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Impact of Genetic Variability in the Serotonin Transporter, Tryptophan Hydroxylase-2, and Serotonin $_{2A}$ Receptors on MDMA Use and Impulsivity

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by

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Dedication

To Lilo, with all my love

In loving memory of Dalal Ezzeddine

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Impact of Genetic Variability in the Serotonin Transporter, Tryptophan Hydroxylase-2, and Serotonin $_{2A}$ Receptors on MDMA Use and Impulsivity

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Ecstasy [MDMA; (±)-3,4-methylenedioxymethamphetamine] is popular for positive effects including enhanced mood and empathy, despite potential deleterious physical and psychological consequences. The serotonin (5-HT) system plays a prominent role in the neurochemical and behavioral effects of MDMA, and preferentially targets 5-HT neurons in humans, although this substituted amphetamine does have significant actions on other monoamine systems. Some negative effects of MDMA use, including mood and cognitive dysfunction, may involve the 5-HT system, and are often treated with 5-HTergic antidepressants. The involvement of specific 5-HT genes, including tryptophan hydroxylase-2 (TPH-2), 5-HT transporter (5-HTT), and 5-HT_{2A} receptor (5-HT_{2A}R) in the effects of MDMA is incompletely understood. Our initial study compared the prevalence of polymorphisms leading to reduced gene expression in those genes among MDMA users versus controls, and assessed the relationship between these polymorphisms and impulsivity. As compared to control groups, MDMA users were less likely to carry 5-HTT SS or 5-HT_{2A}R A1438G GG/T102C CC genotypes thought to lead to decreased gene expression. No relationship was found between genotype and impulsivity, however drug-naïve controls had lower impulsivity levels than either MDMA or polydrug users. The differential distribution of 5-HTT and 5-HT_{2A}R 1438G/ T102C suggests that these genes contribute to individuals' choices of MDMA versus other drugs. The second study examined the relationship between TPH-2, 5-HTT, and 5-

$HT_{2A}R$ polymorphisms, and self-reported MDMA intake or impulsivity in moderate-to-heavy MDMA users. Decreased MDMA use was reported by individuals carrying genotypes associated with reduced gene expression (TPH-2 TT, 5-HTT SS, 5-$HT_{2A}R$ A1438G GG/T102C CC), and 5-HTT genotype-dependent changes in impulsivity were found. Our studies are the first to show a differential allelic distribution between MDMA users and controls, and demonstrate the relationship between TPH-2, 5-HTT and 5-$HT_{2A}R$ A1438G/T102C polymorphisms and MDMA intake. Individuals carrying TPH-2 TT, 5-HTT SS, 5-$HT_{2A}R$ A1438G GG/T102C CC may represent a subset of MDMA users at higher risk to develop MDMA-related adverse events, including depression. Further, this group may display poorer response to antidepressants and decreased retention in treatment given elevated impulsivity levels. Thus, our findings may have important implications for diagnosis and treatment of MDMA-dependent individuals.

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List of Abbreviations

Barratt impulsiveness scale-11	BIS
Diffusion tensor imaging	DTI
Diagnostic and statistical manual for mental disorders, fourth edition	DSM-IV
Dopamine	DA
Drug Enforcement Administration	DEA
European Monitoring Centre for Drugs and Drug Addiction	EMCDDA
3,4-methylenedioxymethamphetamine	MDMA
National Institute of Drug Abuse	NIDA
Norepinephrine	NE
Positron Emission Tomography	PET
Polymerase Chain Reaction	PCR
RefSNP ID accession number	rs
Structured interview for DSM-IV	SCID
Selective serotonin reuptake inhibitors	SSRI
Serotonin	5-HT
Serotonin$_{2A}$ receptor	5-HT$_{2A}$R
Serotonergic	5-HTergic
Serotonin transporter	5-HTT
Single nucleotide polymorphism (base substitutions involving A, T, C, or G)	SNP
Tryptophan hydroxylase-2	TPH-2

Single alleles are represented by A, T, C or G; a *genotype* combining two alleles, one maternal and one paternal, will written as AA, AG, etc.

A SNP will be referred to by its position number, preceded by the wild type allele and followed by the substituted allele, e.g. T102C

Chapter 1: Introduction

GENERAL OVERVIEW & EPIDEMIOLOGY

The substituted amphetamine 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) is a popular drug of abuse. MDMA was synthesized and patented by Merck in 1912 as a chemical intermediary (Freudenmann, Oxler et al. 2006), but not used in further drug development. Recreational use of MDMA was reported in the 1970's (Gaston 1972) and peaked in the 1990's. In addition to recreational use, MDMA was touted as an adjunct for psychotherapy, and reported to facilitate communication, "improve understanding of life" and increase empathy (Greer and Tolbert 1986; Greer and Tolbert 1998; Parrott 2007). Therapeutic use of MDMA was mostly based on anecdotal evidence rather than rigorous trials (Doblin 2002), but it is interesting to note that the effects of MDMA that make it valuable in psychological treatments are the same that maintain its popularity as an drug of abuse. In 1986, concerns about MDMA-induced neurotoxicity in animals led to its re-classification as a schedule I drug (Federal Register 1986). This classification is given to drugs with a high abuse potential and no accepted medical use. However, the popularity of MDMA as an illicit drug continued an unabated rise until recently. The stabilization in usage trends, followed by mild decline in use (2006-2007) among youth in the US appears to be related to an increased awareness of the possible negative effects of MDMA (Johnston 2007).

MDMA is currently the most commonly used *club drug*, in reference to the setting (rave clubs) in which the use was initially reported and still occurs frequently. But MDMA has certainly expanded beyond the rave sub-culture, and is now used in multiple settings (Fendrich, Wislar et al. 2003). Results from the National Household Survey on Drug Use and Health indicates a prevalence of lifetime use of 4.6% and a mean age at initiation of 20 years (SAMHSA 2006). Prevalence of use is highest in the 15-24 age groups, and MDMA use is associated with an increased risk of alcohol and other illicit drug consumption (Strote, Lee et al. 2002; Parrott 2006).

The key appeal of MDMA as a recreational drug resides in its ability to enhance mood and evoke euphoria, elicit empathy and connectedness to others, and increase energy (Vollenweider, Gamma et al. 1998; Liechti, Geyer et al. 2001). Users also report that MDMA elicits alterations in auditory and visual perception, but does not evoke hallucinations per se. The ability of MDMA to evoke both stimulant and hallucinogen-like effects has prompted calls for a separate classification as an "entactogen", a word meaning "the touch within", in reference to the ability of users to get in touch with their feelings (Nichols 1986).

Well-known for the positive effects associated with its use, MDMA can nevertheless lead to negative, even life-threatening consequences. These may include cardiac arrhythmias, hypertension, hyperthermia, hyponatremia, liver problems, seizures, coma, and even death (Schifano, Oyefeso et al. 2003; Schifano, Oyefeso et al. 2003; Schifano 2004; Schifano, Corkery et al. 2006). The majority of MDMA-related adverse events are mild to moderate in severity, and MDMA administration in controlled laboratory settings has not been associated with serious events (Vollenweider, Gamma et al. 1998; Liechti, Gamma et al. 2001). Most emergency room visits secondary to MDMA-related problems are due to collapse, loss of consciousness, palpitations, dizziness or weakness, and anxiety (Liechti, Kunz et al. 2005). Other rare but serious events such as atrial fibrillation (Madhok, Boxer et al. 2003), seizures, paralysis (Goldstein, Mordish et al. 2006) and coma have been reported (Rosenson, Smollin et al. 2007). Conditions in clubs where MDMA is often used, with high ambient temperatures and concomitant polysubstance consumption, may amplify MDMA-related adverse events and lead to severe complications. This is true in particular of hyperthermia and hyponatremia, that may lead to the development of seizures or coma.

Overall, MDMA-related medical complications and fatalities have risen sharply in the past decade (Schifano, Corkery et al. 2006). From 1997 to 2006, MDMA was estimated to account for 4.1% of all drug related deaths, a three to four fold increase from its mid-1990's level (Schifano 2004; Schifano, Corkery et al. 2006). The increase may be

the result of its continued popularity and more widespread use, but may also be the result of increased awareness of its deleterious effects among health care workers. Unfortunately, awareness is not matched by advances in management of MDMA-related complications; treatment is usually aimed at symptomatic relief, and is not MDMA-specific. Further, vulnerability factors for the development of MDMA-related adverse events, whether genetic or epidemiological, are not fully understood.

MECHANISM OF ACTION OF MDMA AND THE CONTRIBUTION OF TRYPTOPHAN HYDROXYLASE, 5-HT TRANSPORTER, AND 5-HT_{2A}R:

MDMA binds to monoamine transporters, inducing non-exocytotic release of 5-HT, dopamine (DA), and norepinephrine (NE), with relatively more release of 5-HT than DA or NE (5-HT>NE>DA) (Stone, Stahl et al. 1986; Gibb, Stone et al. 1987; Rudnick and Wall 1992). 5-HT is synthesized in raphe nuclei cells, which project to almost all areas of the brain (Koella 1969; Snyder and Bennett 1976). 5-HT is involved in cognition, emotional and behavioral control, and acts through a variety of 5-HT receptors currently divide into seven classes (5-HT_1 to5-HT_7) (Hannon and Hoyer 2008). The proper functioning of the 5-HT system in the brain depends upon the integrity of three mechanisms: 5-HT synthesis and degradation, removal from the synaptic cleft, and proper functioning of 5-HT receptors. Following is an overview of three genes involved in 5-HT homeostasis (tryptophan hydroxylase, 5-HT transporter, and the 5-HT_{2A} receptor), their contribution to the effects of MDMA, and the potential role of genetic polymorphisms in this respect.

Tryptophan Hydroxylase-2 (TPH-2):

The rate limiting enzyme in the biosynthesis of serotonin is tryptophan hydroxylase, which catalyzes the conversion of tryptophan to 5-hydroxy-tryptophan. Two isoforms of the enzyme are known, TPH-1 and TPH-2, which share 71% amino acid

homology (Walther, Peter et al. 2003). TPH-2 is brain-specific, whereas TPH-1 is exclusively expressed in peripheral tissues (Zill, Buttner et al. 2004). In rats, acute administration of MDMA causes the loss of TPH activity (Stone, Stahl et al. 1986; Schmidt and Taylor 1990) particularly in the cortex and the hippocampus. New TPH-2 enzyme synthesis is required to overcome the effects of MDMA, and recovery of TPH activity is mediated by an increase in TPH mRNA expression (Garcia-Osta, Del Rio et al. 2004). In rats, MDMA-induced 5-HT depletion can be reversed by administration of the 5-HT precursor, L-5-hydroxytryptophan (Wang, Baumann et al. 2007). Longer-term impact of MDMA use on TPH-2 is not known, and human studies on TPH-2 and MDMA are lacking.

A single nucleotide polymorphism (SNP), rs 4570625 (at position 703, G→T substitution), located in the TPH-2 promoter region, may cause alterations in TPH function; rs 4570625 is inherited as part of a haplotype block, along with rs11178997 and rs11178998 (Scheuch, Lautenschlager et al. 2007). The G to T substitution is associated with a 10-22% reduction in promoter activity of the TPH-2 gene, thus reduced TPH-2 gene expression (Scheuch, Lautenschlager et al. 2007). The G and T alleles are co-dominant, hence carriers of the TT genotype would be expected to have the lowest level of TPH-2 expression. In humans, G703T variability has been shown to modulate amygdala responsiveness to emotional stimuli, with carriers of the T allele experiencing greater amygdala activation in response to happy or sad, but not neutral, stimuli (Canli, Congdon et al. 2005). Further, carriers of the TT genotype displayed impaired executive function (Reuter, Kuepper et al. 2007), lower impulse control represented by low harm avoidance scores (Reuter, Ott et al. 2007), and more emotional dysregulation (Gutknecht, Jacob et al. 2007; Herrmann, Huter et al. 2007) than carriers of GT or GG genotypes.

In the context of MDMA use, and given the need to regenerate new TPH enzyme following MDMA administration, a reduction in promoter function of TPH-2 may slow down, or even hinder recovery from MDMA-induced 5-HT depletion. However, no

studies in humans have yet focused on the influence of TPH-2 polymorphisms on the extent of 5-HT depletion, MDMA intake or related behaviors such as impulsivity.

Serotonin transporter (5-HTT):

The 5-HT transporter (5-HTT) is a member of the SLC6 gene family, the neurotransmitter sodium symporter family (Rudnick 2006). Reuptake by 5-HTT is the first step in terminating neurotransmitter action at extracellular receptor sites. MDMA binds to 5-HTT, causing 5-HT release into the synaptic space (Stone, Stahl et al. 1986). 5-HTT is the site of action of other illicit drugs (cocaine, amphetamines) as well as psychoactive medications, such as selective serotonin reuptake inhibitors antidepressants (SSRI) used to treat depression (Filip, Frankowska et al. 2005; Alessandro and Kato 2008).

Two polymorphisms that lead to lower levels of 5-HTT expression have been identified. A 44 base pair insertion/deletion in the promoter region of 5-HTT reduces transcriptional efficiency of the gene (Lesch, Bengel et al. 1996). The short allele "S" leads to decreased transcription of the serotonin transporter gene when compared to the long allele "L". A second polymorphism leading to reduced 5-HTT gene expression was reported within the L allele: a single base substitution A→G (rs25531), leads to two functional subtypes, L_A and L_G (Hu, Lipsky et al. 2006). L_A is high expressing, while L_G causes low gene expression, comparable in level to the S allele (Hu, Lipsky et al. 2006). The S, L_G and L_A alleles are co-dominant in action, thus carriers of the SS or SL_G genotypes have the lowest level of 5-HTT gene expression, whereas carriers of the L_AL_A genotype have the highest. Most literature has referred to L_A as L, whereas L_G is grouped with the S allele for analysis. Genotype LL is associated with a higher level of 5-HTT in platelets, postmortem brain, and living brain (Little, McLaughlin et al. 1998; Greenberg, Tolliver et al. 1999).

The effects of genetic variability in 5-HTT have been widely studied, and 5-HTT is now known to be a key locus of susceptibility for anxiety, depression and impulsive

behaviors (Caspi, Sugden et al. 2003; Canli and Lesch 2007; Paaver, Nordquist et al. 2007). Indeed, carriers of 5-HTT SS have an increased susceptibility to depression upon stressful life events (Caspi, Sugden et al. 2003). Subjective effects of MDMA are attenuated by blockade of 5-HTT (Liechti, Gamma et al. 2001). MDMA users carrying the SS genotype have been shown to have higher depression scores and more abnormalities in emotional processing than non-MDMA users with the same genotype (Roiser, Cook et al. 2005), whereas MDMA users carrying the LL genotype were comparable to non-MDMA users in terms of depression scores and emotional processing testing (Reneman, Schilt et al. 2006). Thus, the 5-HTT SS genotype appeared to confer a certain vulnerability to neurocognitive sequelae of MDMA. Further, in MDMA users, a significant reduction in cortical and sub-cortical 5-HTT density has been shown, with the extent of reduction possibly being dose dependent (McCann, Szabo et al. 1998), (Buchert, Thomasius et al. 2004). Non-uniform recovery of 5-HTT density may occur with abstinence (Reneman, Booij et al. 2001; Buchert, Thomasius et al. 2004).

The relationship between 5-HTT genetic variability and long-term 5-HTergic regulation is not well understood. There is preliminary evidence that 5-HTT SS and TPH-2 TT have additive effects on impulse control (Herrmann, Huter et al. 2007), but this has not been studied in the context of MDMA use. The influence of genetic variability in TPH-2 and 5-HTT on MDMA use patterns (quantity, frequency of intake) is also unknown, and is a focus of this project, as the extent of MDMA use may affect impulse control and neurocognitive sequelae of MDMA.

Serotonin $_{2A}$ receptor subtype (5-HT$_{2A}$R):

5-HT exerts its actions through the 5-HT receptors, a family of membrane bound receptors. 5-HT receptors belong to the G-protein coupled receptor family, with the exception of 5-HT$_3$ which is a ligand gated ion channel (reviewed in (Hoyer, Hannon et al. 2002)). 5-HT$_{2A}$R mediates some of the MDMA-induced behavioral responses and hyperthermia in rats and humans (Reneman, Endert et al. 2002; Bull, Hutson et al. 2003;

Bull, Hutson et al. 2004; Herin, Liu et al. 2005). Most of the effects of MDMA on 5-HT_{2A}R are thought to be mediated by the increased 5-HT concentration in the synaptic space upon MDMA administration. However, MDMA also has moderate efficacy/ low affinity at 5-HT_{2A}R (Gudelsky, Yamamoto et al. 1994; Nash, Roth et al. 1994). Human MDMA users display significantly lower 5-HT_{2A}R binding ratios on Positron Emission Tomography (PET) than controls in all cortical areas, with time-dependent recovery. 5-HT_{2A}R density positively correlated with duration of abstinence (Reneman, Endert et al. 2002).

MDMA-induced cell death in cortical neuronal cultures is modulated by 5-HT_{2A}R, with protective effects of selective 5-HT_{2A}R antagonists (Capela, Ruscher et al. 2006). MDMA-induced apoptosis is potentiated under hyperthermic conditions. The effect of MDMA on core body temperature is of direct consequence for human users, as the conditions at clubs and rave parties (high ambient temperature, physical exertion, dehydration), where MDMA is often used, may exacerbate MDMA-related adverse events.

Genetic polymorphisms in both the promoter and coding regions of 5-HT_{2A}R have been identified. Promoter polymorphism A1438G (rs 6311, ss7939), an A→G substitution(Spurlock, Heils et al. 1998), affects promoter activity, and therefore, levels of expression of 5-HT_{2A}R. The A allele is associated with significantly greater promoter activity compared to the G allele (Parsons, D'Souza et al. 2004), leading to greater levels of expression of 5-HT_{2A}R. The A1438G polymorphism is thought to alter promoter activity by virtue of its proximity to binding sites of transcription factors (Myers, Airey et al. 2007). A1438G is in linkage disequilibrium with T102C, a single base pair substitution (T→C) located in the non-coding region of exon 1. T102C does not lead to changes in protein coding (Arranz, Munro et al. 1998). The T allele of T102C (and, thus, the A allele at position 1438) is associated with higher 5-HT_{2A}R binding in postmortem brain samples (Turecki, Briere et al. 1999) and in human platelets (Khait, Huang et al. 2005). Carriers of the CC genotype of T102C (and thus the lower-expression form of

A1438 G, GG) were found to have increased impulsivity levels (Bjork, Moeller et al. 2002) and a higher likelihood of a past history of mood disorders (depression) with suicidal ideation (Du, Bakish et al. 2000), and nicotine dependence (do Prado-Lima, Chatkin et al. 2004). The relationship between variability at A1438G and MDMA use patterns has not been explored yet.

NEUROTOXICITY

MDMA administration leads to depletion of brain 5-HT ((Battaglia, Yeh et al. 1987; Schmidt 1987; O'Hearn, Battaglia et al. 1988), reviewed in(Baumann, Wang et al. 2007)) and reduction in markers of 5-HT nerve ending integrity (5-HTT). In humans, effects are limited to axons, sparing cell bodies (Commins, Vosmer et al. 1987; Ricaurte and McCann 2001; Ricaurte and McCann 2001). Neurotoxic damage appears to target fine fibers originating from the dorsal raphe nuclei, with little to no effect on beaded fibers originating from the median raphe nuclei (Paris and Cunningham 1992). The development and extent of MDMA-induced damage depend on the mode of drug administration, dose, and species. Neurotoxicity is predominantly 5-HTergic in humans, rats, but dopaminergic in mice (Green, Mechan et al. 2003). Variability in 5-HTergic genes may modulate individual ability to recover from 5-HT depletion. This is particularly true in the case of allelic variants leading to reduced gene expression, such as 5-HTT SS, TPH-2 TT, and 5-HT_{2A}R A1438G GG.

In animal studies, the neurotoxic effects of MDMA are potentiated by a variety of conditions mirroring typical human MDMA intake: MDMA-induced damage is amplified by higher ambient temperatures (McCann, Ricaurte et al. 2001; Green, Sanchez et al. 2004), and co-administration of other substances, including caffeine (McNamara, Kerans et al. 2006), methamphetamine (Clemens, Cornish et al. 2005), and ethanol (Izco, Orio et al. 2007). Most MDMA tablets sold on the US market lack in purity and contain other substances (Tanner-Smith 2006), and MDMA use tends to occur in overheated environments with extensive physical activity (dancing). Thus, based on the existing

body of knowledge, it is safe to conclude that typical human MDMA use patterns would maximize the potential for MDMA-induced damage (Parrott 2006).

