

Alcohol and cannabis co-use and longitudinal gray matter volumetric changes in early and late adolescence

Xi Luo^{1,*}, James J. Yang¹, Anne Buu², Elisa M. Trucco^{3,4}, Chiang-Shan R. Li^{5,6,7,*}

¹ Department of Biostatistics and Data Science, The University of Texas Health Science Center at Houston, Houston, TX

² Department of Health Promotion and Behavioral Sciences, The University of Texas Health Science Center at Houston, Houston, TX

³ Department of Psychology, Florida International University, Miami, FL

⁴ Department of Psychiatry, University of Michigan, Ann Arbor, MI

⁵ Department of Psychiatry, Yale University School of Medicine, New Haven, CT

⁶ Department of Neuroscience, Yale University School of Medicine, New Haven, CT

⁷ Wu Tsai Institute, Yale University, New Haven, CT

(Running title: alcohol, cannabis, and GMV)

*Address correspondence to

Dr. Xi Luo, Department of Biostatistics and Data Science, The University of Texas Health Science Center at Houston, Houston, TX 77030; TEL: 713-500-9589; FAX: 713-500-9525; Email: xi.luo@uth.tmc.edu

or

Dr. C.-S. Ray Li, Connecticut Mental Health Center S112, 34 Park Street, New Haven, CT 06519; TEL: 203-974-7354; Email: chiang-shan.li@yale.edu

Key words: adolescence, brain development, longitudinal, gray matter volume (GMV), National Consortium on Alcohol and Neurodevelopment in Adolescence (NCANDA), alcohol and cannabis co-use

Manuscript info:

Abstract: 241 words

Main text: 3921 words

Tables: 4

Figures: 3

Supplement: 1

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as doi: [10.1111/adb.13208](https://doi.org/10.1111/adb.13208)

Abstract

Background. Previous studies have characterized the impact of substance use on cerebral structure and function in adolescents. Yet, the great majority of prior studies employed a small sample, presented cross-sectional findings, and omitted potential sex differences.

Methods. Using data based on 724 adolescents (370 females) curated from the NCANDA study, we investigated how gray matter volumes (GMVs) decline longitudinally as a result of alcohol and cannabis use. The impacts of alcohol and cannabis co-use and how these vary across assigned sex at birth and age were examined. Brain imaging data comprised the GMVs of 34 regions of interest and the results were evaluated with a Bonferroni correction.

Results. Mixed-effects modeling showed faster volumetric declines in the caudal middle frontal cortex, fusiform, inferior frontal, superior temporal (STG), and supramarginal (SMG) gyri, at -0.046 to -0.138 cm³/year in individuals with prior-year alcohol and cannabis co-use, but not those engaged in alcohol or cannabis use only. These findings cannot be explained by more severe alcohol use among co-users. Further, alcohol and cannabis co-use in early vs. late adolescence predicted faster volumetric decline in the STG and SMG across assigned sex at birth.

Conclusions. Findings highlight the longitudinal impact of alcohol and cannabis co-use on brain development, especially among youth reporting early adolescent onset of use. Volumetric decline was noted in cortical regions in support of attention, memory, executive control, and

social cognition, suggesting the pervasive effect of alcohol and cannabis co-use on brain development.

Introduction

Impact of alcohol and cannabis use on mental health

Individuals engaged in substance use earlier in life are at greater risk of developing substance use disorders and sustaining cognitive deficits (1, 2). Those who use multiple substances, including alcohol and cannabis co-use, are particularly vulnerable to health consequences (3, 4). Adolescents reporting alcohol and cannabis co-use, but neither use alone, were more likely to engage in non-suicidal self-injury on a given day (5). Co-use of alcohol and cannabis was associated with heavier use of substances, more use-related harms, and symptoms of psychosis in young adulthood (6). Moreover, a report conducted by the National Study on Drug Use and Health found that higher frequency of alcohol or cannabis co-use conferred risk for both alcohol use disorder (AUD) and cannabis use disorder (CUD)(7). Thus, alcohol and cannabis co-use may have a more deleterious impact on the brain compared to single use.

Cognitive and cerebral markers of alcohol and cannabis use

Many studies have examined how substance use impacts cognitive and neural development among adolescents (8). Adolescent cannabis users relative to non-users demonstrated poorer attention, learning, memory, and aspects of executive functioning (9, 10). For example, cannabis use was associated with bilateral prefrontal cortical thinning in a dose-dependent manner over a 5-year period (11). Further, lifetime use was not correlated with baseline thickness, suggesting that cortical thinning reflected cumulative effects of cannabis

consumption. Cannabis use at age 17 was also associated with diminished general cognitive ability at age 23 (12). Among youth, cannabis and alcohol co-use relative to alcohol or cannabis use alone were related to diminished attentional capacity (13) and compromised integrity of frontolimbic tracts (14). Other studies indicate that AUD, but not CUD, is associated with altered regional activations during cognitive and affective challenges in adolescence (15, 16). Notably, while studies have largely associated cannabis and alcohol co-use with negative health and social outcomes, some findings suggest that the combined use of cannabis and alcohol use, may have "protective" effects on neurobiological outcomes, relative to alcohol use alone (17-19).

Taken together, these studies support the distinct impact of cannabis use on brain development and the greater impact of alcohol use on brain structure and function (20). Still, the effects of co-use of these substances on the developing brain remains unclear.

The effects of early vs. late use on brain and behavior

Substance use initiated at an early age likely has a more serious impact on mental health (21). Greater impairment in executive functioning has been demonstrated among adolescent compared to adult frequent cannabis users, and abstinence had a weaker impact on alleviating craving in adolescents compared to adults (22). Earlier onset of cannabis use was associated with more severe impairment in verbal intelligence and executive function (23). Both academic and social functions were greater among high school seniors who initiated substance use at a later vs. earlier age (24). Individuals 16 to 20 years of age who engaged in early (< 16 years of age)

alcohol and cannabis co-use were less likely to successfully transition to adult roles (25).

Adolescent cannabis use elevated the risk of psychosis, more severe positive symptoms (e.g., hallucinations), and altered gray matter volume (GMV) in a cerebellar network implicated in the pathogenesis of schizophrenia (26). Thus, age of initiation is critical in understanding the impact of alcohol and cannabis co-use on the brain.

Differences across assigned sex at birth relating to effects of alcohol and cannabis use

Assigned sex at birth may also modulate the impact of cannabis and alcohol use on brain and behavior. One study found that, although young adult females smoked less cannabis while drinking, they experienced the same acute pharmacological and subjective effects as males (27). Men and women with AUD showed different patterns of gray and white matter volumetric reduction (28); for instance, only males showed reduced amygdala size compared to controls (29). Moreover, early initiation of cannabis use potentially resulted in more spatial working memory deficits in female than in male adolescents (30). Thus, a substantial body of evidence supports sex differences in the impact of alcohol and/or cannabis use on brain and behavior.

Longitudinal impacts of alcohol and cannabis use

Adolescent alcohol use accelerated developmental decreases in GMV, particularly in the frontal and cingulate cortices, and decelerated increases in white matter volumes (31). Fewer longitudinal studies exist examining the impact of cannabis use on the brain. Exceptions include

prior work showing that onset and frequency of cannabis use in early adolescence and young adulthood was associated with declining neurocognitive functions (23, 32). Moreover, adolescent cannabis use frequency increased significantly over five biannual assessments along with impairment in motivation (33). Additional work is necessary to understand the longitudinal impact of cannabis use, alcohol use, and co-use of these substances on the brain.

The present study

This study investigates how GMVs decline longitudinally as a result of alcohol and cannabis use among adolescents enrolled in the National Consortium on Alcohol and Neurodevelopment in Adolescence (NCANDA) study. More specifically, the impact of alcohol and cannabis co-use was compared to alcohol and cannabis use alone, as well as non-users. Further, we investigated how these associations varied across assigned sex at birth and across age (early vs. late adolescents). We hypothesized that alcohol and cannabis co-use would have a greater impact on cerebral GMVs and that this association would vary across males and females and figure more prominently in early vs. late adolescents.

