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Adolescent Brain Surface Area Pre- and Post-Cannabis and Alcohol Initiation

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ABSTRACT. Objective: Changes in gray matter volume and thickness are associated with adolescent alcohol and cannabis use, but the impact of these substances on surface area remains unclear. The present study expands on previous findings to examine the impact of alcohol and cannabis on surface area before and after use initiation. **Method:** Scans for 69 demographically similar youth were obtained at baseline (ages 12–14 years; before substance use) and at 6-year follow-up (ages 17–21 years). Participants were classified into three groups based on substance use: alcohol use initiators (ALC, $n = 23$), alcohol and cannabis use initiators (ALC+CU, $n = 23$), and individuals with minimal substance use (<3 lifetime alcohol and 0 marijuana use episodes; CON, $n = 23$). For each hemisphere, group differences in surface area across time (pre- and post-substance use initiation) and significant group-by-time interactions were examined individually for 34 cortical regions using repeated measures analysis of covariance. A vertex-wise analysis assessed group differences in surface area percent change. **Results:** A significant group-by-time interaction was found in three regions, bilateral medial orbitofrontal cortices and right insula. Although all regions showed decreases in surface area over time ($ps < .05$), a more substantial decrease was identified in the ALC group. Of note, the right medial orbitofrontal cortex survived the conservative vertex-wise analyses ($p < .001$), as a more substantial decrease was found in the ALC compared to the ALC+CU group in this region. **Conclusions:** Surface area in the medial orbitofrontal cortex may be a useful intermediate phenotype for exploring the mechanisms underlying the effects of substance use on brain development. (*J. Stud. Alcohol Drugs, 79,* 835–843, 2018)

ADOLESCENCE IS A PERIOD OF significant morphometric and functional brain maturation, including decreases in gray matter and increases in white matter volume (Giedd, 2004, 2008; Gogtay et al., 2004). Given these extensive maturational changes, the adolescent brain may be particularly vulnerable to the effects of alcohol and cannabis (Jacobus & Tapert, 2013; Squeglia et al., 2009).

Cross-sectional imaging studies have hinted at a relation between adolescent substance use and altered brain development. Alcohol use during adolescence is associated with volumetric reductions of the prefrontal cortex (De Bellis et al., 2005; Medina et al., 2008), cerebellum (Lisdahl et al., 2013), and hippocampus (De Bellis et al., 2000; Nagel et al., 2005), and early cannabis use is associated with enhanced frontal and temporal gray/white matter contrast, cortical thickening, and decreased gyrification (Filbey et al., 2015). Another study found reduced gyrification that was more widely distributed in prefrontal cortex in adolescent cannabis users, whereas reductions in surface area were more subtle and limited to the left ventral lateral and ventral medial prefrontal cortex (Shollenbarger et al., 2015). Yet, it is impossible to disentangle pre-existing structural differences from substance-induced effects in these cross-sectional designs.

Longitudinal studies have observed both pre- and post-substance use differences in brain structure among youth who later transition to substance use (Squeglia & Gray, 2016). In a recent study by Squeglia et al. (2016), several regions of cortical abnormalities were significant predictors of moderate to heavy substance use initiation by age 18. Pre-existing volume differences in the prefrontal cortex, anterior cingulate cortex, and nucleus accumbens have also predicted alcohol-related behaviors in adolescence (Cheetham et al., 2014; Squeglia et al., 2014; Urošević et al., 2015; Whelan et al., 2014). With respect to cannabis, smaller orbitofrontal volume in early adolescence was found to predict initiation of cannabis use over a 4-year follow-up period (Cheetham et al., 2012). Thus, pre-existing neural characteristics may increase risk for later substance use. Progression into heavier substance use, however, also influences neural development and structural maturation (Pfefferbaum et al., 2017; Squeglia et al., 2015).

