MAX}}$ and AUC occurring in the “high dose” condition.\(^{57}\) This finding is likely due to inhibition of the $O$-demethylation reaction leading to increased MDA generation by $N$-demethylation. As HMA is a downstream product of MDMA metabolism, ($\text{MDMA} \rightarrow \text{MDA} \rightarrow \text{HHA} \rightarrow \text{HMA}$), the
dose dependent differences in MDA $C_{\text{MAX}}$ are reflected in HMA $C_{\text{MAX}}$, such that the “high dose” condition had a larger HMA $C_{\text{MAX}}$.\textsuperscript{57}

**Stereoselective Metabolism**

The stereochemistry of MDMA influences the stereochemistry of its resulting metabolic products. Enzymes involved with MDMA metabolism, notably the cytochrome P450 family, are stereoselective.\textsuperscript{51} Consequently, the $R$ and $S$ configurations are eliminated from the body at different rates, and may possess different activities. Because of these differences, plasma concentrations ratios of MDMA and its metabolites (such as HHMA, HMMA, and MDA) differ over time. The proximal cause of MDMA-induced toxicity remains a topic of debate, and one idea suggests that the metabolic products of MDMA breakdown may be more damaging than MDMA itself, a hypothesis which will be revisited subsequently. A better understanding of MDMA stereochemistry and of the properties of its metabolites may prove relevant. Meyer *et al.* believe that many pharmacokinetic parameters differ between racemates in part because of the stereoselective nature of CYP2C19 and CYP2D6,\textsuperscript{51} among others. Pharmacokinetic parameters such as $C_{\text{MAX}}$ differed between racemates, such that the $R$ configurations of MDMA and HHMA achieved a significantly higher $C_{\text{MAX}}$.\textsuperscript{50} Conversely, the $S$ configuration of HMMA and MDA achieved significantly higher $C_{\text{MAX}}$.\textsuperscript{50} The two MDMA racemates do not show the same dose-concentration curves.\textsuperscript{50} Five days after administering 1.6 mg/kg of racemic MDMA to ten healthy volunteers, Schwaninger *et al.* found significant differences in the quantities and proportions of relevant chiral compounds which were excreted in urine. Of the compounds considered (including the
administered drug as well as metabolic products), a median of 21% of all excreted products were in the \( R \) configuration, while a median of 17% were in the \( S \) configuration.\(^{50}\) In the 2 days following administration of racemic MDMA, the \( S \) configuration was cleared from body plasma more rapidly, a result of the stereoselectivity of metabolic processes.\(^{50}\) The \( R \) configurations of MDMA and HHMA were excreted in significantly higher quantities, while the \( S \) configuration of HMMA was excreted in significantly higher quantities.\(^{50}\) No significant difference was found in the R/S proportion of excreted MDA.\(^{50}\) Interestingly, the R/S proportion of excreted compounds changed over time. Several compounds, including MDA and HMMA, were largely eliminated in \( S \) configuration in the first 24 hours following drug administration, but increasing amounts of \( R \) enantiomers appeared over time.\(^{50}\) The authors note significant variability in the proportions and stereochemistry of excreted metabolites over time between subjects, but the final R/S ratios for excreted metabolites between subjects was somewhat more consistent\(^{50}\), suggesting that individual metabolic differences may impact the rate and progression of metabolism rather than the nature of the final metabolic product. Over time, within subjects, the size of the dose did not have any effect on the disposition of the enantiomers produced.\(^{50}\) In vitro experimentation with human liver enzymes has shown a preference for the formation of \( S \)-HHMA, but these results are contradicted by in vivo results. The reason for this contradiction remains unclear. In vivo, at \( C_{\text{MAX}} \) there was instead more \( R \)-HHMA than \( S \)-HHMA.\(^{50}\) HHMA is an intermediary metabolite, and usually is subject to further metabolism in one of two possible ways, both of which involve phase II metabolic processes\(^{19,50}\). HHMA may be conjugated to produce
HHMA sulfate, or it may be instead subject to $O$-methylation by catechol-$O$-methyl transferase (COMT). Both of these metabolic processes exhibited a preference for $S$ enantiomers in vitro. Such a finding could account for the higher $C_{\text{MAX}}$ found for $R$-HHMA, as the $S$ form may be preferentially consumed by metabolism.

**Factors Affecting Quality and Consistency of Kinetic Findings**

It is important to note that pharmacokinetic parameters differ widely between species, and even between individuals. The study performed by Kolbrich et al. was the first to present data on a diverse cohort (paying attention to differences between sexes and races) on a longer timescale, and the authors examine pharmacokinetics following either a placebo (lactose), a low dose (1.0 mg/kg), or a high dose (1.6 mg/kg). MDMA was formulated as a salt, and the subjects remained seated in a quiet room for the duration of the experiment. $T_{1/2}$, $AUC_{\infty}$, $V_d/F$, and $CL_F$ were calculated using noncompartmental models. Ultimately, 17 subjects completed the study. Data obtained with a “low dose” was compared to data obtained with a ”high dose” within the same subject. Because pharmacokinetic variables can change from person to person, the importance of having a large and diverse sample size cannot be understated.

Table 4: Demographics of Study Participants

<p>| | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Mean Age ± SD</td>
<td>21.5 Yrs ± 2.5</td>
</tr>
<tr>
<td>Age Range</td>
<td>18 – 27 Yrs</td>
</tr>
<tr>
<td>Mean Weight ± SDf</td>
<td>76.7 kg ± 17.8 kg</td>
</tr>
<tr>
<td>Male/Female</td>
<td>10/7</td>
</tr>
</tbody>
</table>

The sample used by Kolbrich et al. contained a reasonable number of white individuals as well as black individuals, but only one Hispanic was included in the study. Additional

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*e* For more information on the methods used, see Kolbrich et al., 2008

*f* There was no significant difference in weight between males and females.
studies will be required in order to elucidate ethnic differences in the metabolism of MDMA, but the data presented here remains a good source for establishing baseline pharmacokinetic parameters of MDMA.