Methods and Materials

Participants and assessments

Data from the NCANDA study, a five-site, longitudinal neuroimaging study of adolescents at different developmental stages was examined (see (34)). The NCANDA study

followed an accelerated longitudinal design to assess a sample of youth aged 12-21 (49% male according to assigned sex at birth; 64% White; > 50% at risk for heavy drinking) at baseline and annually for up to 9 years (35). A total of 724 participants who completed the baseline assessment and at least one follow-up visit at year 1, 2, and 3 (679, 625, and 563 participants, respectively) were included in the current study. Participants meeting criteria for any substance use disorder according to DSM IV (4th edition, 1994) at baseline were excluded. **Table 1** summarizes baseline demographics. Participants were classified into two developmental stages – early (< 16 years of age at baseline) and late (≥ 16) adolescents.

Substance use assessments

The Customary Drinking and Drug Use Record (CDDR; Brown et al., 1998) was administered at each wave. The number of days drinking, using marijuana, and the number of cigarettes smoked in the past year were used to quantify participants' historical substance use. With skewed distributions, these variables were recoded as binary variables (0=did not use; 1=used) to form four groups at each wave: non-use controls (N), alcohol-only (A), cannabis-only (C), or alcohol and cannabis (A+C) users. Smoking status during the previous year was included as a covariate in models.

Imaging data processing

At each visit, MRI scans were acquired on a 3-Tesla GE (Discovery MR750) or Siemens (TIM Trio) scanner. The imaging protocols and data preprocessing procedures were described previously (36, 37). The cerebral cortex was segmented into 34 bilateral regions of interest (ROIs) using the Desikan-Killiany atlas (38). Total volumes of each ROI (bilateral) served as the dependent variable.

Statistical analysis

Demographic and clinical variables

Descriptive statistics summarize participants' demographics at baseline, and substance use status at baseline and across each follow-up assessment. Chi-square and t-tests were used to examine associations between the variables and developmental stage (early vs. late) and assigned sex at birth. All analyses were conducted using R 3.5.2.

Substance use and longitudinal volumetric changes

Linear mixed effects models were employed to assess how longitudinal volumetric changes of each ROI were associated with each substance use subtype. For each ROI, a main model was fit where the longitudinal GMVs were entered as outcomes and assigned sex at birth, follow-up year (t), substance use subtype (A_t), and two interaction terms between follow-up year and assigned sex at birth and substance use subtype, respectively, were entered as predictors:

$$E(\text{ROI Vol}_t) = \text{sex} \cdot \beta_s + t \cdot \beta_t + \text{sex} \cdot t \cdot \beta_{st} + A_t \cdot \vec{\beta}_a + \text{sex} \cdot A_t \cdot \vec{\beta}_{sa} + A_t \cdot t \cdot \vec{\beta}_{at} + \text{Covariates} \cdot \vec{\beta}_c + u \quad (1)$$

where E denotes the expectation for each participant's ROI Vol $_t$ at each follow-up year t , and A_t substance use subtype as assessed at visit t . Covariates included baseline age, baseline age squared, race, social-economic status, intra-cranial volume (ICV), study site, smoking status at each visit, and the interactions between baseline age and follow-up year, and intercepts u for each participant entered as random effects.

To test the associations between substance use and annual rates in volumetric changes of each ROI, F tests were used to examine the overall statistical significance of the interaction of follow-up year and substance use subtype. Namely, we tested the null hypothesis that all $\vec{\beta}_{at}$ coefficients in (1) are zero or that alcohol and cannabis use does not impact the annual volume changes. The F test p -values were adjusted using a Bonferroni correction for 34 ROIs, and those showing corrected p -values < 0.05 were retained for further analysis. We employed t -tests to examine pair-wise differences in annual rates across assigned sex at birth and between substance use subtypes. Statistical significance was assessed by two-sided p -values < 0.05 , after Bonferroni corrections, and corrected p -values between 0.05 and 0.06 were considered marginally significant.

Developmental age and assigned sex at birth modulation of the effects of substance use on longitudinal volumetric changes

To examine the developmental patterns among the neurotypical participants, linear regression was adopted to test the association between annual volume changes and age for each

ROI using only non-user data. To assess the moderating effects of developmental stage (i.e., early vs. late adolescence) and assigned sex at birth, a second model was fit for each ROI showing longitudinal volume changes by including two three-way interaction terms as predictors, one between follow-up year, substance use subtype and developmental stage, and the other between follow-up year, substance use subtype, and assigned sex at birth:

$$\begin{aligned}
 E(\text{ROI Vol}_t) = & \text{sex} \cdot \beta_s + t \cdot \beta_t + \text{sex} \cdot t \cdot \beta_{st} + A_t \cdot \vec{\beta}_a + \text{sex} \cdot A_t \cdot \vec{\beta}_{sa} + A_t \cdot t \cdot \vec{\beta}_{at} \\
 & + \text{stage} \cdot \beta_d + \text{stage} \cdot A_t \cdot \vec{\beta}_{da} + \text{stage} \cdot t \cdot \beta_{dt} + \text{stage} \cdot A_t \cdot t \cdot \vec{\beta}_{dat} \\
 & + \text{sex} \cdot A_t \cdot t \cdot \vec{\beta}_{sat} + \text{Covariates} \cdot \vec{\beta}_c + u
 \end{aligned} \tag{2}$$

As with the main model (1), F tests were employed to examine in (2) the null hypotheses of all components $\vec{\beta}_{dat} = 0$ or $\vec{\beta}_{sat} = 0$, respectively. That is, we tested if developmental stage or sex separately modulated the impact of alcohol and cannabis use on annual volume changes.

To assess the robustness of the ROI findings, we also evaluated Model (1) and (2) without smoking status as a covariate.

The effects of alcohol and cannabis use metrics

Alcohol and cannabis co-use, but not alcohol or cannabis use alone, showed significantly faster volumetric declines over time (see Results). As the co-use group showed more severe alcohol consumption than those within the alcohol use alone group, one possibility is that these findings may reflect alcohol use severity. To investigate this possibility, we examined the effects of number of drinking (N_A) and cannabis (N_C) use days in the prior year on the volumetric changes of the ROIs using a Wilcoxon rank sum test to compare these continuous measures

between developmental stages, assigned sex at birth, and substance use groups at each visit. In mixed effects modeling, we replaced categorical substance use types A_t in the main [Equation (1)] and modulation [Equation (2)] model by the continuous alcohol and cannabis use measures and their interaction (N_A , N_C , $N_A \cdot N_C$). Similarly, we used t tests to examine the significance of the coefficients of predictors that involved N_A , N_C , or $N_A \cdot N_C$.

Results

Demographic and clinical variables and volumetrics at baseline

Table 1 shows the summary statistics at baseline for the whole cohort and separately for the two developmental stages. Early and late adolescents did not show significant differences in ICV, socio-economic status, assigned sex at birth or race distributions (p -values > 0.158). Significantly more participants smoked at baseline and during follow-ups in late adolescence (p -values < 0.001). The late adolescent group was also comprised of significantly more alcohol and cannabis users across subtypes at baseline and during follow-up (p -values < 0.001). The two groups showed significant volumetric differences at baseline except for the entorhinal, pericalcarine, and transverse temporal cortex (p -values > 0.116). Females and males did not show significant differences in age, developmental stage, baseline smoking, or substance use types at baseline or during follow-up (**Table S1**). At baseline, males showed larger ICV and ROI volumes (p -values < 0.001).

[Table 1 about here]

The effects of substance use on longitudinal volumetric changes

Overall, prior-year alcohol, cannabis use, and co-use were significantly associated with annual volumetric changes in five ROIs: caudal middle frontal cortex (cMFC, Bonferroni corrected p -value = 0.013), fusiform gyrus (FG, p -value = 0.006), inferior frontal gyrus, pars opercularis (IFGpo, p -value = 0.022), superior temporal gyrus (STG, p -value = 0.002), and supramarginal gyrus (SMG, p -value < 0.001; see **Figure 1** and **Table 2**). Females showed significantly faster volume declines in the STG than males (female – male = $-0.060 \text{ cm}^3/\text{year}$, p -value = 0.020, Bonferroni corrected; **Table 2**). Excluding smoking status as a covariate did not change these statistically significant findings (**Supplementary Table S2**). The results for all ROIs are summarized in **Supplementary Table S3**.

