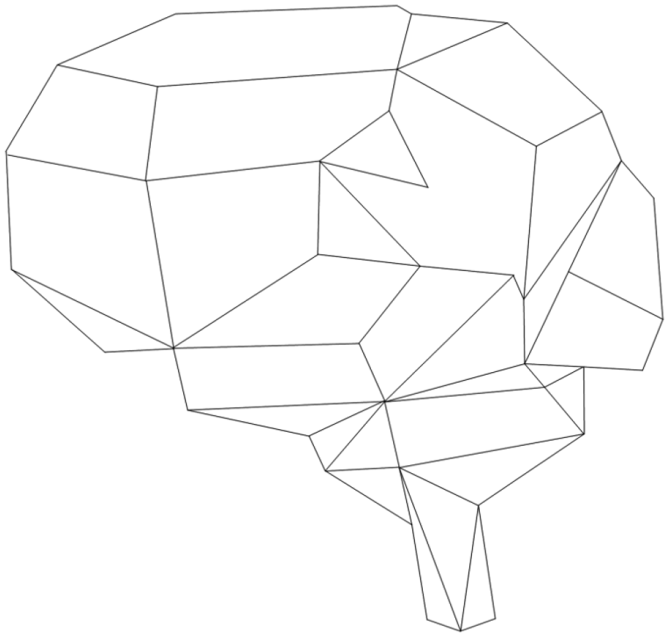
Chapter 8

MEMORY PERFORMANCE AND HIPPOCAMPUS STRUCTURE AFTER INFREQUENT RECREATIONAL STIMULANT USE IN YOUTH



Submitted as:
Schweren LJS, Giedd J, Castro N, Bartsch H, Squeglia LM, Meloy MJ, Tapert S. Memory performance and hippocampus structure after infrequent recreational stimulant use in youth.

ABSTRACT

Stimulant use has been associated with memory problems and hippocampal changes in adult recreational users. Animal studies have shown that the developing brain may be especially vulnerable to these effects, but studies in human adolescents are scarce. Here, we selected three demographically matched groups of youth from a prospective neuroimaging study, based on their substance use patterns. Participants (N=98, M_{AGE}=14.7) were stimulant-naïve at baseline, and classified at ~5 year follow-up as either nonuser controls (n=31), non-stimulant users (e.g., marijuana/alcohol, n=33), or stimulant-users (n=34). Neuropsychological testing and high-resolution magnetic resonance imaging were collected at each time point. Across these ~5 years of late adolescence from baseline to follow-up, performance on a verbal learning and memory test improved for non-stimulant users (+5.9%, p_{time}=0.023) but not for stimulant users (-1.3%, p_{time}=0.413; $p_{group\text{-}by\text{-}time}$=0.032). Improvement for nonuser controls was not significant (+3.2%, p_{time}=0.181). Developmental trajectories of hippocampal volume were not different between groups. These findings suggest some very subtle yet detectable disadvantages for youth who used recreational stimulant drugs, even on a limit basis (i.e., one to two instances).

INTRODUCTION

A substantial proportion of recreational stimulant users (i.e., non-dependent users of amphetamine, methamphetamine, 3,4-methylenedioxy-methamphetamine [MDMA or ecstasy], cocaine, and prescription stimulants including methylphenidate and d-amphetamine) initiate stimulant use before the age of eighteen. Prevalence estimates of adolescent stimulant use range from 0.9 to 9.1 percent, with MDMA and cocaine use being most common in the U.S. (Frieden et al., 2014; Johnston et al., 2015; SAMHSA, 2014). Up to 65% of youngsters associate no risks with low-frequency recreational stimulant use (Johnston et al., 2015), although the long-term effects of adolescent recreational stimulant use on brain development are largely unknown.

Stimulants are indirect monoaminergic agonists, modulating the availability of extracellular serotonin, norepinephrine, and dopamine in the brain. Monoaminergic receptors are abundantly expressed in the hippocampus, which receives afferent dopaminergic projections from the ventral tegmental area and serotonergic projections from the raphe nuclei. Animal studies have shown that chronic stimulant exposure results in impaired hippocampal neurogenesis, a process of stem cell renewal thought to be important in learning and memory (Eisch & Harburg, 2006). Chronic high-dose stimulant exposure has been associated with a wide range of electrophysiological and molecular changes in the hippocampus, including reduced long-term potentiation (Onaivi et al., 1996), reduced serotonergic and dopaminergic receptor binding (Armstrong & Noguchi, 2004), and memory impairments (Belcher et al., 2005) that may persist up to several months after exposure (van Nieuwenhuizen et al., 2010). Human studies have mostly focused on chronic cocaine and methamphetamine use in severely addicted adults. Brain changes including enlarged striata and widespread decreased grey matter volume, particularly in frontal, temporal, and hippocampal regions (Berman et al., 2008; Ersche et al., 2013), and impaired memory and learning have been reported in these patients (Potvin et al., 2014; Scott et al., 2007).