**Racial and Sexual Differences in Metabolism**

Mainly due to variations in the genes encoding the various hepatic enzymes which break down MDMA, there are differences in metabolism between individuals of differing genetic heritage. The gene encoding CYP2D6, a hepatic cytochrome involved in each of the relevant demethylation reactions, may contain as many as 50 different alleles.\(^5^7\) CYP2D6 activity is considered to be highly variable, and individuals may be classified as ultrarapid, extensive, intermediate, or poor metabolizers on the basis of their CYP2D6 activity.\(^6^0\) 1.4% of blacks were classified as poor metabolizers, while 4.5% of blacks were classified as ultrarapid metabolizers. 5-10% of whites may be poor metabolizers, and fewer than 5% of whites were ultrarapid metabolizers.\(^5^7\) COMT activity also shows high variability between groups of differing ethnicities. These differences may offer one explanation as to the outliers found in many pharmacokinetic studies of MDMA metabolism.\(^1^1,5^7\)

Differences in the way MDMA is metabolized by males and females have also been described, and Kolbrich *et al.* expected to find “significant gender differences in MDMA pharmacokinetics…”; MDMA T\(_{1/2}\) was significantly longer in females in the “high dose” condition as compared to males. Kolbrich *et al.* found a greater MDMA C\(_\text{MAX}\) for females, and the same is true for MDA.\(^5^7\) AUC\(_\infty\) was also found to be greater in females than in males for both MDMA and MDA. It is important to note that such
differences were only significant in the “low dose” testing condition, although MDMA AUC$_\infty$ was trending towards significance in the “high dose” condition. This finding suggests that there are differences in the way MDMA is initially metabolized by the two genders, but that these differences may eventually become negligible. Concerning HMMA, C$_{\text{MAX}}$ was significantly lower in females in the “low dose” condition, and AUC$_\infty$ was found to be significantly lower in females in both the “low dose” and “high dose” conditions. This finding may be due to more rapid saturation/inhibition of $O$-demethylenation reactions in females as compared to males, or perhaps alternatively to differences in COMT activity between sexes. One consequence of the gender-based gap in metabolic activity might be a significant difference in MDMA-related adverse reactions.

V. Long-Term Effects and Neurotoxicity

Investigational Difficulties

Investigation of a compound like MDMA has many challenges. It is sometimes difficult to elucidate a clear cause of death in reported cases of MDMA-related fatalities, and there are many considerations that hamper efforts to draw definitive conclusions on the properties of MDMA. Perhaps the most salient is the nature of drug users; they use other drugs, which presents a confound. Describing this problem, McCann et al. suggest that “MDMA users, as a group, used more recreational drugs than control subjects and exposure to other drugs could have played a role in the cognitive or SERT deficits found.” The statistics also support the notion that many MDMA users are polydrug users. Considering individuals seeking treatment for a drug problem in 2010, “ecstasy
was mentioned as the primary drug by 1% or less (almost 1000 clients in total) of reported treatment entrants in all European countries. In humans, experimental data can be difficult to interpret for additional reasons; it can be more difficult to control the amount of time in between drug exposure and experimentation and it is more difficult to normalize the degree of MDMA exposure between subjects. On some occasions, the subjects themselves are not able to definitively quantify how much MDMA they have taken, and people do not always tell the truth. In some cases, using a human subject is simply not possible or ethical. Making conclusive investigation more difficult, as described above, there can be differences between individuals with regard to MDMA metabolism and the body’s tolerance for the drug and its metabolites. In one case, an individual was found to have an MDMA \( C_P \) of 7.720 \( \mu g/ml \) after having taken 42 pills. De Letter et al. suggest that blood concentrations above 1.000 \( \mu g/ml \) may be potentially lethal, and that levels at or below 0.6000 \( \mu g/ml \) may be capable of causing toxicity. The individual in this case complained of a hangover, and experienced hypertension and tachyycardia. The reported plasma concentrations are more than seven times larger than what may have been a lethal concentration for many, yet the individual felt like he had a hangover. While this represents an extreme scenario, it is presented as an example of the tremendous variability in the effect of and tolerance to MDMA, and as a demonstration of the need for further research. De Letter et al. offer their finding of 76 MDMA-related fatalities, wherein \( C_{P,MDMA} \) ranged from as low as 0.040 \( \mu g/ml \) to as high as 18.500 \( \mu g/ml \). It is therefore difficult to identify an \( LD_{50} \) which would be of any practical clinical utility. Of major concern, the range of doses taken recreationally overlap with
those that produce toxicity or fatality. Further complicating matters, MDMA and its metabolites tend to redistribute themselves and even chemically react post-mortem, limiting the utility of autopsy. Because of the tremendous variability in MDMA tolerance, and the potential confounds present in many MDMA users as well as in cases of fatality, achieving a consensus on meaningful clinical variables can be difficult. The applicability of animal research is limited as well; findings based on animal research cannot be easily applied to human beings, as differing metabolisms, morphologies, and tolerances to MDMA limit the human relevance of any conclusions drawn. Studying the long-term effects of MDMA use is challenging in animals with different lifespans, but the study of human subjects presents its own challenges, beyond stricter ethical standards.