The Netherlands XTC study, a large-scale prospective study of the effects of MDMA, using diffusion tensor imaging (an imaging tool ideally suited to reveal brain microstructural white matter organization), concluded that even minimal use of MDMA impacts axonal architecture (de Win, Reneman et al. 2007). This finding suggests that MDMA may cause neurotoxicity in humans at low doses, with the caveat that the changes found could represent a form of neuroadaptation, and not toxicity per se (Jager, de Win et al. 2008). Taken in stride with other results, existing knowledge supports the concerns that heavy use of MDMA, may translate to neurotoxicity in humans, whereas incidental intake may carry little risk (Jager, de Win et al. 2007). However, many questions remain about what defines "heavy" use, about individual susceptibility to MDMA-induced damage, and about genetic factors contributing to such susceptibility. It thus becomes important to identify factors associated with heavier MDMA use and epidemiological, genetic or psychological correlates of such use patterns.

MDMA DEPENDENCE AND NEUROCOGNITIVE DEFICITS:

The first reports of dependence to MDMA were anecdotal (Jansen 1999), and MDMA was long-perceived by most as a benign, non-addictive drug. Diagnosis of MDMA dependence is easily overlooked in clinical samples as MDMA does not have its separate diagnostic category in DSM-IV, and percentages of clinically significant patters (abuse or dependence according to the Diagnostic and Statistical manual of psychiatric disorders, DSM-IV) of use are low. However, longitudinal studies have shown that a small subset of MDMA users reliably develop abuse and dependence. The Early Developmental Stages of Psychopathology Study, a prospective naturalistic study focusing on adolescents reported a cumulative lifetime incidence of 0.6% for dependence and 1% for abuse for MDMA (von Sydow, Lieb et al. 2002). By applying strict DSM-IV

diagnostic algorithms, 34% of MDMA users in general adolescent substance user samples met criteria for MDMA abuse, and 43% met criteria for MDMA dependence (Cottler, Womack et al. 2001). In moderate to heavy self-report MDMA user groups, the percentage of individuals meeting criteria for abuse/dependence on MDMA reaches 60-70% (Thomasius, Petersen et al. 2005). The relationship between neurotoxicity and meeting diagnostic criteria for MDMA abuse/dependence has not been fully investigated, nor is the development of neurotoxicity in humans unequivocally proven as it is in animals; nevertheless, various lines of evidence suggest that the subgroup of MDMA users reporting high cumulative lifetime use and experiencing problems with MDMA as indicated by meeting DSM-IV criteria are at highest risk of suffering the sequelae of MDMA use.

Heavy MDMA users display a characteristic pattern of deficits on tests involving visuospatial orientation tasks (Fisk, Montgomery et al. 2005) or working memory tests (Wareing, Murphy et al. 2004). Impairment is also noted on verbal memory tests, motor function, and attention/vigilance tests (Morgan, McFie et al. 2002; Halpern, Pope et al. 2004) (Hanson and Luciana 2004), with reports of impairment lasting up to seven years after heavy use (Soar, Parrott et al. 2004). Proper assessment and diagnosis of MDMA abuse or dependence is crucial, as neurocognitive deficits relate to the extent of MDMA use, with subjects meeting DSM-IV criteria for those entities exhibit more severe deficits than casual MDMA users (Hanson and Luciana 2004). The relationship between the severity of neurocognitive deficits and MDMA-induced neuronal damage has not been fully elucidated yet, though some evidence suggests the magnitude of memory impairment may be related to altered 5-HT transmission (Reneman, Booij et al. 2000). Thus, although the involvement of 5-HTT, TPH-2, and 5-HT_{2A}R in mediating some effects of MDMA has been well-established, there are still gaps in knowledge of the contribution of these genes to behaviors or personality characteristics important to MDMA use. One such area is impulsivity, thought of as “acting without thinking”, or

acting without regard to consequences. Impulsivity is regulated by the 5-HT system (as detailed below), and is not completely characterized in the context of MDMA.

A CLOSER LOOK AT IMPULSIVITY AND ITS POTENTIAL ROLE IN MDMA USE

Impulsivity is a core symptom in many psychiatric disorders, and represents a vulnerability factor for the development of addictive disorders in general (Kreek, Nielsen et al. 2005; Verdejo-Garcia, Lawrence et al. 2008). Impulsivity has been defined as a "predisposition toward rapid, unplanned reactions to internal and external stimuli without regard to negative consequences of these reactions to the impulsive individual or others" (Moeller, Barratt et al. 2001). Impulsivity is a heterogeneous clinical construct ranging from impulsive decision making to intolerance of delay gratification (Winstanley, Eagle et al. 2006). Various methods to measure impulsivity have been designed, including laboratory and self-report measures. Baseline impulsivity is subject to significant genetic regulation, but an individual's impulsivity levels may be affected throughout the course of a lifetime by various experiences, including drug use (Allen, Moeller et al. 1998) or psychotherapy (Paris 2008). In the case of MDMA, baseline levels of impulsivity are not significantly associated with initiation of use of MDMA (de Win, Schilt et al. 2006), but an increased level of impulsivity was noted in a prospective cohort of young MDMA users following initiation of use (de Win, Reneman et al. 2007). While some studies have indicated that MDMA use is associated with increased behavioral impulsivity and impaired decision-making compared to polydrug non-MDMA users and drug-naïve controls (Morgan, Impallomeni et al. 2006; Quednow, Kuhn et al. 2007), other studies have failed to replicate these findings, and indicate similar levels of impulsivity among MDMA and other drug users (Hanson, Luciana et al. 2008). Interpretation of the results is limited by the type of drugs used by the control groups and the type of tests used to assess impulsivity levels. Taken together, the literature seems to indicate that elevated impulsivity is associated with increased drug use and poorer outcome in general (Allen,

Moeller et al. 1998; Hanson, Luciana et al. 2008), and, in the case of MDMA users, may be associated with increased MDMA-induced sequelae. An increased level of impulsivity, whether at baseline or resulting from drug use, may worsen the drug problem and decrease treatment retention. Indeed, individuals with higher impulsivity levels are more likely to drop out of treatment than their less impulsive counterparts (Moeller, Dougherty et al. 2001).

Impulsivity is regulated by the 5-HT system (Paaver, Nordquist et al. 2007; Walderhaug, Magnusson et al. 2007). Elevated levels of impulsivity have been associated with lower levels of 5-HT and its metabolites in the CNS, as in individuals with a history of violent suicides (reviewed in (Nomura and Nomura 2006)). Further, artificial decreases in 5-HT levels in the brain are accompanied by increased impulsivity (as in during an acute tryptophan depletion state), and this effect is modulated by 5-HTT genotypic variation (Walderhaug, Magnusson et al. 2007).

Animal studies show that impulsivity levels are modulated partially by 5-HT_{2A}R and 5-HT_{2C}R, which exert opposing actions (Winstanley, Theobald et al. 2004; Ross, Herin et al. 2006) and by 5-HTT. Human studies support the notion that the 5-HT system regulates impulsive behaviors, and provide evidence for the contribution of 5-HTT, 5-HT_{2A}R genetic variability in this respect; carriers of 5-HT_{2A}R 102 CC (that is, A1438G GG) genotype had a greater incidence of mood, substance use disorders, and impulsive patterns on cognitive testing compared to carriers of CT or TT genotype (Bjork, Moeller et al. 2002). Carriers of the short allele of 5-HTT (S) display more impulsivity when compared to LL carriers (Paaver, Nordquist et al. 2007). Additionally, MDMA users who are carriers of the 5-HTT SS show altered decision making when compared to carriers of non-SS genotypes (Roiser, Rogers et al. 2006). The variation is noted in the way the different groups weigh, and are attuned to, probabilities of gain and loss. Thus, carriers of genotypes associated with decreased gene expression of 5-HTT and 5-HT_{2A}R appear to be at higher risk for impulsivity, and at higher risk of experiencing MDMA-related sequelae. Based on the current knowledge, it is reasonable to expect an increase in certain

aspects of impulsive responding in the context of MDMA-induced 5-HT depletion. However, the intricacies of regulation of impulsivity by the 5-HTergic system render such a conclusion too simplistic, and the effect of various genetic variants in 5-HTergic genes on impulsive behaviors has to be carefully determined.

In summary, the present studies sought to explore the relationship of variants in TPH-2, 5-HTT, and 5-HT_{2A}R leading to lower gene expression on two behavioral areas likely to affect prognosis in human MDMA users: impulsivity and MDMA intake patterns. To this effect, we conducted an association study to examine the prevalence of alleles leading to lower gene expression in TPH-2, 5-HTT, and 5-HT_{2A}R, and the relationship of those alleles to impulsivity levels in MDMA users, polydrug users and controls. We then focused on the relationship of those alleles on MDMA intake and frequency in MDMA users. In the first study, we show that, while impulsivity levels are not significantly different among MDMA and polydrug users, MDMA users are less likely to be carriers of alleles leading to reduced gene expression of 5-HT_{2A}R A1438G (GG) and 5-HTT (SS). We further demonstrate, in the second study, that alleles of TPH-2, 5-HTT, and 5-HT_{2A}R leading to reduced gene expression do indeed modulate MDMA intake, and that carriers of these genetic variants use less MDMA than their counterparts carrying normal to high-expression alleles. These findings are the first to highlight the contribution of TPH-2, 5-HTT, and 5-HT_{2A}R genotypes in the choice of MDMA versus other drugs (chapter 2), and modulation of MDMA intake in humans (chapter 3), and emphasize the need to study the effect of these gene variants further.

Chapter 2: Prevalence of TPH-2, 5-HTT, & 5-HT_{2A}R alleles leading to decreased gene expression in MDMA users, polydrug users and drug-naïve controls and their relationship to impulsivity

INTRODUCTION

Susceptibility to development of drug abuse and dependence results from of the interaction between genes and environmental stressors, such as family conflict, abuse, or job loss (Kreek, Nielsen et al. 2005). Substantial contribution of genetic factors likely underlies individual differences in vulnerability the development of drug abuse, either in as substance-specific susceptibility factors, as in the case of cell adhesion genes, shown to be dysregulated in methamphetamine dependence (Uhl, Drgon et al. 2008), or as general risk factors for development of substance abuse in response to life stressors (Koob and Kreek 2007).

Candidate gene analysis has emerged as one scientific approach to determine susceptibility or protective genes for drug use based on pathophysiological hypotheses. Using candidate gene analysis, the prevalence of specific genetic variants in single or multiple genes is studied in drug user groups of interest compared to control groups. This approach is advantageous because it allows linking genes variants of interest to behavioral manifestations, such as impulsivity or severity of drug use. Preliminary evidence of genetic susceptibility factors for heroin dependence, cocaine & stimulant dependence has been published (Saiz, Garcia-Portilla et al. 2008) (Nielsen, Barral et al. 2008) (Nomura, Ujike et al. 2006; Williams, LaForge et al. 2007), but the role of genetic factors in MDMA use has not been studied yet.

The substituted amphetamine 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) is a popular illicit drug. MDMA is considered a "club drug", a term used to refer to drugs primarily used by young adults at dance clubs and raves. Club drug use is still rampant in these settings, but is also increasing in other social settings (Fendrich, Wislar et al. 2003). MDMA is the most widely used club drug in Europe and the US (EMCDDA 2007; Johnston 2007), and is popular for its stimulant and hallucinogen-like properties

(Nichols 1986). Chronic use and/or high doses of MDMA can result in adverse physical and psychological adverse effects, including cognitive and memory deficits (Hanson and Luciana 2004; Soar, Parrott et al. 2004; Thomasius, Petersen et al. 2005). Additionally, MDMA intake has been shown to increase impulsivity levels in users (de Win, Reneman et al. 2007). Impulsivity, characterized by acting without regard to consequences, represents a vulnerability factor for drug addiction in general (Winstanley, Theobald et al. 2004; Kreek, Nielsen et al. 2005; Walderhaug, Magnusson et al. 2007). Thus, the finding of increased impulsivity in the following MDMA use is of critical importance, as increased impulsivity levels are associated with risk-taking behaviors including unsafe sexual practices and reckless driving (Teese and Bradley 2008; Velez-Blasini 2008). There are somewhat conflicting results as to the level of impulsivity in MDMA users compared to other drug users, with earlier studies reporting that MDMA users are more impulsive than individuals using other drugs (Morgan 1998; Morgan, Impallomeni et al. 2006), and more recent evidence contradicting those findings, and showing equivalent impulsivity levels in MDMA users when compared to other drug users (Hanson, Luciana et al. 2008).

The mechanism of action of MDMA involves non-exocytotic release of monoamines (serotonin (5-HT), dopamine (DA), and norepinephrine (NE)) (Berger, Gu et al. 1992; Crespi, Mennini et al. 1997), with effects on 5-HT being greater in magnitude than DA or NE (Yamamoto, Nash et al. 1995; Kankaanpaa, Meririnne et al. 1998). Among 5-HTergic genes involved in the actions of MDMA, three genes directly involved in the molecular and behavioral effects of MDMA are of interest in this study: tryptophan hydroxylase-2 (TPH-2), 5-HT transporter (5-HTT), and the 5-HT_{2A} receptor (5-HT_{2A}R). Polymorphisms leading to decreased gene expression for each of the above genes have been identified. A single base pair substitution in the TPH-2 promoter gene at position 703 (G→T) leads to reduced gene expression in the TPH-2 gene (Zill, Buttner et al. 2004; Scheuch, Lautenschlager et al. 2007). Two promoter polymorphisms in the 5-HTT gene (a 44-base pair insertion/deletion leading to a “short” versus “long” allele (S vs L), and an

A→G single nucleotide substitution in the long allele (L_A versus L_G). The S and L_G alleles lead to reduced expression of the 5-HTT gene (Lesch, Bengel et al. 1996; Hu, Lipsky et al. 2006). Similarly, a promoter polymorphism in 5-HT_{2A}R (single base-pair substitution A→G at position 1438) is associated with decreased gene expression (Parsons, D'Souza et al. 2004; Myers, Airey et al. 2007). Past literature has referred to a single base pair substitution (T→C) at position 102, that is in complete linkage disequilibrium with A1438G, but has no functional significance (Spurlock, Heils et al. 1998). The effects of T102C variability documented in the literature, such as increased binding in human post-mortem brains (Turecki, Briere et al. 1999) and platelets (Khait, Huang et al. 2005) can thus be attributed to A1438G variability rather than T102C variability.

Impulsivity is regulated by different components of the 5-HTergic system, a fact of direct relevance to the field of MDMA research, as 5-HT is the neurotransmitter system predominantly affected by MDMA. There is evidence that genetic variability in 5-HTT modulates risk assessment and choices on a gambling task in MDMA users (Roiser, 2005), and that the alleles leading to decreased gene expression of TPH-2 (allele T) (Herrmann, Huter et al. 2007) and 5-HT_{2A}R A1438G/ T102C (alleles G/C) are associated with impaired executive functioning and increased impulsivity levels (Bjork, Moeller et al. 2002). However, the impact of those gene variants on impulsivity in MDMA users has not been studied.

We have chosen to use a candidate analysis approach to study the prevalence of alleles leading to decreased expression of TPH-2, 5-HT_{2A}R and 5-HTT in MDMA users, and link those gene variants to impulsivity in the context of MDMA. In this project, we hypothesized that alleles leading to reduced expression of TPH-2, 5-HT_{2A}R and 5-HTT, will be less prevalent in MDMA users compared to polydrug users and controls. The second aim of this project is to clarify discrepancies in impulsivity levels between polydrug users and MDMA users. We hypothesized impulsivity levels to be comparable between MDMA users and polydrug users, and expected a relationship between TPH-2,

5-HT_{2A}R A1438G and 5-HTT genotypes on impulsivity, with alleles leading to reduced expression of TPH-2, 5-HT_{2A}R and 5-HTT being associated with higher impulsivity levels.

METHODS

Subjects were recruited from 2002 to 2006 at the Substance Abuse Research Clinic, the University of Texas-Health Science Center-Houston. MDMA users consisted of non-treatment seeking individuals above 21 years of age, with a moderate to heavy MDMA use history. Inclusion criteria consisted of self-report moderate to heavy MDMA uses (greater than 20 lifetime uses), and a negative urine drug for any substances (excluding MDMA) at intake. Polydrug users (the equivalent of a sick-control group) consisted of individuals above 21 years of age, with a self- report of drug use excluding MDMA. Drug naïve controls reported no prior history of drug use, except for social alcohol use or cigarette smoking.

Diagnosis:

Axis I diagnoses were made using the Structured Clinical Interview for DSM-IV (SCID). The SCID is a diagnostic instrument initially developed for psychiatric research by Spitzer (Spitzer, Davies et al. 1990; Spitzer and Siegel 1990), and updated for DSM-IV (First, Donovan et al. 1996). The SCID was used to screen for DSM-IV Axis I disorders. Other information obtained from all participating subjects included background information (standard demographic and socioeconomic information), use of medications (use of prescription medications which have an effect on the central nervous system other than drugs of abuse was exclusionary), and a family history summary (psychiatric illness including alcohol and drug dependence). Information about drug use was collected using a drug history questionnaire, previously validated and used at our center, the drug history questionnaire was used as the primary tool for quantification of past drug use (current and lifetime consumption). MDMA users were further asked about details of their MDMA use, including lifetime consumption, usual number of tablets used per session, drugs usually used in conjunction with MDMA, and weekly, monthly, and yearly use frequencies.

The Barratt Impulsiveness Scale, version 11 (BIS) (Patton, Stanford et al. 1995) a 30 item self-report questionnaire which has been extensively used in several previous studies on impulsivity and aggression (Cherek, Moeller et al. 1997; Allen, Moeller et al. 1998), constituted the primary measure of impulsivity in this study. The BIS consists of three subscales yielding separate subscores (motor, attentional, and non-planning) that are summed into a total BIS score representing a global impulsivity score.

Exclusion criteria for all groups:

Subjects were excluded if they had current or past DSM-IV Axis I diagnosis of mood, psychosis, anxiety or other disorders (excluding substance abuse/dependence for MDMA and polydrug user groups, as above), any serious non-psychiatric medical illness requiring ongoing medical treatment or which could affect the central nervous system. Subjects' use of any medication which could affect the central nervous system, a positive HIV test, an I.Q. below 70 on the Wechsler Adult Scale of Intelligence, or a positive pregnancy test, as well as pacemakers, metal or electromechanical implants or metallic foreign bodies, were also exclusion criteria. (The study recruitment served an imaging component for which individuals had to be metal-free).

DNA Collection & Processing:

Blood samples were obtained by venipuncture, collected in EDTA tubes. Leucocytes (buffy coats) were separated and stored at -80°C until DNA extraction, which was done using the PureGene genomic DNA isolation kit (Gentra Systems, Minneapolis, MN). DNA quantitation was performed using PicoGreen® dsDNA Quantitation Kit (Molecular Probes, Eugene, Oregon). Genes of interest were amplified by PCR using a thermostable HotStar Taq enzyme (Qiagen, Valencia, CA) for TPH-2 and 5-HT_{2A}R. The FailSafe™ PCR System (Epicenter Biotechnotology, Madison, Wisconsin) was used for 5-HTT. PCR conditions, primers used are listed in tables 1&2. Characterization of PCR products was done by band detection on 2% agarose gel electrophoresis first, followed by amino acid sequencing to confirm amplimer identity.

Table 1: Primers used for PCR

Gene/ Polymorphism	Forward primer	Reverse primer
TPH-2/ G703T	CTCTGCATAGAGGCATCACAGG	TGCTGATGGGAGGGATAAGATC
5-HTT	TCCCTGTACCCCTCCTAGGATCGCT	TCTAGGTGGCACCAGAATCCCGCG
5-HT_{2A}R/ T102C	CTTCTACACCTCATCTGCTAC	CTCCTTACTTCATCTCCAGGA
5-HT_{2A}R/ A1438G	ACCACAGACGTGCCTAGCCA	CACAGTGCCACTTACCTACC
5-HT_{2A}R/ H452Y, A447V	CAATACCGGCTTTGGCCTACA	GTGGAAGGCACACTGAGCAA
5-HT_{2A}R/ I197V	GCCTCCATCATGCACCTCTG	GACAGTTCGTGCTTTCTGAAA
5-HT_{2A}R/ T25N	CAA GTTCTGGCTTAGACATGG	ACTGGACAGTCGACTCTGAA

Table 2: PCR conditions

Different PCR conditions were used for each polymorphism to optimize yield. Details of PCR cycles are detailed below.