The literature on the developmental effects specific to alcohol and cannabis co-use is sparse. Jacobus et al. (2016) examined the impact of concurrent cannabis and alcohol use on brain structure development before substance use and after use onset among adolescents who primarily used alcohol, alcohol and cannabis, and minimal to no substances (0–5 substance use days) over a 6-year follow-up period. Overall findings showed a more substantial decrease in cortical thickness in controls and alcohol initiators in several bilateral regions compared with those who used alcohol and cannabis

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by follow-up. Group differences at baseline were observed between adolescents who used alcohol by follow-up and those who used alcohol and cannabis. The authors concluded that pre-existing differences before initiation of alcohol and cannabis use and cannabis-related alterations may contribute to altered cortical thickness development.

The interpretation of gray matter volume alterations is complicated without examining its individual components. More recent advances in neuroimaging research allow for the examination of the lower-order surface-based components of gray matter volume, including cortical thickness and surface area. These components are genetically and phenotypically independent of each other (Panizzon et al., 2009; Winkler et al., 2010) and follow distinct developmental trajectories (Raznahan et al., 2011; Tamnes et al., 2017; Wierenga et al., 2014). Although less research has been conducted on surface area in contrast to cortical thickness, findings generally support decreases in both of these measures during adolescence (Tamnes et al., 2017). Surface-based measures have been shown to be more sensitive at detecting gray matter alterations than voxel-based measures used to estimate volume (Hutton et al., 2009), and may provide information regarding the impact of substance use on brain development that would otherwise go undetected with robust volumetric measures.

The goal of the present study was to examine the impact of concurrent cannabis and alcohol use on brain surface area pre-and-post substance use initiation. Adolescents were first assessed at ages 12–14 years and re-assessed approximately 6 years later. Based on previous findings (Jacobus et al., 2016; Shollenbarger et al., 2015), we hypothesized more subtle decreases in surface area across all lobes with greatest decreases in frontal and parietal regions for individuals who had initiated both alcohol and marijuana use over the 6-year follow-up period compared with those who initiated alcohol use only or no substance use by follow-up.

Method

Participants

Adolescents (N = 69; ages 12–14 years at enrollment) with minimal substance use experience were recruited from local San Diego schools and followed for ~6 years (ages 17–21 years at follow-up) as part of a larger ongoing longitudinal study examining youth at risk for substance use disorders. The current sample has been previously described (Jacobus et al., 2016). All participants provided informed consent (or assent if under age 18, with consent from their guardians).

Exclusionary criteria at baseline included a history of prenatal exposure to alcohol (>2 drinks a week) or illicit drugs, premature birth (i.e., born before the 35th gestational week), history of Axis I disorder based on the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (American Psychiatric Association, 2000), history of head trauma or loss of consciousness (>2 minutes), neurological or chronic medical illness, learning or intellectual disability, parental history of psychotic disorder, contraindication to MRI procedures, inadequate comprehension of English, non-correctable sensory impairments, left handedness, and use of psychoactive medications. Eligible participants underwent neuroimaging and comprehensive interviews to assess for demographic information (e.g., age, education, household income), substance use, psychopathology, as well as general life functioning and behavior at the baseline and follow-up appointments.

Participants were classified into three groups based on their reported substance use over the follow-up period, and cutoffs were determined a priori (Jacobus et al., 2016). Adolescents were included in the cannabis and alcohol initiation group (ALC+CU; n = 23) if they reported more than 50 cumulative cannabis use days (roughly representing greater than monthly use since initiation) and greater than 20 cumulative lifetime alcohol use episodes by follow-up. Participants with primarily alcohol use and minimal cannabis use were selected for inclusion in the alcohol initiation group (ALC; n = 23) to further understand the contribution of cannabis use on structural brain changes. Inclusion in the ALC group required more than 20 cumulative lifetime alcohol use episodes and fewer than 40 cumulative cannabis use episodes by follow-up. Adolescents in the control group (CON; n = 23) reported fewer than three lifetime alcohol use episodes and no cannabis or other substance use episodes by follow-up. Of note, substance use reported in Table 1 mostly occurred within 12–36 months of follow-up.