**Biological and Chemical Deficits in Humans**

Following MDMA administration in humans, a dose-dependent decrease has been found in 5-HT transporter sites as well as in 5-HT receptors, markers for 5-HT, and 5-HIAA levels. This decrease persists even several weeks after ceasing to use the drug, and it is not clear how long a complete recovery may take, or if it is possible. This finding was first made in rats, but also holds true in humans. More recent studies have confirmed the finding in humans using an array of techniques including single photon emission computed tomography (SPECT), and positron emission tomography (PET). These changes are persistent and region specific, with the greatest losses found in regions of the HPC, striatum, and PFC. In order to assess functional damage, subjects may be exposed to stressful conditions which specifically seek to challenge the systems which may have been damaged. Such a test is especially sensitive to even small
deficits. In a study by McCann et al., MDMA users who had been abstinent for at least three weeks as well as MDMA-naïve controls were subjected to challenge by the mixed 5-HT agonist meta-chlorophenylpiperazine (m-CPP), and the plasma levels of prolactin and cortisol were measured subsequently. The MDMA using group was found to have significantly lower levels of both hormones. Concerning the previously described MDMA-induced changes in baseline cortisol levels, more long term neuroendocrine changes seem to exist. In regular MDMA users who had abstained for 3 weeks prior to testing, Gerra et al. found that baseline levels of cortisol as well as levels of ACTH were significantly higher than in controls. The authors exposed the subjects to stressful conditions, and consequently found significantly decreased levels of ACTH and cortisol in the abstinent MDMA users, as compared to controls, potentially indicating a blunted response to stress. Taken together, these findings support the conclusion that there is a persistently diminished ability to deal with stress, which results from MDMA use. This finding differs from that seen in the acute response to MDMA administration described previously, where users had an elevated baseline cortisol level, but the magnitude of the change in response to stress was the same as in controls. Gerra et al. suggest that regular MDMA usage, by altering brain 5-HT (and possibly DA) neurons, may cause neuroendocrine dysfunction.
Biological and Chemical Deficits in Animals

The previously described deficits in response to 5-HT challenge observed in humans may not be a finding exclusive to humans; as research has found deficits in reaction time and progressive ratio task in MDMA-exposed rhesus monkeys following challenge with m-CPP. Morton et al., also report a diminished number of axonal projections from the dorsal raphe nucleus, an observation made in rodents. Mueller et al. report that, in rodents, the amount of 5-HT depletion generated was found to correlate positively with the CMAX of MDMA and MDA. MDMA AUC, and not MDA AUC, was found to correlate with brain 5-HT deficits. Like MDMA, MDA is a known brain 5-HT neurotoxin. It is not currently possible to pinpoint which pharmacokinetic parameter is most proximately related to neurotoxicity. Of great significance are the lasting changes in the density of the SERT. It is often difficult to assess particular long-term deficits, as well as the extent of recovery, in human subjects. As discussed, this necessitates increased reliance on animal research, although the applicability of the findings to humans remains uncertain. Investigating the long-term effect of MDMA on 5-HT1B receptors in rats, Aguirre et al. have found opposite changes in receptor density in different regions of the brain. They found an increased density in the cortex, but a reduced density in the dorsal raphe nucleus. If the downregulation observed in the cortex was a response to elevated levels of 5-HT, then the upregulation observed in the dorsal raphe may be due to decreased or levels of 5-HT. Given that the 5-HT1 family of receptors often functions as an autoreceptor, it is not surprising to find that this receptor is upregulated; the effect of this upregulation is to make the neuron more
sensitive to the 5-HT it is releasing, so that release can be more tightly controlled. The upregulation may be a response to long-term deficits. Further work by the same group has also noted an increase of 5-HT$_{1A}$ receptors in the rat hypothalamus, a region critical for regulation of body temperature.$^{115}$ By contrast, the changes noted in the dorsal raphe nucleus were not always replicated in subsequent studies. Also looking in rats, McGregor $et$ $al.$ failed to find any difference in 5-HT$_{1A}$ receptor density in the dorsal raphe. Using SPECT and the radioactive 5-HT$_{2A}$ ligand $[^{123}]$I$R$91150, Reneman $et$ $al.$ quantified cortical receptor density in rats who were recently exposed to MDMA, as well as in abstinent rats. Cortical 5-HT$_{2A}$ receptor density was lower in the rats who had recently used MDMA, but was actually higher in the abstinent group.$^{116}$ This difference may be due to a compensatory upregulation in 5-HT$_{2A}$ expression consequent to diminished levels of 5-HT. In the group recently exposed to MDMA, the large amount of synaptic 5-HT available for binding may have acutely caused downregulation of the receptor.$^{52,116}$ In rats, MDMA has also been found to cause long-term decreases in tryptophan hydroxylase activity in several regions, notably the hippocampus (HPC), hypothalamus, and cortex.$^{52,117,118}$ Such effects of MDMA use in animals are not limited to rats. Using the radioactive ligand$[^{11}]$C$Mc$N5652 (which has an affinity for SERT transporters) in conjunction with PET scanning, deficits in markers for 5-HT have been observed in baboons at least 40 days after exposure to MDMA.$^{119}$
**Deficits in SERT Density**

SERT density is easily quantifiable, and reports of decreases in SERT density consequent to MDMA exposure have been described in many species, including humans, baboons, and rats.

Figure 5: Autoradiograms Showing SERT Density

Though the SERT density reductions described in Figure 5 were observed in rats, similar findings which have been previously described have also been made in non-humane primates, including baboons. In a study using ‘matched controls’ with respect to other recreationally abused drugs in order to account for to polydrug use (as mentioned above), McCann *et al.* were able to quantify SERT density using two different radiotracers in

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\[ \text{Adapted from McGregor *et al.*, 2003. Used with permission.} \]
conjunction with PET. In this particular study, the subjects (on average) had last taken MDMA 4.74 months prior, and the most commonly taken dosage averaged across subjects was 1.79 pills per exposure. The average number of lifetime exposures was 96.96 per subject.\textsuperscript{98}

Figure 6\textsuperscript{h}: PET Scans Displaying SERT Density in a Human Subject\textsuperscript{98}

SERT density is indicated by brightness. It is apparent from either radiotracer used that MDMA causes a reduction in the density of SERT expression. McCann \textit{et al.} expanded on these findings several years later, further quantifying reductions in SERT density in particular regions of interest within the brain. This subsequent investigation used PET in conjunction with the radioactive SERT ligand \textsuperscript{[11]}C DASB. In this study, the authors intentionally chose subjects who had a history of taking at least 2 doses of MDMA less than 12 hours apart.\textsuperscript{97} Subjects were abstinent from all drugs for two weeks prior to their

