5-HTTLPR Genotype Moderates the Effects of Past Ecstasy Use on Verbal Memory Performance in Adolescent and Emerging Adults: A Pilot Study

Natasha E. Wright[1], Judith A. Strong[2], Erika R. Gilbart[1], Skyler G. Shollenbarger[1], Krista M. Lisdahl[1] *

1 Department of Psychology, University of Wisconsin-Milwaukee, Milwaukee, WI, United States of America,
2 Department of Anesthesiology, University of Cincinnati, Cincinnati, OH, United States of America

* krista.medina@gmail.com

Abstract

Objective

Ecstasy use is associated with memory deficits. Serotonin transporter gene (5-HTTLPR) polymorphisms have been linked with memory function in healthy samples. The present pilot study investigated the influence of 5-HTTLPR polymorphisms on memory performance in ecstasy users, marijuana-using controls, and non-drug-using controls, after a minimum of 7 days of abstinence.

Method

Data were collected from 116 young adults (18–25 years-old), including 45 controls, 42 marijuana users, and 29 ecstasy users, and were balanced for 5-HTTLPR genotype. Participants were abstinent seven days prior to completing memory testing. Three MANCOVAs and one ANCOVA were run to examine whether drug group, 5-HTTLPR genotype, and their interactions predicted verbal and visual memory after controlling for gender, past year alcohol use, other drug use, and nicotine cotinine levels.

Results

MANCOVA and ANCOVA analysis revealed a significant interaction between drug group and genotype ($p = .03$) such that ecstasy users with the L/L genotype performed significantly worse on CVLT-2 total recall ($p = .05$), short ($p = .008$) and long delay free recall ($p = .01$), and recognition ($p = .006$), with the reverse pattern found in controls. Ecstasy did not significantly predict visual memory. 5-HTTLPR genotype significantly predicted memory for faces ($p = .02$); short allele carriers performed better than those with L/L genotype.

Conclusions

5-HTTLPR genotype moderated the effects of ecstasy on verbal memory, with L/L carriers performing worse compared to controls. Future research should continue to examine individual differences in ecstasy's impact on neurocognitive performance as well as relationships with neuronal structure. Additional screening and prevention efforts focused on adolescents and emerging adults are necessary to prevent ecstasy consumption.

Introduction

The lifetime prevalence rate of ecstasy (MDMA, or 3,4-methylenedioxymethamphetamine) use among adolescents is 11.3% [1], with girls reporting more use [2]. Neurocognitive developmental changes are abundant in adolescence and last into emerging adulthood [3], with changes in the prefrontal cortex and the limbic system leading to increased vulnerability to risky behavior and the neurotoxic effects of drug use (see [4]). Some suggest that due to this malleable time period of brain development, later life events may not be able to correct for inadequate development during teen years [5]. Therefore, it is important to understand the neurocognitive impact of ecstasy use, and to help determine at-risk groups who may experience poorer outcomes.

Exposure to MDMA, the major component in ecstasy or Molly, impacts reuptake of serotonin (5-HT), and to a lesser extent norepinephrine and dopamine [6–8]. MDMA has been implicated in loss of serotonin in rats and humans [8–15]. This may be due, in part, to the ability of MDMA to reverse serotonin transporters through stimulating serotonin efflux, resulting in an overabundance of serotonin being released at once [16], as well as doubling the amount of 5-HTT expressed several hours after MDMA consumption [8]. In animal studies, MDMA has been shown to be particularly neurotoxic to the serotonergic system [8–10, 13]. Serotonergic recovery has been shown with at least a year of abstinence, though the axons exhibited alternate patterns of functioning compared to controls [9], even after 7 years in squirrel monkeys [10]. Serotonin neurotoxicity has also been demonstrated in humans; in a positron emission tomography (PET) study of ecstasy users, Kish and colleagues [11] found decreased serotonin transporter binding throughout the cortex and specifically in the hippocampus in comparison to controls, even after a minimum of 45 days abstinence. Decreased binding was further predictive of worse verbal memory performance. Other PET studies have found similar reductions in 5-HT binding across cortical sites [12] and availability, including in the hippocampus [17]. Therefore, converging lines of evidence have suggested that the hippocampus may be particularly vulnerable to ecstasy-related damage [11, 18–22]. Consistent with this hypothesis, adolescent ecstasy users show abnormal functional activation of the hippocampus during a verbal working memory task [23]. While there may be some recovery of 5-HT neurons with abstinence [24], Ando and colleagues [25] found that the hippocampus and thalamus recovered less than the rest of the cortex.

Consistent with these hippocampal abnormalities, four meta-analyses have concluded that ecstasy exposure is linked with reduced verbal memory, with medium to large effect sizes [26–29]. For example, Kalechstein and colleagues [27] found deficits in attention, verbal and nonverbal memory and learning, motor speed, and executive functioning systems in ecstasy users, with the largest effect size on verbal learning and memory (Cohen's *d* = .73) and nonverbal learning and memory the next largest (Cohen's *d* = .58). More specifically, with two exceptions [30–31], studies have found verbal learning and memory deficits in ecstasy users compared to

controls [20, 28, 32–37]. Ecstasy users also self-report more memory deficits in comparison to non-ecstasy drug users and controls [38]. However, at least two studies only found memory deficits within the context of polydrug use, not specific to ecstasy consumption [39, 40]. Results regarding visual memory are more limited, with fewer studies investigating how it is affected by ecstasy exposure, though those that have found mixed results. Rosier and colleagues [41] found no significant differences in visual memory performance among ecstasy users when compared to controls, however, several other studies have found visual memory deficits related to ecstasy exposure [19, 32, 42].

Inconsistencies in the literature on memory function in ecstasy users may be explained by individual differences in genes that code for serotonin transporters, as there has been minimal investigation into the potential moderating capacity of serotonin transporter gene (*5-HTTLPR*). In particular, *5-HTTLPR* has been associated with memory function (*5-HTTLPR*) [11, 43]. The short (S) allele of *5-HTTLPR* has decreased reuptake of serotonin transporter (SERT, or 5-HTT) compared to the long (L) allele, perhaps due to having 40% fewer SERT binding sites [44]. L/L genotype carriers also have higher densities of 5-HTT as well as increased 5-HTT expression in comparison to S carriers [8, 45]. Due to downregulation, this may result in overall reduced 5-HT signaling [46], and the S allele has been linked with increased risk for affective disorders [47] and greater depressive symptoms [48]. However, at least one study has found that in the presence of a stressor (family discord), those with the L/L 5-HTTLPR genotype demonstrated significantly higher rates of anxiety [49].

In healthy controls, we found that the S allele was associated with larger left hippocampal volumes, poorer verbal memory and greater depressive symptoms in females, demonstrating a gender specific functional disadvantage [43]. The L/L genotype has also been found to be protective against postpartum depression symptoms [50], while S carriers with chronic stress have had increased depression symptoms in young adults at-risk for depression [48]. However, only a few studies have examined the interaction between *5-HTTLPR* genotype and ecstasy use. In ecstasy users, carriers of the S/S allele have exhibited deficits in verbal fluency [51] as well as decreased cardiovascular and more sedative subjective effects following consumption [52]. S carrier ecstasy users have also been found to have increased risk for mood disorder symptoms [53]. In regards to memory, *5-HTTLPR* genotype moderated the effects of ecstasy on visual memory [41–42]. Cuyas and colleagues [42] found that lifetime ecstasy use negatively correlated with visual memory, and users with *5-HTTLPR* S alleles exhibited poorer performance. Rosier and colleagues [41] similarly found in an adult sample that S carriers trended towards significantly worse visual memory and decision making than healthy controls. However, other studies have found that in the presence of depression, the L allele may confer a functional disadvantage [54–55]. Ecstasy users with L/L genotype have also been found to have greater changes in 5-HTT gene expression, though these results are absent of full statistical analyses due to limited power [8]. Therefore, there is preliminary evidence that the *5-HTTLPR* genotype may moderate the effects of ecstasy on cognition.