Measures

Substance use. The Customary Drinking and Drug Use Record (Brown et al., 1998) was used to assess quantity and frequency of lifetime (defined as cumulative use in days) and past-year alcohol, cannabis, and other drug use at both baseline and annual substance use assessments.

MRI acquisition and data processing

All participants were scanned using the same 3.0 Tesla CXK4 short bore Excite-2 magnetic resonance system (General Electric, Milwaukee, WI) with an eight-channel phase array head coil. Scan parameters and data processing for this sample have been previously described (Jacobus et al., 2016). Briefly, all MRI images were processed using FreeSurfer v5.1 (http://surfer.nmr.mgh.harvard.edu/). Following cross-sectional processing, data were fed through FreeSurfer's longitudinal stream, allowing for estimates that are unbiased with respect to any time point (Reuter et al., 2012). This includes the creation of a within-subject template space and

Table 1. Demographic characteristics of study participants

Variable	CON		ALC		ALC+CU	
	M (*SD*) (*n* = 23)	Range	*M* (*SD*) (*n* = 23)	Range	*M* (*SD*) (*n* = 23)	Range
Age, baseline*,[a]	13.4 (0.6)	12.3–14.5	13.8 (0.6)	12.3–14.9	13.9 (0.6)	12.8–14.9
Age, follow-up*,[a]	18.8 (0.9)	17.3–21.3	19.2 (0.8)	18.1–21.2	19.5 (0.9)	18.1–21.2
Interscan interval, years	5.4 (0.8)		5.5 (0.8)		5.6 (0.7)	
% White	82		87		70	
% Male	56.5		56.5		56.5	
Grade point average, follow-up	3.5 (0.5)		3.6 (0.5)		3.4 (0.7)	
Household income, follow-up	161.2K (101.4)		151.8K (60.1)		193.2K (128.0)	
Maternal education, years	16.2 (2.6)		16.4 (1.7)		16.0 (1.8)	
% Alcohol abuse/dependence, follow-up	0		0		4.3	
% Cannabis abuse/dependence, follow-up	0		0		39.1	
Age at alcohol initiation	16.3 (2.1)		16.1 (1.3)		15.1 (1.1)	
Age at cannabis initiation	–		16.6 (1.7)		15.7 (1.7)	
Lifetime alcohol use days, baseline	0.04 (0.2)	0–1	0.04 (0.2)	0–1	0.4 (0.4)	0–5
Lifetime alcohol use days, follow-up[a,b]	0.34 (0.8)	0–3	127.2 (119.8)	20–523	217.0 (228.6)	29–929
Binge drinking episodes, follow-up*,[a]	0.0 (0.0)		55.8 (76.2)	1–300	89.0 (118.2)	2–485
Lifetime peak drinks on an occasion*,[a,b]	0.7 (1.9)	0–8	11.3 (3.6)	5–20	10.6 (3.0)	6–15
Lifetime cannabis use days, baseline	0.0 (0.0)		0.0 (0.0)		0.2 (0.7)	0–3
Lifetime cannabis use days, follow-up*,[a,c]	0.0 (0.0)		9.4 (11.1)	0–37	426.0 (475.9)	53–1,720
Lifetime other drug use days, baseline	0.0 (0.0)		0.0 (0.0)		0.0 (0.0)	
Lifetime other drug use days, follow-up*,[a,c]	0.0 (0.0)		5.1 (13.3)	0–62	90.0 (131.5)	0–356

Notes: CON = controls (individuals with minimal substance use); ALC = alcohol use initiators; ALC+CU = alcohol and cannabis use initiators; K = $1,000. Bonferroni corrected pairwise comparisons: [a]ALC+CU > CON; [b]ALC > CON; [c]ALC+CU > ALC. Other drug use was defined as any substance use excluding alcohol, cannabis, and nicotine and tobacco products.
*$p < .05$.