\textsuperscript{b} Adapted from McCann \textit{et al.}, 2005. Used with permission
assessment. Ligand/SERT binding was assessed using distribution volume ratios (DVRs), a linear function of receptor availability.\textsuperscript{121}

Figure 7: DASB Binding by Brain Region\textsuperscript{97}

![Graph showing DASB DVR for different brain regions](image)

Figure displaying DASB DVR ± SD. # indicates $p \leq 0.05$; * indicates $p \leq 0.01$; ** indicates $p \leq 0.001$; *** indicates $p \leq 0.0001$. The regions investigated include MB midbrain, Amyg amygdala, HPC hippocampus, TH thalamus, CD caudate, Put putamen, DPFC dorsolateral prefrontal cortex, OC occipital cortex, OFC orbitofrontal cortex, PC parietal cortex, TC temporal cortex, ACC anterior cingulate cortex, PCC posterior cingulate cortex, Dpons dorsal pons, and Vpons ventral pons.

Loss of SERT density is apparent in every cortical region assessed, as well as in the HPC\textsuperscript{97} and the amygdala, as would be expected given the previously described deficits in memory and higher cognition. Based on clinical observations, “the amygdala and the prefrontal cortex… are not only involved in processing emotions, but also participate in

\textsuperscript{1} Adapted from McCann \textit{et al.}, 2008. Used with permission.
the complex neural processing responsible for rational thinking." McCann et al. found that the correlation between SERT density and memory was actually stronger in controls than in MDMA users, and suggest that a compensatory response involving neuroplasticity may be working within the experimental group.97

**Functional Consequences in Humans**

Even after years of abstinence from MDMA, numerous deficits remain.8 These deficits include memory problems and cognitive deficits, psychiatric disorders, altered sleep architecture105, altered appetite, and reduced sexual interest.8 Retrospective memory deficits were the first memory problems to be identified122, with related deficits including diminished ability to perform immediate and delayed recall tasks being discovered subsequently.123–125 Deficits in prospective memory have also been reported126–128, and these appear to be dose-dependent, such that heavy MDMA users experienced a greater deficit than regular or infrequent users129. Deficits in cognition are most obvious in the execution of complex tasks; performance on simpler tasks (including reaction time and basic attention) is not impaired.130 McCann et al. first reported these deficits in 1999, but their existence and nature has been confirmed by subsequent research using a variety of cognitive measures.131–133 Examples of the type of complex task impacted by prior MDMA usage include complex decision tasks, such as the Brixton Spatial Anticipation task.4,133 Those who use MDMA risk developing cognitive deficits in logical reasoning, executive processing, and emotional intelligence.4 One study by Halpern et al. was able to isolate a group of individuals with minimal exposure to other neurotoxic substances,
and one interpretation\(^\dagger\) of their data\(^3\) indicates the presence of the previously mentioned deficits, implicating MDMA exposure as the salient factor.\(^{30k}\)

Pathological changes in sleep architecture are another feature of MDMA use. 5-HT is just one neurotransmitter which influences sleep and circadian rhythms\(^{55(p141),134}\), and since MDMA is a known 5-HT neurotoxin\(^{105}\) with long-term effects, it is likely that patterns of sleep and circadian rhythms may become disrupted due to MDMA.

Furthermore, it is possible that sleep disruptions may lead to other cognitive deficits if normal sleeping patterns are disrupted for extended periods. Such progression may explain deficits observed in abstinent MDMA users.\(^{105}\) In clinical studies with humans, abstinent MDMA users were found to have significantly less total sleep (both REM and NREM)\(^\dagger\) with significantly less stage 2 sleep.\(^{135}\) In a follow-up study of 25 age and gender matched abstinent MDMA users, the previously described reduction in stage 2 sleep failed to reach significance.\(^{136}\) While it is not clear why this result failed to reach significance in a subsequent study, possible explanations include differences in the amount of MDMA administered and the duration of abstinence from MDMA.

Additionally, the authors found significant increases in both stage 3 and stage 4 sleep in the experimental group. They also reported increased sleep disordered breathing (apneas and hypopneas)\(^{105,136}\) In another paradigm of the same study, the subjects were also exposed to the mixed 5-HT m-CPP. The result was an increase in time spent in stage 4

\(^\dagger\) See page 6 for more details on the controversy surrounding this study’s findings.

\(^k\) This previously described study was met with several responses after reportedly observing an absence of MDMA-induced neurotoxicity. We have applied A.C. Parrott’s interpretation of the data gathered by Halpern et al.

\(^\dagger\) REM, or Random Eye Motion, is a phase of the sleep cycle. NREM, or Non Random Eye Motion, refers to all phases of sleep where REM is not occurring.
sleep within the experimental group, but a decrease in time spent in stage 4 sleep within the control group. This paradoxical finding supports the idea that MDMA leads to major changes in the control of sleeping patterns, and that the function of 5-HT neurons in MDMA users differs from that in controls.\textsuperscript{105,136}

**Functional Consequences in Animals**

Lifetime deficits in social behavior, associated with decreased levels of 5-HIAA, have been observed in rats.\textsuperscript{8} In a study by Fone \textit{et al.}, the authors gave male rats a large (7.5 mg/kg) dose of MDMA twice daily for 3 days. When considering the rats’ behavior 12-29 days following the final injection, the authors found a 41% decrease in social behaviors.\textsuperscript{137} A similar finding was also reported by McGregor \textit{et al.}. In this particular study, male rats were divided into 3 groups of 12 rats each: a high dose group, a low dose group, and a vehicle (control) group. Considering the half-life of MDMA in rats, drug administration methodologies were meant to be as similar as possible to human consumption patterns. The high dose group received an injection every hour for 4 hours over two consecutive days; each injection was 5 mg/kg. The low dose group received one 5 mg/kg dose in total.\textsuperscript{120} Social behavior was assessed ten weeks later.