As ecstasy users rarely only use ecstasy, marijuana use co-morbidity is a common issue in studying the unique effects of ecstasy [56]. The presence of executive functioning deficits that are not accounted for by polydrug use is debated [39, 42, 57], though the two drugs have been shown to interact with gender in glial and serotonergic cells [58] and several studies have found deficits in ecstasy users that were above and beyond that of controls and marijuana users [20, 36, 42, 59–60]. Independently, marijuana has been associated with memory deficits [39, 61–65]. There is also some evidence that marijuana may interact with *5-HTTLPR*. The short allele has been shown to be more closely related to bipolar disorder diagnosis in marijuana users [66], and marijuana users with S/S genotype performed worse on a risk-taking task compared to S/S controls, though verbal memory was not assessed [67]. Therefore, there appears to

be some overlap between the effects of ecstasy and marijuana on memory function and the two substances may uniquely interact with *5-HTTLPR*, necessitating the inclusion of a marijuana control group.

The present pilot study was conducted to investigate the independent and interactive influences of ecstasy use and *5-HTTLPR* polymorphisms on memory functioning, while controlling for comorbid marijuana use. It was predicted that ecstasy use and the *5-HTTLPR* genotype would interact in predicting verbal and visual memory impairments, with S carriers demonstrating the greatest memory deficits in comparison to healthy controls and L/L genotypes [41–43].

Materials and Methods

Participants

One-hundred sixteen participants were recruited through fliers and advertisements in community newspapers. Forty-four controls, 43 marijuana users, and 29 ecstasy users, were balanced for *5-HTTLPR* genotype (28 S carrier controls, 28 S carrier marijuana users, 17 S carrier ecstasy users), fitting with larger population estimates of the distribution of S carriers to L/L genotype in mostly white samples [68]. Seventy-two participants (62%) were white. Nine (31%) of the ecstasy users met DSM IV criteria for ecstasy dependence. Exclusion criteria included co-morbid independent Axis I disorders, major medical or neurologic disorders, prenatal issues (e.g., gestation > 35 weeks) or prenatal exposure to alcohol (>4 drinks/day or >7 drinks/week) or illicit drugs (>10 uses), and excessive other-drug use (>50 uses of any drug category except nicotine, alcohol, marijuana, and ecstasy in lifetime). Control subjects had fewer than 3 lifetime ecstasy uses and 10 lifetime marijuana uses. Marijuana group subjects had fewer than 6 ecstasy uses and had used marijuana more than 50 times lifetime. Ecstasy users were so grouped if they had reported more than 10 lifetime ecstasy uses during initial screening; upon more detailed collection of substance use history, it was revealed that not all participants met this initial inclusion criterion and therefore those in the ecstasy group have a minimum of 7 lifetime uses. Participants were required to remain abstinent from alcohol or drug use for seven days prior to the study session.

Procedure

The University of Cincinnati Institutional Review Board approved all aspects of this study. Advertisements were placed in newspapers and on college campuses to recruit participants. Interested participants called in and, after providing oral consent, completed a phone screen using a semi-structured interview to assess for Axis I disorders [69]. If eligible and after obtaining informed written consent, eligible participants completed the parent imaging genetics study (PI: Lisdahl, 1R03 DA027457) in either one or two sessions. Those with substance use histories completed the psychological questionnaires, drug use interview, neuropsychological battery, and MRI scan in two sessions (typically 2–3 days apart). Those with minimal use completed the study in one session. Participants were paid $160 for two sessions ($110 for one) and received parking reimbursement, local substance treatment resources and images of their brain.

Biological samples. Participants underwent a urine toxicology screen using the One Step Drug Screen Test, a breathalyzer test, and female participants were administered a pregnancy test. Those who tested positive for drugs and/or alcohol except cannabis and nicotine were excluded. Metabolite levels of THC were examined for participants that tested positive for marijuana using mass spectrometry testing; session 2 THC:creatinine metabolite ratios were

subtracted from session 1 total ratios to ensure there were no increases or current use during the week of memory testing (see [70]).

Drug use. Ecstasy and other drug use was measured using the Timeline Follow-Back (TLFB). A modified version of the *Time-Line Follow-Back* [69, 71] technique was used, which utilizes memory cues of common holidays and personal events to measure frequency of drug use over the past year (assessed month-by-month for one year). Additionally, a semi-structured interview was administered to measure frequency/quantity of lifetime drug use [69]. For each drug category, participants were asked their average weekly use for each year of use. The following drug categories were assessed: ecstasy or Molly, alcohol, marijuana, sedatives (e.g., downers, ketamine, GHB), stimulants (amphetamine, methamphetamine, cocaine, crack cocaine), hallucinogens (mushrooms, PCP, LSD, peyote), opioids (heroin, opium), and inhalants (nitrous oxide, paint, glue, household cleaners, gas). The participant's drug use was measured in standard units (tablets for ecstasy; standard drinks for alcohol; joints for marijuana).

Memory. The California Verbal Learning Test—2nd edition (CVLT-2) [72] was used to measure verbal memory. Participants are read 20 words belonging to four semantic categories and asked to recall as many as possible across five learning trials. They are then read a 20-word distracter list. After a short delay, they are asked to recall the first list given cues about semantic categories. After a 20-minute delay, free and cued recall, and recognition ability are measured. The Rey Osterrieth Complex Figure Test (RCFT) [73] and the Faces I and Faces II subtests from the Wechsler Memory Scale-III (WMS-III) [74] were used to assess visual memory. For the RCFT, participants first copied a unique picture containing various shapes and markings, then drew the figure again from memory immediately and after a delay. For the WMS-III Faces tasks, during the initial presentation, participants were exposed to a series of 24 target faces. Immediately following, participants were shown a second series of 48 faces (24 old, 24 new) and asked to recognize the target faces (Faces I). After a delay, participants were shown another series of 48 faces and were again asked to identify the target faces that were from the initial presentation (Faces II).

Genotyping. As described in Price et al. [43], Dr. Judith Strong performed the genotyping for *5-HTTLPR* L/S promoter polymorphism [75]. The amplification products were digested with restriction enzyme HpaII in order to analyze the functional A/G SNP (rs25531) within the L/S promoter polymorphism. L alleles with G at the rs 25531 site were scored as S alleles. Details of the amplification method and primers used were as in Thompson et al. [76].

Data analysis. ANOVAS and chi-squares were run to examine potential demographic differences between drug groups and genotype. To assess the independent and interactive effects of ecstasy use and *5-HTTLPR* status on verbal and visual memory, MANCOVAs were run to examine whether groups (controls, MJ users, and ecstasy users) differed on 1) verbal memory (total recall, short delay free recall, and long delay free recall), 2) visual figure memory (immediate, delayed recall), and 3) visual faces memory (immediate, delayed recall). All memory variables included in each of the three MANCOVAs were significantly correlated within their own domains (i.e., verbal memory, object memory, and faces memory; r's>.70). All variables were normally distributed, except for verbal memory recognition. Therefore, after logarithmically transforming the variable, we ran verbal memory recognition as a separate ANCOVA. We did not adjust for multiple comparisons as this is a small pilot study and such a correction would increase the likelihood of Type II error too significantly. Covariates included gender, cotinine levels (nicotine metabolite), past year alcohol use (total number of standard drinks), and total other drug use (all episodes combined). Because nicotine and alcohol use have previously been shown to influence verbal memory performance [77, 78] and due to the polydrug use which frequently occurs with MDMA use, cotinine, past year alcohol use, and other drug use were chosen as covariates, though they were not found to influence results in the present study.