Less is known about the effects of occasional illicit stimulant use. In an animal model of recreational stimulant use, single-episode binge exposure to stimulants has induced serotonin depletion and memory deficits lasting up to several weeks (Biezonski & Meyer, 2010) or months (McGregor et al., 2003) after exposure. In human recreational users of MDMA (Murphy et al., 2012) and prescription stimulant misuse (Reske et al., 2010), deficits in verbal memory have been found. Neuroimaging studies, however, have yielded mixed results, partially due to methodological and participant group differences. Reduced frontal grey matter volume (Cowan et al., 2003; Daumann et al., 2011) and changes in striatal structure and function (De Win et al., 2008; Mackey et al., 2014) have been reported. Structural changes in the

hippocampus have not been found in whole-brain studies (Cowan et al., 2003; Daumann et al., 2011; Mackey et al., 2014) nor when the hippocampus was specifically targeted as a region of interest (Koester et al., 2012). By contrast, recreational stimulant users did show reduced blood-oxygen-level dependent (BOLD)-response in the left hippocampus and parahippocampal gyrus compared to nonusers during memory encoding (Becker et al., 2013; Roberts et al., 2009). In sum, memory deficits and reduced hippocampal activity during memory tasks have been seen in occasional stimulant users, but structural hippocampal changes have not been observed.

The sparse literature regarding the effects of occasional stimulant use on hippocampal structure and memory has limitations. First, the majority of studies addressing memory performance, and all investigations of brain structure, in occasional stimulant users had a cross-sectional design. Any alterations or deficiencies reported in this group may have existed prior to stimulant exposure, potentially indicating a risk factor for, rather than a consequence of, recreational stimulant use. Second, three out of four structural neuroimaging studies in occasional users utilized voxel-based morphometry (VBM) techniques, optimized to detect volumetric changes in contiguous grey matter structures such as the cortex. Non-volumetric features, such as surface morphology of the hippocampus, may develop independent of volumetric changes, and may be more sensitive to subtle changes. Moreover, subcortical and limbic structures are composited of both grey and white matter. The absence of macroscopic grey matter changes does not preclude the presence of changes in white matter microstructure. In fact, a recent study found indications of loss of hippocampal myelin proteins and tissue integrity in patients with mild memory impairments, in the absence of volumetric changes (Granziera et al., 2015).

Generalizability of stimulant effects in young adult occasional users to adolescents is uncertain. The mechanism by which stimulant abuse causes hippocampal damage in adult heavy users and memory deficits in adult occasional users likely applies to adolescents as well. During adolescence, however, the brain undergoes a series of complex developmental changes, rendering the adolescent brain especially vulnerable to external influences such as substance exposure and toxicity (Andersen & Navalta, 2011). Major modifications of brain architecture during adolescence include widespread cortical thinning (Giedd et al., 2009), and increasing myelination of cortico-cortical connections (Uda et al., 2015). Hippocampus volume and entorhinal cortical thickness typically reach their peak before adolescence, after which the adolescence phase is characterized by relative stability (Krogsrud et al., 2014; Tamnes et al., 2014; Wierenga et al., 2014). By contrast, hippocampal white matter continues to develop throughout adolescence and into early adulthood, with

increasing fractional anisotropy (FA) and decreasing mean diffusivity (MD; Bava et al., 2010; Tamnes et al., 2010), both indicative of increased myelination. Ongoing development may sensitize the adolescent brain to lasting changes, i.e., may increase the severity of structural brain changes after stimulant use, and/or may increase the likelihood of structural changes after less frequent stimulant exposure. Such age-dependent effects have previously been reported in rats, that exhibited memory deficits and hippocampal shape changes after low-dose stimulant exposure during adolescence, but not after exposure at later age (van der Marel et al., 2014). In humans, adolescent but not adult exposure to MDMA predicted 5-HT transporter density in midbrain (Klomp et al., 2012).

In this prospective longitudinal neuroimaging study, we investigated changes in hippocampus structure and verbal memory performance in adolescent recreational stimulant users. Changes in the stimulant users group were compared to typical developmental changes in a matched group of adolescents not using any substances, and to changes in a matched group of non-stimulant substance users (e.g., users of alcohol, marijuana, and/or cigarette smokers). We hypothesized that adolescent stimulant users would present with subtle yet detectable signs of memory impairment and changes in hippocampal structure, i.e., hippocampal total volume reduction and/or localized surface changes over time and decreased hippocampal white matter integrity, compared to both control groups.