image from the two cross-sectionally processed time points (baseline and follow-ups) using a consistent robust inverse registration method (Reuter et al., 2010). Each time point is then re-processed using the within-subject unbiased template (Reuter et al., 2012). A rater blind to participant characteristics visually inspected the data to correct any errors made during the cortical reconstruction process. All longitudinal runs were checked for quality, and no editing was necessary. Following inspection, an automated parcellation procedure divided each hemisphere into 34 standard-gyral based neuroanatomical regions using the Desikan–Killiany atlas that is built into the FreeSurfer processing pipelines (Desikan et al., 2006). The surface area (mm^2) for each time point was estimated and exported for region of interest analyses. The symmetrized percent change (SPC) in surface area was calculated at each vertex. A smoothing Gaussian Kernel with a full-width half maximum of 15 mm was used for this calculation. This smoothing level was chosen to increase signal-to-noise ratio for the vertex-wise analysis as recommended in within-subject SPC unbiased longitudinal image analysis using FreeSurfer (Reuter et al., 2012) and research on adolescent brain development (e.g., Tamnes et al., 2017). The SPC provides a measure of the rate of surface area change with respect to the average surface area across time points. The estimated total intracranial volume (eTIV) was also computed and included as an a priori covariate in accordance with previous studies and standardized approaches to account for global measures of brain size (Barnes et al., 2010; Jacobus et al., 2016; Mills et al., 2016).

Data analyses

Group comparisons with regard to demographic characteristics and substance use variables were examined using chi-square tests and analyses of variance. Two sets of longitudinal analyses were conducted to compare changes in surface area pre- and post-substance use initiation between groups.

Region of interest analysis of surface area. To expand on the work by Jacobus et al. (2016), we examined changes in surface area using the same methodological approach previously outlined. For each a priori cortical region (34 per hemisphere), a repeated-measures analysis of covariance was conducted with the average surface area as the dependent variable, time as the within-subject factor, and group as the between-subject factor. Nuisance covariates included eTIV and age, as both have been shown to correlate with surface area (Barnes et al., 2010). These covariates were centered on the grand mean to improve interpretability of our findings and more accurately estimate the impact of substance use on brain development while controlling for sample heterogeneity confounding factors. The main effect of group, time, and their interaction were evaluated, and alpha was set at .05. Significant interaction and group effects were followed up with pairwise comparisons using Bonferroni correction ($\alpha = .05/3 = .017$). Analyses were performed using SPSS.

Vertex-wise analysis of symmetrized percent change in surface area. Secondary more conservative analyses to assess group differences in the SPC in surface area over time

Table 2. Surface area values and post hoc comparisons for all significant between-group differences identified

	Group surface area in mm^2 *M* (*SE*)			Comparison *p* value		
Variable	CON (*n* = 23)	ALC (*n* = 23)	ALC+CU (*n* = 23)	CON > ALC	CON > ALC+CU	ALC+CU > ALC
L superior frontal gyrus	7,942.35 (106.76)	7,710.79 (102.36)	7,514.00 (104.75)	N.S.	.022†	N.S.
L superior parietal gyrus	5,825.62 (103.20)	5,381.98 (98.52)	5,827.77 (101.26)	.009*	N.S.	.007*
R superior parietal gyrus	5,774.39 (94.00)	5,542.12 (90.12)	5,946.78 (92.23)	N.S.	N.S.	.007*
L entorhinal cortex	418.69 (16.70)	382.13 (16.01)	445.14 (16.39)	N.S.	N.S.	.022†
R entorhinal cortex	318.05 (16.87)	300.73 (16.18)	370.15 (16.56)	N.S.	N.S.	.011*
R inferior temporal gyrus	3548.11 (59.75)	3,345.28 (57.29)	3,387.57 (68.62)	.055†	N.S.	N.S.
L fusiform	3,666.12 (75.51)	3,420.84 (69.53)	3,655.04 (71.15)	.056†	N.S.	.068†