Table 1. Demographics by Drug Group and Genotype.

	Controls (n = 44)		MJ (n = 43)		Ecstasy (n = 29)	
	% or M (SD) Range		% or M (SD) Range		% or M (SD) Range	
	S carrier	L/L	S carrier	L/L	S carrier	L/L
5-HTTLPR Genotype	28	16	28	15	17	12
	(64%)	(36%)	(65%)	(35%)	(59%)	(41%)
Age	21.3	20.5	21.3	20.8	22.2	22.2
	(2.2)	(2.3)	(2.3)	(2.4)	(2.1)	(2.2)
	18–25	18–25	18–25	18–25	18–25	19–25
Education	14.1	13.3	13.2	13.9	13.6	13.0
	(1.8)	(1.4)	(1.8)	(2.2)	(2.0)	(1.6)
	11–18	11–16	9–17	12–19	11–18	11–17
Reading Score	97.8	104.4	102.9	103.7	100.1	97.4
	(10.4)	(10.1)	(14.5)	(15.9)	(10.6)	(13.3)
	78–120	89–120	73–133	80–134	82–115	67–112
Gender (% female)	50%	56%	32%	47%	35%	33%
% White	57%	56%	71%	53%	71%	58%
*[a] Past Year Ecstasy Use	0	0	0.1	0.1	36.9	14.3
	(0)	(0)	(0.3)	(0.5)	(62.3)	(12.0)
			0–1	0–2	0–255	1–41
* [a] Lifetime Ecstasy Use (tablets)	0	0	0.5 (1.2)	0.8	448	303.8
	(0)	(0)	0–5	(1.7)	(1062.5)	(672.3)
				0–6	7–4026	11–2202
* [b] Past Year Marijuana Use (joints)	.3	.8	468.6	406.2	845.4	1025.3
	(1.4)	(1.7)	(830.0)	(410)	(1731.3)	(1334.4)
	0–7	0–5	10–3895	4–1662	0–7343	12–4179
* [b] Past Year Alcohol Use (standard drinks)	69.5	95.3	293.0	360.5	394.2	179.2
	(79.4)	(212.8)	(288.9)	(482.1)	(435.1)	(243.7)
	0–320	0–878	0–914	7–1724	0–1426	0–884
* [b] Cotinine Level	1.2	1.9	4.0	3.6	3.8	4.3
	(1.7)	(2.7)	(2.3)	(2.6)	(2.4)	(2.3)
	0–6	0–6	0–6	0–6	0–6	1–6
* [a] Other Drug Use	0.04	0.3	11.0	2.3	111.1	159.7
	(0.2)	(0.8)	(32.5)	(3.1)	(198.7)	(302.3)
	0–1	0–3	0–170	0–10	0–827	0–1038

Notes: M = mean; SD = standard deviation.

*p < .05

a = ecstasy users significantly greater than MJ users and controls

b = MJ users and ecstasy users significantly greater than controls

doi:10.1371/journal.pone.0134708.t001

Results

Demographics

Drug groups did not differ significantly on gender [x^2 = 2.5, p = .3], age [$F_{(2,113)}$ = 2.9, p = .06], ethnicity [x^2 = 9.7, p = .5], education [$F_{(2,113)}$ = 1.0, p = .4], reading ability [$F_{(2,113)}$ = 1.0, p = .4], income [$F_{(2,113)}$ = 0.7, p = .5], serotonin genotype [x^2 = 0.5, p = .8], or past year cigarette use [$F_{(2,113)}$ = 2.9, p = .06] (see Table 1). As genetic results are often influenced by

ethnicity [79], we confirmed our findings through running the same analyses using only white subjects; results remained unchanged. Drug groups differed on recent nicotine use (cotinine level) [F(2,112) = 14.3, p < .001], past year ecstasy [F(2,113) = 13.8, p < .001], alcohol [F(2,113) = 8.2, p < .001], marijuana [F(2,113) = 9.3, p < .001], and other drug use (including ecstasy) [F(2,106) = 10.87, p < .001]; cotinine, alcohol, and other drug use are controlled for in the MANCOVA analysis (see Table 1). Post-hoc analysis revealed that the ecstasy and marijuana groups both differed from controls, but not from each other, in their past year alcohol, marijuana, and nicotine use (*p*'s>.10). Ecstasy users reported significantly more other drug use, especially hallucinogens, although this was limited to no more than 25 times in their lifetime.

Genotype groups also did not differ on gender [x^2 = .55, *p* = 0.46], age [F(1,114) = 1.1, p = .3], ethnicity [x^2 = 4.66, *p* = 0.46], education [F(1,114) = 0.4, p = .5], reading ability [F(1,114) = 0.6, p = .4], income [F(1,114) = 0.02, p = .9], or past year alcohol [F(1,114) = 0.1, p = .8], marijuana [F(1,114) = 0.1, p = .8], nicotine [F(1,114) = 1.8, p = .2], and other drugs [F(1,114) = 0.4, p = .5]. Further, when looking only at the ecstasy group, the two genotype groups did not differ on gender [x^2 = .01, *p* = 0.6], age [F(1,27) = .01, p = .9], ethnicity [x^2 = 2.5, *p* = 0.5], education [F(1,27) = 0.7, p = .4], reading ability [F(1,27) = 0.4, p = .6], income [F(1,27) = 0.6, p = .4], or past year alcohol [F(1,27) = 2.4, p = .13], marijuana [F(1,27) = 0.1, p = .8], nicotine [F(1,27) = 2.4, p = .14], and other drugs [F(1,27) = 0.3, p = .6], past year [F(1,27) = 1.5, p = .2] or lifetime [F(1,27) = .2, p = .7] ecstasy use, nor did they differ on length of abstinence from ecstasy [F(1,25) = 0.01, p = .9].

Frequency/Quantity of substance use

In ecstasy users, mean lifetime ecstasy use was 388.3 tablets (SD = 909.9; range 7–4026), with a mean use of 27.5 tablets (SD = 49.0; range 0–255) in the past year. Ecstasy users also used marijuana an average of 919.8 joints in the past year (SD = 1555.8; range 0–7343). In marijuana using controls, mean lifetime ecstasy use was 0.6 tablets (SD = 1.4). Marijuana users' mean lifetime marijuana use was 1450.1 joints (SD = 3136.4; range 10–17821), with a mean use of 446.8 joints (SD = 706.9) in the past year. All participants demonstrated negative toxicology tests for all drugs, including MDMA, and reduced levels of THC from session one to two, demonstrating at least one week of abstinence from all drugs. The ecstasy users were abstinent from ecstasy for an average of 75 days (SD = 71; range 7–274).