Table 5\textsuperscript{m}: Social Interactions Following MDMA Administration in Rats\textsuperscript{120}

<table>
<thead>
<tr>
<th>Group</th>
<th>Time Spent Interacting [Secs] (SEM)</th>
<th>Number of Interactions (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>120.63 (8.05)</td>
<td>56.25 (3.27)</td>
</tr>
<tr>
<td>Low dose</td>
<td>72.98 (4.03)$§$</td>
<td>47.00 (3.42)</td>
</tr>
<tr>
<td>High dose</td>
<td>56.31 (4.85)$★$</td>
<td>42.67 (2.28)$§$</td>
</tr>
</tbody>
</table>

$★$: Significant with respect to low dose group. $§$: Significant with respect to vehicle group.

\textsuperscript{m} Adapted from McGregor \textit{et al.}, 2003. Used with permission.
McGregor *et al.* also found significantly reduced levels of 5-HT and 5-HIAA in the PFC, striatum, HPC, and amygdala.\(^{120}\) These results clearly demonstrate the lasting effects of MDMA use on social behaviors and on the 5-HT system, even weeks after the cessation of MDMA use. In addition to social deficits, one study by Balogh *et al.* looked at the effects of MDMA on a number of parameters, including sleep and circadian rhythms, in rats. As might be expected upon administration of an amphetamine analog, patterns of sleep and motor activity were disrupted for at least 6 hours following a single systemic dose of MDMA to drug-naïve rats. The effect was much more pronounced in rats that had been pretreated with MDMA three weeks prior; circadian sleep and motor activity rhythms were disrupted for about 5 days.\(^{105,138}\) Alterations in motor activity, wakefulness, and slow-wave (deep) sleep remained apparent a full month after MDMA administration, the last data collection point.\(^{105,138}\) Reaching the same finding as previous research\(^{96}\), there was also a reduction in paroxetine binding to SERTs located on neurons in the cortex.\(^{105,138}\)

**VI. Hypotheses on the Etiology of MDMA-Mediated Neurotoxicity**

**The Role of MDMA Metabolites**

The mechanism behind neurotoxicity remains unclear, as neither MDMA nor its metabolites MDA, HHMA, and HMMA were found to produce serotonin toxicity when injected into the rat brain.\(^{57}\) Mueller *et al.* administered 20 mg/kg of MDMA to rats orally, measuring brain concentrations of MDMA and metabolites at various time points. Only MDMA and MDA were found in the brain at every time point considered.\(^{103}\)
Furthermore, the concentrations of MDMA and MDA in the brain were found to significantly correlate with their respective plasma concentrations; this was not true for other MDMA metabolites such as HHMA. Neither HHMA nor HMMA were detectable in significant quantities in the brain, leading Mueller et al. to hypothesize that neither of these substances readily cross the blood-brain barrier, and that there is a negligible amount of brain metabolism producing these substances. Further research has been done on the compounds HHMA and HHA, and in both cases the authors concluded that neither compound could be wholly responsible for the neurotoxicity produced by MDMA. Regardless, the finding that a direct injection of MDMA into the brain fails to produce toxicity has led to questions about the roles of MDMA metabolites created elsewhere in the body. As mentioned previously, hepatic metabolism aims to conjugate MDMA or its metabolites to hydrophilic moieties, so that they can be excreted in the urine. Mueller et al. note previous work suggesting that some products of hepatic metabolism may be neurotoxic; “glutathione and N-acetylcysteine conjugates of catechol metabolites of MDMA and MDA have been identified and implicated in MDMA neurotoxicity”. The catechol metabolites of MDMA may (HHMA, HHA) become oxidized, forming their respective quinones. These quinones are able to form adducts with thiol-containing compounds, including glutathione. One such metabolite, 5-(N-acetylcystein-S-yl)-3,4-dihydroxymethamphetamine (5-NAC-HHMA) has been suggested as a possible cause of toxicity.

Mueller et al. injected 5-NAC-HHMA at two different doses directly into the striatum of rats. Modest, non-significant reductions in indole levels were found, but were
not dose-dependent and could not be blocked by pretreatment with fluoxetine, which is known to prevent 5-HT toxicity. Injection of an MDMA metabolite such as 5-NAC-HHMA into a particular area of the brain may not adequately model the conditions that produce toxicity. This is offered on the basis that the complex interaction of neurotransmitters (5-HT, DA, etc.) may be necessary, and a locally administered injection may not be an accurate model of such an interaction. Furthermore, compounds causing MDMA-like toxicity have not been demonstrated to form quinones as a result of metabolism. Quinone formation is a necessary component of the toxic metabolite hypothesis. The significance of 5-NAC-HHMA and other metabolites in the production of MDMA-induced neurotoxicity is a topic of continued investigation.

The Role of Reactive Oxygen Species

Although MDMA is a 5-HT neurotoxin, its effects on other neurotransmitters may be important when considering the mechanism by which toxicity is produced, especially since DA release has been found to correlate with the degree of SERTs lost. As noted by others, MDMA is “messy.” Sprague et al. note that the production of reactive oxygen species (ROS) is necessary to produce 5-HT toxicity in rats. ROS production may occur as a result of deamination or autoxidation of DA; DA is a relatively reactive compound. Since DA release has been found to correlate with the degree of SERTs lost, this hypothesis is especially attractive, although it may be just one part of a larger mechanism, or completely coincidental. Investigators continue to debate the veracity of the claim that MDMA-induced toxicity is a result of ROS, and some have likened the situation to that of the chicken and the egg. Jones et al. have
demonstrated that the observed increase in ROS production takes place in cells possessing the SERT.\textsuperscript{146} DA is not the only potential cause of ROS generation.