Notes: Values are adjusted for baseline total intracranial volume and age (mean centered). *P* values surviving multiple comparisons (Bonferroni corrected $p < .017$) are highlighted by *. †indicates pairwise comparison was only marginally significant, $p < .1$; L = left; R = right; N.S. = not significant.

were conducted for each hemisphere using the two-stage longitudinal general linear model within FreeSurfer (Reuter et al., 2012). Results were corrected for multiple comparisons using a Monte Carlo permutation (10,000 iterations) with a cluster-based threshold of $p < .05$. The use of SPC minimizes the impact of individual variation and thus eTIV and age were not included as covariates (Reuter et al., 2012). Significant group effects were followed up with pairwise comparisons using Bonferroni correction ($\alpha = .05 / 3 = .017$). Exploratory Pearson's correlations were conducted to assess whether percent change in surface area was associated with substance use estimates (i.e., days of cannabis/alcohol use between baseline and follow-up, number of binge drinking episodes between baseline and follow-up, and age at initiation of alcohol and cannabis use) among the substance users only (i.e., ALC+CU and ALC, $n = 46$). Correlations between cannabis use variables were examined within the ALC+CU group only.

Results

Demographics

Demographic information is summarized in Table 1. Group differed in age at baseline and follow-up (ps < .05), such that the ALC+CU group was slightly older (5 months at baseline and 9 months at follow-up) than the CON. Groups were also found to differ on substance use variables at follow-up as expected (ps < .05). No other significant demographic differences were observed.

Region of interest analysis of surface area

Main effect of time. Across groups, we found a significant main effect of time (baseline > follow-up) on surface area across most regions (ps < .05), except for the right entorhinal cortex ($p = .706$), left entorhinal cortex ($p = .106$), and left temporal pole ($p = .08$).

Main effect of group. Regions showing a significant main effect of group on surface area and corresponding pairwise comparisons are presented in Table 2.

Within the frontal lobe, a significant main effect of group on surface area was found in the left superior frontal gyrus, $F(2, 64) = 3.85$, $p = .026$. Post hoc pairwise comparisons revealed marginally significant greater surface area for the CON group compared with the ALC+CU group ($p = .022$).

Within the parietal lobe, a main effect of group was also found in the left, $F(2, 64) = 6.72$, $p = .002$, and right, $F(2, 64) = 5.06$, $p = .009$, superior parietal gyrus. Specifically, CON and ALC+CU had greater left superior parietal surface area compared with the ALC group (ps < .010), whereas ALC+CU demonstrated greater surface area than ALC ($p = .007$) in the right hemisphere in this cortical region.

Within the temporal lobes, a significant main effect of group was found in the left entorhinal cortex, $F(2, 64) = 3.91$, $p = .025$. Post hoc comparisons showed greater surface area in the ALC+CU group compared with the ALC group that was only marginally significant after Bonferroni correction ($p = .022$). The same pattern was found in the right entorhinal gyrus, $F(2, 64) = 4.82$, $p = .011$ (ALC+CU > ALC, $p = .011$). A main effect of group was found in the right inferior temporal gyrus, $F(2, 64) = 3.14$, $p = .05$, such that across time points the CON group displayed marginally significant greater surface area than the ALC group ($p = .055$). A significant main effect of group was found in the left fusiform, $F(2, 64) = 3.95$, $p = .024$, whereby marginally significant greater surface was observed in the CON group compared with the ALC group ($p = .056$). A similar pattern was observed between the ALC+CU and ALC group for this region (CON > ALC, ALC+CU > ALC; $p = .062$).