Verbal memory

Ecstasy Use. MANCOVA analysis revealed that after controlling for recent nicotine, alcohol and other drug use, ecstasy group status did not independently predict verbal memory. ANCOVA analysis of verbal memory recognition revealed that after controlling for recent nicotine, alcohol and other drug use, ecstasy group status did not independently predict verbal memory. Genotype. Similarly, genotype did not independently predict verbal memory performance. Ecstasy**5-HTTLPR* Genotype. A significant interaction between ecstasy group status and *5-HTTLPR* genotype [Pillai's trace = .12, F(6,206) = 2.28, p = .04] was observed in predicting CVLT-2 verbal memory. Post-hoc analysis revealed that *5-HTTLPR* genotype interacted with ecstasy group to predict total recall [F(2,104) = 2.99, p = .05], short delay free recall [F(2,104) = 5.27, p = .007], long delay free recall [F(2,104) = 4.88, p = .009; see Fig 1]. Similarly, in examining the ANCOVA analysis, ecstasy group interacted with *5-HTTLPR* genotype in predicting total recognition discriminability [F(2,104) = 4.73, p = .01; see Fig 1]. In the non-using controls, the S allele was associated with poorer verbal memory in each domain while in the ecstasy users, the L/L genotypes performed worse than S carriers and both control groups.
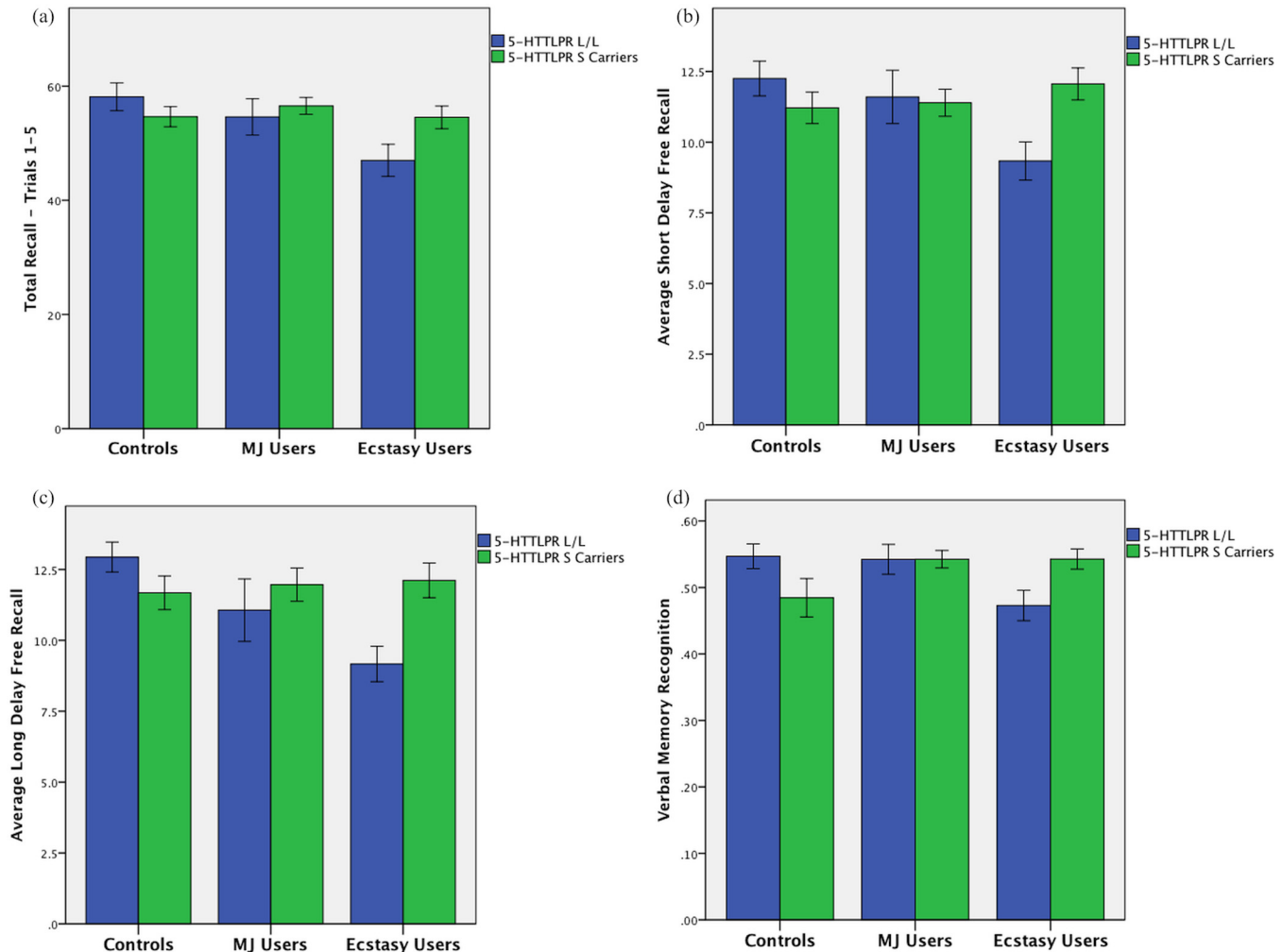
Fig 1. Average Verbal Memory Performance According to Drug Group and 5-HTTLPR Genotype. *5-HTTLPR* genotype interacted with ecstasy group to predict (a) total recall of the first 5 trials; (b) average short delay free recall; (c) average long delay (20 minute) verbal recall; and (d) average recognition. In the non-using controls, the S allele was associated with poorer verbal memory while in the ecstasy users, the L/L genotypes performed worse than S carriers and both control groups.

doi:10.1371/journal.pone.0134708.g001

Visual memory

Ecstasy Use. MANCOVA analysis revealed that after controlling for recent nicotine, alcohol and other drug use, ecstasy group status did not independently predict RCFT visual memory. Genotype. *5-HTTLPR* genotype predicted WMS Faces performance [Pillai's Trace = .08, F $(2,103)$ = 4.3, p = .01]. Post-hoc analysis revealed that *5-HTTLPR* genotype predicted Faces total recall I [F$(1,104)$ = 6.2, p = .01] and II [F$(1,104)$ = 8.7, p = .004]. Examination of estimated marginal means (see Table 2) revealed that S carriers performed better on both Faces I and II recall compared to L/L genotype carriers. Ecstasy* *5-HTTLPR* Genotype. No significant interactions were revealed between ecstasy group status and *5-HTTLPR* genotype.

Discussion

The aims of the current study were to examine the independent and interactive effects of ecstasy (MDMA) exposure and *5-HTTLPR* genotype on verbal and visual memory in abstinent

Table 2. Estimated Marginal Means for Verbal and Visual Memory Performance by Drug Group and Genotype.

	Controls		MJ		Ecstasy	
	M (SE)		*M* (SE)		*M* (SE)	
Verbal Memory	S carrier	L/L	S carrier	L/L	S carrier	L/L
Immediate Total Raw	54.7 (2.0)	58.7 (2.4)	57.2 (1.9)	54.7 (2.5)	54.4 (2.4)	47.2 (3.0)
Short Delay	11.2 (.6)	12.4 (.7)	11.8 (.5)	11.8 (.7)	12.0 (.7)	9.0 (.8)
Long Delay	11.5 (.6)	13.0 (.8)	12.3 (.6)	11.3 (.8)	12.2 (.8)	9.0 (1.0)
Recognition	.48 (.020)	.55 (.024)	.55 (.019)	.55 (.025)	.55 (.025)	.47 (.030)
Visual Memory						
RCFT Immediate Recall	19.4 (1.3)	20.1 (1.6)	20.4 (1.3)	20.5 (1.6)	18.6 (1.6)	19.8 (1.9)
RCFT Delayed Recall	18.6 (1.2)	18.9 (1.5)	19.5 (1.2)	20.7 (1.5)	18.1 (1.5)	19.5 (1.8)
WMS Faces I	37.4 (.9)	37.2 (1.0)	38.5 (1.0)	38.4 (.9)	40.6 (1.3)	37.4 (1.1)
WMS Faces II	39.1 (.8)	37.5 (.9)	38.5 (.9)	38.3 (.8)	41.3 (1.2)	37.7 (.9)

Notes: *M* = mean; SE = standard error

doi:10.1371/journal.pone.0134708.t002

adolescents and emerging adults. The findings demonstrated that *5-HTTLPR* status moderated the impact of ecstasy use on verbal memory function in youth. As hypothesized, in controls, the *5-HTTLPR* S allele was associated with poorer verbal memory. However, ecstasy and marijuana users demonstrated the opposite pattern, the *5-HTTLPR* L/L genotype was associated with inferior verbal memory performance. Results further revealed that *5-HTTLPR* genotype predicted memory for faces; S carriers performed superior to individuals with the L/L genotype.