METHODS

Procedure

This study was part of an ongoing neuroimaging study of adolescents at familial risk for substance use problems. A total of 295 adolescents 12 to 14 years of age, who had had no or minimal exposure to substances, were recruited through local middle schools. The sample was enriched for adolescents with a family history of substance use disorders. Assessment included interviews and questionnaires regarding substance use history, background, family history, and mental health functioning, all obtained from the adolescent, a biological parent or legal guardian, and a close relative, as well as neuropsychological testing and a magnetic resonance imaging (MRI) session for the adolescent. After enrollment, participants were administered substance use interviews by phone every six months, and were invited for complete assessment including an MRI scan annually. All scans included in the current study were obtained between 2005 and 2015 on the same 3T scanner. The study protocol was approved by the University of California, San Diego, Human

Research Protections Program. Adolescents and their parents (while the adolescent was <18 years) signed informed assent and consent, respectively, at each assessment.

Participants

Exclusion criteria at study enrollment included the presence of any DSM-IV axis I disorder, mental retardation or learning disabilities, a history of chronic medical illness or head trauma, prenatal alcohol or illicit drug exposure, and contra-indication to MRI (for details, see Squeglia et al., 2015). Participants who had successfully completed at least two 3T structural MRI scans, reported no psychostimulant use prior to the first scan, and had no medical use of prescription psychostimulants at any time (e.g., for ADHD) were selected for this study. When more than two successful 3T measurements were available, one baseline and one follow-up measurement were selected such that 1) baseline represented the earliest valid 3T measurement, 2) for participants reporting stimulant use, follow-up represented the first follow-up measurement after the last instance of stimulant use [to maximize the amount of stimulant exposure captured, while minimizing the delay between exposure and scan], and 3) for participants who did not report stimulant use, follow-up duration optimally matched the average follow-up duration across participants who did report stimulant use. Average follow-up interval was 5.1 years (range: 0.8-8.9 years).

Of all eligible participants (n=199), 35 reported stimulant use between baseline and follow-up (17.6%, 'stimulant-users' or 'STIM'). One participant reporting a pattern of regular rather than occasional stimulant use (>10 instances of stimulant use per year) was excluded. Of those participants reporting no stimulant use (i.e., controls), 71 reported no or minimal substance use between baseline and follow-up ('nonuser controls' or NU-CON, 35.7%, reporting ≤1 instance of being drunk, using marijuana or other drugs, or smoking cigarettes, per year), and 93 reported substance use, but not stimulant use ('non-stimulant controls' or NS-CON, 46.7%). Next, both control groups were optimally matched to the stimulant-users group. Participants were one-to-one matched on baseline age (+/- 1SD), follow-up duration (+/- 1SD), and sex, the latter criterion being dropped if necessary (n=2). Both control groups were group-level matched to the STIM group regarding family history of substance use problems. Furthermore, the NS-CON group was group-matched to the STIM group regarding the use of non-stimulant substances between baseline and follow-up. The latter matching was only partially successful (Table 1). For one stimulant-user, no match could be found in the NS-CON group, and for three stimulant-users no match could be found in the NU-CON group. The final sample consisted of 34 participants in the STIM group, 33 participants in the NS-CON group, and 31 participants in the NU-CON group.

TABLE 1. Demographic and substance use characteristics of nonuser controls, non-stimulant controls, and stimulant users

	NONUSER (*n*=31)		NONUSER (*n*=31)		NONUSER (*n*=31)		STIM *vs.* NU-CON	STIM *vs.* NOSTIM	Assoc. with stim use freq.
	M	SD	M	SD	M	SD	T / Chi²	T / Chi²	r / T
% male	51.6		63.6		58.8		0.341	0.163	-0.429
% with family history of SUD	51.6		63.6		50.0		0.017	1.268	-0.961
Baseline age	14.5	1.8	14.8	1.7	19.4	1.9	-0.796	-0.207	-0.078
Follow-up interval in years	4.7	1.8	5.3	1.8	5.4	1.8	-1.643	-0.322	-0.098
# CVLT sessions (BL-FU)	1.2	1.2	1.8	1.4	1.3	1.3	-0.333	1.562	-0.163
# instances of alcohol use (before BL)	0.0	0.0	4.0	13.1	1.3	3.8	-	1.138	0.093
# instances of substance use (before BL)	0.0	0.0	4.5	15.8	8.3	24.5	-	-0.747	0.084
Freq. of alcohol intoxication (times/year, BL-FU)	0.0	0.0	13.7	16.1	25.7	25.2	-	-2.332*	0.239
Freq. of marijuana use (times/year, BL-FU)	0.0	0.0	28.1	50.7	53.0	65.0	-	-1.752	0.008
Freq. of tobacco use (cigarettes/year, BL-FU)	0	0	326	991	106	222	-	1.245	0.269
Days since last substance use (FU)	-	-	6.2	5.8	5.0	3.7	-	1.001	0.065