[Figure 1 and Table 2 about here]

Associations between annual volumetric changes of these five ROIs and substance use subtypes were examined. Compared with non-users, alcohol and cannabis co-users showed significantly faster volumetric declines in the cMFC (A+C – N = $-0.094 \text{ cm}^3/\text{year}$, Bonferroni corrected p -value < 0.001), FG (-0.106 , p -value < 0.001), IFGpo (-0.046 , p -value = 0.003), STG (-0.131 , p -value < 0.001), and SMG (-0.138 , p -value < 0.001). Alcohol and cannabis co-use was also associated with faster SMG volume declines than cannabis-only use, though only with marginal significance (-0.280 , p -value = 0.053). Other group differences were not significant. These findings are summarized in **Table 3**.

[Table 3 about here]

Modulation by assigned sex at birth and developmental age

Annual volume changes vs. age among female and male controls are shown in **Figure 2** for the five ROIs identified from the main model. Only the slope for SMG among females was significant (p -value = 0.044, Bonferroni corrected; > 0.313 , all other slopes).

After Bonferroni corrections, assigned sex at birth did not modulate the effects of substance use subtype on volumetric changes (p -values > 0.125).

[Figure 2 and Table 4 about here]

F tests showed that developmental stage significantly modulated the associations between substance use and annual volume change in the STG (p -value = 0.004, Bonferroni corrected) and SMG (p -value = 0.014) but not any other regions (**Table 4**). Alcohol and cannabis co-use vs. non-use among early adolescents predicted significantly faster volume declines only in the STG (females: $-0.210 \text{ cm}^3/\text{year}$, Bonferroni corrected p -value = 0.022; males: $-0.368 \text{ cm}^3/\text{year}$, p -value $< 10^{-7}$) and SMG (females: $-0.218 \text{ cm}^3/\text{year}$, p -value = 0.020; males: $-0.300 \text{ cm}^3/\text{year}$, p -value $< 10^{-4}$) (**Figure 3, Supplementary Table S4**). In contrast, both females and males in late adolescence did not show significant associations between alcohol and cannabis co-use and volume decline rates in any regions (corrected p -values > 0.832). Excluding smoking status as a covariate did not change these statistically significant findings (**Supplementary Table S5**). Among alcohol and cannabis co-users, the early vs. late adolescent group also showed faster

declines for both regions and across assigned sex at birth. However, the differences were only marginally significant (Bonferroni corrected p -value < 0.06) (**Supplementary Table S4**).

[Figure 3 about here]

Analysis of continuous alcohol and cannabis use measures

The number of drinking and cannabis use days were significantly higher in the late versus the early adolescent group for all visits (**Supplementary Table S6**), but none were significantly different between females and males after Bonferroni correction (**Supplementary Table S7**).

The number of drinking days was also significantly higher in the A+C vs. A and vs. C group across all visits, but the number of cannabis use days was not significantly different between A+C and C for any visit, after Bonferroni correction (**Supplementary Table S8**).

The longitudinal models showed that none of the quantitative use measures or their interaction terms were significant in modulating longitudinal volume change rates for the cMFC, FG, IFCpo, STG, or SMG (all p -values > 0.157 , uncorrected) across developmental period.

Discussion

The current study revealed two main findings. First, alcohol and cannabis co-use, but not alcohol or cannabis use alone, is associated with a significant longitudinal regional volumetric decline. Importantly, this cannot be accounted for by more severe alcohol use in the co-use group. Second, alcohol and cannabis co-use in early adolescence predicted significantly faster

volumetric decline in the superior temporal gyrus and supramarginal gyrus, in both females and males. In contrast, no longitudinal volumetric changes were noted for late adolescents for any substance use groups. Taken together, these findings suggest that alcohol and cannabis co-use is a distinct phenotype and that the effects of alcohol and cannabis use figure more prominently on the brain when onset occurs during early adolescence.

The effects of alcohol and cannabis co-use on volumetric decline in adolescents

Individuals engaged in alcohol and cannabis co-use relative to non-drug users showed significantly faster volumetric declines in the caudal middle frontal cortex, fusiform gyrus, inferior frontal gyrus, pars opercularis, superior temporal gyrus, and supramarginal gyrus. These brain regions are implicated in attention, cognitive control, and emotion processing (39), suggesting a broad impact of alcohol and cannabis co-use on cognitive and affective functions. Cannabis use alone vs. non-use did not appear to incur volumetric decline, consistent with relatively minor effects of cannabis exposure on cognitive functioning. Surprisingly, alcohol use alone vs. non-use did not demonstrate significant differences in volumetric decline. This finding appears at odds with a vast literature demonstrating volumetric deficits from heavy drinking both in adults (28, 29, 40-42) and adolescents [see (8) for a review]. For instance, prior work indicates smaller GMVs in brain regions, including the hippocampus (43), and left frontal, temporal and parietal cortices (44) in adolescents engaged in problem alcohol use. However, it is important to note that our approach aimed to identify a longitudinal pattern of changes rather than cross-

sectional effects. Thus, alcohol misuse may manifest in diminished GMVs in a given year, but not show a significant annual decrement over time.

Indeed, brain regions may increase or decrease in GMVs during development (45). For instance, while more children demonstrate an increase in GMVs in the rostral anterior cingulate and superior temporal gyrus with development, the opposite is true of the cuneus and rostral middle frontal gyrus (45). To account for these developmental changes, including U- or inverted U-shaped patterns, of GMVs, we accounted for the effects of age and age squared in mixed effects modeling. Further, by modeling the GMV changes from prior to current year, we were able to query the yearly difference irrespective of overall patterns. Thus, the findings of volumetric decline here suggest that alcohol and cannabis co-use impedes or even reverses the typical trend of age-related volumetric increases for some brain regions and accelerates the trend of age-related decreases for others. The findings support the importance in modeling brain volumes longitudinally, which may yield insight on brain development that eludes cross-sectional findings.

Developmental period but not assigned sex at birth modulates the effects of alcohol and cannabis co-use on volumetric decline in adolescents

Consistent with prior work (46), alcohol use and alcohol and cannabis co-use, but not cannabis use alone, was significantly more prevalent in the late than early adolescent group. The early but not late adolescent group demonstrated volumetric decline due to alcohol and cannabis

co-use. This suggests that early adolescence is a developmental period characterized by increased vulnerability to alcohol and cannabis co-use.

Alcohol and cannabis co-use during early adolescence predicted significantly faster volumetric decline in the superior temporal gyrus and supramarginal gyrus, in both females and males. Across all substance use subtypes, females showed significantly faster volume declines in the superior temporal gyrus (STG). However, assigned sex at birth did not modulate the effects of substance use subtype on volumetric changes. Thus, assigned sex at birth demonstrated a limited modulating effect on the impact of alcohol and cannabis use on brain development. This finding does not negate the importance of studying assigned sex at birth differences in the influences of substance use, which may transpire earlier or only unfold later in the lifespan.

General implications of the findings

Adolescence represents a developmental period marked by significant behavioral vulnerability and complex structural and functional changes in the brain (47). The current study highlights the importance of characterizing the longitudinal patterns of brain changes during adolescence. The current work not only adds to the literature of cross-sectional findings but also suggests the possibility of cross-sectional studies misrepresenting the effects of substance use, which are likely non-linear and area-dependent. It would be of particular interest to investigate how longitudinal brain changes support cognitive and behavioral development across this critical

lifespan. The longitudinal dataset of the NCANDA and Adolescent Brain Cognition and Development projects provide ample opportunities to this end.

Limitations, other considerations, and conclusions

A number of limitations need to be considered for the study. First, substance use data were self-reported and thus were subject to recall bias. Second, there are many other clinical variables, including childhood adversity, that can influence brain volume changes. A much larger longitudinal dataset would be required to thoroughly address the impact of these other factors on brain development. Third, the current findings were based on alcohol and cannabis use as categorical variables. Yet, findings modeling these variables continuously did not yield significant results. This likely reflects the non-linear compounding effects of alcohol and cannabis co-use. For example, the STG volume showed annual decline of $-0.131 \text{ cm}^3/\text{year}$ (Table 3) with prior-year alcohol and cannabis co-use, but statistically non-significant changes of $0.112 \text{ cm}^3/\text{year}$ and $-0.041 \text{ cm}^3/\text{year}$ for cannabis and alcohol use only, respectively. Thus, the volumetric decline in co-use cannot be explained simply by the sum of the changes in cannabis and alcohol use alone.

Although we demonstrated longitudinal brain changes, this is a not a randomized trial and the findings should not be taken to imply causality of the effects of alcohol and cannabis use. That is, substance use precedes structural brain changes but temporal order alone is not sufficient to determine causation. Thus, alternative explanations should be explored further.