This study lends further evidence that ecstasy users demonstrate significant verbal memory deficits in new learning, retention, and recognition of verbal material with relatively intact visual memory [11, 20, 32–33, 35–37, 57], but only in L/L ecstasy users. The current sample of ecstasy-users used an average of 27 ecstasy tablets in the past year (80% using 25 or fewer tablets in the past year), demonstrating that compared to other drugs of abuse, relatively low levels of exposure may result in verbal memory deficits. For example, marijuana studies include participants who use marijuana several times a week to multiple times a day, resulting in hundreds of uses over a year. Further, consistent with our prior study [20], the ecstasy users were abstinent from ecstasy for an average of 2.5 months (minimum 7 days), suggesting that verbal memory deficits may continue despite significant periods of abstinence. These converging lines of evidence warrant consistent screening for ecstasy consumption in schools and universities with clear warnings that even recreational use of ecstasy may result in significant verbal memory problems, particularly in those with specific genotypes (here, L/L serotonin transporter genotype).

We previously [43] reported that in healthy controls, females with the S allele had poorer verbal memory, larger hippocampal volumes, and greater depressive symptoms compared to the L/L genotype, suggesting that the S allele may be associated with reduced 5-HT signaling in healthy samples. In support of this, the current study demonstrated that non-drug using individuals with the S allele performed more poorly on verbal memory tasks. This supports the previous studies demonstrating that the S allele was associated with poorer downstream serotonin signaling [44], which may place one at risk for poorer verbal memory or increased risk for serotonin-related psychiatric disorders [47]. The current study also identified a significant at-risk group for negative outcomes; those who had the L/L serotonin transporter genotype and used ecstasy recreationally demonstrated the most robust verbal memory deficits. This is in contrast

with our original hypothesis, based on prior findings [41, 53], that the S allele would confer a functional disadvantage in the drug users. The current findings suggest that the downstream functional relationship between *5-HTTLPR* genotype and memory may be different in drug users, or the drugs directly impacted the serotonin system, altering the functional consequence of the genotype. This may also be due to the increased 5-HTT gene expression in ecstasy users with the L/L genotype, compared to S carrier ecstasy users [8], which may result in greater downstream downregulation of the serotonin system following repeated use. Similar findings have been reported in depression [54–55] and those with significant family discord [49]. For example, individuals with late-onset depression and the *5-HTTLPR* L/L genotype demonstrated significantly smaller hippocampal volumes while those with early-onset depression demonstrated the opposite pattern. On balance, Frodl and colleagues [54] found reduced gray matter volumes in PFC and limbic regions, including the hippocampus in *5-HTTLPR* S allele carriers in controls, while the opposite was found in depressed patients. In summary, it is possible that damaging a robustly active serotonin system may result in increased functional consequences, or drugs such as ecstasy interact with the *5-HTTLPR* genotype in a unique manner [8]. Converging evidence suggests a link between *5-HTTLPR* genotype, hippocampal structure and memory, although this relationship may be moderated by the presence of a serotonin stressor (i.e., mood disorders, family discord, or ecstasy exposure). Additional research is needed to examine the impact of ecstasy and marijuana on hippocampal serotonin signaling across *5-HTTLPR* genotypes in youth.

Similar to Rosier et al. [41] and Medina et al. [20], the current study did not find significant visual memory deficits in ecstasy users. Also, this study found a functional advantage to the *5-HTTLPR* S allele in predicting facial memory performance. Similarly, Rosier and colleagues found that independent of ecstasy use, volunteers with the S/S genotype performed better than those with the L/L genotype on a visual planning task. In addition, Anderson and colleagues [80] found that in a healthy sample S carriers performed better on a task of visual working memory. Perhaps, then, if the L/L allele is related to increased serotonin signaling, then this increased signaling may be related to improved verbal memory but worse visual memory in otherwise healthy individuals. These findings provide preliminary evidence suggesting a role for the *5-HTTPLR* genotype in visual memory for faces and calls into question the idea that the S allele is always a risk allele. This finding needs to be confirmed with additional studies focused on visual processing and memory.

Replication in science is important. Candidate gene studies in particular tend to have greater issues in replicability [81]. Importantly, *5-HTTLPR* has been associated with serotonin diseases. For example, an analysis of the *5-HTTLPR* haplotype utilizing genome-wide association study (GWAS) data (n = 1505 cases and 2168 controls) found that the S allele of *5-HTTLPR* is associated with major depressive disorder [82]. However, a recent GWAS mega-analysis examining genetic predictors of MDD did not reveal significant findings, which was attributable to poor power [83]. Therefore, additional GWAS and candidate gene studies are needed to examine the downstream endophenotypes associated with the *5-HTTLPR* gene in healthy and serotonin-stressed subgroups (e.g., ecstasy users, those with MDD) [84]. Future studies should seek to replicate this particular endophenotypic finding.

The present findings are limited by a few important factors. First, due to low sample sizes we were unable to examine whether gender or ethnicity moderate the relationships between *5-HTTLPR* genotype and memory function [36, 68]; either gender or ethnicity may interact with *5-HTTLPR* genotype in predicting memory function and should be assessed in future studies. Second, a common issue in ecstasy studies is polydrug use. While we controlled for other drug use, alcohol, and nicotine use and included a marijuana control group, it is still difficult to assess the unique effects of ecstasy used *simultaneously* with other drugs. Third, while

we had a fairly large sample, we did not have enough power to separately assess L/S or S/S genotype. This may obscure some of the potential differences between polymorphisms. Other genes also may have a unique influence on memory due to interactions with the serotonin system, such as *COMT* [51] or *BDNF* [85]. Larger datasets that combine imaging genetics data and drug use information are needed to examine potential genetic epistasis. Replication of these results is also necessary; we were unable to replicate this study within an independent sample in the present paper due to logistical issues in recruiting new participants, but this will be an important step in future studies. Due to the skewed distribution of use, we were unable to assess the potential dose-dependent relationship between drug use and memory with genotype. Finally, we did not test for the purity of ecstasy or Molly, and therefore other substances may have been included in the pills (most often caffeine and other stimulants); however, reports from online testing in the United States reports show that the majority of ecstasy or Molly tablets do contain MDMA [86], with purity even higher in non-domestic ecstasy tablets [87].

Conclusions

In summary, the present study investigated the independent and interactive effects of ecstasy use and serotonin transporter gene (*5-HTTLPR*) on verbal and visual memory. We found that *5-HTTLPR* genotype significantly moderated ecstasy's impact on verbal memory. While in controls the L allele tends to be a protective factor, it appears to be a risk factor in ecstasy poly-drug users. These findings are particularly interesting when considering the novelty of this study and the level of difficulty in recruiting this population. Further, results demonstrate that even recreational ecstasy use is associated with verbal memory deficits, particularly in those with the *5-HTTLPR* L allele. This may be especially true in developing adolescents and emerging adults. Scientists developing large, longitudinal, multi-site imaging genetics studies are encouraged to consider the impact of genetics, and ecstasy exposure on the developing brain, as well as assessing the influence of gender.

Supporting Information

S1 File. Dataset.
(XLSX)

Author Contributions

Conceived and designed the experiments: KML. Performed the experiments: KML. Analyzed the data: NEW KML ERG SGS. Contributed reagents/materials/analysis tools: KML JS. Wrote the paper: NEW KML ERG SGS.