BL baseline; FU follow-up; NU-CON nonuser control group; NS-CON non-stimulant control group; STIM stimulant users group; CVLT California Verbal Learning Test; SUD substance use disorder; * $p<0.05$

Substance use

Self-reported frequency of substance use was assessed with the Customary Drinking and Drug Use Record (CDDR; Brown et al., 1998). Reports from a second informant (e.g., parent, sibling, or friend) were collected for confirmation. The CDDR assesses the use of stimulants, as well as alcohol, marijuana, barbiturates, hallucinogens, inhalants, opiates, benzodiazepines, ketamine, gamma-hydroxybutyric acid (GHB), phencyclidine (PCP, or 'angel dust'), and cigarette smoking. Stimulant use could be reported on the following items: amphetamine-type stimulants (ATS, *"speed, crystal, meth, Ritalin, Adderall, stimulant pills, performance enhancing, ripped fuel, ephedrine, diet pills"*), cocaine (*"coke, blow, crack"*), MDMA/ecstasy (*"MDMA, MDA, E, X, Rolls, Molly"*), and *"misuse of prescription drugs."* Participants were classified as stimulant users if they reported ≥1 instance of ≥1 stimulant type between baseline and follow-up. Annual stimulant use frequency was calculated as the total number of instances of stimulant use (of any type) between baseline and follow-up, divided by follow-up duration. Lifetime stimulant use frequency was calculated by dividing the lifetime number of stimulant use instances by age at follow-up. Substance use frequencies were also calculated for each stimulant type separately, and for alcohol (instances of 'being drunk'), marijuana, and cigarettes.

Memory performance

Verbal learning and memory performance was assessed using the California Verbal Learning Test for Children (CVLT-C; Delis et al., 1994) in the first four years of the study and the CVLT-II adult version (Delis et al., 2000) in later years. A list of common words belonging to word categories was presented to the participant five times. After the fifth trial, an interference list of new words was read once, and the participant was asked to recall items from the interference list. After a 20-minute delay, during which testing continued with nonverbal tasks, a delayed recall of the original list was requested. The delayed recall memory score (CVLT-LD) is defined as the percentage of words correctly stated after the 20-minute interval. CVLT-LD has been associated with variation in hippocampus volume in healthy populations, and is sensitive to changes in adolescent memory performance after substance exposure (Ashtari et al., 2011; Brown et al., 2000; Wright et al., 2015).

MRI data acquisition

All neuroimaging data reported here were collected with the same 3T General Electric (Milwaukee, Wisconsin) scanner with an 8-channel phase-array head coil, at

the University of California, San Diego, Keck Center for Functional MRI. Eight high-bandwidth receivers for ultrashort repetition times reduced signal distortion and signal dropout. Imaging included a sagittal high-resolution 3D T1-weighted structural acquisition (FOV=24 cm, 0.94×0.94×1 mm voxels, 176 slices, TR=20 ms, TE=4.8 ms; flip angle=12°). Diffusion-weighted imaging was added later in the study; 66% (n=65) of participants received at follow-up an axial diffusion-weighted single-shot dual spin echo acquisition (FOV = 24 cm, matrix size = 96x96; 53 interleaved slices, slice thickness = 2.5 mm; b=1000, 30 diffusion directions plus 2 b_0 reference images, TR=12000, optimized TE).

MRI analyses

Left and right hippocampi were segmented from the original T1 images through the automated FMRIB integrated registration and segmentation tool pipeline (FIRST) of the FMRIB Software Library (FSL; Patenaude et al., 2011), with default settings. FIRST registers each scan to standard space (MNI152 with 1x1x1 mm resolution), and then integrates shape and intensity information into a Bayesian, probabilistic framework to accurately segment 15 subcortical structures, including the bilateral hippocampi. FIRST's output consists of volumetric representation of the bilateral hippocampi, as well as a mesh representation of their surface. Images were visually inspected after each processing step (skull-stripping, registration to standard space, and automated segmentation), and settings were manually adapted where needed. Surface meshes were filled to allow spatial statistics on the hippocampus surface shape, with global scaling to remove volumetric effects. Statistical modeling was performed in FSL Permutation Analysis of Linear Models (PALM; Winkler et al., 2014) with threshold-free cluster enhancement (1000 permutations) for cluster inference, and with family-wise error (FWE) adjustment for multiple testing.