Gene/ Polymorphism	Enzyme	Activation tº/time	Denaturation tº/time	Annealing tº/time	Elongation tº/time	Activation tº/time
TPH-2 G703T 5-HT_{2A}R A1438G	HotStar Taq	95ºC/ 15’	94ºC/30”	54ºC/30”	72ºC/1’	72ºC/10’
5-HT_{2A}R T102C	HotStar Taq	95ºC/ 15’	94ºC/30”	58ºC/30”	72ºC/1’	72ºC/10’
5-HTT	FailSafe™ PCR System	95 °C / 2’	95 °C/ 30”	65 °C/ 30”	68 °C/ 45”	68 °C/ 10’

Statistical analysis:

Analysis was conducted using the Number Crunching Statistical Software (NCSS, 2006, Kaysville, Utah). Bonferroni correction for type I error was done based on the family wise error rate. Thus, a test was considered significant if $\alpha < 0.05/(\#\text{tests per analysis family})$. Allele/genotype prevalence was compared using chi-square testing. When assumptions for chi-square testing were not met, a Fisher’s two-tailed exact test was used. Further analyses were conducted with genotypes grouped in two tiers (e.g. CC versus non-CC).

Since the two 5-HT_{2A}R promoter polymorphism studied (T102C, A1438G) are in linkage disequilibrium, only one was included in the analysis to decrease the number of multiple testing corrections needed. 5-HTT alleles were analyzed based on functionality of the allele, with L_G grouped with the S allele L_A. Thus, SL_G= SS, whereas L_AL_A= LL. BIS scores were compared using univariate ANOVA, then with ANCOVA using age, ethnicity and education as covariates as these differed among groups.

Calculations of Hardy-Weinberg equilibrium:

As a quality check method, genotype frequencies were tested for Hardy-Weinberg equilibrium using the HWSIM program. Comparison of observed versus expected genotypes was performed using a goodness of fit test (chi-square). Exact testing with a Monte-Carlo permutation (10,000 permutations) was used when assumptions for chi-square testing were violated (Yale University).

Data Missingness:

Genotype completion rates were compared between groups. Missingness rates differed for 5-HTT (χ^2=20.67, p=0.000005); polydrug users and drug-naïve controls had significantly more missing values than MDMA users. Therefore, a sensitivity analysis was conducted to determine the possibility of data imputation (Wayman 2003). Briefly, eight imputed data sets were created. Complete/imputed sets were then compared. The pattern of missingness was not ignorable and thus, missing data was not replaced. This may limit the generalizability of the any comparisons involving HTT among those two groups, as the drug naïve group had no individuals with the LL genotype.

RESULTS

Comparison of baseline characteristics of MDMA users, polydrug users and drug-naïve controls:

Baseline demographic characteristics are reported in Table 3. MDMA users, polydrug users, and drug-naïve controls were similar in gender distribution, but differed in age, ethnicity and educational attainment. Age was not normally distributed. MDMA users were significantly younger than drug-naïve controls and polydrug users (ANOVA, $F_{(1,145)}$= 14.85, p=0.00005; Kruskal Wallis, H=17.2, df=2, *p*=0.0002). Ethnicity differed overall among groups (χ^2= 14.4, p=0.025). Specifically, polydrug users included more subjects of African-American ethnicity than MDMA users (χ^2 = 12.2, *p*= 0.007). In contrast, no difference in ethnic distribution was found between MDMA users and drug-naïve controls (χ^2=3.23, p=0.35). Drug naïve controls included more subjects with a

college degree compared to both drug users groups (χ^2=12.2, p=0.007). MDMA and polydrug users did not differ in overall self-report patterns of drug use (detailed in table 4), with the exception of polydrug users reporting significantly more alcohol use than MDMA users. No gender differences were noted in any of the above characteristics.

Table 3: Baseline characteristics

Demographic characteristics of MDMA, polydrug users and drug-naïve controls are presented below. Significance level was set at 0.01 (0.05/5, Bonferroni-corrected). Gender, education and ethnicity were compared using χ^2 testing. Age was compared among the three groups using a one-way ANOVA.

Group	MDMA users	Drug-naïve controls	Polydrug users
Number of subjects	85	26	38
Gender *n(%)*			
Male	62 (72.9)	13 (50)	27 (71.1)
Female	23 (27.1)	13 (50)	11 (28.1)
*Age *mean(sd)*	24.4 (4.8)	33.3 (9.5)	29.1 (9.7)
*Education *n(%)*			
Less than HS	7 (8.2)	0	9 (30)
Highschool	26 (30.6)	1 (6.3)	12 (40)
Part college	37 (43.5)	8 (50)	7 23.3)
College & up	5 (5.9)	7 (43.7)	2 (6.7)
**Ethnicity *n(%)*			
Caucasian	29 (34.1)	6 (26.1)	3 (8.1)
African-American	39 (45.9)	1 (47.8)	28 (75.7)
Hispanic	11 (12.9)	3 (13.0)	3 (8.1)
Other	3 (3.5)	3 (13.0)	3 (8.1)

*significant at $p<0.01$
** $p=0.02$

Differences in Prevalence for 5-HTergic Polymorphisms:

The raw data for prevalence of TPH2, 5-HTT, and 5-HT_{2A}R polymorphisms are listed below (Table 5). MDMA users were less likely than controls to be carriers of the 5-HT_{2A}R T102C CC/ A1438G GG genotype, whereas control groups were more likely to carry the non-CC/non-GG genotype (overall χ^2=8.78, p=0.01). This finding was more pronounced when MDMA users were compared to drug-naïve controls alone (χ^2=7.96, *p*=0.004). However, the difference in prevalence only trended towards significance when MDMA users were compared to polydrug users alone (χ^2=3.24, *p*= 0.07).

MDMA users were less likely to be carriers of the 5-HTT SS genotype compared to controls (χ^2=7.98, p=0.01) who were more likely to carry a non-SS genotype. Results were comparable when MDMA users were compared to polydrug users alone (χ^2=6.9, p=0.008), but not when MDMA users were compared to drug-naïve controls (χ^2=2.3, *p*=0.13). No difference was found in the prevalence of TPH-2 among MDMA users, polydrug users, or drug-naïve controls (overall χ^2= 2.72, *p*= 0.3).

Table 4: Comparison of drug use between MDMA and polydrug users

	MDMA users *n(%)*	Polydrug users *n(%)*	Statistical test
Cannabis	37 (80.4)	34 (100)	Fisher's exact test, p=0.006
Alcohol	23* (50)	34* (100)	Fisher's exact test, p =0.000001
Cocaine	17 (37)	13 (38.2)	χ^2=0.01, p=0.9
Benzodiazepines	4* (8.7)	12* (35.3)	Fisher's exact test, p=0.003
Methamphetamine	11 (23.9)	1 (2.9)	Fisher's exact test, p=0.01
Hallucinogens	13 (28.3)	2 (5.9)	Fisher's exact test, p=0.01
Opiates	7 (15.2)	7 (20.6)	χ^2=0.39, p=0.5

* Significant at <0.006

Self-report use of various drug classes was compared among MDMA and polydrug users using χ^2 tests. Significance level was set at p= 0.006 after Bonferroni correction (0.05/8). A Fisher's exact test was used when assumptions for χ^2 were not met (cell size <5).

Table 5: 5-HT gene polymorphisms and their distribution among MDMA, polydrug users, and drug naïve control groups

	MDMA			Polydrug			Drug-naïve		
5-HT_{2A}R promoter polymorphisms									
T102C	CC	CT	TT	CC	CT	TT	CC	CT	TT
	26	49	10	16	19	3	14	8	3
A1438G	GG	GA	AA	GG	GA	AA	GG	GA	AA
	20	48	7	17	18	3	11	10	4
TPH-2 promoter polymorphism									
G703T	GG	GT	TT	GG	GT	TT	GG	GT	TT
	39	38	7	15	18	4	8	15	1
5- HTT promoter polymorphism									
$L=L_A=L_G$	LL	LS	SS	LL	LS	SS	LL	LS	SS
Analysis based on morphology	21	34	26	3	7	14	2	8	6
$L=L_A$, L_G=S	LL	LS	SS	LL	LS	SS	LL	LS	SS
Analysis based on functionality	11	34	36	0	6	18	1	5	11

Comparison of impulsivity scores among MDMA users, polydrug users and drug-naïve controls

Self-report impulsivity levels measured by BIS scores and subscores were compared among groups (Table 6). BIS scores were significantly higher among MDMA users and polydrug users compared to drug naïve controls on the non-planning subscore (ANOVA, $F_{(2,125)}$= 4.31, p=0.01), on motor subscore (ANOVA, $F_{(2,125)}$=6.85, p=0.001), attentional subscore (ANOVA $F_{(2,125)}$=6.63, p=0.002), and BIS total score (ANOVA, $F_{(2,125)}$=8.56, p=0.0003). After controlling for age, ethnicity and education, group differences persisted in motor score (ANCOVA, $F_{(2,106)}$= 3.95, p=0.02) and total BIS score ($F_{(2,106)}$=3.51, p=0.03). Scores did not differ significantly among MDMA and polydrug user groups (Figure 1).

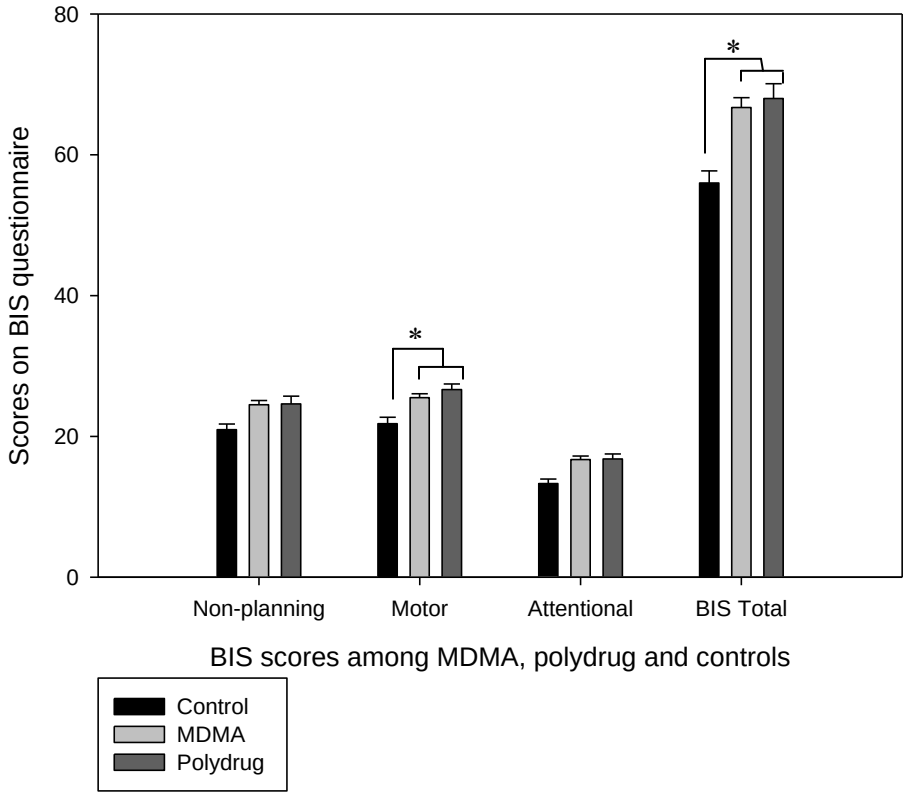
Controlling for age, ethnicity and education, an analysis of covariance failed to show a relationship between TPH-2, 5-HTT or 5-HT_{2A}R genotype and impulsivity scores. However, as shown in figures 2 to 4, the relationship of genotype to impulsivity

levels was not uniform across groups and gender, although the differences did not reach statistical significance. No relationship between gender and BIS total scores (ANCOVA, $F_{(2,106)}$=0.42, p=0.6) and other subscores was detected, although females appeared to have a lower impulsivity level than males (Figure 5).

Table 6: Impulsivity levels by group-BIS scores and subscores

Group / Score *(mean)(sd)*	MDMA users *(n=83)*	Polydrug users *(n=23)*	Drug-naïve controls *(n=22)*
Motor score	25.5 (5)	26.6 (4.3)	21.8 (4.2)
Non-planning score	24.5 (5.5)	24.6 (5.1)	20.9 (3.7)
Attentional score	16.7 (4.4)	16.8 (3.4)	13.3 (3.0)
Total BIS score	66.7 (12.5)	68.0 (9.7)	56.0 (7.9)

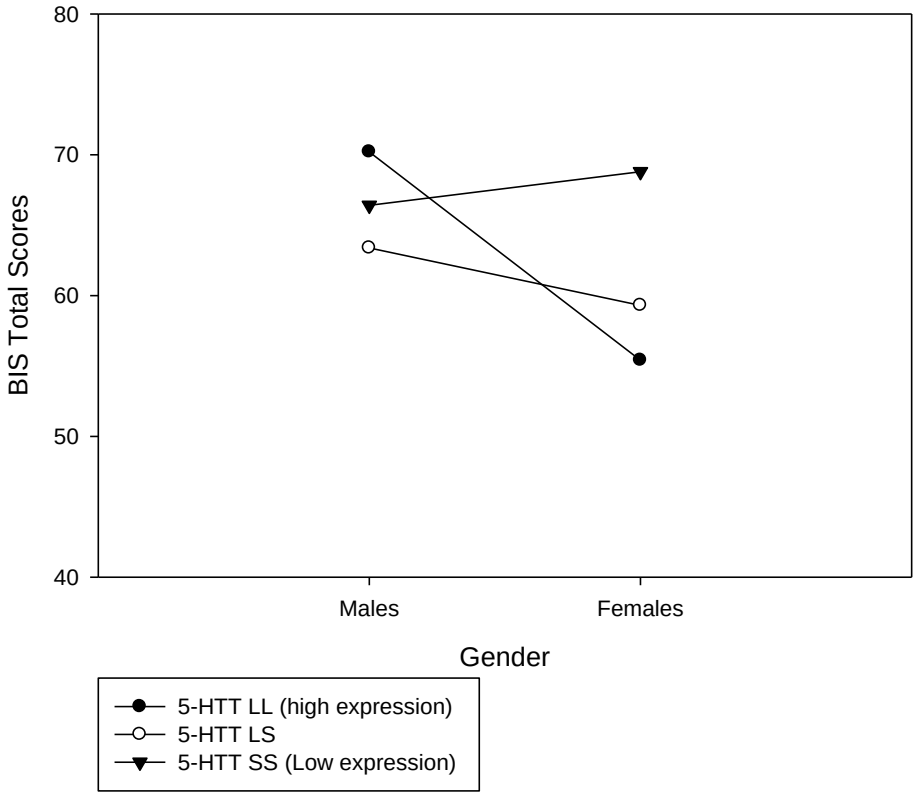
Figure 1: BIS Impulsivity Score MDMA users, polydrug users, and drug-naïve controls



*p<0.05

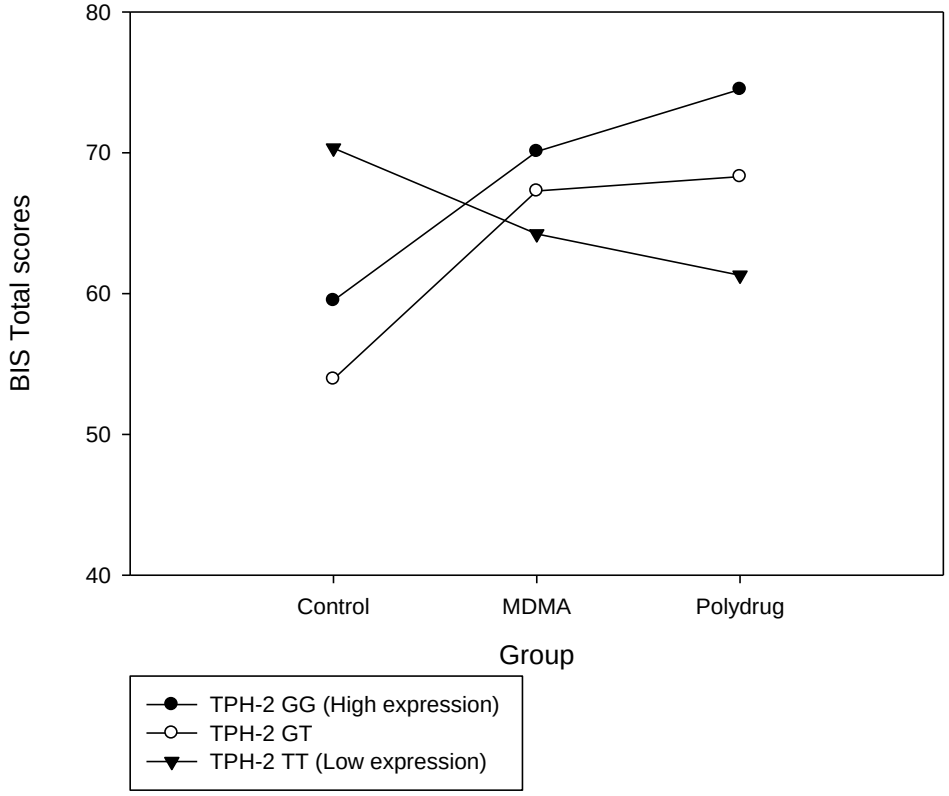
Impulsivity levels were compared among all three groups by an ANCOVA controlling for education, age and ethnicity. MDMA and polydrug users were more impulsive than drug-naïve controls.

Figure 2: Relationship between 5-HTT Variability and impulsivity levels stratified by gender in MDMA users, polydrug users, and drug-naïve controls



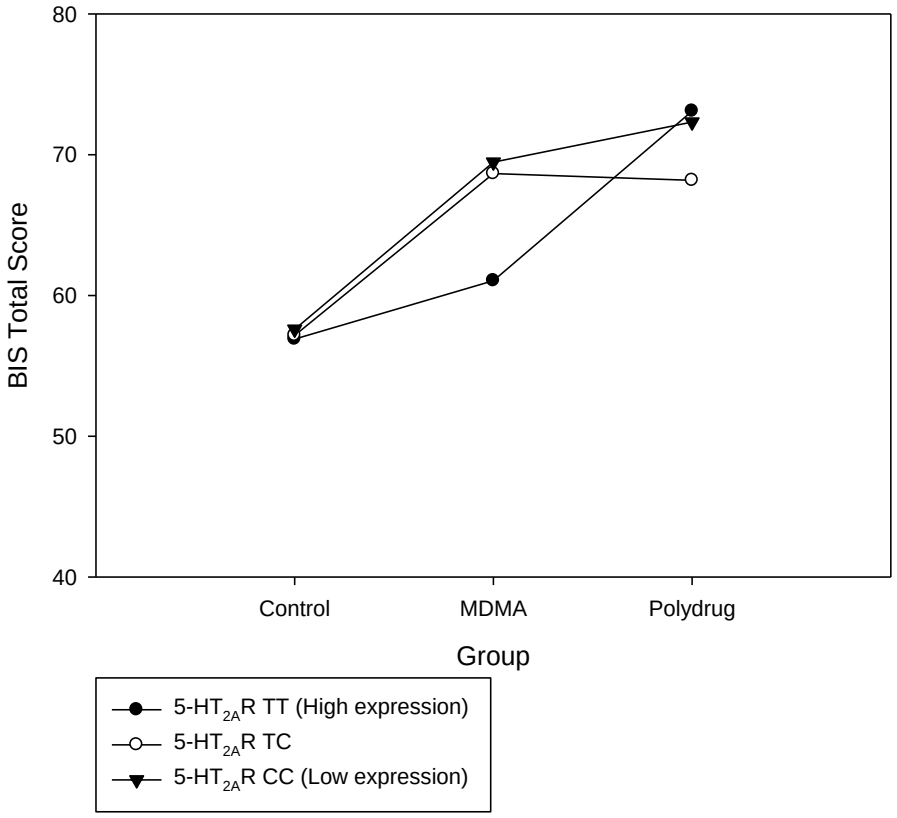
5-HTT genotype appeared to modulate LL impulsivity levels differentially among genders. Error bars were omitted for clarity. While male carriers of 5-HTT LL had higher impulsivity levels than males carrying non-LL genotypes, the opposite trend was found for females. This finding suggests the need to study the relationship between gender and impulsivity further.