Group × Time interaction. Regions showing a significant group-by-time interaction are presented in Figure 1 and Table 3. A significant group-by-time interaction effect was found in the left medial orbitofrontal cortex, $F(2, 64) = 5.06$, $p = .009$ (Figure 1a). A significant decrease in surface area between baseline and follow-up for the ALC ($p < .001$) and ALC+CU groups ($p < .001$) was identified. In contrast, youths in the CON group showed no significant difference in surface area between baseline and follow-up scans ($p =$

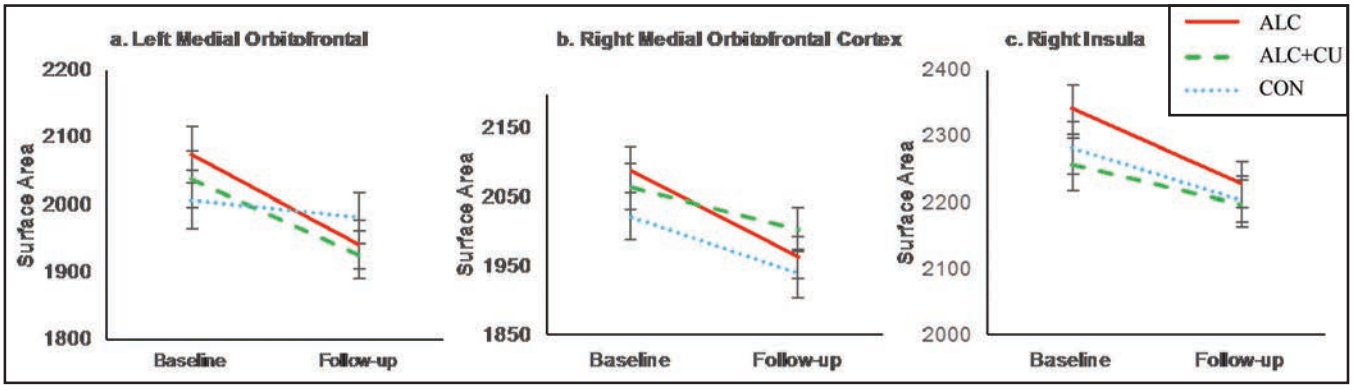

FIGURE 1. Regions showing a significant group-by-time interaction in surface area controlling for baseline estimated total intracranial volume and age

.287). A significant group-by-time interaction effect was also found in the right medial orbitofrontal cortex, $F(2, 64) = 4.15$, $p = .02$ (Figure 1b). Post hoc pairwise comparisons revealed a significant decrease in surface area over time for all groups (ps < .001). Inspection of the mean difference surface area within each showed that the ALC group had the greatest decrease ($\Delta M = 125.66$, $SE = 16.013$) in comparison to ALC+CU ($\Delta M = 61.68$, $SE = 16.39$) and CON ($\Delta M = 83.22$, $SE = 16.70$). No significant group differences in surface were observed at either baseline or follow-up. A significant group-by-time interaction for the right insula, $F(2, 64) = 3.82$, $p = .027$ (Figure 1c), showed a similar pattern; although all groups showed a significant decrease in surface area from baseline to follow-up (ps < .001), a more substantial decrease was found in the ALC group ($\Delta M = 113.40$, $SE = 13.88$) in contrast to the ALC+CU ($\Delta M = 60.37$, $SE = 14.20$) and CON groups ($\Delta M = 77.66$, $SE = 14.47$), and no significant differences between groups were observed at either time point.

Symmetrized percent change in surface area. Results from the secondary vertex-wise analysis revealed group differences in the SPC in surface area in the right medial orbitofrontal cortex ($p < .001$), after correction for multiple comparisons. Post hoc pairwise comparisons revealed greater reduction in surface area between baseline and follow-up scans (after 6–8 years) in the ALC group compared with the ALC+CU initiation group only ($p = .011$). No differences were observed between the substance use groups and CON.

Exploratory SPC correlational analyses. No significant bivariate correlations were found between substance use measures (number of cannabis/alcohol use days between baseline and follow-up, number of binge drinking episodes between baseline and follow-up, and age at cannabis and alcohol initiation) and percent change in surface area in the right medial orbitofrontal cortex.