Diffusion scans (follow-up only) were processed through an automated pipeline provided by the Multimodal Imaging Laboratory (MMIL) of the University of California, San Diego, integrating diffusion-, T2-, and high-resolution T1-weighted acquisitions. Diffusion-weighted volumes were registered to the b_0 reference volume by rigid body registration. Eddy current distortions were removed, and magnetic susceptibility artifacts were minimized using a nonlinear b_0-unwarping method that enables highly accurate registration with the T1-weighted images. Next, a diffusion tensor was fitted. Transformation matrices resulting from intra-modal registrations (b_0 to T1) were used to transform hippocampal segmentation masks to diffusion space. Average fractional anisotropy (FA) and mean diffusivity (MD) within these hippocampal masks were extracted. In addition, T1-T2 ratio, indicative of myelin content (Glasser & van Essen, 2011), within the FreeSurfer hippocampal

segmentation mask was calculated as the average T1-weighted voxel intensity (normalized to the corresponding bias-field image to reduce artifacts), divided by the average T2-weighted voxel intensity from the b_0 diffusion image (adjusted for voxel intensity of cerebral spinal fluid in the same image, to reduce scanner- or upgrade-specific effects).

Statistical testing

In SPSS linear mixed effect models, we predicted memory performance and hippocampal volume from time (baseline vs. follow-up), group (STIM vs. NS-CON, or STIM vs. NU-CON), and time-by-group-interaction, while including baseline age, sex, and follow-up duration as covariates. The effect of interest is captured in the time-by-group interaction, testing whether memory performance/hippocampus volume changed differently over time for stimulant-users compared to controls. For the vertex-wise analysis of the hippocampal surface, the same longitudinal model was implemented in PALM. In the absence of diffusion measurements at baseline, follow-up hippocampal FA, MD, and T1-T2 ratio were predicted from group (STIM vs. NS-CON, and STIM vs. NU-CON), including the covariates sex and age at follow-up.

Second, we performed generalized additive modeling (GAM) regression analyses using the mgcv-package in R (Wood, 2011), to assess associations between stimulant use frequency and each outcome measure (memory performance, hippocampus volume, FA, MD, and T1-T2 ratio) within the STIM group. Memory performance and hippocampus volume at follow-up were predicted from sex, baseline age, follow-up duration, baseline memory performance/hippocampus volume, and frequency of alcohol intoxication, marijuana use, cigarette smoking, and stimulant use between baseline and follow-up. The same model was again implemented in PALM to assess hippocampal surface changes associated with stimulant use frequency. The cross-sectional outcomes FA, MD, and T1-T2 were predicted from sex, age at follow-up, and lifetime frequency of alcohol intoxication, marijuana use, cigarette smoking, and stimulant use. Each of the substance use frequency predictors were positively skewed, with the majority of participants reporting infrequent use, hence the predictors were log-transformed. In R, stimulant use frequency was added as a smooth regression term with no restrictions to the link function, to allow detection of non-linear associations.

For each significant stimulant effect, we evaluated the influence of potential confounders in a second step by adding the following additional covariates to the model: 1) an effect-by-sex interaction term, 2) an effect-by-age interaction term, 3) for contrasts not including the NU-CON group: number of days since last substance

use instance, to account for potential acute substance effects, and 4) any demographic variable associated with stimulant use.

For memory performance, alpha was divided by two ($\alpha=0.05/2=0.025$; two case-control contrasts) to adjust for multiple testing. For the hippocampus measures, alpha was divided by six ($\alpha=0.05/6=0.008$; two times three outcome measures [memory performance, and hippocampus volume, shape, and microstructure]). Power to detect case-control differences meeting this alpha-level given our limited sample size was low, i.e., 0.67 for differences of large effect size (Cohen's $d=0.8$) and 0.22 for differences of medium effect size (Cohen's $d=0.5$). Preliminary outcomes meeting nominal significance are therefore also reported ($\alpha=0.05$).

TABLE 2. Cumulative lifetime stimulant use and stimulant use frequency between baseline and follow-up, within the stimulant users group.

			Lifetime use (instances)				Frequency (instances/year)			
	N	%	M	SD	Median	Range	M	SD	Median	Range
Stimulants (any type)	34	100.0	9.0	12.9	3.5	1-60	1.7	2.2	0.8	0.2-9.6
MDMA	20	58.8	3.5	3.5	2.0	1-14	0.7	0.8	0.4	0.1-2.9
ATS	16	47.1	10.2	14.3	6.5	1-60	1.9	2.3	1.6	0.2-9.6
Cocaine	14	41.2	5.2	6.7	3.0	1-23	1.0	1.2	0.4	0.1-3.8