Figure 3: Relationship between TPH-2 Variability and impulsivity levels in MDMA users, polydrug users, and drug-naïve controls



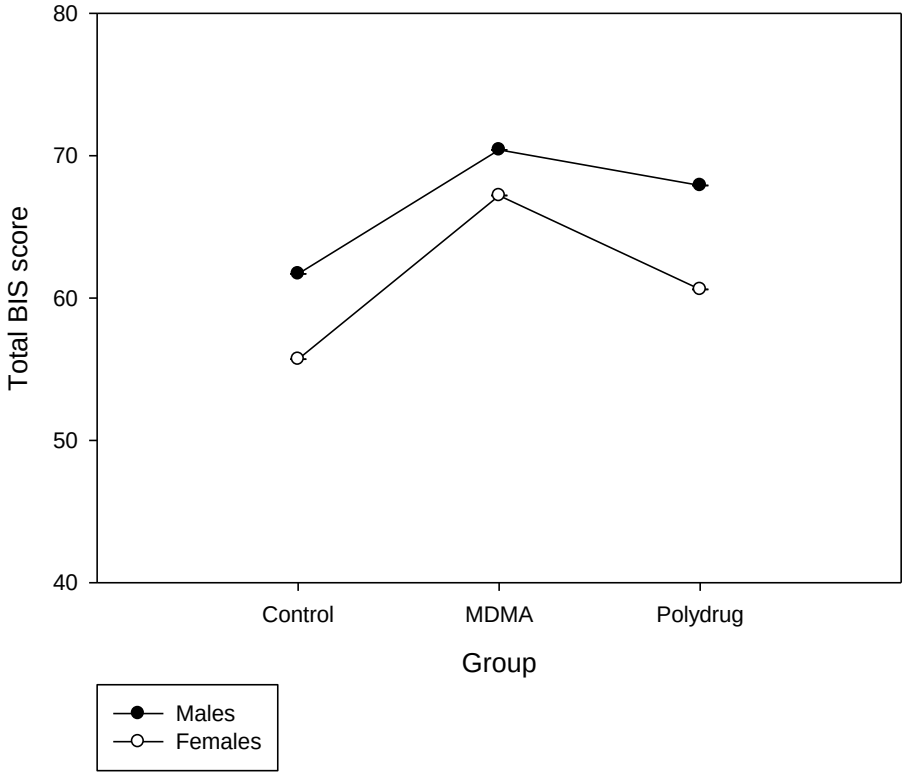
BIS total scores were compared using an ANCOVA, controlling for ethnicity, education and age. Error bars were omitted for clarity. Carriers of TPH-2 TT genotype belonging to the control groups reported higher impulsivity levels than carriers of non-TT genotypes. However, the opposite was true for MDMA and polydrug users, where the TPH-2 TT genotype was associated with lower impulsivity level. This finding was non-significant, but suggests the effect of TPH-2 polymorphism may depend on the drugs used by an individual.

Figure 4: Relationship between 5-HT_{2A}R T102C/A1438G variability and impulsivity levels in MDMA users, polydrug users, and controls



Impulsivity levels as measured by total BIS scores were compared among MDMA, polydrug users, and controls as a function of 5-HT_{2A}R T102C/A1438G polymorphism using ANCOVA. Error bars were omitted for clarity. The effect of 5-HT_{2A}R T102C/A1438G polymorphism is not uniform between MDMA and polydrug users. Although this relationship was not significant, it suggests a more complex relation between genotype and drugs than previously known.

Figure 5: Relationship between Total BIS Scores, Group and Gender



Impulsivity scores were compared using a two-way ANOVA. As shown in the figure, there was a trend for female subjects to have lower impulsivity scores than males, although this finding did not reach statistical significance. Error bars are omitted for clarity.

DISCUSSION

The present study sought to compare the prevalence of allelic variants of TPH-2, 5-HTT and 5-HT_{2A}R leading to reduced gene expression among MDMA users versus two control groups (polydrug users, drug naïve), and investigate the relationship between those alleles and self-reported impulsivity levels. The genes TPH-2, 5-HTT and 5-HT_{2A}R were chosen based on their prominent role in the molecular and behavioral effects of MDMA. Congruent with our hypothesis, alleles leading to decreased gene expression of 5-HTT and 5-HT_{2A}R A1438G were less prevalent in MDMA users compared to both control groups. Impulsivity levels were comparable among MDMA and polydrug users, yet higher than drug-naïve controls.

The differential allele prevalence between groups suggests that the alleles studied (5-HTT S, and 5-HT_{2A}R T102C/A1438G C/G) may be associated with a decreased propensity to use MDMA relative to other drugs. No prior studies have documented differential allelic expression in MDMA users, and our findings raise the question of whether 5-HT_{2A}R T102C/A1438G CC/GG play a role in drug choices in humans (choosing MDMA versus other drugs), and if so, whether the effects is MDMA-specific. Decreased prevalence of the GG genotype of 5-HT_{2A}R A1438G in heroin dependent individuals compared to controls was reported recently (Saiz, Garcia-Portilla et al. 2008); taken together with our finding that MDMA users are less likely to be carriers of A1438G GG, this similarity between heroin and MDMA users suggests that the effects of the GG genotype of 5-HT_{2A}R A1438G on drug use may not be MDMA-specific.

The findings of decreased 5-HTT SS genotypes among MDMA users compared to polydrug users and controls has not been replicated in the literature, but parallels the finding with 5-HT_{2A}R discussed above. The sample size in this study was not large enough to ascertain whether there are any additive effects of alleles leading to reduced gene expression (5-HT_{2A}R A1438G/T102C CC/GG, and 5-HTT SS), although evidence from prior studies suggest this may be the case for certain 5-HTergic genes, as TPH-2 TT and 5-HTT SS have additive effects on impulsive decision-making (Herrmann, Huter et

al. 2007). Thus, our results constitute preliminary results that need to be investigated further in larger samples.

In contrast to the differences in genotype detected among groups, we failed to find differences in impulsivity levels between MDMA and polydrug users. Despite inconsistencies in the early literature on impulsivity in the context of MDMA, our finding of comparable impulsivity levels among MDMA and polydrug user groups is in line with recent findings. Hanson et al (Hanson, Luciana et al. 2008), in a carefully matched sample of MDMA and polydrug users, and using a composite impulsivity score that was created based on results of the tests utilized, found no difference in impulsivity levels among MDMA and other drug users on a variety of impulsivity measures. Discrepant findings in previous studies may have resulted from variations in drugs used by the polydrug control groups. For example, the first studies reporting elevated impulsivity levels in MDMA users recruited polydrug users with little to no cocaine or amphetamine use (Morgan 1998). Additionally, the use of a wide variety of instruments to measure impulsivity makes results from various studies difficult to compare. Last but not least, a synergistic effect between lowered 5-HT levels and novel situations on impulsivity has been shown (Walderhaug, Landro et al. 2007), raising the possibility that self-report, questionnaire-type measures may not adequately capture the full span of MDMA-related impulsivity changes.

The main limitation of this study is the relatively small sample size in the control groups. The small sample size limited the prospects of studying additional exploratory interactions among the genotypes of interest, and limited the possibility of making meaningful conclusions about gender differences. This issue was further complicated by the low frequency of some alleles, such as TPH-2 (T), which limited statistical power. Lastly, change in equipment by our core laboratory led to a higher rate of missing data for 5-HTT among control groups, perhaps limiting the generalizability of our findings regarding 5-HTT.

In summary, this study is the first to demonstrate that MDMA users are less likely to carry 5-HTT (SS), and 5-HT_{2A}R T102C/A1438G CC/GG than polydrug users or drug-naïve controls, and highlights the need to study the contribution of genotype to choices MDMA use versus other drugs. The study also hints to gender and genotype influences on impulsivity which warrant further investigation.

Chapter 3: Relationship between alleles leading to reduced expression of TPH-2, 5-HTT, & 5-HT_{2A}R T102C/A1438G and MDMA use, impulsivity in moderate to heavy MDMA users

INTRODUCTION

MDMA is a popular drug of abuse widely used by teenagers and young adults (SAMHSA 2006; EMCDDA 2007), with complex pharmacological actions targeting monoamine neurotransmitter systems. MDMA has stimulant effects and hallucinogen-like effects, leading to altered visual, auditory and time perception, but is unlikely to produce frank hallucinations (Nichols 1986). Preclinical literature indicates that MDMA predominantly affects the 5-HTergic system and causes global 5-HT depletion (Schmidt 1987; Green, Mechan et al. 2003; Baumann, Wang et al. 2007), and data from human studies, although more limited, is largely in agreement with preclinical evidence (Reneman, Booij et al. 2000; Reneman, Booij et al. 2001; Reneman, Endert et al. 2002; de Win, Reneman et al. 2004).

Three genes, TPH-2, 5-HTT, and 5-HT_{2A}R are among 5-HTergic genes that mediate behavioral and molecular effects of MDMA. Polymorphisms leading to decreased gene expression for each of those genes have been identified. Single nucleotide polymorphisms in TPH-2 (G→T) (Zill, Buttner et al. 2004) and 5-HT_{2A}R (T→C at position 102, A→G at position 1438) are associated with decreased levels of gene expression (Spurlock, Heils et al. 1998; Parsons, D'Souza et al. 2004; Myers, Airey et al. 2007). Two polymorphisms in the 5-HTT gene promoter are associated with decreased expression levels. The first is a 44 base-pair deletion in the promoter leading to a "short" allele (S). The S allele leads to lower gene expression compared to the "long" allele, L (Lesch, Bengel et al. 1996). Decreased 5-HTT expression levels also result from a single base pair substitution (A→G) in the promoter resulting in the L_G allele that has comparable expression levels to the S allele (Hu, Lipsky et al. 2006).

MDMA users report increased tenderness and affection towards others, improved mood, increased energy (Vollenweider, Gamma et al. 1998), and increased sexual

arousal, but may also complain of nervousness, irritability, and paranoid feelings (Baylen and Rosenberg 2006). Concern about harmful effects of MDMA stems from various lines of evidence highlighting its potential to cause 5-HTergic neurotoxicity (Battaglia, Yeh et al. 1987; Schmidt 1987; O'Hearn, Battaglia et al. 1988), negative physical and psychological sequelae (Schifano 2004; Baylen and Rosenberg 2006), as well as memory and cognitive deficits (Reneman, Booij et al. 2000; Soar, Parrott et al. 2004; Thomasius, Petersen et al. 2005). Individual determinants of the development of negative sequelae following MDMA use are poorly understood, and have not been studied in light of genetic variability in TPH-2, 5-HTT, and 5-HT_{2A}R.

Little is known about factors promoting the initiation and maintenance of MDMA use in humans. MDMA use, as with other drug use, results from the interaction of genes and environmental factors (Koob and Kreek 2007; Koob and Le Moal 2008); however, in the field of MDMA research, most studies have focused on epidemiological factors influencing use, while the genetic underpinnings of the response to MDMA in humans are largely unknown.

Evidence that genetic variability impacts drug intake comes from alcoholism studies. Adolescents carrying 5-HTT LL (the allele leading to higher level of 5-HTT gene expression), were found to have an increased alcohol consumption and decreased sensitivity to the effects of alcohol (Hinckers, Laucht et al. 2006). While this has not been studied yet for MDMA, surveys of MDMA users indicate that following initial use of MDMA, an important factor associated with heavy use include having used more than one MDMA pill upon initiation of use (Sterk, Theall et al. 2007). The link between the amount used and the level of response to MDMA is unknown, as are potential contributing genetic factors.

Among the behavioral correlates of MDMA use, impulsivity has also received attention in the context of MDMA. Impulsivity, defined as a predisposition to action without foresight (Moeller, Barratt et al. 2001), constitutes a vulnerability factor for the development of drug dependence (Verdejo-Garcia, Lawrence et al. 2008). Impulsivity is

a multi-factorial concept, and tasks measuring discrete processes drawing on different neural circuits only correlate weakly with each other (Evenden 1999). Thus, although a large body of literature documents the relation between lowered 5-HT levels in the CNS (Nomura, Ujike et al. 2006) and increased impulsivity, the effect of lowering 5-HT levels in the CNS using tryptophan depletion, a widely used laboratory strategy (Bell, Hood et al. 2005), is limited to certain types of impulsive responding (Clark, Roiser et al. 2005).

Baseline impulsivity levels are not associated with initiation of MDMA use, but increases in impulsivity were found after MDMA use in a prospective cohort study (de Win, Schilt et al. 2006), raising questions about interactions between MDMA-induced 5-HT depletion and the effects of MDMA. Polymorphisms associated with decreased levels of gene expression in key 5-HTergic genes (TPH-2 T allele, 5-HTT S allele, and 5-HT_{2A}R A1438G/T102C C allele) are associated with increased impulsivity and impaired executive functioning (Bjork, Moeller et al. 2002; Paaver, Nordquist et al. 2007; Reuter, Ott et al. 2007).

The aim of this study was to address the effect of genetic variability of TPH-2, 5-HTT, and 5-HT_{2A}R A1438G/T102C on MDMA use patterns and self-report impulsivity levels. We have previously shown (Chapter 2) that MDMA users expressed less 5-HTT SS, and 5-HT_{2A}R T102C CC/ A1438G GG compared to controls, suggesting that alleles leading to decreased gene expression, and subsequent altered 5-HTergic transmission may modulate drug preference. Whether those alleles also affect MDMA use patterns and impulsivity among MDMA users is unknown. Thus, this study sought to clarify the relationship of TPH-2 TT, 5-HTT SS, and 5-HT_{2A}R A1438G/ T102C CC/GG on MDMA intake and impulsivity, and hypothesized that these alleles will be associated with lower MDMA use and higher impulsivity levels.

METHODS

Non-treatment seeking individuals (males or females) above 21 years of age, with a moderate to heavy (greater than 20 lifetime uses) MDMA use history were recruited via

newspaper advertisements and flyers in rave clubs. The MDMA use questionnaire, designed for this study, was used to get information about cumulative lifetime MDMA intake, usual amount per sitting, maximal amount taken in one sitting, date of last use, and frequency of use per week, month, and year. This questionnaire also queried about other drug usually used with MDMA. Subjects were excluded if they had a co-morbid diagnosis other than that of alcohol or cannabis abuse or a serious non-psychiatric medical illness requiring ongoing medical treatment or which could affect the central nervous system. Other exclusion criteria included use of any medication which could affect the central nervous system, a positive HIV test, I.Q. below 70 on the Wechsler Adult Scale of Intelligence, or a positive pregnancy test.

The Barratt Impulsiveness Scale (BIS-11) (Patton, Stanford et al. 1995), a 30 item self-report questionnaire which has been used in several previous studies on impulsivity and aggression (Cherek, Moeller et al. 1997; Allen, Moeller et al. 1998) was used as the primary measure of impulsivity in this study. The BIS consists of three subscales: motor, attentional, and non-planning scores.

DNA was extracted from lymphoblastoid cell lines using the PureGene genomic DNA isolation kit (Gentra Systems, Minneapolis, MN), and quantitated using PicoGreen® dsDNA Quantitation Kit (Molecular Probes, Eugene, Oregon). TPH-2 G703T, 5-HT_{2A}R A1438G/T102C were amplified by PCR using a thermostable HotStar Taq enzyme (Qiagen, Valencia, California). The FailSafe™ PCR System (Epicenter Biotechnotology, Madison, Wisconsin) was used for 5-HTT. Hardy Weinberg equilibrium was checked by HMSWIM, a specialized software for this purpose (Yale University). All genes studied were in Hardy-Weinberg equilibrium.

Statistical analysis was conducted using the Number Crunching Statistical Software (NCSS, 2006, Kaysville, Utah). BIS scores were analyzed by one-way ANOVA, and the effect of genotype on impulsivity was studied using the General Linear Model function of NCSS (for 2-way ANOVA). MDMA use parameters (lifetime cumulative intake, maximum amount ever taken, usual amount per sitting, frequency per

week, month and year, and days since last use) were non-normally distributed, therefore a Kruskal-Wallis non-parametric ANOVA was used to analyze genotype effects on MDMA use parameters. Further analyses were conducted for genotypes combined in two tiers (TPH-2 TT versus non-TT, 5-HTT SS versus non-SS, and 5-HT_{2A}R A1438G GG versus non-GG): a t-test was used for this purpose. When assumptions for t-test were not met, a Mann-Whitney-U rank sum test was used. Please note that the two polymorphisms of 5-HT_{2A}R, T102C and A1438G, are in linkage disequilibrium, thus are always inherited together. Only the A1438G allele is reported, to decrease the number of corrections needed for multiple testing. Results for 5-HTT are reported as S or L, with alleles S and L_G combined as S, and L_A reported as L.

RESULTS

A total of 85 individuals with a history of heavy MDMA use were recruited, with a mean of ~228 lifetime MDMA use. The sample consisted of 63 males and 22 females, with a mean age of 24.4 years (±4.8). Most subjects identified themselves as African-American (45.9%), and 34% as Caucasian. Almost half the sample reported some college education or completion of a college degree (49.4%). Males and females were similar on all demographic and MDMA use variables examined, and differed only in the maximal amount used per sitting, although this finding was no longer significant after Bonferroni correction (Wilcoxon rank sum test, z=-2.29, p=0.02). Details of self-reported MDMA use are in listed in Table 7. Most subjects reported other drug use concomitant with MDMA, and details are listed in Table 8.

Relationship between TPH-2, 5-HTT and 5-HT_{2A}R A1438G polymorphisms on amount of MDMA used:

The raw data are presented in Table 9. A trend for association between lower MDMA use and alleles leading to lower gene expression (TPH-2 TT, 5-HTT SS, and A1438G GG) was noted. Cumulative lifetime intake trended towards significance when stratified by 5-HTT genotype ($F_{(2,71)}$= 2.98, p=0.057; Kruskal Wallis, H= 5.84, df= 2, *p*=

0.053) with LL carriers reporting higher lifetime use than SS or SL carriers. A similar finding was noted for maximal amount used per sitting and 5-HTT genotype ($F_{(2,73)}$=2.57, p=0.08; Kruskal Wallis, H=5.9, df= 2, *p*= 0.052). Results for A1438G and TPH-2 paralleled those for 5-HTT, with alleles leading to reduced gene expression being associated with decreased use of MDMA, but the relationship did not reach significance (*p*= 0.22 for TPH-2, and *p*= 0.1 for A1438G). Figures 6&7 depict the relation between the alleles of interest and MDMA use variables.

Relationship between TPH-2, 5-HTT and 5-HT_{2A}R A1438G polymorphisms on frequency of MDMA use:

Use frequency differed significantly by 5-HTT genotypes, with SL carriers reporting lower use than LL carriers for use per month ($F_{(2,47)}$=6.47, p=0.003; Kruskal Wallis H=6.6, df=2, *p*= 0.03), or use per year ($F_{(2,34)}$=6.56, p= 0.004; Kruskal Wallis H= 8.29, df=2, *p*= 0.02). SS carriers reported intermediate use frequency values, lower than LL but higher than SL carriers.

Carriers of the 5-HT_{2A}R A1438G GG genotype had lower use frequencies than AG or AA carriers, but this finding did not reach statistical significance ($F_{(2,36)}$= 2.72, *p*=0.07; Kruskal Wallis H=3.17, df=2, *p*=0.2). Although the relationship did not reach statistical significance, the magnitude of difference in use frequencies needs to be noted, as individual carrying the 5-HT_{2A}R A1438G AA genotype (i.e. genotype leading to normal to high gene expression) reported ~ 3 times as much use of MDMA per year relative to GG carriers. For TPH-2, MDMA use frequency per week, month and year displayed a similar trend to 5-HTT and 5-HT_{2A}R A1438G that failed to reach statistical significance, with carriers of TT genotype (genotype leading to reduced TPH-2 expression) reporting less use than carriers of GG and TG genotypes (see figure 8).