In summary, this work demonstrates the longitudinal impact of alcohol and cannabis co-use on volumetric brain development and more significant impact with onset of use in early than in late adolescence and potentially in females than in males. Volumetric decline was noted in cortical regions in support of attention, memory, executive control, and social cognition, suggesting potentially pervasive influences of alcohol and cannabis co-use on brain development. Alcohol and cannabis co-use may represent a distinct phenotype of importance to addiction neuroscience and medicine. More research is needed to address how these volumetric markers predict alcohol and cannabis use and co-use during a critical period in the developmental lifespan (35).

Acknowledgement

This study is supported by NIH grant R01EB022911 (Luo), R01DA051922 (Li), R01DA049154 (Buu), and U54 MD012393 (Trucco). NCANDA data collection was supported by NIH Grants AA021697, AA021697-04S1, AA021695, AA021692, AA021696, AA021681, AA021690, AA021691. The Institutional Review Board (IRB) of the local collection sites approved the data collection in accordance with Department of Health and Human Services regulations at 45 CFR Part 46.

Author Contributions

XL, AB, and CL were responsible for the study concept and design. XL, JJY, and AB contributed to the acquisition and management of data. XL and JJY performed the statistical analysis. JJY, AB, and CL assisted with data analysis and interpretation of findings. XL and CL drafted the manuscript. EMT provided critical feedback and contributed to the final version of the manuscript. All authors critically reviewed content and approved the final version for publication.

Disclosures

The authors report no financial interests or potential conflicts of interest.

References

1. Blest-Hopley G, Colizzi M, Giampietro V, Bhattacharyya S. Is the Adolescent Brain at Greater Vulnerability to the Effects of Cannabis? A Narrative Review of the Evidence. *Front Psychiatry*. 2020;11:859. Epub 2020/10/03. doi: 10.3389/fpsy.2020.00859. PubMed PMID: 33005157; PMCID: PMC7479242.
2. Sorkhou M, Bedder RH, George TP. The Behavioral Sequelae of Cannabis Use in Healthy People: A Systematic Review. *Front Psychiatry*. 2021;12:630247. Epub 2021/03/06. doi: 10.3389/fpsy.2021.630247. PubMed PMID: 33664685; PMCID: PMC7920961.
3. Linakis JG, Thomas SA, Bromberg JR, Casper TC, Chun TH, Mello MJ, Richards R, Ahmad F, Bajaj L, Brown KM, Chernick LS, Cohen DM, Dean JM, Fein J, Horeczko T, Levas MN, McAninch B, Monuteaux MC, Mull CC, Grupp-Phelan J, Powell EC, Rogers A, Shenoi RP, Suffoletto B, Vance C, Spirito A, Pediatric Emergency Care Applied Research N. Adolescent alcohol use predicts cannabis use over a three year follow-up period. *Subst Abus*. 2021:1-6. Epub 2021/07/09. doi: 10.1080/08897077.2021.1949665. PubMed PMID: 34236277.
4. Yurasek AM, Aston ER, Metrik J. Co-use of Alcohol and Cannabis: A Review. *Curr Addict Rep*. 2017;4(2):184-93. Epub 2017/06/01. doi: 10.1007/s40429-017-0149-8. PubMed PMID: 32670740; PMCID: PMC7363401.
5. Sellers CM, Diaz-Valdes A, Oliver MM, Simon KM, O'Brien KHM. The relationship between alcohol and cannabis use with nonsuicidal self-injury among adolescent inpatients: Examining the 90 days prior to psychiatric hospitalization. *Addict Behav*. 2021;114:106759. Epub 2020/12/19. doi: 10.1016/j.addbeh.2020.106759. PubMed PMID: 33338906; PMCID: PMC7792986.
6. Thompson K, Holley M, Sturgess C, Leadbeater B. Co-Use of Alcohol and Cannabis: Longitudinal Associations with Mental Health Outcomes in Young Adulthood. *Int J Environ Res Public Health*. 2021;18(7). Epub 2021/04/04. doi: 10.3390/ijerph18073652. PubMed PMID: 33807491; PMCID: PMC8037602.
7. Waddell JT. Between- and within-group effects of alcohol and cannabis co-use on AUD/CUD in the NSDUH 2002-2019. *Drug Alcohol Depend*. 2021;225:108768. Epub 2021/05/29. doi: 10.1016/j.drugalcdep.2021.108768. PubMed PMID: 34049100.
8. Ewing SW, Sakhardande A, Blakemore SJ. The effect of alcohol consumption on the adolescent brain: A systematic review of MRI and fMRI studies of alcohol-using youth. *Neuroimage Clin*. 2014;5:420-37. Epub 2014/01/01. doi: 10.1016/j.nicl.2014.06.011. PubMed PMID: 26958467; PMCID: PMC4749850.
9. Coronado C, Wade NE, Aguinaldo LD, Mejia MH, Jacobus J. Neurocognitive Correlates of Adolescent Cannabis Use: An Overview of Neural Activation Patterns in Task-Based Functional MRI Studies. *J Pediatr Neuropsychol*. 2020;6(1):1-13. Epub 2021/01/12. doi: 10.1007/s40817-020-00076-5. PubMed PMID: 33425663; PMCID: PMC7793547.

10. Figueiredo PR, Tolomeo S, Steele JD, Baldacchino A. Neurocognitive consequences of chronic cannabis use: a systematic review and meta-analysis. *Neuroscience and biobehavioral reviews*. 2020;108:358-69. Epub 2019/11/13. doi: 10.1016/j.neubiorev.2019.10.014. PubMed PMID: 31715191.
11. Albaugh MD, Ottino-Gonzalez J, Sidwell A, Lepage C, Juliano A, Owens MM, Chaarani B, Spechler P, Fontaine N, Rioux P, Lewis L, Jeon S, Evans A, D'Souza D, Radhakrishnan R, Banaschewski T, Bokde ALW, Quinlan EB, Conrod P, Desrivieres S, Flor H, Grigis A, Gowland P, Heinz A, Ittermann B, Martinot JL, Paillere Martinot ML, Nees F, Papadopoulos Orfanos D, Paus T, Poustka L, Millenet S, Frohner JH, Smolka MN, Walter H, Whelan R, Schumann G, Potter A, Garavan H, Consortium I. Association of Cannabis Use During Adolescence With Neurodevelopment. *Jama Psychiat*. 2021. Epub 2021/06/17. doi: 10.1001/jamapsychiatry.2021.1258. PubMed PMID: 34132750; PMCID: PMC8209561.
12. Ross JM, Ellingson JM, Rhee SH, Hewitt JK, Corley RP, Lessem JM, Friedman NP. Investigating the causal effect of cannabis use on cognitive function with a quasi-experimental co-twin design. *Drug Alcohol Depend*. 2020;206:107712. Epub 2019/11/23. doi: 10.1016/j.drugalcdep.2019.107712. PubMed PMID: 31753729; PMCID: PMC7179798.
13. Wade NE, Bagot KS, Tapert SF, Gruber SA, Filbey FM, Lisdahl KM. Cognitive Functioning Related to Binge Alcohol and Cannabis Co-Use in Abstinent Adolescents and Young Adults. *Journal of studies on alcohol and drugs*. 2020;81(4):479-83. Epub 2020/08/18. PubMed PMID: 32800084; PMCID: PMC7437560.
14. Wade NE, Thomas AM, Gruber SA, Tapert SF, Filbey FM, Lisdahl KM. Binge and Cannabis Co-Use Episodes in Relation to White Matter Integrity in Emerging Adults. *Cannabis Cannabinoid Res*. 2020;5(1):62-72. Epub 2020/04/24. doi: 10.1089/can.2018.0062. PubMed PMID: 32322677; PMCID: PMC7173670.
15. Aloï J, Blair KS, Crum KI, Bashford-Largo J, Zhang R, Lukoff J, Carollo E, White SF, Hwang S, Filbey FM, Dobbertin M, Blair RJR. Alcohol Use Disorder, But Not Cannabis Use Disorder, Symptomatology in Adolescents Is Associated With Reduced Differential Responsiveness to Reward Versus Punishment Feedback During Instrumental Learning. *Biol Psychiatry Cogn Neurosci Neuroimaging*. 2020;5(6):610-8. Epub 2020/04/18. doi: 10.1016/j.bpsc.2020.02.003. PubMed PMID: 32299790; PMCID: PMC7286796.
16. Blair RJR, Bajaj S, Sherer N, Bashford-Largo J, Zhang R, Aloï J, Hammond C, Lukoff J, Schwartz A, Elowsky J, Tyler P, Filbey FM, Dobbertin M, Blair KS. Alcohol Use Disorder and Cannabis Use Disorder Symptomatology in Adolescents and Aggression: Associations With Recruitment of Neural Regions Implicated in Retaliation. *Biol Psychiatry Cogn Neurosci Neuroimaging*. 2021;6(5):536-44. Epub 2021/03/14. doi: 10.1016/j.bpsc.2020.11.016. PubMed PMID: 33712378; PMCID: PMC8113085.
17. Karoly HC, Ross JM, Ellingson JM, Feldstein Ewing SW. Exploring Cannabis and Alcohol Co-Use in Adolescents: A Narrative Review of the Evidence. *J Dual Diagn*. 2020;16(1):58-74.