References

1. Johnston LD, O'Malley PM, Bachman JG, Schulenberg JE. Monitoring the Future national survey results on drug use, 1975–2011. Volume I: Secondary school students. Ann Arbor: Institute for Social Research, The University of Michigan; 2012.

2. Wu P, Liu X, Pham TH, Jin J, Fan B, Jin Z. Ecstasy use amongst US adolescence from 1999 to 2008. Drug and Alcohol Dependence 2010; 112:33–38. doi: 10.1016/j.drugalcdep.2010.05.006 PMID: 20570447

3. Gogtay N, Giedd JN, Lusk L, Hayashi KM, Greenstein D, Vaituzis AC, et al. Dynamic mapping of the human cortical development during childhood through early adulthood. PNAS 2004; 101:8174–9. PMID: 15148381

4. Bava S, Tapert SF. Adolescent brain development and the risk for alcohol and other drug problems. Neuropsychology Review 2010; 20(4):398–413. doi: 10.1007/s11065-010-9146-6 PMID: 20953990

5. Blakemore SJ, Choudhury S. Development of the adolescent brain: Implications for executive function and social cognition. Journal of Child Psychology and Psychiatry 2006; 47(3–4):296–312. PMID: 16492261

6. Parrott AC. Human psychopharmacology of Ecstasy (MDMA): A review of 15 years of empirical research. Hum. Psychopharmacol. 2001; 16:557–77. PMID: 12404536

7. Verrico CD, Miller GM, Madras BK. MDMA (Ecstasy) and human dopamine, norepinephrine, and serotonin transporters: Implications for MDMA-induced neurotoxicity and treatment. Psychopharmacology (Berl). 2007; 189(4):489–503.

8. Yubero-Lahoz S, Ayestas MA, Blough BE, Partilla JS, Rothman RB, de la Torre R, et al. Effects of MDMA and analogs on plasma 5-HT: Relevance of 5-HT transporters in blood and brain. European Journal of Pharmacology 2014; 674:337–44.

9. Fischer C, Hatzidimitriou J, Wlos J, Katz J, Ricaurte G. Reorganization of ascending 5-HT axon projections in animals previously exposed to the recreational drug (+)3,4-methylenedioxymethamphetamine (MDMA, "ecstasy). The Journal of Neuroscience 1995; 15:5476–85. PMID: 7643196

10. Hatzidimitriou G, McCann UD, Ricaurte GA. Altered serotonin innervation patterns in the forebrain of monkeys treated with (+)3,4-methylenedioxymethamphetamineseven years previously: Factors influencing abnormal recovery. The Journal of Neuroscience 1999; 19:5096–107. PMID: 10366642

11. Kish SJ, Lerch J, Furukawa Y, Tong J, McCluskey T, Wilkins D, et al. Decreased cerebral cortical serotonin transporter binding in ecstasy users: a positron emission tomography/[11C]DASB and structural brain imaging study. Brain 2010; 133:1779–97. doi: 10.1093/brain/awq103 PMID: 20483717

12. McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte RA. Positron emission tomographic evidence of toxic effect of MDMA ("Ecstasy") on brain serotonin neurons in human beings. Lancet 1998; 352:1433–7. PMID: 9807990

13. Perrine SA, Ghoddusi F, Michaels MS, Hyde EM, Kuhn DM, Galloway MP. MDMA administration decreases serotonin but not N-acetylaspartate in the rat brain. Neurotoxicology; 31(6):654–61. doi: 10.1016/j.neuro.2010.08.005 PMID: 20800616

14. Semple DM, Ebmeier KP, Glabus MF, O'Carroll RE, Johnstone EC. Reduced in vivo binding to the serotonin transporter in the cerebral cortex of MDMA ('ecstasy') users. The British Journal of Psychiatry 1999; 175:63–9. PMID: 10621770

15. Urban NB, Girgis RR, Talbot PS, Kegeles LS, Xu X, Frankle WG, et al. Sustained recreational use of ecstasy is associated with altered pre and postsynaptic markers of serotonin transmission in neocortical areas: a PET study with [11C]DASB and [11C]MDL100907. Neuropsychopharmacology 2012; 37 (6):1465–73. doi: 10.1038/npp.2011.332 PMID: 22353758

16. Rudnick G, Wall SC. Molecular mechanisms of "ecstasy" [3,4-methylenedioxy-methamphetamine (MDMA)]: Serotonin transporters are targets for MDMA-induced serotonin release. Proceedings of the National Academy of Sciences USA 1992; 89:1817–21.

17. Buchert R, Thomasius R, Wilke F, Peterson K, Nebeling B, Obrocki J, et al. A voxel-based pet investigation of the long-term effects of "ecstasy" consumption on brain serotonin transporters. American Journal of Psychiatry 2004; 161:1181–9. PMID: 15229049

18. Fox HC, McClean A, Turner JJ, Parrott AC, Rogers R, Sahakian BJ. Neuropsychological evidence of a relatively selective profile of temporal dysfunctionin drug-free MDMA ("ecstay") polydrug users. Psychopharmacology 2002; 162(2):203–14. PMID: 12110998

19. Gouzoulis-Mayfrank E, Thiumm B, Rezk M, Hensen G, Daumann J. Memory impairment suggests hippocampal dysfunction in abstinent ecstasy users. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2003; 27:819–27.

20. Medina KL, Shear PK, Corcoran K. Ecstasy (MDMA) exposure and neuropsychological functioning: A polydrug perspective. Journal of the International Neuropsychological Society 2005; 11:753–65. PMID: 16248911

21. Sharkey J, McBean DE, Kelly PA. Alterations in hippocampal function following repeated exposure to the amphetamine derivative methylenedioxymethamphetatmine ("Ecstasy"). Psychopharmacology 1991; 105(1):113–118. PMID: 1684057

22. Sprague JE, Preston AS, Leifheit M, Woodside B. Hippocampal serotonergic damage induced by MDMA (ecstasy): Effects of spatial learning. Physiol Behav 1993; 79:1281–7.

23. Jacobsen LK, Mencl WE, Pugh KR, Skudlarski P, Krystal JH. Preliminary evidence of hippocampal dysfunction in adolescent MDMA ("ecstasy") users: Possible relationship to neurotoxic effects. Psychopharmacology (Berl) 2004; 173:383–90.

24. Reneman L, Booij J, de Bruin K, Reitsma JB, de Wolff FA, Gunning WB, et al. Effects of dose, sex, and long-term abstention from use on toxic effects of MDMA (ecstasy) on brain neurons. Lancet 2001; 358:1864–9. PMID: 11741626

25. Ando RD, Andori C, Kirilly E, Molnar E, Kovacs GG, Ferrington L, et al. Acute SSRI-induced anxiogenic and brain metabolic effects are attenuated 6 months after initial MDMA-induced depletion. Behavioural Brain Research 2010; 207:280–9. doi: 10.1016/j.bbr.2009.10.011 PMID: 19840819

26. Laws KR, Kokkalis J. (2007). Ecstasy (MDMA) and memory function: a meta-analytic update. Human Psychopharmacology (Berl). 2007; 22,:381–8.

27. Kalechstein AD, de la Garza R, Mahoney JJ, Fantegrossi WE, Newtown TF. MDMA use and neurocognition: A meta-analytic review. Psychopharmacology 2007; 189:531–7. PMID: 17082969

28. Verbaten MN. Specific memory deficits in ecstasy users? The results of a meta-analysis. Hum Psychopharm. 2003; 167:85–96.

29. Zakzanis KK, Campbell Z, Jovanovski D. The neuropsychology of ecstasy (MDMA) use: A quantitative review. Hum Psychopharm. 2007; 22:427–35.