Jones \textit{et al.} have found that two specific glutathione adducts (specifically the thioethers 5-(Glutathion-S-yl)-α-methyldopamine and 2,5-bis(glutathion-S-yl)-α-methyldopamine, which are glutathione adducts of HHMA and HHA, respectively) are potent generators of ROS. Thioether-metabolites may oxidize the cell membrane via a ROS-mediated mechanism. Jones \textit{et al.} also found that these two compounds cause the uptake of DA into cells possessing SERT.\textsuperscript{141,146} The DA may then become reactive as previously discussed, creating multiple sources of ROS. In this model, the glutathione adducts are responsible for the uptake of DA into cells possessing SERT. The DA then causes an increase in ROS, which may damage the neuron. The increase in ROS is both time and concentration dependent.\textsuperscript{146} Capela \textit{et al.} corroborate these findings, suggesting that the thioether metabolites of HHMA and HHA may be far more neurotoxic than their parent compounds.\textsuperscript{147} Additionally, MDMA and MDA themselves have been shown to cause a somewhat less pronounced uptake of DA into cells expressing SERT.\textsuperscript{146}

In addition to the uptake of DA into cells expressing SERTs, the thioether metabolites of HHMA and HHA, products of conjugation with glutathione or \textit{N}-acetylcysteine, produced a concentration-dependent delayed neuronal death, with accompanying activation of caspase 3.\textsuperscript{147,148} This finding strengthens the relevance of the correlation between DA release and 5-HT neurotoxicity, and offers a possible explanation. As previously mentioned, Mueller \textit{et al.} found that direct injection of 5-\textit{N}-acetylcysteine-HHMA did not produce the expected amount of 5-HT toxicity when
injected directly into the striatum of a rat.\textsuperscript{103} This result weakens the hypothesis that this metabolite is responsible for producing toxicity, although it is possible that the MDMA-induced toxicity ordinarily observed in humans is a result of the synergistic action of a number of metabolites as well as the parent compound (MDMA) itself. Such synergistic effects may produce toxicity by cooperatively increasing the oxidative load beyond the cell’s ability to cope, or through other mechanisms. DA is extremely reactive and may produce ROS, and as discussed, activation of 5-HT\textsubscript{2A} may lead to increased DA synthesis.\textsuperscript{149,150} Capela et al. succinctly described the hypothesis: “5-HT2A-receptor stimulation produces intracellular oxidative stress that leads to neuronal apoptosis accompanied by caspase 3 activation.”\textsuperscript{148} Adding to the oxidative burden, it is possible that the extreme degree of cellular activity caused by the activity of MDMA contributes to neurotoxicity, and some have proposed that this effect mediates the increased production of ROS.\textsuperscript{8}

**Dopamine, Temperature, and MDMA**

The extent of the deficits in SERT caused by MDMA seems to positively correlate with the amount of DA released\textsuperscript{1,142,143}. One explanation for this phenomenon suggests that activation of the 5-HT\textsubscript{2A} receptor leads to increased production of DA. The previously described action of MDMA at 5-HT\textsubscript{2A} receptors\textsuperscript{10} may activate the receptors; activation of 5-HT\textsubscript{2A}, either by the endogenous ligand 5-HT or by MDMA may lead to increased DA synthesis\textsuperscript{149,150}, partially explaining the observed correlation.\textsuperscript{6} Furthermore, in rats, extracellular DA levels as well as the extent of serotonergic toxicity

\textsuperscript{8} MDMA produces 5-HT toxicity, and causes release of 5-HT. Release of 5-HT causes increased dopamine synthesis through action at this receptor.
caused by MDMA administration were found to be reduced by pre-treatment with 5-HT$_{2A}$ antagonists.\textsuperscript{1,151,152} Malberg \textit{et al.} administered α-methyl-\textit{p}-tyrosine (AMPT), an inhibitor of tyrosine hydroxylase, to reduce DA levels.\textsuperscript{151} Consequently, they observed a reduction in the neurotoxicity produced by MDMA. The other significant finding was that pretreatment with AMPT, followed by administration of MDMA, produced a marked and long-lasting hypothermia in the rats.\textsuperscript{151} As discussed, research by Brown and Kiyatkin as well as others has demonstrated the neuroprotective effects of lower temperatures, such that higher temperatures exacerbate MDMA-induced toxicity.\textsuperscript{8,71} Since 5-HT$_{2A}$ antagonists were found to cause hypothermia, it is possible that hypothermia, and not reduced DA levels, was responsible for the observed neuroprotection. In a study by Yuan \textit{et al.}, both vesicular and cytosolic DA were depleted using AMPT and reserpine (which inhibits VMAT).\textsuperscript{153} Upon administration of MDMA, the rats were prevented from experiencing hypothermia by increasing the temperature of the environment. The neuroprotective effects which were previously thought to derive from reduced levels of DA disappeared,\textsuperscript{153} indicating that the neuroprotection observed by Malberg \textit{et al.} and others was actually due to hypothermia, not lower DA levels. One important conclusion that can be drawn from these findings implicates DA in the generation of hyperthermia, a hypothesis which has been suggested by multiple studies.\textsuperscript{154,155} Hyperthermia might result from activation of D$_1$ receptors by DA.\textsuperscript{52,156}

\textbf{Effects Relating to Temperature}

The role of temperature in MDMA-induced neurotoxicity has been well documented. Working with rats, Brown and Kiyatkin administered 9 mg/kg of MDMA at
a variety of temperatures, in settings meant to mimic the ways in which humans self-administer MDMA. Brown et al. were especially interested in the effects on the nucleus accumbens (NACC) and the HPC. At 23 °C, MDMA produced a moderate hyperthermia; the temperature gains in the NACC and HPC were greater than those found in muscle, suggesting that the brain’s metabolic activity may be to blame for the increase in temperature. The temperature increase in these two brain regions was also more rapid than in muscle. When social interaction with a female was added to the experimental condition, the hyperthermia observed in the NACC and HPC was significantly potentiated. When MDMA was administered at 29 °C, hyperthermia pushed the temperature in the brain to its biological limit (over 41 °C), resulting in fatality. Of further concern is the notion that hyperthermia potentiates neurotoxicity. This is yet another reason why small variations in dose or environment can lead to large changes in the severity of the outcome. As noted by A.C. Parrott, elevated temperatures (including those caused by an elevated ambient temperature) can worsen serotonergic toxicity, while lower temperatures afford some degree of neuroprotection.