Epub 2019/09/15. doi: 10.1080/15504263.2019.1660020. PubMed PMID: 31519143; PMCID: PMC7007306.

18. Scott JC, Wolf DH, Calkins ME, Bach EC, Weidner J, Ruparel K, Moore TM, Jones JD, Jackson CT, Gur RE, Gur RC. Cognitive functioning of adolescent and young adult cannabis users in the Philadelphia Neurodevelopmental Cohort. *Psychol Addict Behav.* 2017;31(4):423-34. Epub 2017/04/18. doi: 10.1037/adb0000268. PubMed PMID: 28414475; PMCID: PMC5468477.
19. Jacobus J, McQueeney T, Bava S, Schweinsburg BC, Frank LR, Yang TT, Tapert SF. White matter integrity in adolescents with histories of marijuana use and binge drinking. *Neurotoxicol Teratol.* 2009;31(6):349-55. Epub 2009/07/28. doi: 10.1016/j.ntt.2009.07.006. PubMed PMID: 19631736; PMCID: PMC2762024.
20. Infante MA, Nguyen-Louie TT, Worley M, Courtney KE, Coronado C, Jacobus J. Neuropsychological Trajectories Associated with Adolescent Alcohol and Cannabis Use: A Prospective 14-Year Study. *J Int Neuropsychol Soc.* 2020;26(5):480-91. Epub 2019/12/12. doi: 10.1017/S1355617719001395. PubMed PMID: 31822320; PMCID: PMC7205577.
21. Jordan CJ, Andersen SL. Sensitive periods of substance abuse: Early risk for the transition to dependence. *Dev Cogn Neurosci.* 2017;25:29-44. Epub 2016/11/15. doi: 10.1016/j.dcn.2016.10.004. PubMed PMID: 27840157; PMCID: PMC5410194.
22. Gorey C, Kuhns L, Smaragdi E, Kroon E, Cousijn J. Age-related differences in the impact of cannabis use on the brain and cognition: a systematic review. *Eur Arch Psychiatry Clin Neurosci.* 2019;269(1):37-58. Epub 2019/01/27. doi: 10.1007/s00406-019-00981-7. PubMed PMID: 30680487; PMCID: PMC6394430.
23. Castellanos-Ryan N, Pingault JB, Parent S, Vitaro F, Tremblay RE, Seguin JR. Adolescent cannabis use, change in neurocognitive function, and high-school graduation: A longitudinal study from early adolescence to young adulthood. *Dev Psychopathol.* 2017;29(4):1253-66. Epub 2016/12/30. doi: 10.1017/S0954579416001280. PubMed PMID: 28031069; PMCID: PMC5403347.
24. Williams GC, Battista K, Leatherdale ST. An examination of how age of onset for alcohol, cannabis, and tobacco are associated with school outcomes in grade 12. *Addict Behav.* 2020;102:106215. Epub 2019/12/01. doi: 10.1016/j.addbeh.2019.106215. PubMed PMID: 31785476.
25. Tucker JS, Rodriguez A, Davis JP, Klein DJ, D'Amico EJ. Simultaneous trajectories of alcohol and cannabis use from adolescence to emerging adulthood: Associations with role transitions and functional outcomes. *Psychol Addict Behav.* 2021. Epub 2021/04/30. doi: 10.1037/adb0000744. PubMed PMID: 33914560.
26. Penzel N, Antonucci LA, Betz LT, Sanfelici R, Weiske J, Pogarell O, Cumming P, Quednow BB, Howes O, Falkai P, Upthegrove R, Bertolino A, Borgwardt S, Brambilla P, Lencer R, Meisenzahl E, Rosen M, Haidl T, Kambeitz-Ilankovic L, Ruhrmann S, Salokangas RRK, Pantelis C, Wood SJ, Koutsouleris N, Kambeitz J, Consortium P. Association between age of cannabis

initiation and gray matter covariance networks in recent onset psychosis.

Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology. 2021;46(8):1484-93. Epub 2021/03/05. doi: 10.1038/s41386-021-00977-9. PubMed PMID: 33658653; PMCID: PMC8209059.

27. Wright M, Wickens CM, Di Ciano P, Sproule B, Fares A, Matheson J, Mann RE, Rehm J, Shuper PA, George TP, Huestis MA, Stoduto G, Le Foll B, Brands B. Sex differences in the acute pharmacological and subjective effects of smoked cannabis combined with alcohol in young adults. *Psychol Addict Behav.* 2021;35(5):536-52. Epub 2021/06/04. doi: 10.1037/adb0000749. PubMed PMID: 34081489.

28. Rossetti MG, Patalay P, Mackey S, Allen NB, Batalla A, Bellani M, Chye Y, Cousijn J, Goudriaan AE, Hester R, Hutchison K, Li CR, Martin-Santos R, Momenan R, Sinha R, Schmaal L, Sjoerds Z, Solowij N, Suo C, van Holst RJ, Veltman DJ, Yucel M, Thompson PM, Conrod P, Garavan H, Brambilla P, Lorenzetti V. Gender-related neuroanatomical differences in alcohol dependence: findings from the ENIGMA Addiction Working Group. *Neuroimage Clin.* 2021;30:102636. Epub 2021/04/16. doi: 10.1016/j.nicl.2021.102636. PubMed PMID: 33857771; PMCID: PMC8065340.

29. Grace S, Rossetti MG, Allen N, Batalla A, Bellani M, Brambilla P, Chye Y, Cousijn J, Goudriaan AE, Hester R, Hutchison K, Labuschagne I, Momenan R, Martin-Santos R, Rendell P, Solowij N, Sinha R, Li CR, Schmaal L, Sjoerds Z, Suo C, Terrett G, van Holst RJ, Veltman DJ, Yucel M, Thompson P, Conrod P, Mackey S, Garavan H, Lorenzetti V. Sex differences in the neuroanatomy of alcohol dependence: hippocampus and amygdala subregions in a sample of 966 people from the ENIGMA Addiction Working Group. *Transl Psychiatry.* 2021;11(1):156. Epub 2021/03/06. doi: 10.1038/s41398-021-01204-1. PubMed PMID: 33664226; PMCID: PMC7933136.

30. Noorbakhsh S, Afzali MH, Boers E, Conrod PJ. Cognitive Function Impairments Linked to Alcohol and Cannabis Use During Adolescence: A Study of Gender Differences. *Front Hum Neurosci.* 2020;14:95. Epub 2020/04/23. doi: 10.3389/fnhum.2020.00095. PubMed PMID: 32317950; PMCID: PMC7154290.

31. El Marroun H, Klapwijk ET, Koevoets M, Brouwer RM, Peters S, Van't Ent D, Boomsma DI, Muetzel RL, Crone EA, Hulshoff Pol HE, Franken IHA. Alcohol use and brain morphology in adolescence: A longitudinal study in three different cohorts. *The European journal of neuroscience.* 2021;54(6):6012-26. Epub 2021/08/15. doi: 10.1111/ejn.15411. PubMed PMID: 34390509.

32. Ajmera N, Collins PF, Weiss H, Luciana M. Initiation of Moderately Frequent Cannabis use in Adolescence and Young Adulthood is Associated with Declines in Verbal Learning and Memory: A Longitudinal Comparison of Pre- versus Post-Initiation Cognitive Performance. *J Int Neuropsychol Soc.* 2021;27(6):621-36. Epub 2021/07/16. doi: 10.1017/S1355617721000631. PubMed PMID: 34261549.