MDMA 3,4-methylenedioxy-methamphetamine, ATS amphetamine-type stimulants

RESULTS

Ninety-eight participants (57 males, 58.1%) were included in three sex- and age-matched groups (Table 1). Average age was 14.7 years at baseline and 19.8 years at follow-up. Despite optimal matching, participants in the STIM group reported more frequent alcohol intoxication between baseline and follow-up compared to the NS-CON group (25.7 instances/year *vs.* 13.7 instances/year). Frequency of marijuana use and cigarette smoking did not differ between the groups. Substance use other than stimulants, alcohol, marijuana, or nicotine included hallucinogens ($n=24$), opiates ($n=8$), benzodiazepines ($n=7$), inhalants ($n=5$), and ketamine ($n=3$). At follow-up, 97 percent of participants in the combined STIM and NS-CON group had used substances (either alcohol or drugs) within 30 days prior to study participation, with an average of 6 days since their most recent use. The STIM group included users of amphetamine-type stimulants ($n=16$, 47.1%), cocaine ($n=14$, 41.2%), and MDMA ($n=20$, 58.8%), with 38% ($n=13$) of stimulant-users reporting more than one stimulant type. The average frequency of stimulant use (any type) was 1.7 instances

per year (range: 0.2-9.6), adding up to on average 9 lifetime instances (range: 1-60; Table 2).

Verbal memory performance

The time-by-group interaction effect for stimulant users versus non-stimulant controls reached marginal significance (p=0.032; Figure 1). Whereas CVLT-LD memory performance improved in the NS-CON group ($M_{BASELINE}$=79.9%, $M_{FOLLOW-UP}$=85.8%, β_{TIME}=0.424, p_{TIME}=0.023), test performance was stable in the STIM group ($M_{BASELINE}$=81.5%, $M_{FOLLOW-UP}$=80.2%, β_{TIME}=-0.145, p_{TIME}=0.413). Change in performance for the NU-CON group resembled that of the NS-CON group but was not significant ($M_{BASELINE}$=82.3%, $M_{FOLLOW-UP}$=85.5%, β_{TIME}=0.251, p=0.181). The time-by-group interaction effect was not significant for stimulant users versus nonuser controls (p=0.127). At baseline, the STIM group performed similar to the NS-CON group (p=0.587) and the NU-CON group (p=0.295). At follow-up the STIM group performed worse compared to the NU-CON group (p=0.024) but not compared to the NS-CON group (p=0.095). The difference in slope between stimulant users and non-stimulant controls did not interact with sex (p=0.746) or age (p=0.173), and remained unchanged when accounting for potential acute substance use effects ($p_{DAYS-SINCE-USE}$=0.389; $p_{STIMvs.NOSTIM}$=0.029) and frequency of alcohol intoxication ($p_{ALC-FREQ}$=0.430; $p_{STIMvs.NOSTIM}$=0.033). Memory performance was not associated with stimulant use frequency between baseline and follow-up within the stimulant-users group (p=0.518).

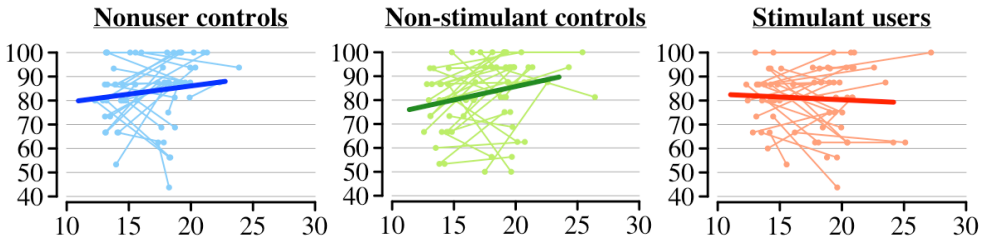


FIGURE 1. Change in CVLT delayed free recall performance (y-axis: percentage correctly recalled) between baseline and follow-up (x-axis: age) in nonuser controls (blue), non-stimulant controls (green), and stimulant users (red).

Test performance is likely to improve as a result of exposure to the CVLT materials. Between baseline and follow-up, participants performed the CVLT between 0 and 6 times (M=1.33, SD=1.26), with no difference between groups. The average delay between the last CVLT session (either baseline or an intermediate assessment) and the follow-up session was 3.1 years (SD=1.6), with no difference between groups.

Eighty-three percent of participants had been administered the children's version at baseline and the adult version at follow-up, with no difference between groups.

Hippocampal volume

Left and right hippocampal volumes changed little between baseline and follow-up in all three groups. Changes in hippocampal volumes over time in the STIM group did not differ from those in the NU-CON group (left: p=0.122; right: p=0.057) or the NS-CON group (left: p=0.092; right: p=0.678). Across groups, changes over time were not significant (left: β_{TIME}=-0.034, p=0.460; right: β_{TIME}=-0.058, p=0.296), and there were no between-group differences in hippocampus volume at baseline or follow-up. Within the STIM group, stimulant use frequency was not associated with left or right hippocampus volume (left: p=0.121; right: p=0.415).