30. Croft RJ, Mackay AJ, Mills ATD, Gruzelier JGH. The relative contributions of ecstasy and cannabis to cognitive impairment. Psychopharmacology (Berl) 2000; 153:373–9.

31. Halpern JH, Sherwood AR, Hudson JI, Gruber S, Kozin D, Pope HG Jr. Residual neurocognitive features of long-term ecstasy users with minimal exposure to other drugs. Addiction 2011; 106(4):777–86. doi: 10.1111/j.1360-0443.2010.03252.x PMID: 21205042

32. Bolla KI, McCann UD, Ricaurte GA. Memory impairment in abstinent MDMA ("Ecstasy") users. American Academy of Neurology 1998; 51:1532–7.

33. Curran HV, Travill RA. Mood and cognitive effects of ± 354-niethylenedioxymethainphetaniine (MDMA, 'ecstasy'): week-end 'high' followed by mid-week low. Addiction 1997; 92(7):821–31. PMID: 9293041

34. Morgan MJ, McFie L, Fleetwood LH, Robinson JA. Ecstasy (MDMA): Are the psychological problems associated with its use reversed by prolonged abstinence? Psychopharmacology (Berl). 2002; 159:294–303.

35. Parrott AC, Lees A, Garnham NJ, Jones M, Wesnes K. Cognitive performance in recreational users of MDMA or 'ecstasy': evidence for memory deficits. Journal of Psychopharmacology 1998; 12(1):79–83. PMID: 9584971

36. Price JS, Shear P, Lisdahl KM. Ecstasy exposure and gender: Examining components of verbal memory function. PLoS One 2014; 9(12):e115645. doi: 10.1371/journal.pone.0115645 PMID: 25545890

37. Reneman L, Booij J, Schmand B, van den Brink W, Gunning WB. Memory disturbances in "Ecstasy" users are correlated with an altered brain serotonin neurotransmission. Psychopharmacology 2000; 148:322–4. PMID: 10755746

38. Rodgers J, Buchanan T, Scholey AB, Heffernan TM, Ling J, Parrott AC. Patterns of drug use and the influence of gender on self-reports of memory ability in ecstasy users: A web-based study. Journal of Psychopharmacology 2003; 17:389–96. PMID: 14870950

39. Hanson KL, Luciana M. Neurocognitive impairments in MDMA and other drug users: MDMA alone may not be a cognitive risk factor. Journal of Clinical and Experimental Neuropsychology 2010; 32(4):337–49. doi: 10.1080/13803390903042361 PMID: 20397296

40. Jager G, de Win MM, Vervaeke HK, Schilt T, Kahn RS, van der Brink W, et al. Incidental use of ecstasy: No evidence for harmful effects on cognitive brain function in a prospective fMRI study. Psychopharmacology 2007; 193:403–14. PMID: 17476480

41. Rosier JP, Rogers RD, Cook LJ, Sahakian BJ. The effect of polymorphism at the serotonin transporter gene on decision-making, memory, and executive function in ecstasy users and controls. Psychopharmacology 2006; 188(2):213–27. PMID: 16941121

42. Cuyas E, Verdejo-Garcia A, Fagundo AB, Khymenets O, Rodriguez J, Cuenca A et al. The influence of genetic and environmental factors among MDMA users in cognitive performance. PLoS One 2011; 6 (11):e27206. doi: 10.1371/journal.pone.0027206 PMID: 22110616

43. Price JS, Strong J, Elliassen J, McQueeny T, Miller M, Padula CB, et al. Serotonin transporter gene moderates associations between mood, memory and hippocampal volume. Behavioural Brain Research 2013; 242:158–65. doi: 10.1016/j.bbr.2012.11.013 PMID: 23266326

44. Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996; 274:1527–31. PMID: 8929413

45. Lesch K, Mossner R. Genetically driven variation in serotonin uptake: is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? Biological Psychiatry 1998; 44:179–92. PMID: 9693390

46. Williams RB, Marchuk DA, Gadde KM, Barefoot JC, Grichnik K, Helms MJ, et al. Serotonin-related gene polymorphisms and central nervous system serotonin function. Neuropsychopharmacology 2003; 28:533–41. PMID: 12629534

47. Lasky-Su JA, Faraone SV, Glatt SJ, Tsuang MT. Meta-analysis of the association between two polymorphisms in the serotonin transporter gene and affective disorders. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 2005; 133:110–5.

48. Starr LR, Hammen C, Conway CC, Raposa E, Brennan PA. Sensitizing effect of early adversity on depressive reactions to later proximal stress: Moderation by polymorphisms in serotonin transporter and corticotropin releasing hormone receptor genes in a 20-year longitudinal study. Development and Psychopathology 2014; 26:1241–54. doi: 10.1017/S0954579414000996 PMID: 25422958

49. Laucht M, Treutlein J, Blomeyer D, Buchmann AF, Schmid B, Becker K, et al. Interaction between the 5-HTTLPR serotonin transporter polymorphism and environmental adversity for mood and anxiety psychopathology: evidence from a high-risk community sample of young adults. International Journal of Neuropsychopharmacology 2009; 2:737–47.

50. Zhang X, Wang L, Huang F, Li J, Xiong L, Xue H, et al. Evaluation of the promoter region polymorphism (*5-HTTLPR*) in the serotonin transporter gene in females with postpartum depression. Experimental and Therapeutic Medicine 2015; 9:245–9. PMID: 25452810

51. Fagundo AB, Cuyas E, Verdejo-Garcia A, Khymenets O, Langhor K, Martin-Santos R, et al. The influence of 5-HTT and COMT genotypes on verbal fluency in ecstasy users. Journal of Psychopharmacology 2010; 24(9);1381–93. doi: 10.1177/0269881109354926 PMID: 20080926

52. Pardo-Lozano R, Farre M, Yubero-Lahoz S, O'Mathuna B, Torrens M, Mustata C. Clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"): The influence of gender and genetics (CYP2D6, COMT, 5-HTT). PLoS One 2012; 7(10):e47599. doi: 10.1371/journal.pone.0047599 PMID: 23112822

53. Martin-Santos R, Torrens M, Poudevida S, Langohr K, Cuyas E, Pacifici R, et al. 5-HTTLPR polymorphism, mood disorders and MDMA use in a 3-year follow-up study. Addiction Biology 2009; 15:15–22.

54. Frodl T, Koutsouleris N, Bottlender R, Born C, Jäger M, Mörgenthaler M, et al. Reduced gray matter brain volumes are associated with variants of the serotonin transporter gene in major depression. Molecular Psychiatry 2008; 13:1093–101. doi: 10.1038/mp.2008.62 PMID: 19008895

55. Taylor WD, Steffens DC, Payne ME, MacFall JR, Marchuk DA, Svenson IK, et al. Influence of serotonin transporter promote region polymorphisms onhippocampal volumes in late-life depression. Archives of General Psychiatry 2005; 62:537–44. PMID: 15867107

56. Martins SS, Mazzotti G, Chilcoat HD. Trends in ecstasy use in the United States from 1995–2001: Comparison with marijuana users and association with other drug use. Experimental and Clinical Psychopharmacology 2005; 13(3):244–52. PMID: 16173888

57. Lamers CTJ, Bechara A, Rizzo M, Ramaekers JG. Cognitive function and mood in MDMA/THC users, THC users and non-drug using controls. Journal of Psychopharmacology 2006; 20(2):302–11. PMID: 16510488

58. Lopez-Rodriquez AB, Llorente-Berzal A, Garcia-Segura LM, Viveros M. Sex-dependent long-term effects of adolescent exposure to THC and/or MDMA on neuroinflammation and serotoninergic and cannabinoid systems in rats. British Journal of Pharmacology 2013; 171(6):1435–47.