Differences in Toxicity and Cause of Death Between Genders

Data from multiple sources, both clinical and pre-clinical, suggest a sexually dimorphic pattern of pathology produced by MDMA. Adult females appear more sensitive to the acute and sub-acute physiological and psychological effects of MDMA, as well as to the long-term changes in 5-HT systems. Considering the pharmacological differences in metabolism previously discussed, it is not surprising that women are also

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For a comprehensive review of the sexual dimorphisms associated with MDMA, see Allott and Redman, 2007.
more likely to experience adverse responses to MDMA, when given the same bolus dose as a male. Individuals with lower body weights, as are found in many women, may be more susceptible to hyponatraemia.\textsuperscript{4} Analyzing 1407 cases of MDMA-related hyponatraemia, Rosenson \textit{et al.} found women were significantly more represented than men.\textsuperscript{95} Considering individuals who attended a particular Dutch “rave” in 2010, Van Dijken \textit{et al.} analyzed the plasma sodium concentration of 63 subjects who had taken MDMA. The authors found mild hyponatraemia in 25\% of females, but only 3\% of males, even though there was no significant difference in the rate of pill consumption between individuals.\textsuperscript{4,158}

\textbf{VII. Conclusions}

The chemistry, disposition and metabolism, and biological effects of MDMA have been detailed. Potential adverse effects, including serotonin neurotoxicity, have also been discussed. Exploring the history of MDMA, we have seen the ways in which the pattern of MDMA use is changing, such that it is now more commonly taken in private homes than in nightclubs or concert venues. At the same time, the frequency of MDMA-related hospitalizations has risen. There appear to be two vocal groups with strong opinions regarding MDMA; one group advocates its use as a novel psychotherapeutic adjunct, while the other emphases its potential fatal properties. The more likely risks associated with MDMA include the described long-term serotonergic deficits and pathological consequences, but the etiology of these effects is not well understood, and not all of the effects associated with MDMA use have been well described in humans. In
other words, the public discourse on MDMA is confusing and frequently misrepresented. As noted by A.C. Parrott, “Perhaps the most important [issue] is the very misleading message for the general public (at-risk youngsters in particular) that MDMA is safe for human consumption, and that MDMA can help solve your personal problems”. The confusing state of public discourse in many ways reflects the confusing state of current scientific opinion on MDMA. Until more details relating to MDMA’s mechanism of action, particularly the mechanisms underlying MDMA-induced 5-HT injury and associated functional consequences, those who wish to use MDMA should proceed with caution, with due considerations of risks and benefits.

The range of responses to a given dose of MDMA can be highly variable between individuals, and because its metabolism is non-linear, small changes in dose can lead to large changes in plasma concentrations. This effect may be expected to increase the chance for errors in dosing, and is another reason recreational use of MDMA should be done cautiously. Further contributing to the unpredictable effects of “ecstasy” use, it is impossible to know what kinds of impurities are contained in a dose of the drug bought illegally. MDMA may not even be present. The need for continued research on the effects and mechanisms associated with MDMA is more pressing than ever.
### APPENDIX

Appendix 1a: Mean $C_{\text{MAX}}$, $T_{\text{MAX}}$, and AUC$_{\infty}$ for MDMA & HMMA$^{57,p}$

<table>
<thead>
<tr>
<th></th>
<th>MDMA</th>
<th>HMMA</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>$C_{\text{MAX}}$ (ng/ml)</td>
<td></td>
<td></td>
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<tr>
<td>Overall</td>
<td>$26.0 \pm 4.6$</td>
<td>$71.5 \pm 8.5$</td>
</tr>
<tr>
<td>Median</td>
<td>$23.8$</td>
<td>$70.0$</td>
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<tr>
<td>Range</td>
<td>$15.4$ to $32.8$</td>
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<tr>
<td>$T_{\text{MAX}}$ (h)</td>
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<tr>
<td>Overall</td>
<td>$2.4 \pm 0.2$</td>
<td>$1.8 \pm 0.1$</td>
</tr>
<tr>
<td>Median</td>
<td>$2.2$</td>
<td>$1.8$</td>
</tr>
<tr>
<td>Range</td>
<td>$1.7$ to $3.7$</td>
<td>$1.3$ to $2.3$</td>
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<tr>
<td>AUC$_{\infty}$ (ng/ml/h)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>$23.6 \pm 4.8$</td>
<td>$96.3 \pm 14.8$</td>
</tr>
<tr>
<td>Median</td>
<td>$21.8$</td>
<td>$93.6$</td>
</tr>
<tr>
<td>Range</td>
<td>$18.3$ to $27.2$</td>
<td>$66.3$ to $135.3$</td>
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$^p$ Adapted from Kolbrich et al., 2008. Used with permission.
### Appendix 1b: Mean C\textsubscript{MAX}, T\textsubscript{MAX}, and AUC\textsubscript{∞} for MDMA & HMMA (continued)

<table>
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<td>(n = 7)</td>
<td>(n = 6)</td>
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<tr>
<td>Mean + SE</td>
<td>246.0 ± 841.4</td>
<td>396.1 ± 297.7</td>
<td>198.3 ± 176.5</td>
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<td>286.67</td>
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<td>Range</td>
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<td>2349.9 - 4492.9</td>
<td>783.6 - 1740.8</td>
<td>458.6 - 2950.2</td>
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<td>(n = 10)</td>
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<tr>
<td>Mean + SE</td>
<td>1420.4 ± 101.1</td>
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<td>Median</td>
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<td>Mean + SD</td>
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<tr>
<td>Overall</td>
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<td>(n = 17)</td>
<td>(n = 16)</td>
<td>(n = 16)</td>
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<td>Mean + SD</td>
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<td>(n = 17)</td>
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### LIST OF JOURNAL ABBREVIATIONS