33. Pacheco-Colon I, Hawes SW, Duperrouzel JC, Gonzalez R. Evidence Lacking for Cannabis Users Slacking: A Longitudinal Analysis of Escalating Cannabis Use and Motivation among Adolescents. *J Int Neuropsychol Soc.* 2021;27(6):637-47. Epub 2021/07/16. doi: 10.1017/S135561772000096X. PubMed PMID: 34261556; PMCID: PMC8289119.
34. Brown SA, Brumback T, Tomlinson K, Cummins K, Thompson WK, Nagel BJ, De Bellis MD, Hooper SR, Clark DB, Chung T, Hasler BP, Colrain IM, Baker FC, Prouty D, Pfefferbaum A, Sullivan EV, Pohl KM, Rohlfing T, Nichols BN, Chu W, Tapert SF. The National Consortium on Alcohol and NeuroDevelopment in Adolescence (NCANDA): A Multisite Study of Adolescent Development and Substance Use. *Journal of studies on alcohol and drugs.* 2015;76(6):895-908. Epub 2015/11/13. doi: 10.15288/jsad.2015.76.895. PubMed PMID: 26562597; PMCID: PMC4712659.
35. Morales AM, Boyd SJ, Mackiewicz Seghete KL, Johnson AJ, De Bellis MD, Nagel BJ. Sex Differences in the Effect of Nucleus Accumbens Volume on Adolescent Drinking: The Mediating Role of Sensation Seeking in the NCANDA Sample. *Journal of studies on alcohol and drugs.* 2019;80(6):594-601. Epub 2019/12/04. PubMed PMID: 31790349; PMCID: PMC6900990.
36. Pfefferbaum A, Kwon D, Brumback T, Thompson WK, Cummins K, Tapert SF, Brown SA, Colrain IM, Baker FC, Prouty D, De Bellis MD, Clark DB, Nagel BJ, Chu W, Park SH, Pohl KM, Sullivan EV. Altered Brain Developmental Trajectories in Adolescents After Initiating Drinking. *Am J Psychiatry.* 2018;175(4):370-80. Epub 2017/11/01. doi: 10.1176/appi.ajp.2017.17040469. PubMed PMID: 29084454; PMCID: PMC6504929.
37. Sullivan EV, Brumback T, Tapert SF, Brown SA, Baker FC, Colrain IM, Prouty D, De Bellis MD, Clark DB, Nagel BJ, Pohl KM, Pfefferbaum A. Disturbed Cerebellar Growth Trajectories in Adolescents Who Initiate Alcohol Drinking. *Biol Psychiatry.* 2020;87(7):632-44. Epub 2019/10/28. doi: 10.1016/j.biopsych.2019.08.026. PubMed PMID: 31653477; PMCID: PMC7061065.
38. Desikan RS, Segonne F, Fischl B, Quinn BT, Dickerson BC, Blacker D, Buckner RL, Dale AM, Maguire RP, Hyman BT, Albert MS, Killiany RJ. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage.* 2006;31(3):968-80. Epub 2006/03/15. doi: 10.1016/j.neuroimage.2006.01.021. PubMed PMID: 16530430.
39. Gazzaniga MS, Ivry RB. *Cognitive Neuroscience. The biology of the mind, Fifth Edition.* 2014.
40. Mackey S, Allgaier N, Chaarani B, Spechler P, Orr C, Bunn J, Allen NB, Alia-Klein N, Batalla A, Blaine S, Brooks S, Caparelli E, Chye YY, Cousijn J, Dagher A, Desrivieres S, Feldstein-Ewing S, Foxe JJ, Goldstein RZ, Goudriaan AE, Heitzeg MM, Hester R, Hutchison K, Korucuoglu O, Li CR, London E, Lorenzetti V, Luijten M, Martin-Santos R, May A, Momenan R, Morales A, Paulus MP, Pearson G, Rousseau ME, Salmeron BJ, Schluter R, Schmaal L, Schumann G, Sjoerds Z, Stein DJ, Stein EA, Sinha R, Solowij N, Tapert S, Uhlmann A, Veltman D, van Holst R, Whittle S, Wright MJ, Yucel M, Zhang S, Yurgelun-Todd D, Hibar DP, Jahanshad N, Evans A, Thompson PM, Glahn DC,

- Conrod P, Garavan H, Group EAW. Mega-Analysis of Gray Matter Volume in Substance Dependence: General and Substance-Specific Regional Effects. *Am J Psychiatry*. 2019;176(2):119-28. Epub 2018/10/20. doi: 10.1176/appi.ajp.2018.17040415. PubMed PMID: 30336705; PMCID: PMC6427822.
41. Meda SA, Hawkins KA, Dager AD, Tennen H, Khadka S, Austad CS, Wood RM, Raskin S, Fallahi CR, Pearlson GD. Longitudinal Effects of Alcohol Consumption on the Hippocampus and Parahippocampus in College Students. *Biol Psychiatry Cogn Neurosci Neuroimaging*. 2018;3(7):610-7. Epub 2018/04/24. doi: 10.1016/j.bpsc.2018.02.006. PubMed PMID: 29680476; PMCID: PMC6062479.
42. Rando K, Hong KI, Bhagwagar Z, Li CS, Bergquist K, Guarnaccia J, Sinha R. Association of frontal and posterior cortical gray matter volume with time to alcohol relapse: a prospective study. *Am J Psychiatry*. 2011;168(2):183-92. Epub 2010/11/17. doi: 10.1176/appi.ajp.2010.10020233. PubMed PMID: 21078704; PMCID: PMC3668974.
43. Nagel BJ, Schweinsburg AD, Phan V, Tapert SF. Reduced hippocampal volume among adolescents with alcohol use disorders without psychiatric comorbidity. *Psychiatry Res*. 2005;139(3):181-90. Epub 2005/08/02. doi: 10.1016/j.psychresns.2005.05.008. PubMed PMID: 16054344; PMCID: PMC2270700.
44. Fein G, Greenstein D, Cardenas VA, Cuzen NL, Fouche JP, Ferrett H, Thomas K, Stein DJ. Cortical and subcortical volumes in adolescents with alcohol dependence but without substance or psychiatric comorbidities. *Psychiatry Res*. 2013;214(1):1-8. Epub 2013/08/07. doi: 10.1016/j.psychresns.2013.06.001. PubMed PMID: 23916536; PMCID: PMC4203651.
45. Vijayakumar N, Allen NB, Youssef G, Dennison M, Yucel M, Simmons JG, Whittle S. Brain development during adolescence: A mixed-longitudinal investigation of cortical thickness, surface area, and volume. *Hum Brain Mapp*. 2016;37(6):2027-38. Epub 2016/03/08. doi: 10.1002/hbm.23154. PubMed PMID: 26946457; PMCID: PMC6867680.
46. Wellman RJ, Dugas EN, O'Loughlin EK, Sylvestre MP, Pelekanakis A, O'Loughlin J. Natural course of co-use of alcohol and cannabis in adolescents and young adults in Montreal, Canada. *Psychol Addict Behav*. 2021. Epub 2021/07/30. doi: 10.1037/adb0000766. PubMed PMID: 34323529.
47. Konrad K, Firk C, Uhlhaas PJ. Brain development during adolescence: neuroscientific insights into this developmental period. *Dtsch Arztebl Int*. 2013;110(25):425-31. Epub 2013/07/11. doi: 10.3238/arztebl.2013.0425. PubMed PMID: 23840287; PMCID: PMC3705203.

Figure and Table Legends

Figure 1. Brain regions that showed statistically significant associations, after Bonferroni corrections, between annual brain volume changes and alcohol and/or cannabis use 1-year prior, as demonstrated by the main mixed model (see also Table 2). Colors correspond to uncorrected p-values. CMF: caudal middle frontal cortex; Fusiform: Fusiform gyrus; PO: inferior frontal gyrus, pars opercularis.

Figure 2. Annual volume changes (cm^3) vs. age among female (F) and male (M) controls (no alcohol or cannabis use ever) of the five statistically significant regions as shown in Table 2: caudal middle frontal cortex, fusiform gyrus, inferior frontal gyrus (IFG), pars opercularis, superior temporal gyrus, and supramarginal gyrus. Solid and dashed lines show linear regressions and their 95% confidence intervals. The fitted lines are negative showing decreasing volumes year after year. Only the slope for supramarginal gyrus among females was marginally significant (Bonferroni corrected p-value = 0.044; Bonferroni p-values > 0.313 for all other slopes).