Relationship between TPH-2, 5-HTT and 5-HT_{2A}R A1438G polymorphisms on impulsivity measures:

BIS scores and subscores were normally distributed. Heterozygous individuals (carriers of 5-HTT SL) had the lowest BIS scores, followed by carriers of 5-HTT SS then 5-HTT LL (see figure 9 & table 10). This finding reached statistical significance for the effect of 5-HTT genotypes on BIS total score (F=6.72, p=0.002) and attentional subscore (F=7.12, p=0.001). For TPH-2, TT carriers had lower BIS scores than TG or GG carriers, but the trend was not significant ($F_{(2,79)}$=0.24, *p*=0.78). For 5-HT_{2A}R A1438G, carriers of the GG and AG genotypes reported higher impulsivity levels than carriers of the normal to high expression allele AA. This finding did not reach statistical significance ($F_{(2,80)}$=1.11, *p*=0.3).

Both genders had similar patterns of BIS scores by genotypes. A two-way ANOVA examining the effects of gender and 5-HTT genotype showed a significant effect of 5-HTT ($F_{(2,73)}$=3.65, p=0.03) but not of gender ($F_{(2,73)}$=1.1, p=0.29). No interaction was noted between gender and genotype.

Relationship between MDMA use frequency to impulsivity levels and amounts of MDMA used:

As shown in figure 10, most subjects reported using less than 10 days per month (Mean = 5.5 days/month, median = 3 days/ month, range 0-30, standard deviation= 6.0). users reporting a frequency of use <8 days per/month were considered as “casual” users, whereas users reporting a higher frequency were termed “heavy users”. Heavy users reported higher impulsivity levels than casual users (t=3.5, *p*=0.0009), and more than 3 times more cumulative lifetime MDMA use than casual users (mean= 616 lifetime uses for heavy users versus 177 lifetime uses for casual users, t= 1.78, *p*=0.08, Mann-Whitney-U z= 0.08, p=0.9).

Table 7: MDMA use amounts and frequency

	N	Mean	Standard Deviation	Range
Cumulative Lifetime MDMA use	77	228.1	578	20-5000
Amount per sitting	38	2.1	1.4	0.25-7
Max amount taken in one sitting	78	6.1	5.8	1-28
Use per week	61	2.4	1.7	0-7
Use per month	51	5.6	6.0	0-30
Use per year	39	71.7	79.9	0-365
Last use (in days)	78	40.8	129.7	1-1095

Table 8: Concomitant drug use with MDMA

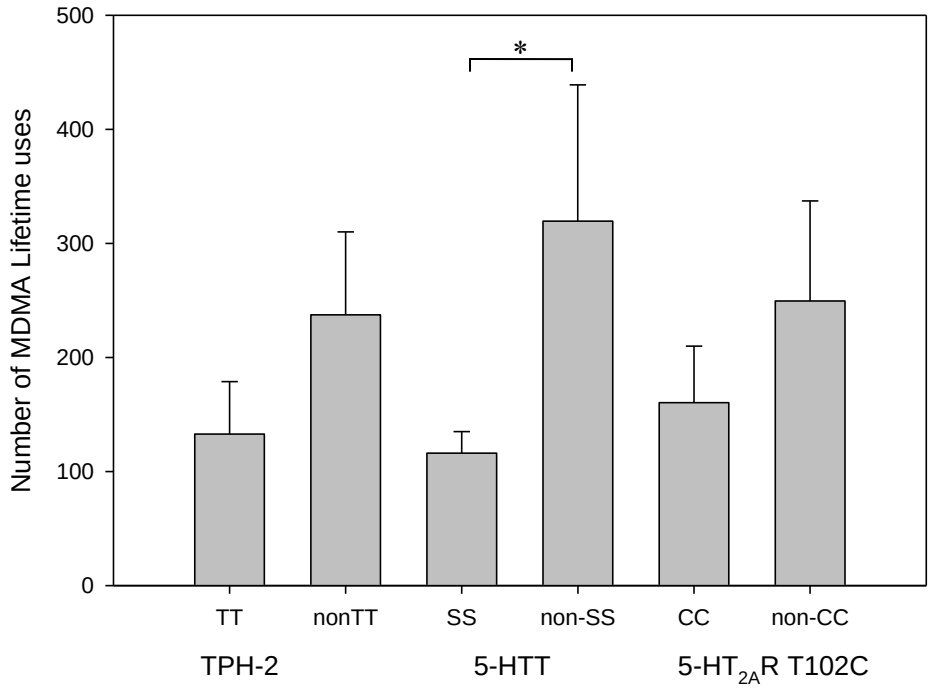
Drug (by group)	**Self-report Use** ***n (%)***
Cannabis	36 (80)
Alcohol	22 (48.9)
Benzodiazepines	4 (8.9)
Cocaine	16 (35.6)
Opiates	6 (13.3)
Methamphetamine	11 (24.4)
Hallucinogens	12 (26.7)

Table 9: MDMA use variables per genotype

MDMA use variable	Per genotype *Mean (n)(se)*								
	TPH-2			5-HTT			5-HT_{2A}R A1438G/T102C		
	GG	GT	TT	LL	LS	SS	TT	TC	CC
Cumulative lifetime intake	286.5 (36) (97.7)	185.3 (34) (100.6)	132.8 (7) (221.7)	607.3 (11) (174)	217.4 (31) (103.7)	116.1 (32) (102)	221.7 (9) (194.7)	260.8 (45) (87)	160.7 (24) (119.2)
Maximum amount taken	7.2 (36) (0.9)	5.4 (35) (0.9)	3.9 (7) (2.2)	8.4 (10) (1.2)	6.9 (32) (0.9)	4.5 (34) (0.9)	6.6 (8) (2.0)	6.6 (48) (0.8)	4.8 (23) (1.2)
Usual amount per sitting (# tablets)	2.1 (16) (0.3)	2.1 (19) (0.3)	1.5 (4) (0.7)	2.2 (4) (0.6)	2.1 (17) (0.3)	1.7 (16) (0.3)	2.5 (6) (0.6)	2.0 (27) (0.3)	1.6 (7) (0.5)
Ecstasy/ week	2.8 (27) (0.3)	2.3 (28) (0.3)	1.3 (6) (.7)	3.4* (10) (0.5)	1.9* (23) (0.3)	2.5* (25) (0.3)	3.5 (6) (0.7)	2.3 (39) (0.3)	2.3 (16) (0.4)
Ecstasy/ month	6.2 (25) (1.2)	5.2 (22) (1.3)	3.75 (4) (3.1)	10.9* (8) (1.8)	3.1* (20) (1.2)	5.2* (22) (1.1)	8 (7) (2.3)	5.6 (31) (1.1)	3.8 (14) (1.6)
Ecstasy/ year	85.6 (18) (19.1)	61 (19) (18.6)	48 (2) (57.3)	142.4* (7) (25.4)	29.6 (14) (18.0)	68.5 (16) (16.8)	145 (4) (38.3)	74.1 (23) (16.0)	42.5 (12) (22.1)

*$p<0.05$

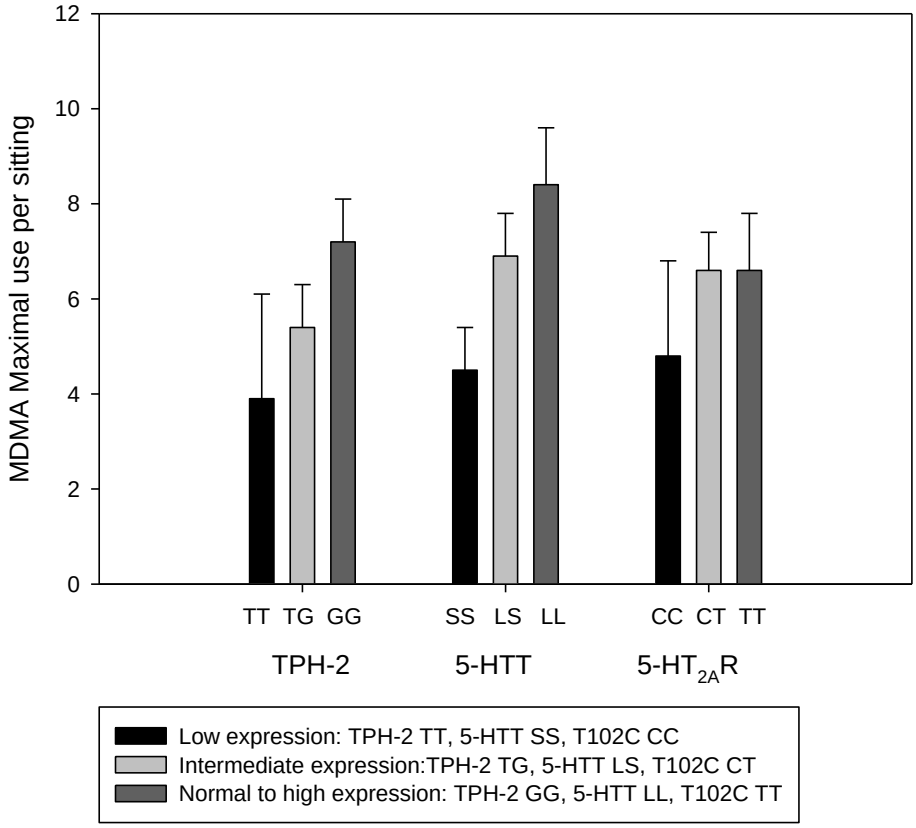
Figure 6: Effect of Effect of TPH-2, 5-HTT, and 5-HT_{2A}R T102C/A1438G polymorphisms on MDMA cumulative lifetime intake



*p<0.05,

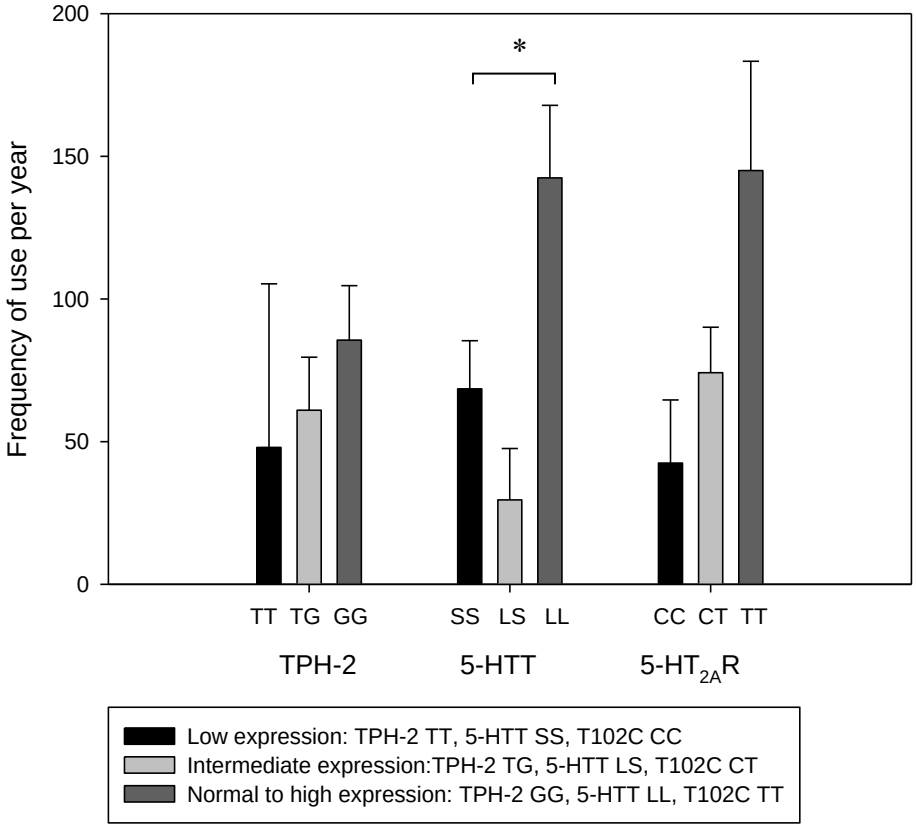
Cumulative lifetime use was compared using t-test or Mann-Whitney U when appropriate for TPH-2 (TT versus non-TT), 5-HTT (SS versus non-SS), and 5-HT_{2A}R T102C/A1438G (CC versus non-CC, GG versus non-GG). The difference between 5-HTT genotypes was significant whereas the results did not reach statistical significance with TPH-2 and 5-HT_{2A}R T102C/A1438G. All three genes are shown on the same figure to illustrate the similarity in trend across genes.

Figure 7: Relationship between TPH-2, 5-HTT, and 5-HT_{2A}R T102C/A1438G polymorphisms on maximal amounts of MDMA used per sitting



Maximal amount per sitting was compared using t-test or Mann-Whitney U when appropriate for TPH-2 (TT versus non-TT), 5-HTT (SS versus non-SS), and 5-HT_{2A}R T102C/A1438G (CC versus non-CC, GG versus non-GG). The difference did not reach statistical significance. All three genes are shown on the same figure to illustrate the similarity in trend.

Figure 8: Relationship between TPH-2, 5-HTT, and 5-HT_{2A}R T102C/A1438G polymorphisms on frequency of MDMA use per year



*$p<0.05$

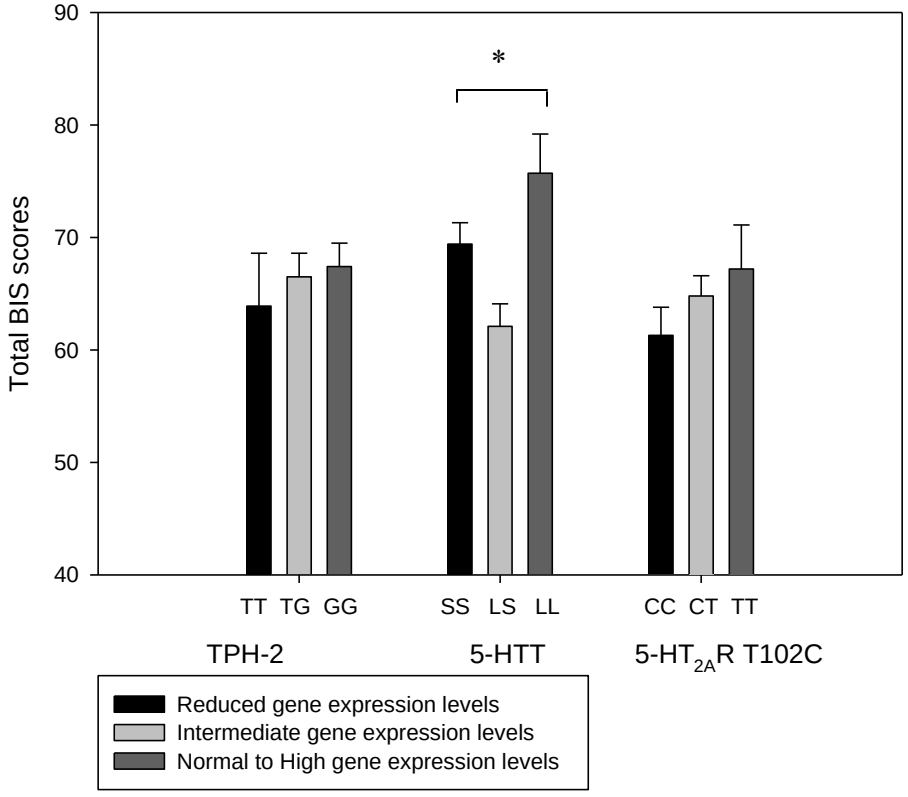
Frequency of use per year was compared using t-test or Mann-Whitney U when appropriate for TPH-2 (TT versus non-TT), 5-HTT (SS versus non-SS), and 5-HT_{2A}R T102C/A1438G (CC versus non-CC). The trend reached significance for 5-HTT only. Carriers of 5-HTT SL had lower MDMA use frequency per year than either homozygous groups (5-HTT SS or LL).

Table 10: BIS scores by genotype

	Per genotype *Mean (n)(se)*								
	TPH-2			5-HTT			5-HT_{2A}R T102C		
	GG	GT	TT	LL	LS	SS	TT	TC	CC
Non-planning score	24.8 (38) (1.0)	24.2 (37) (0.8)	24.6 (7) (2.1)	26.7 (11) (1.5)	23.4 (33) (0.9)	29.5 (35) (0.9)	22.6 (10) (1.8)	24.7 (47) (0.8)	24.8 (26) (1.1)
Attentional score	16.7 (38) (0.7)	17.0 (37) (0.8)	15 (7) (0.8)	29.5 (11) (1.6)	23.8 (33) (0.8)	26.1 (35) (0.8)	14.5 (10) (0.8)	17.3 (47) (0.7)	16.6 (26) (0.8)
Motor score	25.8 (38) (0.9)	25.3 (37) (0.6)	24.3 (7) (1.8)	19.4 (11) (1.3)	14.9 (33) (0.7)	18.8 (35) (0.7)	24.2 (10) (1.1)	25.2 (47) (0.8)	26.6 (26) (1.0)
Total BIS score	67.4 (38) (2.1)	66.5 (37) (2.1)	63.9 (7) (4.7)	75.7* (11) (3.5)	62.1* (33) (2.0)	69.4* (35) (1.9)	61.3 (10) (3.9)	64.8 (47) (1.8)	67.2 (25) (2.5)

*$p<0.05$

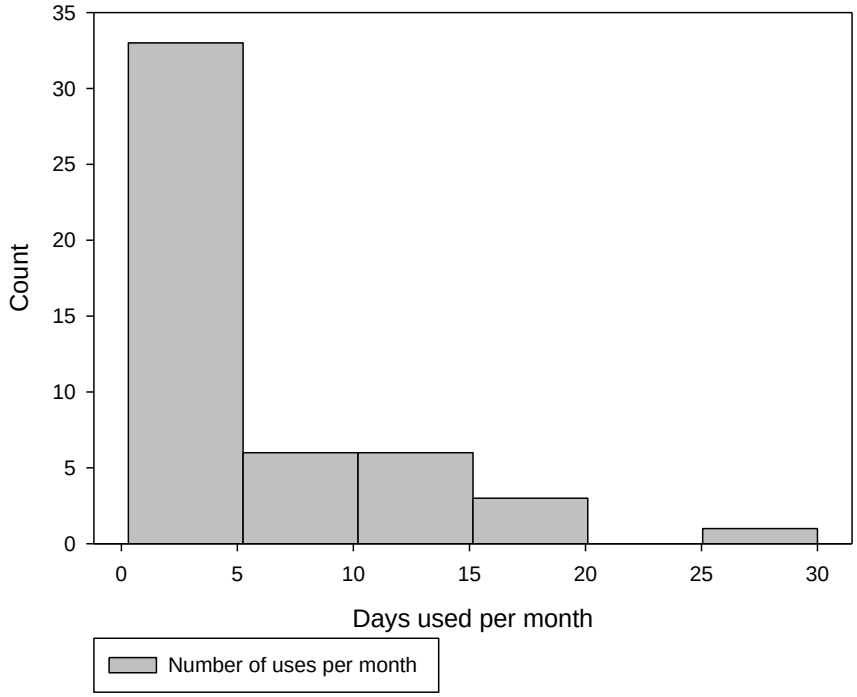
Figure 9: Relationship between TPH-2, 5-HTT, and 5-HT_{2A}R A1438G/ T102C Polymorphisms on Impulsivity Levels



*p<0.05

The relationship between impulsivity levels measured by total BIS scores and TPH-2, 5-HTT and 5-HT_{2A}R T102C/A1438G genotypes was examined using a univariate ANOVA for each gene. All three genes are shown on the same figure to illustrate the similarity in trend across genes. Carriers of 5-HTT SL had lower MDMA use frequency per year than either homozygous groups (5-HTT SS or LL).

Figure 10: MDMA self-report use frequency



As shown above, most MDMA users reported a frequency of use of less than 8 days per month. Such use frequency is compatible with occasional weekend use during parties or social occasions. Heavier MDMA users, in contrast, reported using up to 30 days per month, suggesting use is not related to social activities or classical “rave” parties.

DISCUSSION

This study sought to investigate the relationship between alleles leading to lower gene expression of TPH-2, 5-HTT, and 5-HT_{2A}R and MDMA use, impulsivity levels in MDMA users. Congruent with the overarching hypothesis of this project, genotypes associated with lower gene expression (TPH-2 TT, 5-HTT SS, and 5-HT_{2A}R T102C/A1438G CC/GG) were associated with decreased MDMA use and higher impulsivity levels. MDMA users carrying TPH-2 TT, 5-HTT SS, or 5-HT_{2A}R T102C/A1438G CC/GG reported less MDMA use than their counterparts carrying normal to high expression alleles. This pattern was demonstrated across all three genes studied, and all MDMA parameters surveyed.