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Nephrol. Dial. Transplant.  
Nephrology, Dialysis, Transplantation: Official Publication of the European Dialysis and Transplant Association - European Renal Association

Neuroscience and Biobehavioral Reviews

Neurotox. Res.  
Neurotoxicity Research

NIDA Res. Monogr.  
NIDA Research Monograph

Open Addict. J.  
The Open Addiction Journal

Pharmacol. Biochem. Behav.  
Pharmacology, Biochemistry, and Behavior

Pharmacol. Ther.  
Pharmacology and Therapeutics

Prog. Neurobiol.  
Progress in Neurobiology

Psychiatry Res.  
Psychiatry Research

Psychopharmacology (Berl)  
Psychopharmacology (Berlin)

QJM Mon. J. Assoc.  
Quarterly Journal of Medicine: Monthly Journal of the Association of Physicians

ScientificWorldJournal  
The Scientific World Journal

Swiss Med. Wkly.  
Swiss Medical Weekly

Ther. Drug Monit.  
Therapeutic Drug Monitoring

Toxicology and Applied Pharmacology

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33. Parrott AC. Residual neurocognitive features of ecstasy use: a re-interpretation of Halpern et al. (2011) consistent with serotonergic neurotoxicity. *Addict Abingdon*


CURRICULUM VITAE
ALEXANDER IAN HELFAND

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New York, NY ahelfand@bu.edu
Born 1989

EDUCATION
Boston University, Boston, MA.
Master of Science, Medical Science (September 2012 - Current)
Bachelor of Arts, Psychology (May 2012)

AWARDS AND PUBLICATIONS
Student Research Award
Boston University
Honored twice consecutively. (09/11, 01/12)


RESEARCH
Research Coordinator & Trainee (Psychiatry),
Johns Hopkins University School of Medicine, Baltimore, MD,
Laboratories of Dr. Una McCann and Dr. George Ricaurte (6/13-Present)
• Attended 8th annual Johns Hopkins Bayview Research Symposium; Second author for associated poster and abstract. (12/13/13)
• Involved with two clinical studies, evaluating damage to DAT and SERT transporters using PET, MRI, and cognitive testing

Research Assistant,
Boston University,
Laboratories of Dr. Michael Baum & Dr. James Cherry (04/11–08/12)
• Attended Association for Chemoreception Sciences Conference in Huntington Beach, CA. Second author for associated poster and abstract. (4/25/12)
• Researched olfaction, pheromones, motivational systems, and sexual dimorphism in laboratory mice.
• Conducted neurosurgery including tract tracing studies and lesioning. Performed transcardiac perfusions and ovariectomies. Implanted hormone capsules and monitored endocrine state. Provided postoperative care and monitored subject condition and anesthesia levels during surgery. Conducted analysis of brain tissue using

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immunocytochemistry, confocal microscopy, and epifluorescent microscopy. Gathered behavioral data.

- Research supported by Boston University Undergraduate Research Opportunity Program

**TEACHING**
Undergraduate Teaching Assistant (Systems Physiology),
Boston University
(01/12 – 5/12)
- Performed troubleshooting on equipment used to assess biological variables.
- Evaluated performance of other Undergraduate Teaching Assistants.

Undergraduate Teaching Fellow (Experimental Physiological Psychology), Boston University
(01/12–5/12)
- Assisted in designing neuroanatomy practical exam by creating sheep brain dissections and pinning structures.
- Proctored exams, held office hours, and managed attendance records.
- Prepared laboratory solutions and rat dissections for student use. Assisted in teaching fundamentals of microscopy and histology.
- Tested experimental methods independently prior to classroom implementation.
- Aided in classroom exercises and teaching of laboratory sections. Answered student questions.

BIOBUGS
(Biology Inquiry & Outreach with Boston University Graduate Students),
Boston University
(12/11–12/12)
- Taught inner city high school students about epidemiology and disease. Facilitated discussion, aided in management of lab exercises.
- Spoke on group panel designed to educate students on scientific research.
- Explained effective techniques for managing time, work, and stress in college.
- Led high school student groups on ‘Science Wednesdays’ as a part of Boston University Upward Bound Math and Science. Assisted in designing curriculum.

**CLINICAL**
Trainee (Dept. of Psychiatry),
Johns Hopkins University School of Medicine, Baltimore, MD
(06/13–present)
• Volunteer weekly in the Johns Hopkins Bayview Medical Center Emergency Department as a Patient Representative Aide. Responsibilities include interfacing between patients, families of patients, and medical staff.
• Shadow psychiatrist Dr. Una McCann twice a month in the Anxiety Disorders Clinic.

Goddard Riverside ACT Team (Assertive Community Treatment), New York City (06/10-08/10)
• Shadowed psychiatrist Dr. Eric Weitzner. Observed interactions with patients and techniques employed on patients refusing treatment. Made house calls along with social workers, Dr. Weitzner, or other members of the ACT team.
• Aided in patient care by ensuring patients got to medical appointments as scheduled. Ensured patients had adequate supplies of medication and food at home. Engaged patients in discussion and kept track of patient goals.

ACTIVITIES
EMT Certification, Lifeline Institute for EMS & Allied Health Professions, Woburn, MA (01/13-04/13)
• Completed training for EMT certification.

Active Minds, Boston University (01/10-05/11)
• Promoted mental health awareness by hosting events on campus and providing screening for common mental illnesses.
• Chapter President (09/10-05/11). Planned and executed biweekly meetings. Interfaced with faculty and administration. Organized financial records and drafted purchase orders for supplies, requests for funding.
• Named Best Chapter (2010) by Active Minds national organization. Attended national conference (09/10) and took part in meet and greet and introduction of Dr. John Nash.

Student Support Network, Boston University (02/10-05/10)
• Received training from university staff and student health on how to recognize signs of mental distress in peers, including suicidal ideation, eating disorders, and substance abuse problems.