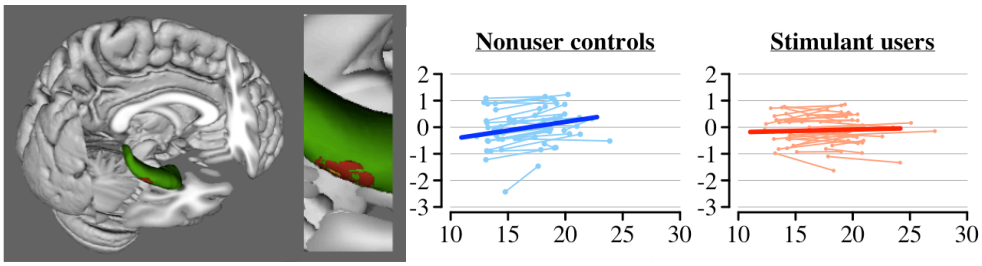


FIGURE 2. Left: Right hippocampal region of significant (pFWE<0.05 in red, pFWE<0.01 in yellow) group-by-time interaction. Right: surface expansion (y-axis) by age (x-axis) in the nonuser control group and the stimulant users group.

Hippocampal surface

Vertex-wise analyses revealed no significant differences in hippocampus surface morphology between stimulant users and nonuser controls, or between stimulant users and non-stimulant controls, at either baseline or follow-up. The time-by-group (STIM *vs.* NU-CON) interaction was significant in a small cluster on the right lateral hippocampus surface (3.5% of surface voxels at p_{FWE}<0.05, 0.4% at p_{FWE}<0.01, Figure 2). Within this cluster, surface expansion over time was found in the NU-CON group (β_{TIME}=0.493, p<0.001) but not in the STIM group (β_{TIME}=0.087, p=0.267). The effect was the same for male and female participants ($p_{TIME*GROUP*SEX}$=0.216), and for participants of different baseline ages ($p_{TIME*GROUP*AGE}$=0.495). Stimulant use frequency was not associated with surface expansion in the identified cluster (p=0.422), nor with surface changes in other vertices. Finally, there were no significant time-by-group

interaction effects for stimulant users *vs.* non-stimulant controls anywhere on the cortical surface.

Hippocampal microstructure

At follow-up, there were no significant between-group differences in hippocampal FA, MD, or T1-T2 ratio between stimulant users and nonuser controls (left: p_{FA}=0.324, p_{MD}=0.824, $p_{T1\text{-}T2}$=0.194; right: p_{FA}=0.121, p_{MD}=0.822, $p_{T1\text{-}T2}$=0.657), or between stimulant users and non-stimulant controls (left: p_{FA}=0.395, p_{MD}=0.212, $p_{T1\text{-}T2}$=0.081; right: p_{FA}=0.467, p_{MD}=0.574, $p_{T1\text{-}T2}$=0.388). Within stimulant-users, none of the microstructure parameters were associated with lifetime stimulant use frequency (left: p_{FA}=0.862, p_{MD}=0.970, $p_{T1\text{-}T2}$=0.241; right: p_{FA}=0.857, p_{MD}=0.483, $p_{T1\text{-}T2}$=0.168).

DISCUSSION

We investigated changes in verbal memory performance and hippocampus structure in adolescent recreational stimulant users over a 5-year period. We found a modest time-by-group interaction effect on CVLT delayed recall score: between baseline and follow-up, memory test performance improved in the non-stimulant control group, but not in the stimulant users group. Second, we found a small cluster on the right lateral hippocampal surface where stimulant users did not show typical surface expansion with increasing age. There was no effect of stimulant use on hippocampal volume or white matter microstructure.

We had hypothesized that stimulant-users would show subtle memory deficits compared to both control groups. In the NS-CON group, delayed recall memory performance improved by 5.9% from baseline to follow-up, in line with practice effects typically seen after repeated CVLT assessment (Woods et al., 2006). The NU-CON group improved by 3.2% over time (non-significant), such that memory performance at follow-up was very similar for the NU-CON and NS-CON groups (85.5% and 85.8%, respectively). The stimulant users, despite having been exposed to the test materials to the same degree, did not benefit from practice. The effect of stimulant use on memory performance was not attributable to pre-existing differences or acute substance effects. Memory deficits after recreational stimulant use had previously been shown in adults, who in most studies reported more than one-hundred lifetime instances of stimulant use (Murphy et al., 2012), which is much higher compared to the average of nine and median of four lifetime instances of stimulant use in our adolescent sample. It is important to note that although memory test performance in the STIM group did not significantly deteriorate over time (-1.3%), the absence of improvement compared to controls was marginally significant.