Figure 3. Annual volumetric changes (higher bars: faster declines) in (A) females and (B) males by substance use group and developmental stage, as shown by the modulation model (Table 4) after covariate adjustment. Annual rates were shown separately by the prior year substance use and developmental stage (Early: early adolescents, Late: late adolescents). Statistically significant differences after Bonferroni correction were marked by horizontal bars. P values, uncorrected: *** <0.001.

Table 1. Descriptive statistics of baseline clinical/gray matter volume variables and alcohol and cannabis use at baseline and during the study period (ever) for all and for early and late group separately.

Table 2. Annual changes (95% confidence intervals) in brain volumes (cm^3) by assigned sex at birth and substance use types, after covariate adjustment in the main model.

Table 3. Differences in annual brain volume changes (cm^3) between substance use groups and controls, after covariate adjustment in the main model.

Table 4. Annual brain volume changes by assigned sex at birth, developmental stages and substance use types as shown in the modulation model.

Table 1. Descriptive statistics of baseline clinical/gray matter volume variables and alcohol and cannabis use at baseline and during the study period (ever) for all and for early and late group separately.

	All (N = 724)	Early (N = 381)	Late (N = 343)	P-value
Demographics				
Age (years)	16.02 ± 2.45	14.06 ± 1.13	18.20 ± 1.49	<0.001
ICV (cm ³)	1,051.25 ± 118.02	1,050.65 ± 117.32	1,051.91 ± 118.95	0.887
Social-econ status (years)	16.86 ± 2.42	16.94 ± 2.48	16.77 ± 2.34	0.321
Assigned sex at birth (female, N)	370 (51.10%)	193 (50.66%)	177 (51.60%)	0.857
Race				0.158
White	523 (72.24%)	264 (69.29%)	259 (75.51%)	
Black	80 (11.05%)	45 (11.81%)	35 (10.20%)	
Others	121 (16.71%)	72 (18.90%)	49 (14.29%)	
Smoking				
Baseline	41 (5.82%)	5 (1.32%)	36 (11.04%)	<0.001
Ever	191 (27.09%)	75 (19.79%)	116 (35.58%)	<0.001
Alcohol/Cannabis use at baseline				
Non-use controls	510 (70.44%)	345 (90.55%)	165 (48.10%)	<0.001
Cannabis	17 (2.35%)	5 (1.31%)	12 (3.50%)	
Alcohol	108 (14.92%)	22 (5.77%)	86 (25.07%)	
Alcohol+Cannabis	89 (12.29%)	9 (2.36%)	80 (23.32%)	
Alcohol/Cannabis use ever				
Non-use controls	175 (24.17%)	138 (36.22%)	37 (10.79%)	<0.001
Cannabis	22 (3.04%)	20 (5.25%)	2 (0.58%)	
Alcohol	164 (22.65%)	56 (14.70%)	108 (31.49%)	
Alcohol+Cannabis	363 (50.14%)	167 (43.83%)	196 (57.14%)	
Volume at baseline (cm³)				
Superior temporal sulcus	5.77 ± 0.91	5.92 ± 0.91	5.60 ± 0.89	<0.001
Caudal anterior cingulate cortex	4.83 ± 0.92	4.91 ± 0.94	4.74 ± 0.89	0.014
Caudal middle frontal cortex	15.13 ± 2.63	15.58 ± 2.62	14.63 ± 2.56	<0.001
Cuneus cortex	6.33 ± 1.04	6.48 ± 1.06	6.17 ± 0.99	<0.001
Entorhinal cortex	3.74 ± 0.70	3.75 ± 0.71	3.72 ± 0.69	0.486
Fusiform gyrus	23.18 ± 3.19	23.66 ± 3.27	22.65 ± 3.01	<0.001
Inferior parietal cortex	32.41 ± 4.65	33.69 ± 4.62	30.99 ± 4.27	<0.001
Inferior temporal gyrus	24.70 ± 3.95	25.38 ± 3.90	23.95 ± 3.89	<0.001
Isthmus-cingulate cortex	6.09 ± 1.06	6.26 ± 1.07	5.90 ± 1.03	<0.001
Lateral occipital cortex	25.48 ± 3.61	26.04 ± 3.51	24.85 ± 3.63	<0.001
Lateral orbital frontal cortex	17.59 ± 2.39	18.18 ± 2.31	16.93 ± 2.30	<0.001

Lingual gyrus	14.90 ± 2.22	15.17 ± 2.28	14.60 ± 2.13	<0.001
Medial orbital frontal cortex	11.72 ± 1.61	12.17 ± 1.61	11.22 ± 1.46	<0.001
Middle temporal gyrus	25.92 ± 3.43	26.65 ± 3.25	25.12 ± 3.45	<0.001
Parahippocampal gyrus	4.89 ± 0.66	4.97 ± 0.65	4.80 ± 0.66	<0.001
Paracentral lobule	8.59 ± 1.29	8.89 ± 1.31	8.27 ± 1.19	<0.001
IFG, pars opercularis	10.61 ± 1.63	10.83 ± 1.59	10.37 ± 1.63	<0.001
IFG, pars orbitalis	5.83 ± 0.86	6.07 ± 0.84	5.57 ± 0.81	<0.001
IFG, pars triangularis	9.42 ± 1.52	9.77 ± 1.47	9.03 ± 1.48	<0.001
Pericalcarine cortex	4.69 ± 0.88	4.71 ± 0.89	4.66 ± 0.88	0.489
Postcentral gyrus	21.32 ± 2.89	21.80 ± 2.79	20.79 ± 2.91	<0.001
Posterior-cingulate cortex	7.77 ± 1.11	8.01 ± 1.10	7.51 ± 1.06	<0.001
Precentral gyrus	30.70 ± 3.69	31.24 ± 3.63	30.10 ± 3.66	<0.001
Precuneus cortex	23.11 ± 3.15	23.87 ± 3.07	22.27 ± 3.03	<0.001
Rostral anterior cingulate cortex	5.62 ± 0.93	5.70 ± 0.96	5.53 ± 0.89	0.012
Rostral middle frontal gyrus	37.85 ± 5.94	39.58 ± 5.86	35.93 ± 5.42	<0.001
Superior frontal gyrus	53.34 ± 6.61	55.24 ± 6.31	51.23 ± 6.31	<0.001
Superior parietal cortex	29.61 ± 4.00	30.55 ± 4.04	28.57 ± 3.69	<0.001
Superior temporal gyrus	26.79 ± 3.20	27.37 ± 3.12	26.16 ± 3.18	<0.001
Supramarginal gyrus	24.93 ± 3.62	25.76 ± 3.60	24.01 ± 3.41	<0.001
Frontal pole	2.32 ± 0.41	2.44 ± 0.41	2.18 ± 0.36	<0.001
Temporal pole	5.36 ± 0.62	5.43 ± 0.64	5.28 ± 0.59	0.001
Transverse temporal cortex	2.32 ± 0.38	2.34 ± 0.38	2.30 ± 0.38	0.116
Insula	14.36 ± 1.69	14.51 ± 1.69	14.18 ± 1.67	0.008

Note: P-values were for t or chi-square tests of the difference between early and late adolescents as appropriate for the variable distributions.

ICV: intra-cranial volume; IFG: inferior frontal gyrus; Early: early adolescents; Late: late adolescents.

Table 2. Annual changes (95% confidence intervals) in brain volumes (cm³) by sex and substance use types, after covariate adjustment in the main model.