Discussion

The results of this study expanded on our previous work (Jacobus et al., 2106) and revealed significant group differences in frontal, parietal, and temporal lobe surface area pre- and post-alcohol and cannabis use initiation in a longitudinal sample of adolescents. The control (CON) and alcohol + cannabis (ALC+CU) groups tended to display greater surface area at both time points as compared with adolescents who primarily only used alcohol (ALC). Furthermore, although all groups showed a decrease in surface area between baseline and follow-up time points in the bilateral medial orbitofrontal cortex and right insula, adolescents in the ALC group generally showed a more substantial decrease in comparison to the CON and ALC+CU groups. A greater decrease in right medial orbitofrontal cortex surface area in

TABLE 3. Surface area values and post hoc comparisons (baseline > follow-up) for all significant Group × Time interaction effects

	Group surface area in mm^2 *M* (*SE*)								
	CON (*n* = 23)		ALC (*n* = 23)		ALC+CU (*n* = 23)		Comparison *p* value Baseline > follow-up		
Variable	Baseline	Follow-up	Baseline	Follow-up	Baseline	Follow-up	CON	ALC	ALC+CU
L medial orbitofrontal cortex	2,006.54 (43.33)	1,979.97 (36.70)	2,073.56 (41.55)	1,940.26 (35.16)	2,037.02 (42.52)	1,926.34 (36.01)	<.001	<.001	N.S.
R medial orbitofrontal cortex	2,022.57 (34.08)	1,939.35 (35.52)	2,089.47 (32.68)	1,963.82 (30.22)	2,065.26 (33.44)	2,003.58 (30.92)	<.001	<.001	<.001
R insula	2,282.59 (39.98)	2,204.93 (35.03)	2,340.82 (38.33)	2,227.43 (33.59)	2,257.85 (39.23)	2,197.48 (34.37)	<.001	<.001	< .001

Notes: Values are adjusted for baseline total intracranial volume and age (mean centered). L = left; R = right; N.S. = not significant.

the ALC versus ALC+CU groups was also observed in the more conservative vertex-wise analysis of SPC, suggesting a reliable and strong substance-related effect in this area.

The medial orbitofrontal cortex is part of the stress and reward system (Montague & Berns, 2002) and is relevant to substance use disorders (Dom et al., 2005). Damage to the orbitofrontal cortex has been associated with greater impulsivity and risk-taking behavior, and impaired goal-directed behavior (Crews & Boettiger, 2009; Volkow & Fowler, 2000). Decreased right medial orbitofrontal cortex volumes have been linked with cannabis-related problems and cannabis initiation (Cheetham et al., 2012; Churchwell et al., 2010), suggesting that orbitofrontal volume may serve as a biomarker for future cannabis use. However, our findings link changes in surface area with alcohol initiation, and not co-occurring cannabis and alcohol use, in young adulthood. We did not see associations between the SPC in surface area in the right medial orbitofrontal cortex and age at initiation of cannabis use. Marginal reductions in orbitofrontal cortex surface area and gyrification among adolescent cannabis users has been previously reported, but after correction for multiple comparisons only gyrification differences remained significant (Shollenbarger et al., 2015). The authors concluded that frequent cannabis use (defined as >25 past-year uses and >50 lifetime joints) may influence cortical folding in the prefrontal cortex to a greater extent than surface area in regions with later surface area development. Thus, it is possible that any unique effects of cannabis and alcohol use on structural brain changes may not be best captured by the surface area measurement.

Our findings of greater reduction in the right insula surface area in the ALC group compared with adolescents in the CON group are in line with previous research that has linked heavy alcohol use (defined as adolescents with a drinking history of at least 10 years or adults meeting criteria for lifetime alcohol dependence) with decreased insular volume and cortical thickness (Heikkinen et al., 2017; Momenan et al., 2012), and general evidence implicating the insula in addiction (for a review, see Goldstein et al., 2009). Comparable to our findings for the medial orbitofrontal cortex, similar trajectories were observed between the right insula surface area for the ALC+CU and CON groups; however, this region did not survive the more conservative statistical approach.