However, there is the imminent risk of type 1 error, hence these findings are preliminary until replicated and should not be over-interpreted.

The effect of stimulant use on memory task performance occurred in the absence of changes in hippocampal volume, microstructure, or shape, with the exception of a small cluster on the right hippocampal surface. The absence of stimulant effects on hippocampus volume is in line with a cross-sectional study in adult recreational users, in which hippocampus volume was not abnormal after infrequent (<5 lifetime instances of MDMA and/or <5 mg of amphetamine) or frequent stimulant use (>100 instances of MDMA and/or >50 mg of amphetamine; Koester et al., 2012). We had expected that microstructure/ diffusion parameters may be more sensitive compared to grey matter volume to detect subtle hippocampal changes, as had been shown in patients with mild memory impairment (Granziera et al., 2015), but we found no association between stimulant use and hippocampal microstructure either. Stimulant use may impact memory performance through changes in brain regions other than the hippocampus. In fact, it has been suggested that gradual memory improvement during adolescence may be facilitated by frontal rather than hippocampal changes (Sowell et al., 2001). The association between adolescent stimulant use, memory performance, and frontal brain changes would be an interesting target for future studies. We wish to emphasize that failure to detect hippocampal changes in the current sample, with low-frequency stimulant use patterns and limited power to detect medium or small effects, does not necessarily imply an absence of hippocampal changes.

In a small cluster on the right lateral hippocampus, a significant time-by-group interaction effect indicated surface expansion in the NU-CON group, but not in STIM group. Prior analyses in adult stimulant users found no significant surface changes after stimulant exposure (Bava et al., 2010). In rats, medial inward vertex displacement was found after adolescent exposure to stimulants, but not after adult exposure (van der Marel et al., 2015). To our best knowledge, adolescent hippocampal surface development has not yet been described. Across the adult lifespan, typical surface changes encompass contractions in medial regions and expansions in lateral regions (Voineskos et al., 2015), hence the absence of expansion in the stimulant users group could be interpreted as a deviation from normal development. However, we warrant cautious interpretation for several reasons. First, the region of significant effect is very small, spanning less than 1% of the hippocampal surface. The functional relevance of such a small alteration is likely very limited. In our sample, surface change within the cluster was not associated with memory test performance. Second, we had hypothesized that surface changes in the STIM group would deviate from those in both control groups. The NS-CON group, however, showed no expansion over time within the cluster. We conclude that adolescent stimulant use could potentially

induce changes in a small cluster on the right hippocampal surface; however, the robustness and relevance of this effect requires further investigation.

The current study presents several novelties. This is the first structural MRI study to investigate the effects of recreational stimulant use on the developing adolescent brain. With up to thirty percent of recreational users initiating stimulant use during adolescence (SAMHSA, 2014), and animal studies reporting amplification of negative stimulant effects when animals are exposed earlier in life (Van Der Marel et al., 2014 and 2015), investigations targeting human adolescents are urgently needed. The limited exposure to stimulants within our sample reflects the frequency of stimulant use in typical adolescent populations, ensuring the validity of our findings. In addition, the current study is the first with a prospective design, allowing for the first time a distinction between stimulant-exposure effects and pre-existing differences. There were limitations as well. First, our sample size provided limited power to detect small effects, and did not allow separate analyses per stimulant type. Differentiation between stimulants types was further limited as all stimulants were aggregated in one questionnaire category. As a result, the STIM group was likely a heterogeneous group, which may have further reduced statistical power. Second, the observational study design, although inevitable, may have introduced a susceptibility bias (e.g., stress may have simultaneously increased the likelihood of stimulant use and elicited memory problems). Furthermore, the use of self- and parent-report substance use interviews, as well as the illicit origin of many substances, may compromise substance use data accuracy. Future studies employing biological markers for substance use (e.g., hair analysis) are needed. Finally, our focus on the hippocampus was firmly based in the literature yet precluded potential findings in other brain regions. The frontal cortex would be an interesting target for future studies.

In sum, we found that changes in memory performance may occur after infrequent recreational stimulant use in adolescents. Furthermore, adolescent stimulant-users may present with subtle changes in hippocampal surface morphology, but the functional significance of such changes needs further investigation. Replication in a larger sample is needed, for instance in the Adolescent Brain Cognitive Development study (ABCD). If replicated, these findings may also have implications for patients with ADHD, who are often prescribed stimulant treatment for extended periods of time, albeit typically at lower dose compared to recreational use. Declarative memory performance has received little attention in ADHD research, and subtle changes in stimulant-treated patients may have gone undetected.