59. Quednow BB, Jessen F, Kuhn KU, Maier W, Daum I, Wagner M. Memory deficits in abstinent MDMA (ecstasy) users: Neuropsychological evidence of frontal dysfunction. Journal of Psychopharmacology 2006; 20:373–84. PMID: 16574711

60. Taurah L, Chandler C, Sanders G. Depression, impulsiveness, sleep, and memory in past and present polydrug users of 3,4-methylenedioxymethamphetamine (MDMA, ecstasy). Psychopharmacology 2014; 231:737–51. doi: 10.1007/s00213-013-3288-1 PMID: 24114426

61. Gonzalez R, Schuster RM, Mermelstein RJ, Vassileva J, Martin EM, Diviak KR. Performance of young adult cannabis users on neurocognitive measures of impulsive behavior and their relationship to symptoms of cannabis use disorders. Journal of Clinical and Experimental Neuropsychology 2012; 34 (9):962–76. doi: 10.1080/13803395.2012.703642 PMID: 22882144

62. Harvey MA, Sellman JD, Porter RJ, Frampton CM. The relationship between non-acute adolescent marijuana use and cognition. Drug and Alc Rev. 2007; 26(3):309–19.

63. Medina KL, Hanson KL, Schweinsburg AD, Cohen-Zion M, Nagel BJ, Tapert SF. Neuropsychological functioning in adolescent marijuana users: Subtle deficits detectable after a month of abstinence. J Int Neuropsychol Soc. 2007; 13(5):807–20. PMID: 17697412

64. Tait RJ, Mackinnon A, Christensen H. Cannabis use and cognitive function: 8-year trajectory in a young adult cohort. Addiction 2011; 106(12):2195–203. doi: 10.1111/j.1360-0443.2011.03574.x PMID: 21749524

65. Takagi M, Lubman DI, Cotton S, Fornito A, Baliz Y, Tucker A, et al. Executive control among adolescent inhalant and cannabis users. Drug and Alcohol Review 2011; 30:629–637. doi: 10.1111/j.1465-3362.2010.00256.x PMID: 21355925

66. De Pradier M, Gorwood P, Beaufils B, Ades J, Dubertret C. Influence of the serotonin transporter gene polymorphism, cannabis and childhood sexual abuse on phenotype of bipolar disorder: A preliminary study. European Psychiatry 2010; 25:323–7. doi: 10.1016/j.eurpsy.2009.10.002 PMID: 20434316

67. Verdejo-Garcia A, Fagundo AB, Cuenca A, Rodriguez J, Cuyas E, Langhor K, et al. COMT val158met and 5-HTTLPR genetic polymorphisms moderate executive control in cannabis users. Neuropsychopharmacology 2013; 38(8):1598–606. doi: 10.1038/npp.2013.59 PMID: 23449176

68. Noskova T, Pivac N, Nedic G, Kazantseva A, Gaysina D, Gaskhutdinova G, et al. Ethnic differences in the serotonin transporter polymorphism (5-HTTLPR) in several European populations. Pro Neuropsychopharmacol Biol Psyciatry 2008; 32(7):1745–9.

69. Lisdahl KL, Price JS. Increased marijuana use and gender predict poorer cognitive functioning in adolescents and emerging adults. J Int Neuropsychol Soc. 2012; 18(4):876–88.

70. Goodwin RS, Darwin WD, Chiang CN, Shih M, Li SH, Huestis MA. Urinary elimination of 11-nor-9-carboxy-delta9-tetrahydrocannabinol in cannbis users during continuously monitored abstinence. J Anal Toxicol. 2008; 32(8):562–9. PMID: 19007504

71. Sobell LC, Sobell MB. Timeline follow-back: A technique for assessing self-reported alcohol consumption. In Litten R. Z., & Allen J. (Eds.), Measuring alcohol consumption: Psychosocial and biological methods. New Jersey: Humana Press; 1992.

72. Delis DC, Kramer JH, Kaplan E, Ober BA. Manual for the California Verbal Learning Test. 2nd edition (CVLT-II). San Antonio, TX: The Psychological Corporation; 2000.

73. Meyers JE, Meyers KR. Rey complex figure test and recognition trial. San Antonio, TX: The Psychological Corporation; 1995.

74. Wechsler D. Wechsler Memory Scale–Third edition manual. San Antonio, TX: The Psychological Corporation; 1997.

75. Kaiser R, Tremblay PB, Roots I, Brockmoller J. Validity of PCR with emphasis on variable number of tandem repeat analysis. Clinical Biochemistry 2002; 35:49–56. PMID: 11937078

76. Thompson RD, Heffner JL, Strong JA, Blom TJ, Anthenelli RM. Relationship between the serotonin transporter polymorphism and obsessive-compulsive alcohol craving in alcohol-dependent adults: a pilot study. Alcohol 2010; 44:401–6. doi: 10.1016/j.alcohol.2010.05.008 PMID: 20598843

77. Gilbertson R., Boissoneault J., Prather R., & Nixon S.J. (2011). Nicotine effects on immediate and delayed verbal memory after substance use detoxification. J Clin Exp Neuropsychol., 33(6), 609–18.

78. Winward J.L., Hanson K.L., Tapert S.F., & Brown S.A. (2014). Heavy alcohol use, marijuana use, and concomitant use by adolescents are associated with unique and shared cognitive decrements. J Int Neuropsychol Soc., 20(8), 784–95.

79. Marchini J, Cardon LR, Phillips MS, Donnelly P. The effects of human population structure on large genetic association studies. Nature Genetics 2004; 36(5):512–7. PMID: 15052271

80. Anderson DE, Bell TA, Awh E. Polymorphisms in the 5-HTTLPR gene mediate storage capacity of visual working memory. Journal of Cognitive Neuroscience 2009; 24(5):1069–76.

81. Hart AB, de Wit H, Palmer AA. Candidate gene studies of a promising intermediate phenotype: Failure to replicate. Neuropsychopharmacology 2013; 38;802–816. doi: 10.1038/npp.2012.245 PMID: 23303064

82. Haenisch B, Herms S, Mattheisen M, Steffens M, Breuer R, Strohmaier J, et al. Genome-wide association data provide further support for an association between 5-HTTLPR and major depressive disorder. Journal of Affective Disorders 2013; 146:438–440. doi: 10.1016/j.jad.2012.08.001 PMID: 22921522

83. Ripke S, Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium, et al. A mega-analysis of genome-wide association studies for major depressive disorder. Mol. Psychiatry 2013; 18(4):497–511. doi: 10.1038/mp.2012.21 PMID: 22472876

84. Hall M-H, Smoller JW. A new role for endophenotypes in the GWAS era: Functional characterization of risk variants. Harv. Rev. Psychiatry 2010; 18:67–74. doi: 10.3109/10673220903523532 PMID: 20047462

85. Mattson MP, Maudsley S, Martin B. BDNF and 5-HT: A dynamic duo in age-related neuronal plasticity and neurodegenerative disorders. Trends Neurosci 2004; 27(10):589–594. PMID: 15374669

86. Tanner-Smith EE. Pharmacological content of tablets sold as "ecstasy": results from an online testing service. Drug Alcohol Depend. 2006; 83(3):247–54. Erratum in: Drug Alcohol Depend. 2008;93(1–2):190. PMID: 16364567

87. Parrott AC. Is ecstasy MDMA? A review of the proportion of ecstasy tablets containing MDMA, their dosage levels, and the changing perceptions of purity. Psychopharmacology (Berl). 2004; 173(3–4):234–41.