Impulsivity levels appeared to be modulated by genetic variability as well, although discrepant findings were noted across TPH-2, 5-HTT, and, 5-HT_{2A}R. Interestingly, individuals carrying 5-HTT LL genotypes had higher impulsivity levels measured by BIS scores than SS carriers, contrarily to our expectations. Carriers of the SL genotype of 5-HTT reported lower impulsivity levels than carriers of either the SS or LL genotypes. This is surprising given that S and L alleles are reported to be co-dominant in action thus one would expect the impulsivity level of SL genotype carriers to be intermediate between those of SS and LL carriers. To our knowledge, only one study (Walderhaug, Magnusson et al. 2007), has reported a similar pattern (i.e. 5-HTT SL having lower impulsivity levels than SS or LL) with 5-HTT variation in normal controls undergoing tryptophan depletion: carriers of 5-HTT SL had lowered impulsive and mood response than carriers of 5-HTT SS or LL. The SL genotype was thus described as "protective" against the effects of tryptophan depletion in females, though the effects were less pronounced in males (Walderhaug, Magnusson et al. 2007). In our study, both genders displayed the same relationship between 5-HTT variability and impulsivity levels.

This study further showed that MDMA intake in humans is modulated by variability in TPH-2, 5-HTT, and 5-HT_{2A}R. Previous pre-clinical research demonstrate

the involvement of 5-HT in the reinforcing effects of psychostimulants (Lile, Wang et al. 2003). In rats, both 5-HTergic and dopaminergic inputs affect the discriminative stimulus of MDMA (Goodwin, Pynnonen et al. 2003). Thus, the alleles studied herein may modulate MDMA intake by inducing variations in individuals' sensitivity and response level of MDMA. This finding is critical given that surveys among MDMA users have determined that the most important factor in repeated use of MDMA after initial experimentation is a positive experience (Sumnall, Cole et al. 2006), whereas abstention from MDMA was related to a negative experience or the expectation of a negative effects. Consequently, TPH-2 TT, 5-HTT SS, and 5-HT_{2A}R A1438G/T102C CC/GG may shape future drug use behavior.

The main limitation of this study is a small sample size that curtailed the possibility of analysis of additive gene effects and gender. It would have been helpful to obtain objective measures of 5-HT levels, as well as inquiries about the subjective effect of MDMA. Barring those limitations, this is the first study to demonstrate that MDMA intake is modulated by genetic variation in TPH-2, 5-HTT and 5-HT_{2A}R. Our findings underscore the importance of conducting further studies to delineate the impact of these gene variants on the course of MDMA use and development of MDMA dependence.

Chapter 4: Conclusions

The studies described herein describe pilot work examining the contribution of 5-HTergic gene variability (TPH-2 (allele T), 5-HTT (S allele, and L_G) and 5-HT_{2A}R A1438G (allele G)/ T102C (allele C) to MDMA use, focusing on the link between the genetic factors of interest and behavior (as manifested by impulsivity and MDMA use patterns). Our results showed that alleles leading to reduced gene expression in 5-HTT and 5-HT_{2A}R A1438G were less prevalent in MDMA users compared to controls, suggesting that variability in those genes may play a role in determining drug preference. We then examined the role of TPH-2, 5-HTT and 5-HT_{2A}R A1438G/T102C variability in modulating MDMA intake, and showed an association between genotypes leading to lower gene expression and decreased MDMA use by self-report.

As presented in prior chapters, the 5-HT system is involved in regulation of impulsive behaviors as well as in the effects of MDMA. Moreover, abundant literature exists in support of the role of 5-HT in mood and anxiety disorders (Nutt 2008; Trivedi, Hollander et al. 2008). Thus, any discussion of the role of 5-HTergic genes in MDMA use would be incomplete without consideration of the frequent comorbidity of anxiety and depression with MDMA use. There is a significant overlap between genes associated with predisposition to depression and genes found in our study to affect MDMA use. Shared biological susceptibility, with genetic influences predisposing to dual diagnoses entities, is a hotly debated topic in psychiatry (Palomo, Kostrzewa et al. 2007), but the possibility that MDMA dependence and depression share a common genetic basis has not been studied.

Depression, as diagnosed by the DSM-IV, the gold standard for diagnosis in American psychiatry, is a clinical syndrome including depressed mood, neurovegetative changes (alterations in appetite, weight, energy, sleep or concentration), lack of enjoyment of life, and possibly recurrent thoughts of death and suicidal ideations (DSM-IV 2000). Low mood, a key symptom in depression, is reported in up to 55% of MDMA

users (Baylen and Rosenberg 2006), with clinically significant depressive symptoms in up to 15% of non-treatment seeking users (Falck, Wang et al. 2006). Anxiety symptomatology may range from pervasive worry throughout the day that impairs functioning at work or home to sudden panic attacks, debilitating enough to make individuals unable to leave their homes for fear of having an attack in public. Anxiety and depression are leading worldwide causes of disability (Kroenke, Spitzer et al. 2007; Brenes, Penninx et al. 2008). Interestingly, individuals with childhood symptoms of anxiety and depression are at increased risk of MDMA use in adulthood (Huizink, Ferdinand et al. 2006). TPH-2, 5-HTT and 5-HT_{2A}R A1438G/ T102C have been shown to contribute to the pathophysiology of 5-HT mediated symptomatology. Thus, these genes may impact both the degree of MDMA use and development of psychiatric comorbidity in the context of MDMA. Taken in the context of the broader 5-HT literature, we believe that genetic variability in TPH-2, 5-HTT and 5-HT_{2A}R A1438G/ T102C contributes to the complex clinical picture that MDMA users often present with, and suggest these polymorphisms, particularly 5-HTT, may have implications for pharmacological treatment of MDMA users with comorbidities.

DIAGNOSTIC IMPLICATIONS OF THE IMPACT OF TPH-2, 5-HTT AND 5-HT_{2A}R A1438G/ T102C POLYMORPHISMS IN MDMA USERS

To aid with treatment planning and provide more accurate prognosis, a distinction is made between drug-induced diagnoses from primary psychiatric symptoms, that is, symptoms occurring without the influence of substances (Spitzer 2004; Spitzer and First 2005). Drugs in general can exacerbate pre-existing psychiatric symptoms, or precipitate symptoms that were non-existent prior to drug use. MDMA use is no exception to this rule, and has been associated with a myriad of mental problems (Thomasius, Petersen et al. 2005; Soar, Turner et al. 2006). Users with a prolonged heavy history of MDMA consumption who meet DSM-IV diagnostic criteria for abuse and dependence algorithms display more problems compared to users who do not meet these criteria (Hanson and

Luciana 2004). The 5-HT system, which constitutes the main target of the effects of MDMA in humans, has a crucial role in modulation of depression, anxiety and impulsivity, all symptoms that have been described as undesirable psychological sequelae of MDMA use.

Lowered levels of 5-HT in the CNS may predispose to depressive mood although the relationship between 5-HT levels and mood is complex (Ruhe, Mason et al. 2007). Acute tryptophan depletion, a dietary technique leading to transient reduction in central 5-HT levels, has been widely used to study the impact of 5-HT levels on mood symptoms (Bell, Hood et al. 2005). Responses to acute tryptophan depletion are modulated by gender, with females developing more depressive symptoms, and males developing more impulsive symptoms, and by 5-HTT variability, with carriers of 5-HTT SS displaying the most intense responses (Ruhe, Mason et al. 2007; Walderhaug, Magnusson et al. 2007). However, depressive symptomatology in MDMA users has never been studied in light of 5-HTT, TPH-2 or 5-HT_{2A}R variability. Results of acute 5-HT depletion studies have to be interpreted with caution, as depressive symptomatology in MDMA users is prominent only in heavy users (greater than 50 lifetime uses) (Falck, Wang et al. 2008), whereas occasional MDMA users may paradoxically experience mood improvement (de Win, Reneman et al. 2007; Falck, Wang et al. 2008).

Among the three genes studied in this project, TPH-2, 5-HTT and 5-HT_{2A}R, 5-HTT has been the most investigated, though evidence for the involvement TPH-2 and 5-HT_{2A}R in mood disorders, specifically depression, is available (Serretti, Benedetti et al. 2005; Perlis 2007). 5-HT_{2A}R A1438G/T102C alleles are associated with clinical depression, and carriers of genotype T102C CC are over-represented among individuals with major depression compared to controls (Choi, Kang et al. 2005).

5-HTT gene variability was found to modulate stress sensitivity independent of drug use (Caspi, Sugden et al. 2003; Canli and Lesch 2007). Preschool age children carrying the 5-HTT SS genotype display more shyness than carriers of SL or LL (Hayden, Dougherty et al. 2007; Hayden, Bodkins et al. 2008). Further, the 5-HTT SS

genotype predisposes children to a more negative processing style, accompanied by more retention of negative information (Hayden, Dougherty et al. 2007). As a result, following stressful life events, individuals carrying 5-HTT SS may be at heightened risk of developing mood symptoms. This may be partially explained by the fact that carriers of the short 5-HTT allele may have higher levels of tonic amygdala activation, and thus may be in a state of "constant vigilance" (Canli and Lesch 2007). Indeed, the 5-HTT genotype has been shown to modulate resting brain function in emotion-related regions in healthy individuals (Rao, Gillihan et al. 2007).

Our results (chapter 2) showed that MDMA users were less likely to be carriers of 5-HTT SS and 5-HT_{2A}R A1438G GG than polydrug users or controls. Carriers of these genotypes (5-HTT SS and 5-HT_{2A}R A1438G GG) belonged to the MDMA group, they reported less use of MDMA compared to their counterparts carrying other genotypes (chapter 3). Taken together with the association between 5-HTT SS and5-HT_{2A}R A1438G GG (T102C CC) with depression, these results raise the possibility that carriers of genotypes leading to altered gene transmission may be at higher risk of developing depression upon MDMA use, or may end up using less MDMA because of their increased sensitivity to its effects. Further studies exploring the interaction between 5-HTT, 5-HT_{2A}R and TPH-2 and mood, sensitivity to positive or adverse effects in MDMA users are thus clearly needed. Additionally, we advocate that clinicians dealing with MDMA users should have a high index of suspicion for the possibility of a co-morbid mood disorder and a low threshold for initiating pharmacological treatment, as MDMA users appear to be at higher likelihood to suffer from dual diagnoses (drug use as well as a primary mood or anxiety disorder).

IMPLICATIONS OF TPH-2, 5-HTT AND 5-HT_{2A}R A1438G/ T102C POLYMORPHISMS IN TREATMENT OF MDMA USERS

Treatment for drug use has long been dominated by psychosocial modalities, with an expanding role for pharmacotherapy of substance abuse and dependence (Swift and Pettinati 2005; Gossop, Stewart et al. 2008). Common psychosocial interventions

include: twelve-step programs, which adopts a supportive approach, motivational interviewing, which aims to increase an individual's motivation for change, group and individual skills training, family psychoeducation regarding the signs and effects of substance use, and individual or group psychotherapy involving cognitive and/or behavioral principles, which aim to increase coping strategies, awareness and self-monitoring (Cleary, Hunt et al. 2008). None of these treatment modalities has been studied specifically for MDMA users. Pharmacological treatments, aiming to block craving for a drug or block its positive effects, are other options for drug treatments, and may be particularly useful for MDMA use when co-morbid with other psychiatric disorders. However, limited data exists in that area (Schmidt and Taylor 1990; Liechti, Geyer et al. 2001; Tancer and Johanson 2007).

Compared to users of other drugs, few MDMA users seek treatment (Maxwell and Spence 2005), although up to half of MDMA users surveyed reported having tried to decrease their use without formal assistance (Topp, Hando et al. 1999). Depression, paranoia and anxiety rank highest among problems experienced by MDMA users and subjectively reported (Baylen and Rosenberg 2006). Of MDMA users who decide to quit, more than half decide to do so because of mental health issues, mostly depression; a majority of individuals who decide to quit MDMA because of mental health reasons seek psychiatric care (~65%) (Verheyden, Maidment et al. 2003).

With the exception of limited laboratory studied, no clinical trials for psychotherapy or pharmacological treatments for MDMA have been conducted. Harm reduction approaches have included pill testing to determine the presence of adulterants (Baggott 2002), however, this approach was found to have unintended consequences of increased rather than decreased use, as users may feel more confident of their tablet purity (Winstock, Wolff et al. 2001). Education about adverse effects of MDMA may help in decreasing use. The stabilization, followed by decline in use of MDMA among youth in the US is believed to be the result of such large-scale interventions among young adults (Johnston 2007).

The effects of two selective serotonin reuptake inhibitors (SSRI), fluoxetine and citalopram, have been studied in MDMA users under controlled laboratory settings. The SSRIs as a class block 5-HT reuptake, raising extracellular 5-HT levels, with different SSRIs have varying pharmacological profiles in addition to their action on 5-HTT, e.g. (Leonard 1992). The effects of two SSRIs on the effects of MDMA in humans have been studied: citalopram and fluoxetine. Citalopram decreased most of the psychological effects of MDMA, as well as MDMA-induced sympathetic activation and cardiovascular effects (Liechti, Baumann et al. 2000; Liechti, Geyer et al. 2001). Fluoxetine similarly attenuated most, but not all, of the subjective effects of MDMA, and attenuated the heart rate increase associated with MDMA-induced sympathetic activation (Tancer and Johanson 2007). The results of these studies suggest that SSRIs could be used to treat MDMA users. However, both studies were preliminary in that they had small sample sizes, and did not include an examination of the effect of 5-HTergic gene variability on response, limiting generalizability of the results.

Genetic polymorphisms in 5-HTT, 5-HT_{2A}R, and possibly TPH-2 predict responses to SSRIs (Serretti, Benedetti et al. 2005; Alessandro and Kato 2008). Carriers of 5-HTT SS treated for depression are less responsive to SSRIs and achieve remission less frequently from their depressive disorder (Alessandro and Kato 2008). Depressed individuals carrying the 5-HT_{2A}R A1438G GG genotype have a better response to citalopram than carriers of non-GG genotypes (Choi, Kang et al. 2005). Taken together, this evidence suggests that SSRI antidepressant may be useful for treatment of MDMA users, with or without co-morbid depression. Longer-term studies are needed to examine the full potential of SSRI treatment for MDMA users.

The fact that SSRI antidepressants do not block all of the subjective effects of MDMA is in line with pre-clinical literature supporting the involvement of the dopaminergic or other systems in the behavioral and molecular effects of MDMA. Haloperidol, a classical antipsychotic medication with dopaminergic antagonist properties, blocks some subjective effects of MDMA and causes dysphoria (Liechti,

Geyer et al. 2001). This underscores the need to study medications with a broader spectrum of action for MDMA treatment, perhaps medications with dual action at 5-HT and dopamine systems.

The possibility of pre-treatment genetic testing is an attractive option that may help to maximize treatment response in MDMA dependent individuals. A pilot study for pre-treatment genetic testing for 5-HTT as part of a depression treatment-algorithm showed that using pre-treatment testing increases the chances of matching patients with medications that will provide them with a higher likelihood of remission (Smits, Smits et al. 2007). While genetic testing is a controversial issue in general, there is some evidence that a relatively high percentage of adolescents (~62%) may be accepting of the idea if the information obtained from testing allows them to uncover susceptibility to addictive disorders (Tercyak, Peshkin et al. 2006). In summary, variability in 5-HTergic genes may modulate the comorbidity of psychiatric problems in MDMA users, as well as the response to treatment for those conditions, and needs to be taken into account when treating MDMA users.

SUMMARY

In conclusion, the present studies demonstrate the importance of genetic variability in 5-HTT, TPH-2 and 5-HT_{2A}R in modulating MDMA use in humans. Our gene association study is the first to show that alleles leading to decreased gene expression in 5-HTT, TPH-2 and 5-HT_{2A}R may, by ways yet unknown, lead individuals to use other drugs preferentially to MDMA, suggesting a genetic basis for drug preference. These same genetic factors confer susceptibility to depression, highlighting the possibility that carriers of 5-HTT SS, TPH-2 TT and 5-HT_{2A}R A1438G GG may represent a vulnerable population for the development of mental health sequelae of MDMA consumption. A second major finding in this study is the impact of 5-HTT SS, TPH-2 TT and 5-HT_{2A}R T102C CC on the amount and frequency of MDMA use, suggesting the need to further study the interaction between altered 5-HTergic

transmission and behavior in humans. In all, these studies provide a firm basis for the contribution of 5-HTT SS, TPH-2 TT and 5-HT_{2A}R A1438G GG to behavioral patterns in MDMA users, and warrant further investigation of the role of those alleles as susceptibility factors in the development of psychiatric comorbidity, as well as potential modulators of treatment response.

Bibliography/References

Alessandro, S. and M. Kato (2008). "The serotonin transporter gene and effectiveness of SSRIs." Expert Rev Neurother **8**(1): 111-20.

Allen, T. J., F. G. Moeller, H. M. Rhoades and D. R. Cherek (1998). "Impulsivity and history of drug dependence." Drug Alcohol Depend **50**(2): 137-45.

Arranz, M. J., J. Munro, M. J. Owen, G. Spurlock, P. C. Sham, J. Zhao, G. Kirov, D. A. Collier and R. W. Kerwin (1998). "Evidence for association between polymorphisms in the promoter and coding regions of the 5-HT2A receptor gene and response to clozapine." Mol Psychiatry **3**(1): 61-6.

Baggott, M. J. (2002). "Preventing problems in Ecstasy users: reduce use to reduce harm." J Psychoactive Drugs **34**(2): 145-62.

Battaglia, G., S. Y. Yeh, E. O'Hearn, M. E. Molliver, M. J. Kuhar and E. B. De Souza (1987). "3,4-Methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [3H]paroxetine-labeled serotonin uptake sites." J Pharmacol Exp Ther **242**(3): 911-6.

Baumann, M. H., X. Wang and R. B. Rothman (2007). "3,4-Methylenedioxymethamphetamine (MDMA) neurotoxicity in rats: a reappraisal of past and present findings." Psychopharmacology (Berl) **189**(4): 407-24.

Baylen, C. A. and H. Rosenberg (2006). "A review of the acute subjective effects of MDMA/ecstasy." Addiction **101**(7): 933-47.

Bell, C. J., S. D. Hood and D. J. Nutt (2005). "Acute tryptophan depletion. Part II: clinical effects and implications." Aust N Z J Psychiatry **39**(7): 565-74.

Berger, U. V., X. F. Gu and E. C. Azmitia (1992). "The substituted amphetamines 3,4-methylenedioxymethamphetamine, methamphetamine, p-chloroamphetamine and fenfluramine induce 5-hydroxytryptamine release via a common mechanism blocked by fluoxetine and cocaine." Eur J Pharmacol **215**(2-3): 153-60.

Bjork, J. M., F. G. Moeller, D. M. Dougherty, A. C. Swann, M. A. Machado and C. L. Hanis (2002). "Serotonin 2a receptor T102C polymorphism and impaired impulse control." Am J Med Genet **114**(3): 336-9.

Brenes, G. A., B. W. Penninx, P. H. Judd, E. Rockwell, D. D. Sewell and J. L. Wetherell (2008). "Anxiety, depression and disability across the lifespan." Aging Ment Health **12**(1): 158-63.

Buchert, R., R. Thomasius, F. Wilke, K. Petersen, B. Nebeling, J. Obrocki, O. Schulze, U. Schmidt and M. Clausen (2004). "A voxel-based PET investigation of the long-term effects of "Ecstasy" consumption on brain serotonin transporters." Am J Psychiatry **161**(7): 1181-9.

Bull, E. J., P. H. Hutson and K. C. Fone (2003). "Reduced social interaction following 3,4-methylenedioxymethamphetamine is not associated with enhanced 5-HT 2C receptor responsivity." Neuropharmacology **44**(4): 439-48.

Bull, E. J., P. H. Hutson and K. C. Fone (2004). "Decreased social behaviour following 3,4-methylenedioxymethamphetamine (MDMA) is accompanied by changes in 5-HT2A receptor responsivity." Neuropharmacology **46**(2): 202-10.

Canli, T., E. Congdon, L. Gutknecht, R. T. Constable and K. P. Lesch (2005). "Amygdala responsiveness is modulated by tryptophan hydroxylase-2 gene variation." J Neural Transm **112**(11): 1479-85.