Region	Sex	Non-use controls	Cannabis	Alcohol	Alcohol+Cannabis	P-value
Caudal middle frontal cortex						<0.001
	Female	-0.361 (-0.394, -0.329)	-0.318 (-0.449, -0.187)	-0.421 (-0.480, -0.362)	-0.455 (-0.510, -0.400)	
	Male	-0.376 (-0.409, -0.342)	-0.332 (-0.463, -0.201)	-0.435 (-0.495, -0.375)	-0.469 (-0.524, -0.415)	
Fusiform gyrus						<0.001
	Female	-0.379 (-0.413, -0.345)	-0.375 (-0.511, -0.239)	-0.439 (-0.501, -0.378)	-0.485 (-0.542, -0.428)	
	Male	-0.367 (-0.402, -0.333)	-0.363 (-0.499, -0.227)	-0.427 (-0.490, -0.365)	-0.473 (-0.530, -0.416)	
IFG, Pars opercularis						<0.001
	Female	-0.188 (-0.204, -0.172)	-0.157 (-0.222, -0.092)	-0.207 (-0.236, -0.178)	-0.234 (-0.261, -0.206)	
	Male	-0.192 (-0.209, -0.175)	-0.161 (-0.226, -0.096)	-0.211 (-0.241, -0.181)	-0.238 (-0.265, -0.210)	
Superior temporal gyrus						<0.001
	Female	-0.399 (-0.441, -0.357)	-0.287 (-0.456, -0.119)	-0.440 (-0.516, -0.364)	-0.530 (-0.601, -0.460)	
	Male	-0.339 (-0.382, -0.297)	-0.228 (-0.396, -0.060)	-0.381 (-0.458, -0.303)	-0.471 (-0.541, -0.400)	
Supramarginal gyrus						<0.001
	Female	-0.615 (-0.658, -0.571)	-0.473 (-0.646, -0.299)	-0.703 (-0.781, -0.624)	-0.752 (-0.825, -0.680)	

Region	Sex	Non-use controls	Cannabis	Alcohol	Alcohol+Cannabis	P-value
	Male	-0.638 (-0.683, -0.594)	-0.496 (-0.670, -0.323)	-0.727 (-0.806, -0.647)	-0.776 (-0.849, -0.704)	

Note: P-values (unadjusted) were for testing the overall associations between annual volume changes and substance use types. Only ROIs with Bonferroni corrected p-values < 0.05 are shown; the results for all ROIs are shown in Supplementary Table S2. Only superior temporal gyrus showed significant annual change differences between females and males (p-value = 0.004, uncorrected). No other regions showed significant differences across assigned sex at birth.

Table 3. Differences in annual brain volume changes (cm³) between substance use groups and controls, after covariate adjustment in the main model.

Region	Substance	Reference	Substance -Reference	P-value
Caudal middle frontal cortex				
	C	N	0.044	0.510
	A	N	-0.060	0.019
	A+C	N	-0.094	<0.001
	A	C	-0.103	0.129
	A+C	C	-0.137	0.042
	A+C	A	-0.034	0.215
Fusiform gyrus				
	C	N	0.004	0.952
	A	N	-0.060	0.023
	A+C	N	-0.106	<0.001
	A	C	-0.064	0.363
	A+C	C	-0.110	0.116
	A+C	A	-0.046	0.108
IFG, pars opercularis				
	C	N	0.031	0.348
	A	N	-0.019	0.132
	A+C	N	-0.046	<0.001
	A	C	-0.050	0.141
	A+C	C	-0.076	0.023
	A+C	A	-0.027	0.052
Superior temporal gyrus				
	C	N	0.112	0.188
	A	N	-0.041	0.208
	A+C	N	-0.131	<0.001
	A	C	-0.153	0.080
	A+C	C	-0.243	0.005
	A+C	A	-0.090	0.011
Supramarginal gyrus				
	C	N	0.142	0.105
	A	N	-0.088	0.009
	A+C	N	-0.138	<0.001
	A	C	-0.230	0.011

Region	Substance	Reference	Substance -Reference	P-value
	A+C	C	-0.280	0.002
	A+C	A	-0.050	0.175

Note: N: non-use controls; C: cannabis; A: alcohol; A+ C: alcohol and cannabis co-use. P-values (unadjusted) were for testing the differences in annual rates. Bonferroni corrected p-values < 0.05 are bolded. All regions in Table 2 were tested but only those with significant p-values are reported here.

Table 4. Annual brain volume changes by sex, developmental stages and substance use types as shown in the moderation model.

Region	Sex	Stage	Non-use controls	Cannabis	Alcohol	Alcohol+Cannabis	Stage Modulation (p-value)	Sex Modulation (p-value)
Caudal middle frontal							0.406	0.597
	Female	Early	-0.356 (-0.394, -0.318)	-0.343 (-0.566, -0.120)	-0.464 (-0.556, -0.371)	-0.453 (-0.543, -0.363)		
	Female	Late	-0.365 (-0.447, -0.283)	-0.168 (-0.469, 0.132)	-0.410 (-0.512, -0.309)	-0.416 (-0.511, -0.321)		
	Male	Early	-0.365 (-0.404, -0.326)	-0.409 (-0.614, -0.203)	-0.447 (-0.544, -0.350)	-0.506 (-0.594, -0.418)		
Fusiform gyrus							0.317	0.383
	Male	Late	-0.374 (-0.457, -0.292)	-0.234 (-0.508, 0.039)	-0.393 (-0.498, -0.288)	-0.468 (-0.559, -0.377)		
	Female	Early	-0.380 (-0.419, -0.340)	-0.381 (-0.612, -0.150)	-0.420 (-0.516, -0.325)	-0.517 (-0.611, -0.423)		
	Female	Late	-0.377 (-0.462, -0.291)	-0.153 (-0.464, 0.159)	-0.388 (-0.493, -0.282)	-0.442 (-0.541, -0.344)		
IFG, pars opercularis							0.115	0.353
	Male	Early	-0.349 (-0.390, -0.308)	-0.474 (-0.687, -0.261)	-0.464 (-0.564, -0.363)	-0.521 (-0.612, -0.429)		
	Male	Late	-0.346 (-0.431, -0.260)	-0.245 (-0.529, 0.038)	-0.431 (-0.540, -0.322)	-0.446 (-0.541, -0.352)		
	Female	Early	-0.191 (-0.210, -0.172)	-0.169 (-0.279, -0.058)	-0.233 (-0.279, -0.187)	-0.239 (-0.284, -0.195)		
Superior temporal							<0.001	0.025
	Female	Late	-0.206 (-0.247, -0.166)	-0.057 (-0.205, 0.092)	-0.201 (-0.251, -0.150)	-0.225 (-0.272, -0.177)		
	Male	Early	-0.186 (-0.205, -0.166)	-0.236 (-0.338, -0.134)	-0.243 (-0.291, -0.194)	-0.266 (-0.310, -0.223)		
	Male	Late	-0.201 (-0.242, -0.160)	-0.124 (-0.260, 0.011)	-0.210 (-0.263, -0.158)	-0.251 (-0.297, -0.206)		
Superior temporal	Female	Early	-0.401 (-0.450, -0.353)	-0.340 (-0.624, -0.056)	-0.473 (-0.591, -0.355)	-0.611 (-0.726, -0.496)		

Region	Sex	Stage	Non-use controls	Cannabis	Alcohol	Alcohol+Cannabis	Stage Modulation (p-value)	Sex Modulation (p-value)
Supramarginal gyrus	Female	Late	-0.454 (-0.558, -0.349)	-0.002 (-0.386, 0.382)	-0.399 (-0.530, -0.269)	-0.414 (-0.536, -0.293)	<0.001	0.308
	Male	Early	-0.298 (-0.348, -0.248)	-0.445 (-0.707, -0.183)	-0.437 (-0.560, -0.313)	-0.666 (-0.779, -0.554)		
	Male	Late	-0.351 (-0.456, -0.245)	-0.107 (-0.456, 0.242)	-0.363 (-0.497, -0.229)	-0.470 (-0.586, -0.354)		
	Female	Early	-0.591 (-0.641, -0.540)	-0.507 (-0.800, -0.213)	-0.768 (-0.889, -0.646)	-0.809 (-0.928, -0.690)		
	Female	Late	-0.569 (-0.678, -0.461)	0.052 (-0.345, 0.448)	-0.595 (-0.729, -0.460)	-0.599 (-0.725, -0.473)		
	Male	Early	-0.600 (-0.652, -0.548)	-0.731 (-1.002, -0.460)	-0.769 (-0.897, -0.641)	-0.899 (-1.016, -0.783)		
	Male	Late	-0.579 (-0.687, -0.470)	-0.173 (-0.533, 0.188)	-0.596 (-0.734, -0.457)	-0.689 (-0.809, -0.569)		

Note: P-values were for F-test whether either developmental stage or assigned sex at birth moderated the associations between annual volumetric changes and substance use. Bonferroni corrected p-values < 0.05 are bolded.