Canli, T. and K. P. Lesch (2007). "Long story short: the serotonin transporter in emotion regulation and social cognition." Nat Neurosci **10**(9): 1103-1109.

Capela, J. P., K. Ruscher, M. Lautenschlager, D. Freyer, U. Dirnagl, A. R. Gaio, M. L. Bastos, A. Meisel and F. Carvalho (2006). "Ecstasy-induced cell death in cortical neuronal cultures is serotonin 2A-receptor-dependent and potentiated under hyperthermia." Neuroscience **139**(3): 1069-81.

Caspi, A., K. Sugden, T. E. Moffitt, A. Taylor, I. W. Craig, H. Harrington, J. McClay, J. Mill, J. Martin, A. Braithwaite and R. Poulton (2003). "Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene." Science **301**(5631): 386-9.

Cherek, D. R., F. G. Moeller, D. M. Dougherty and H. Rhoades (1997). "Studies of violent and nonviolent male parolees: II. Laboratory and psychometric measurements of impulsivity." Biol Psychiatry **41**(5): 523-9.

Choi, M. J., R. H. Kang, B. J. Ham, H. Y. Jeong and M. S. Lee (2005). "Serotonin receptor 2A gene polymorphism (-1438A/G) and short-term treatment response to citalopram." Neuropsychobiology **52**(3): 155-62.

Clark, L., J. P. Roiser, R. Cools, D. C. Rubinsztein, B. J. Sahakian and T. W. Robbins (2005). "Stop signal response inhibition is not modulated by tryptophan depletion or the serotonin transporter polymorphism in healthy volunteers: implications for the 5-HT theory of impulsivity." Psychopharmacology (Berl) **182**(4): 570-8.

Cleary, M., G. Hunt, S. Matheson, N. Siegfried and G. Walter (2008). "Psychosocial interventions for people with both severe mental illness and substance misuse." Cochrane Database Syst Rev(1): CD001088.

Clemens, K. J., J. L. Cornish, K. M. Li, G. E. Hunt and I. S. McGregor (2005). "MDMA ('Ecstasy') and methamphetamine combined: order of administration influences hyperthermic and long-term adverse effects in female rats." Neuropharmacology **49**(2): 195-207.

Commins, D. L., G. Vosmer, R. M. Virus, W. L. Woolverton, C. R. Schuster and L. S. Seiden (1987). "Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain." J Pharmacol Exp Ther **241**(1): 338-45.

Cottler, L. B., S. B. Womack, W. M. Compton and A. Ben-Abdallah (2001). "Ecstasy abuse and dependence among adolescents and young adults: applicability and reliability of DSM-IV criteria." Hum Psychopharmacol **16**(8): 599-606.

Crespi, D., T. Mennini and M. Gobbi (1997). "Carrier-dependent and Ca(2+)-dependent 5-HT and dopamine release induced by (+)-amphetamine, 3,4-methylendioxymethamphetamine, p-chloroamphetamine and (+)-fenfluramine." Br J Pharmacol **121**(8): 1735-43.

de Win, M. M., L. Reneman, G. Jager, E. J. Vlieger, S. D. Olabarriaga, C. Lavini, I. Bisschops, C. B. Majoie, J. Booij, G. J. den Heeten and W. van den Brink (2007). "A prospective cohort study on sustained effects of low-dose ecstasy use on the brain in new ecstasy users." Neuropsychopharmacology **32**(2): 458-70.

de Win, M. M., L. Reneman, J. B. Reitsma, G. J. den Heeten, J. Booij and W. van den Brink (2004). "Mood disorders and serotonin transporter density in ecstasy users--the influence of long-term abstention, dose, and gender." Psychopharmacology (Berl) **173**(3-4): 376-82.

de Win, M. M., T. Schilt, L. Reneman, H. Vervaeke, G. Jager, S. Dijkink, J. Booij and W. van den Brink (2006). "Ecstasy use and self-reported depression, impulsivity, and sensation seeking: a prospective cohort study." J Psychopharmacol **20**(2): 226-35.

do Prado-Lima, P. A., J. M. Chatkin, M. Taufer, G. Oliveira, E. Silveira, C. A. Neto, F. Haggstram, L. C. Bodanese and I. B. da Cruz (2004). "Polymorphism of 5HT2A serotonin receptor gene is implicated in smoking addiction." Am J Med Genet B Neuropsychiatr Genet **128**(1): 90-3.

Doblin, R. (2002). "A clinical plan for MDMA (Ecstasy) in the treatment of posttraumatic stress disorder (PTSD): partnering with the FDA." J Psychoactive Drugs **34**(2): 185-94.

DSM-IV, Ed. (2000). Diagnostic and Statistical Manual of Mental Disorders. Washington, DC, American Psychiatric Association.

Du, L., D. Bakish, Y. D. Lapierre, A. V. Ravindran and P. D. Hrdina (2000). "Association of polymorphism of serotonin 2A receptor gene with suicidal ideation in major depressive disorder." Am J Med Genet **96**(1): 56-60.

EMCDDA (2007). 2007 Annual report on the state of the drugs problem in Europe E. M. C. f. D. a. D. A. (EMCDDA).

Evenden, J. L. (1999). "Varieties of impulsivity." Psychopharmacology (Berl) **146**(4): 348-61.

Falck, R. S., J. Wang and R. G. Carlson (2008). "Depressive symptomatology in young adults with a history of MDMA use: a longitudinal analysis." J Psychopharmacol **22**(1): 47-54.

Falck, R. S., J. Wang, R. G. Carlson and H. A. Siegal (2006). "Prevalence and correlates of current depressive symptomatology among a community sample of MDMA users in Ohio." Addict Behav **31**(1): 90-101.

Federal Register, J. L. (1986). **51**: 36552-63560.

Fendrich, M., J. S. Wislar, T. P. Johnson and A. Hubbell (2003). "A contextual profile of club drug use among adults in Chicago." Addiction **98**(12): 1693-703.

Filip, M., M. Frankowska, M. Zaniewska, A. Golda and E. Przegalinski (2005). "The serotonergic system and its role in cocaine addiction." Pharmacol Rep **57**(6): 685-700.

First, M. B., S. Donovan and A. Frances (1996). "Nosology of chronic mood disorders." Psychiatr Clin North Am **19**(1): 29-39.

Fisk, J. E., C. Montgomery, M. Wareing and P. N. Murphy (2005). "Reasoning deficits in ecstasy (MDMA) polydrug users." Psychopharmacology (Berl) **181**(3): 550-9.

Freudenmann, R. W., F. Oxler and S. Bernschneider-Reif (2006). "The origin of MDMA (ecstasy) revisited: the true story reconstructed from the original documents." Addiction **101**(9): 1241-5.

Garcia-Osta, A., J. Del Rio and D. Frechilla (2004). "Increased CRE-binding activity and tryptophan hydroxylase mRNA expression induced by 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") in the rat frontal cortex but not in the hippocampus." Brain Res Mol Brain Res **126**(2): 181-7.

Gaston, T. (1972). "Identification of 3,4-methylenedioxymethamphetamine." Microgram **5**: 60-63.

Gibb, J. W., D. M. Stone, D. C. Stahl and G. R. Hanson (1987). "The effects of amphetamine-like designer drugs on monoaminergic systems in rat brain." NIDA Res Monogr **76**: 316-21.

Goldstein, L. H., Y. Mordish, I. Abu-Kishak, M. Toledano and M. Berkovitch (2006). "Acute paralysis following recreational MDMA (Ecstasy) use." Clin Toxicol (Phila) **44**(3): 339-41.

Goodwin, A. K., D. M. Pynnonen and L. E. Baker (2003). "Serotonergic-dopaminergic mediation of MDMA's discriminative stimulus effects in a three-choice discrimination." Pharmacol Biochem Behav **74**(4): 987-95.

Gossop, M., D. Stewart and J. Marsden (2008). "Attendance at Narcotics Anonymous and Alcoholics Anonymous meetings, frequency of attendance and substance use outcomes after residential treatment for drug dependence: a 5-year follow-up study." Addiction **103**(1): 119-25.

Green, A. R., A. O. Mechan, J. M. Elliott, E. O'Shea and M. I. Colado (2003). "The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy")." Pharmacol Rev **55**(3): 463-508.

Green, A. R., V. Sanchez, E. O'Shea, K. S. Saadat, J. M. Elliott and M. I. Colado (2004). "Effect of ambient temperature and a prior neurotoxic dose of 3,4-methylenedioxymethamphetamine (MDMA) on the hyperthermic response of rats to a single or repeated ('binge' ingestion) low dose of MDMA." Psychopharmacology (Berl) **173**(3-4): 264-9.

Greenberg, B. D., T. J. Tolliver, S. J. Huang, Q. Li, D. Bengel and D. L. Murphy (1999). "Genetic variation in the serotonin transporter promoter region affects serotonin uptake in human blood platelets." Am J Med Genet **88**(1): 83-7.

Greer, G. and R. Tolbert (1986). "Subjective reports of the effects of MDMA in a clinical setting." J Psychoactive Drugs **18**(4): 319-27.

Greer, G. R. and R. Tolbert (1998). "A method of conducting therapeutic sessions with MDMA." J Psychoactive Drugs **30**(4): 371-9.

Gudelsky, G. A., B. K. Yamamoto and J. F. Nash (1994). "Potentiation of 3,4-methylenedioxymethamphetamine-induced dopamine release and serotonin neurotoxicity by 5-HT2 receptor agonists." Eur J Pharmacol **264**(3): 325-30.

Gutknecht, L., C. Jacob, A. Strobel, C. Kriegebaum, J. Muller, Y. Zeng, C. Markert, A. Escher, J. Wendland, A. Reif, R. Mossner, C. Gross, B. Brocke and K. P. Lesch (2007). "Tryptophan hydroxylase-2 gene variation influences personality traits and disorders related to emotional dysregulation." Int J Neuropsychopharmacol **10**(3): 309-20.

Halpern, J. H., H. G. Pope, Jr., A. R. Sherwood, S. Barry, J. I. Hudson and D. Yurgelun-Todd (2004). "Residual neuropsychological effects of illicit 3,4-methylenedioxymethamphetamine (MDMA) in individuals with minimal exposure to other drugs." Drug Alcohol Depend **75**(2): 135-47.

Hannon, J. and D. Hoyer (2008). "Molecular biology of 5-HT receptors." Behav Brain Res.

Hanson, K. L. and M. Luciana (2004). "Neurocognitive function in users of MDMA: the importance of clinically significant patterns of use." Psychol Med **34**(2): 229-46.

Hanson, K. L., M. Luciana and K. Sullwold (2008). "Reward-related decision-making deficits and elevated impulsivity among MDMA and other drug users." Drug Alcohol Depend.

Hayden, E. P., M. Bodkins, C. Brenner, A. Shekhar, J. I. Nurnberger, Jr., B. F. O'Donnell and W. P. Hetrick (2008). "A multimethod investigation of the behavioral activation system in bipolar disorder." J Abnorm Psychol **117**(1): 164-70.

Hayden, E. P., L. R. Dougherty, B. Maloney, C. Emily Durbin, T. M. Olino, J. I. Nurnberger, Jr., D. K. Lahiri and D. N. Klein (2007). "Temperamental fearfulness in childhood and the serotonin transporter promoter region polymorphism: a multimethod association study." Psychiatr Genet **17**(3): 135-42.

Herin, D. V., S. Liu, T. Ullrich, K. C. Rice and K. A. Cunningham (2005). "Role of the serotonin 5-HT2A receptor in the hyperlocomotive and hyperthermic effects of (+)-3,4-methylenedioxymethamphetamine." Psychopharmacology (Berl) **178**(4): 505-13.

Herrmann, M. J., T. Huter, F. Muller, A. Muhlberger, P. Pauli, A. Reif, T. Renner, T. Canli, A. J. Fallgatter and K. P. Lesch (2007). "Additive effects of serotonin transporter and tryptophan hydroxylase-2 gene variation on emotional processing." Cereb Cortex **17**(5): 1160-3.

Hinckers, A. S., M. Laucht, M. H. Schmidt, K. F. Mann, G. Schumann, M. A. Schuckit and A. Heinz (2006). "Low level of response to alcohol as associated with serotonin transporter genotype and high alcohol intake in adolescents." Biol Psychiatry **60**(3): 282-7.

Hoyer, D., J. P. Hannon and G. R. Martin (2002). "Molecular, pharmacological and functional diversity of 5-HT receptors." Pharmacol Biochem Behav **71**(4): 533-54.

Hu, X. Z., R. H. Lipsky, G. Zhu, L. A. Akhtar, J. Taubman, B. D. Greenberg, K. Xu, P. D. Arnold, M. A. Richter, J. L. Kennedy, D. L. Murphy and D. Goldman (2006). "Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder." Am J Hum Genet **78**(5): 815-26.

Huizink, A. C., R. F. Ferdinand, J. van der Ende and F. C. Verhulst (2006). "Symptoms of anxiety and depression in childhood and use of MDMA: prospective, population based study." Bmj **332**(7545): 825-8.

Izco, M., L. Orio, E. O'Shea and M. I. Colado (2007). "Binge ethanol administration enhances the MDMA-induced long-term 5-HT neurotoxicity in rat brain." Psychopharmacology (Berl) **189**(4): 459-70.

Jager, G., M. M. de Win, I. van der Tweel, T. Schilt, R. S. Kahn, W. van den Brink, J. M. van Ree and N. F. Ramsey (2008). "Assessment of cognitive brain function in ecstasy users and contributions of other drugs of abuse: results from an FMRI study." Neuropsychopharmacology **33**(2): 247-58.

Jager, G., M. M. de Win, H. K. Vervaeke, T. Schilt, R. S. Kahn, W. van den Brink, J. M. van Ree and N. F. Ramsey (2007). "Incidental use of ecstasy: no evidence for harmful effects on cognitive brain function in a prospective fMRI study." Psychopharmacology (Berl) **193**(3): 403-14.

Jansen, K. L. (1999). "Ecstasy (MDMA) dependence." Drug Alcohol Depend **53**(2): 121-4.

Johnston, L. D., O'Malley, P. M., Bachman, J. G. & Schulenberg, J. E. (2007). Monitoring the Future: National Results on Adolescent Drug Use. Overview of Findings 2007.

Kankaanpaa, A., E. Meririnne, P. Lillsunde and T. Seppala (1998). "The acute effects of amphetamine derivatives on extracellular serotonin and dopamine levels in rat nucleus accumbens." Pharmacol Biochem Behav **59**(4): 1003-9.

Khait, V. D., Y. Y. Huang, G. Zalsman, M. A. Oquendo, D. A. Brent, J. M. Harkavy-Friedman and J. J. Mann (2005). "Association of serotonin 5-HT2A receptor binding and the T102C polymorphism in depressed and healthy Caucasian subjects." Neuropsychopharmacology **30**(1): 166-72.

Koella, W. P. (1969). "What is the functional role of central nervous serotonin?" Neurosci Res (N Y) **2**(0): 229-51.

Koob, G. and M. J. Kreek (2007). "Stress, dysregulation of drug reward pathways, and the transition to drug dependence." Am J Psychiatry **164**(8): 1149-59.

Koob, G. F. and M. Le Moal (2008). "Addiction and the brain antireward system." Annu Rev Psychol **59**: 29-53.

Kreek, M. J., D. A. Nielsen, E. R. Butelman and K. S. LaForge (2005). "Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction." Nat Neurosci **8**(11): 1450-7.

Kroenke, K., R. L. Spitzer, J. B. Williams, P. O. Monahan and B. Lowe (2007). "Anxiety disorders in primary care: prevalence, impairment, comorbidity, and detection." Ann Intern Med **146**(5): 317-25.

Leonard, B. E. (1992). "Pharmacological differences of serotonin reuptake inhibitors and possible clinical relevance." Drugs **43 Suppl 2**: 3-9; discussion 9-10.

Lesch, K. P., D. Bengel, A. Heils, S. Z. Sabol, B. D. Greenberg, S. Petri, J. Benjamin, C. R. Muller, D. H. Hamer and D. L. Murphy (1996). "Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region." Science **274**(5292): 1527-31.

Liechti, M. E., C. Baumann, A. Gamma and F. X. Vollenweider (2000). "Acute psychological effects of 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy") are attenuated by the serotonin uptake inhibitor citalopram." Neuropsychopharmacology **22**(5): 513-21.

Liechti, M. E., A. Gamma and F. X. Vollenweider (2001). "Gender differences in the subjective effects of MDMA." Psychopharmacology (Berl) **154**(2): 161-8.

Liechti, M. E., M. A. Geyer, D. Hell and F. X. Vollenweider (2001). "Effects of MDMA (ecstasy) on prepulse inhibition and habituation of startle in humans after pretreatment with citalopram, haloperidol, or ketanserin." Neuropsychopharmacology **24**(3): 240-52.

Liechti, M. E., I. Kunz and H. Kupferschmidt (2005). "Acute medical problems due to Ecstasy use. Case-series of emergency department visits." Swiss Med Wkly **135**(43-44): 652-7.

Lile, J. A., Z. Wang, W. L. Woolverton, J. E. France, T. C. Gregg, H. M. Davies and M. A. Nader (2003). "The reinforcing efficacy of psychostimulants in rhesus monkeys: the role of pharmacokinetics and pharmacodynamics." J Pharmacol Exp Ther **307**(1): 356-66.

Little, K. Y., D. P. McLaughlin, L. Zhang, C. S. Livermore, G. W. Dalack, P. R. McFinton, Z. S. DelProposto, E. Hill, B. J. Cassin, S. J. Watson and E. H. Cook (1998). "Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels." Am J Psychiatry **155**(2): 207-13.

Madhok, A., R. Boxer and D. Chowdhury (2003). "Atrial fibrillation in an adolescent--the agony of ecstasy." Pediatr Emerg Care **19**(5): 348-9.

Maxwell, J. C. and R. T. Spence (2005). "Profiles of club drug users in treatment." Subst Use Misuse **40**(9-10): 1409-26.

McCann, U. D., G. A. Ricaurte and M. E. Molliver (2001). ""Ecstasy" and serotonin neurotoxicity: new findings raise more questions." Arch Gen Psychiatry **58**(10): 907-8.

McCann, U. D., Z. Szabo, U. Scheffel, R. F. Dannals and G. A. Ricaurte (1998). "Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings." Lancet **352**(9138): 1433-7.

McNamara, R., A. Kerans, B. O'Neill and A. Harkin (2006). "Caffeine promotes hyperthermia and serotonergic loss following co-administration of the substituted amphetamines, MDMA ("Ecstasy") and MDA ("Love")." Neuropharmacology **50**(1): 69-80.

Moeller, F. G., E. S. Barratt, D. M. Dougherty, J. M. Schmitz and A. C. Swann (2001). "Psychiatric aspects of impulsivity." Am J Psychiatry **158**(11): 1783-93.

Moeller, F. G., D. M. Dougherty, E. S. Barratt, J. M. Schmitz, A. C. Swann and J. Grabowski (2001). "The impact of impulsivity on cocaine use and retention in treatment." J Subst Abuse Treat **21**(4): 193-8.

Morgan, M. J. (1998). "Recreational use of "ecstasy" (MDMA) is associated with elevated impulsivity." Neuropsychopharmacology **19**(4): 252-64.

Morgan, M. J., L. C. Impallomeni, A. Pirona and R. D. Rogers (2006). "Elevated impulsivity and impaired decision-making in abstinent Ecstasy (MDMA) users compared to polydrug and drug-naive controls." Neuropsychopharmacology **31**(7): 1562-73.

Morgan, M. J., L. McFie, H. Fleetwood and J. A. Robinson (2002). "Ecstasy (MDMA): are the psychological problems associated with its use reversed by prolonged abstinence?" Psychopharmacology (Berl) **159**(3): 294-303.

Myers, R. L., D. C. Airey, D. H. Manier, R. C. Shelton and E. Sanders-Bush (2007). "Polymorphisms in the regulatory region of the human serotonin 5-HT2A receptor gene (HTR2A) influence gene expression." Biol Psychiatry **61**(2): 167-73.

Nash, J. F., B. L. Roth, J. D. Brodkin, D. E. Nichols and G. A. Gudelsky (1994). "Effect of the R(-) and S(+) isomers of MDA and MDMA on phosphatidyl inositol turnover in cultured cells expressing 5-HT2A or 5-HT2C receptors." Neurosci Lett **177**(1-2): 111-5.

Nichols, D. E. (1986). "Differences between the mechanism of action of MDMA, MBDB, and the classic hallucinogens. Identification of a new therapeutic class: entactogens." J Psychoactive Drugs **18**(4): 305-13.

Nielsen, D. A., S. Barral, D. Proudnikov, S. Kellogg, A. Ho, J. Ott and M. J. Kreek (2008). "TPH2 and TPH1: association of variants and interactions with heroin addiction." Behav Genet **38**(2): 133-50.

Nomura, A., H. Ujike, Y. Tanaka, K. Otani, Y. Morita, M. Kishimoto, A. Morio, M. Harano, T. Inada, M. Yamada, T. Komiyama, Y. Sekine, N. Iwata, I. Sora, M. Iyo, N. Ozaki and S. Kuroda (2006). "Genetic variant of prodynorphin gene is risk factor for methamphetamine dependence." Neurosci Lett **400**(1-2): 158-62.

Nomura, M. and Y. Nomura (2006). "Psychological, neuroimaging, and biochemical studies on functional association between impulsive behavior and the 5-HT2A receptor gene polymorphism in humans." Ann N Y Acad Sci **1086**: 134-43.

Nutt, D. J. (2008). "Relationship of neurotransmitters to the symptoms of major depressive disorder." J Clin Psychiatry **69 Suppl E1**: 4-7.

O'Hearn, E., G. Battaglia, E. B. De Souza, M. J. Kuhar and M. E. Molliver (1988). "Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity." J Neurosci **8**(8): 2788-803.

Paaver, M., N. Nordquist, J. Parik, M. Harro, L. Oreland and J. Harro (2007). "Platelet MAO activity and the 5-HTT gene promoter polymorphism are associated with impulsivity and cognitive style in visual information processing." Psychopharmacology (Berl) **194**(4): 545-54.

Palomo, T., R. M. Kostrzewa, R. J. Beninger and T. Archer (2007). "Genetic variation and shared biological susceptibility underlying comorbidity in neuropsychiatry." Neurotox Res **12**(1): 29-42.

Paris, J. (2008). "Clinical trials of treatment for personality disorders." Psychiatr Clin North Am **31**(3): 517-26, viii.

Paris, J. M. and K. A. Cunningham (1992). "Lack of serotonin neurotoxicity after intraraphe microinjection of (+)-3,4-methylenedioxymethamphetamine (MDMA)." Brain Res Bull **28**(1): 115-9.

Parrott, A. C. (2006). "MDMA in humans: factors which affect the neuropsychobiological profiles of recreational ecstasy users, the integrative role of bioenergetic stress." J Psychopharmacol **20**(2): 147-63.

Parrott, A. C. (2007). "The psychotherapeutic potential of MDMA (3,4-methylenedioxymethamphetamine): an evidence-based review." Psychopharmacology (Berl) **191**(2): 181-93.

Parsons, M. J., U. M. D'Souza, M. J. Arranz, R. W. Kerwin and A. J. Makoff (2004). "The -1438A/G polymorphism in the 5-hydroxytryptamine type 2A receptor gene affects promoter activity." Biol Psychiatry **56**(6): 406-10.

Patton, J. H., M. S. Stanford and E. S. Barratt (1995). "Factor structure of the Barratt impulsiveness scale." J Clin Psychol **51**(6): 768-74.

Perlis, R. H. (2007). "Pharmacogenetic studies of antidepressant response: how far from the clinic?" Psychiatr Clin North Am **30**(1): 125-38.

Quednow, B. B., K. U. Kuhn, C. Hoppe, J. Westheide, W. Maier, I. Daum and M. Wagner (2007). "Elevated impulsivity and impaired decision-making cognition in heavy users of MDMA ("Ecstasy")." Psychopharmacology (Berl) **189**(4): 517-30.

Rao, H., S. J. Gillihan, J. Wang, M. Korczykowski, G. M. Sankoorikal, K. A. Kaercher, E. S. Brodkin, J. A. Detre and M. J. Farah (2007). "Genetic variation in serotonin transporter alters resting brain function in healthy individuals." Biol Psychiatry **62**(6): 600-6.

Reneman, L., J. Booij, K. de Bruin, J. B. Reitsma, F. A. de Wolff, W. B. Gunning, G. J. den Heeten and W. van den Brink (2001). "Effects of dose, sex, and long-term abstention from use on toxic effects of MDMA (ecstasy) on brain serotonin neurons." Lancet **358**(9296): 1864-9.

Reneman, L., J. Booij, B. Schmand, W. van den Brink and B. Gunning (2000). "Memory disturbances in "Ecstasy" users are correlated with an altered brain serotonin neurotransmission." Psychopharmacology (Berl) **148**(3): 322-4.

Reneman, L., E. Endert, K. de Bruin, J. Lavalaye, M. G. Feenstra, F. A. de Wolff and J. Booij (2002). "The acute and chronic effects of MDMA ("ecstasy") on cortical 5-HT2A receptors in rat and human brain." Neuropsychopharmacology **26**(3): 387-96.

Reneman, L., T. Schilt, M. M. de Win, J. Booij, B. Schmand, W. van den Brink and O. Bakker (2006). "Memory function and serotonin transporter promoter gene polymorphism in ecstasy (MDMA) users." J Psychopharmacol **20**(3): 389-99.

Reuter, M., Y. Kuepper and J. Hennig (2007). "Association between a polymorphism in the promoter region of the TPH2 gene and the personality trait of harm avoidance." Int J Neuropsychopharmacol **10**(3): 401-4.

Reuter, M., U. Ott, D. Vaitl and J. Hennig (2007). "Impaired executive control is associated with a variation in the promoter region of the tryptophan hydroxylase 2 gene." J Cogn Neurosci **19**(3): 401-8.

Ricaurte, G. A. and U. D. McCann (2001). "Assessing long-term effects of MDMA (Ecstasy)." Lancet **358**(9296): 1831-2.

Ricaurte, G. A. and U. D. McCann (2001). "Experimental studies on 3,4-methylenedioxymethamphetamine (MDA, "ecstasy") and its potential to damage brain serotonin neurons." Neurotox Res **3**(1): 85-99.

Roiser, J. P., L. J. Cook, J. D. Cooper, D. C. Rubinsztein and B. J. Sahakian (2005). "Association of a functional polymorphism in the serotonin transporter gene with abnormal emotional processing in ecstasy users." Am J Psychiatry **162**(3): 609-12.

Roiser, J. P., R. D. Rogers, L. J. Cook and B. J. Sahakian (2006). "The effect of polymorphism at the serotonin transporter gene on decision-making, memory and executive function in ecstasy users and controls." Psychopharmacology (Berl) **188**(2): 213-27.

Rosenson, J., C. Smollin, K. A. Sporer, P. Blanc and K. R. Olson (2007). "Patterns of ecstasy-associated hyponatremia in California." Ann Emerg Med **49**(2): 164-71, 171 e1.

Ross, J. D., D. V. Herin, P. S. Frankel, M. L. Thomas and K. A. Cunningham (2006). "Chronic treatment with a serotonin(2) receptor (5-HT(2)R) agonist modulates the behavioral and cellular response to (+)-3,4-methylenedioxymethamphetamine [(+)-MDMA]." Drug Alcohol Depend **81**(2): 117-27.

Rudnick, G. (2006). "Serotonin transporters--structure and function." J Membr Biol **213**(2): 101-10.

Rudnick, G. and S. C. Wall (1992). "The molecular mechanism of "ecstasy" [3,4-methylenedioxy-methamphetamine (MDMA)]: serotonin transporters are targets for MDMA-induced serotonin release." Proc Natl Acad Sci U S A **89**(5): 1817-21.

Ruhe, H. G., N. S. Mason and A. H. Schene (2007). "Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies." Mol Psychiatry **12**(4): 331-59.

Saiz, P. A., M. P. Garcia-Portilla, C. Arango, B. Morales, S. Martinez-Barrondo, C. Alvarez, G. San Narciso, E. Carreno, V. Alvarez, E. Coto and J. Bobes (2008). "Association between heroin dependence and 5-HT2A receptor gene polymorphisms." Eur Addict Res **14**(1): 47-52.

SAMHSA (2006). National Household Survey on Drug Use and Health.

Scheuch, K., M. Lautenschlager, M. Grohmann, S. Stahlberg, J. Kirchheiner, P. Zill, A. Heinz, D. J. Walther and J. Priller (2007). "Characterization of a Functional Promoter Polymorphism of the Human Tryptophan Hydroxylase 2 Gene in Serotonergic Raphe Neurons." Biol Psychiatry.

Schifano, F. (2004). "A bitter pill. Overview of ecstasy (MDMA, MDA) related fatalities." Psychopharmacology (Berl) **173**(3-4): 242-8.

Schifano, F., J. Corkery, P. Deluca, A. Oyefeso and A. H. Ghodse (2006). "Ecstasy (MDMA, MDA, MDEA, MBDB) consumption, seizures, related offences, prices, dosage levels and deaths in the UK (1994-2003)." J Psychopharmacol **20**(3): 456-63.

Schifano, F., A. Oyefeso, J. Corkery, K. Cobain, R. Jambert-Gray, G. Martinotti and A. H. Ghodse (2003). "Death rates from ecstasy (MDMA, MDA) and polydrug use in England and Wales 1996-2002." Hum Psychopharmacol **18**(7): 519-24.

Schifano, F., A. Oyefeso, L. Webb, M. Pollard, J. Corkery and A. H. Ghodse (2003). "Review of deaths related to taking ecstasy, England and Wales, 1997-2000." Bmj **326**(7380): 80-1.

Schmidt, C. J. (1987). "Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine." J Pharmacol Exp Ther **240**(1): 1-7.

Schmidt, C. J. and V. L. Taylor (1990). "Reversal of the acute effects of 3,4-methylenedioxymethamphetamine by 5-HT uptake inhibitors." Eur J Pharmacol **181**(1-2): 133-6.

Serretti, A., F. Benedetti, R. Zanardi and E. Smeraldi (2005). "The influence of Serotonin Transporter Promoter Polymorphism (SERTPR) and other polymorphisms of the serotonin pathway on the efficacy of antidepressant treatments." Prog Neuropsychopharmacol Biol Psychiatry **29**(6): 1074-84.

Smits, K. M., L. J. Smits, J. S. Schouten, F. P. Peeters and M. H. Prins (2007). "Does pretreatment testing for serotonin transporter polymorphisms lead to earlier effects of drug treatment in patients with major depression? A decision-analytic model." Clin Ther **29**(4): 691-702.

Snyder, S. H. and J. P. Bennett, Jr. (1976). "Neurotransmitter receptors in the brain: biochemical identification." Annu Rev Physiol **38**: 153-75.

Soar, K., A. C. Parrott and H. C. Fox (2004). "Persistent neuropsychological problems after 7 years of abstinence from recreational Ecstasy (MDMA): a case study." Psychol Rep **95**(1): 192-6.

Soar, K., J. J. Turner and A. C. Parrott (2006). "Problematic versus non-problematic ecstasy/MDMA use: the influence of drug usage patterns and pre-existing psychiatric factors." J Psychopharmacol **20**(3): 417-24.

Spitzer, R. L. (2004). "Good idea or politically correct nonsense?" Psychiatr Serv **55**(2): 113.

Spitzer, R. L., M. Davies and R. A. Barkley (1990). "The DSM-III-R field trial of disruptive behavior disorders." J Am Acad Child Adolesc Psychiatry **29**(5): 690-7.

Spitzer, R. L. and M. B. First (2005). "Classification of psychiatric disorders." Jama **294**(15): 1898-9; author reply 1899-900.

Spitzer, R. L. and B. Siegel (1990). "The DSM-III-R field trial of pervasive developmental disorders." J Am Acad Child Adolesc Psychiatry **29**(6): 855-62.

Spurlock, G., A. Heils, P. Holmans, J. Williams, U. M. D'Souza, A. Cardno, K. C. Murphy, L. Jones, P. R. Buckland, P. McGuffin, K. P. Lesch and M. J. Owen (1998). "A family based association study of T102C polymorphism in 5HT2A and schizophrenia plus identification of new polymorphisms in the promoter." Mol Psychiatry **3**(1): 42-9.

Sterk, C. E., K. P. Theall and K. W. Elifson (2007). "Getting into ecstasy: comparing moderate and heavy young adult users." J Psychoactive Drugs **39**(2): 103-13.

Stone, D. M., D. C. Stahl, G. R. Hanson and J. W. Gibb (1986). "The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain." Eur J Pharmacol **128**(1-2): 41-8.

Strote, J., J. E. Lee and H. Wechsler (2002). "Increasing MDMA use among college students: results of a national survey." J Adolesc Health **30**(1): 64-72.

Sumnall, H. R., J. C. Cole and L. Jerome (2006). "The varieties of ecstatic experience: an exploration of the subjective experiences of ecstasy." J Psychopharmacol **20**(5): 670-82.

Swift, R. and H. M. Pettinati (2005). "Choosing pharmacotherapies for the COMBINE Study--process and procedures: an investigational approach to combination pharmacotherapy for the treatment of alcohol dependence." J Stud Alcohol Suppl(15): 141-7; discussion 140.

Tancer, M. and C. E. Johanson (2007). "The effects of fluoxetine on the subjective and physiological effects of 3,4-methylenedioxymethamphetamine (MDMA) in humans." Psychopharmacology (Berl) **189**(4): 565-73.

Tanner-Smith, E. E. (2006). "Pharmacological content of tablets sold as "ecstasy": results from an online testing service." Drug Alcohol Depend **83**(3): 247-54.

Teese, R. and G. Bradley (2008). "Predicting recklessness in emerging adults: a test of a psychosocial model." J Soc Psychol **148**(1): 105-26.

Tercyak, K. P., B. N. Peshkin, L. A. Wine and L. R. Walker (2006). "Interest of adolescents in genetic testing for nicotine addiction susceptibility." Prev Med **42**(1): 60-5.

Thomasius, R., K. U. Petersen, P. Zapletalova, L. Wartberg, D. Zeichner and A. Schmoldt (2005). "Mental disorders in current and former heavy ecstasy (MDMA) users." Addiction **100**(9): 1310-9.

Topp, L., J. Hando, P. Dillon, A. Roche and N. Solowij (1999). "Ecstasy use in Australia: patterns of use and associated harm." Drug Alcohol Depend **55**(1-2): 105-15.

Trivedi, M. H., E. Hollander, D. Nutt and P. Blier (2008). "Clinical evidence and potential neurobiological underpinnings of unresolved symptoms of depression." J Clin Psychiatry **69**(2): 246-58.

Turecki, G., R. Briere, K. Dewar, T. Antonetti, A. D. Lesage, M. Seguin, N. Chawky, C. Vanier, M. Alda, R. Joober, C. Benkelfat and G. A. Rouleau (1999). "Prediction of level of serotonin 2A receptor binding by serotonin receptor 2A genetic variation in postmortem brain samples from subjects who did or did not commit suicide." Am J Psychiatry **156**(9): 1456-8.

Uhl, G. R., T. Drgon, C. Johnson, O. O. Fatusin, Q. R. Liu, C. Contoreggi, C. Y. Li, K. Buck and J. Crabbe (2008). ""Higher order" addiction molecular genetics: convergent data from genome-wide association in humans and mice." Biochem Pharmacol **75**(1): 98-111.

Velez-Blasini, C. J. (2008). "Evidence against alcohol as a proximal cause of sexual risk taking among college students." J Sex Res **45**(2): 118-28.

Verdejo-Garcia, A., A. J. Lawrence and L. Clark (2008). "Impulsivity as a vulnerability marker for substance-use disorders: review of findings from high-risk research, problem gamblers and genetic association studies." Neurosci Biobehav Rev **32**(4): 777-810.

Verheyden, S. L., R. Maidment and H. V. Curran (2003). "Quitting ecstasy: an investigation of why people stop taking the drug and their subsequent mental health." J Psychopharmacol **17**(4): 371-8.

Vollenweider, F. X., A. Gamma, M. Liechti and T. Huber (1998). "Psychological and cardiovascular effects and short-term sequelae of MDMA ("ecstasy") in MDMA-naive healthy volunteers." Neuropsychopharmacology **19**(4): 241-51.

von Sydow, K., R. Lieb, H. Pfister, M. Hofler and H. U. Wittchen (2002). "Use, abuse and dependence of ecstasy and related drugs in adolescents and young adults-a transient phenomenon? Results from a longitudinal community study." Drug Alcohol Depend **66**(2): 147-59.

Walderhaug, E., N. I. Landro and A. Magnusson (2007). "A synergic effect between lowered serotonin and novel situations on impulsivity measured by CPT." J Clin Exp Neuropsychol: 1-8.

Walderhaug, E., A. Magnusson, A. Neumeister, J. Lappalainen, H. Lunde, H. Refsum and N. I. Landro (2007). "Interactive effects of sex and 5-HTTLPR on mood and impulsivity during tryptophan depletion in healthy people." Biol Psychiatry **62**(6): 593-9.

Walther, D. J., J. U. Peter, S. Bashammakh, H. Hortnagl, M. Voits, H. Fink and M. Bader (2003). "Synthesis of serotonin by a second tryptophan hydroxylase isoform." Science **299**(5603): 76.

Wang, X., M. H. Baumann, C. M. Dersch and R. B. Rothman (2007). "Restoration of 3,4-methylenedioxymethamphetamine-induced 5-HT depletion by the administration of l-5-hydroxytryptophan." Neuroscience **148**(1): 212-20.

Wareing, M., P. N. Murphy and J. E. Fisk (2004). "Visuospatial memory impairments in users of MDMA ('ecstasy')." Psychopharmacology (Berl) **173**(3-4): 391-7.

Wayman, J. (2003). Multiple imputation for missing data: what is it and how can I use it? Annual Meeting fo the American Educational Research Association. Chicago, IL.

Williams, T. J., K. S. LaForge, D. Gordon, G. Bart, S. Kellogg, J. Ott and M. J. Kreek (2007). "Prodynorphin gene promoter repeat associated with cocaine/alcohol codependence." Addict Biol **12**(3-4): 496-502.

Winstanley, C. A., D. M. Eagle and T. W. Robbins (2006). "Behavioral models of impulsivity in relation to ADHD: translation between clinical and preclinical studies." Clin Psychol Rev **26**(4): 379-95.

Winstanley, C. A., D. E. Theobald, J. W. Dalley, J. C. Glennon and T. W. Robbins (2004). "5-HT2A and 5-HT2C receptor antagonists have opposing effects on a measure of impulsivity: interactions with global 5-HT depletion." Psychopharmacology (Berl) **176**(3-4): 376-85.

Winstock, A. R., K. Wolff and J. Ramsey (2001). "Ecstasy pill testing: harm minimization gone too far?" Addiction **96**(8): 1139-48.

Yale University, F. C. http://info.med.yale.edu/genetics/kkidd/programs.html**:** Hardy-Weinberg Equilibrium Analyis software.

Yamamoto, B. K., J. F. Nash and G. A. Gudelsky (1995). "Modulation of methylenedioxymethamphetamine-induced striatal dopamine release by the interaction between serotonin and gamma-aminobutyric acid in the substantia nigra." J Pharmacol Exp Ther **273**(3): 1063-70.

Zill, P., A. Buttner, W. Eisenmenger, B. Bondy and M. Ackenheil (2004). "Regional mRNA expression of a second tryptophan hydroxylase isoform in postmortem tissue samples of two human brains." Eur Neuropsychopharmacol **14**(4): 282-4.

Zill, P., A. Buttner, W. Eisenmenger, H. J. Moller, B. Bondy and M. Ackenheil (2004). "Single nucleotide polymorphism and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene in suicide victims." Biol Psychiatry **56**(8): 581-6.

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Publications

Moukaddam NJ, Hirschfeld MA "Intravenous Antidepressants: a Review", Depression and Anxiety, 19, 2004; 1-9

Nidal Moukaddam, Angela Stotts, Anne Hamilton Dougherty, Charles Green, Marc Mooney PhD5 Ann Garcia, Richard A. Meisch, Katherine Cowan, David E. Moody, Joy M. Schmitz, John Grabowski "Opioid Agonist (LAAM) Maintenance Dosing in Heroin Dependence", *in press,* Journal of Addictive Diseases and Their Treatment.