In contrast to research examining the effects of alcohol on adolescent brain development, the neurological effects of cannabis are not as well characterized (Camchong et al., 2017; Filbey et al., 2015; Jacobus et al., 2009, 2013; McQueeny et al., 2011; Orr et al., 2016). Previous studies from our group found cannabis use moderated regional alterations in white matter integrity among binge drinking adolescents in comparison to controls, where the alterations were less pronounced in the cannabis co-users (Jacobus et al., 2009). It has been hypothesized that peripheral inflammation, specifically proinflammatory cytokines in the blood, might in part mediate alcohol-related brain alterations associated with adolescent heavy drinking (Ward et al., 2014). Importantly, studies have suggested that cannabidiol, a nonpsychoactive compound of marijuana, appears to ameliorate some of the negative effects of alcohol use on brain inflammation (Hamelink et al., 2005; Karoly et al., 2018; Liput et al., 2013). Co-use of alcohol and cannabis (compared to single-substance use) may be linked to differential outcomes on neural health and may be one explanation for group differences between ALC and ALC+CU users observed in our study, although more research is needed to better disentangle the effects of co-use on brain development. Given the widely reported sex differences in brain development, it is possible that the impact of cannabis use may differentially affect males and females (Ketcherside et al., 2016). However, interactions between group and sex were not possible to examine in this study given our relatively small sample size and thus limited power to detect additional group effects. Sex and drug use interactions across neurodevelopment is a topic that warrants further research in future studies.

The extent to which interference with the endocannabinoid system during neurodevelopmental results in a deleterious effect on the brain remains unclear, as different imaging estimates and behavioral outcomes are likely influenced by neurobiological interactions between cannabis and other substances (e.g., alcohol) that may vary as a function of age and phase of development (Hammond et al., 2014). However, there is evidence to support the negative sequelae of alcohol and cannabis co-use relative to alcohol use only (e.g., Subbaraman & Kerr, 2015) and thus further research is warranted. In addition, the findings of greater surface area averaged across time in the CON and ALC+CU groups compared to the ALC group may suggest potential pre-existing group differences in the development of surface area that were not accounted for in this study. Future studies with larger sample sizes will need to examine cortical morphology at earlier developmental stages to identify critical periods when differences emerge and factors that may contribute to these differences.

Of note, Jacobus et al. (2016) found 18 regions in which non-users and alcohol initiators showed a more substantial decrease (i.e., thinning) of the cerebral cortex compared to those who initiated alcohol and cannabis. The discrepancies between these findings and the present results are not surprising given that cortical thickness and surface area are genetically and phenotypically independent of each other (Panizzon et al., 2009; Winkler et al., 2010) and follow distinct developmental trajectories by which the onset and timing can vary across the brain (Raznahan et al., 2011; Tamnes et al., 2017; Wierenga et al., 2014).

Strengths of the current study include the unique sample of adolescents assessed both pre- and post-substance use initiation and the focus on co-occurring alcohol and cannabis use. The use of surface area analysis also provides a more

sensitive assessment of potential cerebral cortex abnormalities as compared to volumetric-based measures. Limitations of the study include the relatively smaller sample; however, this is somewhat mitigated by the within-subject design. The less conservative approach to multiple comparison correction in the region of interest analysis, although consistent with previous studies examining cortical thickness and using the same sample (Jacobus et al., 2016), may increase the likelihood of between-group findings; therefore, replication is important given the large number of analyses conducted and the modest effect sizes. Additional time point assessments would also enable modelling of individual growth trajectories. Modest age differences were found between our groups, and it is possible that age-related neurodevelopmental differences contributed to findings. However, average age differences only ranged from 5 to 9 months, and therefore any differential effects of age on surface area between the groups is likely small. Moreover, our assessment of substance use was based on self-report, which may introduce measurement error. Amount of cannabis consumed each day during each episode was not collected in this sample and is a limitation of this study. Quantifying cannabis use is challenging given the number of ways in which it is consumed and variation in potency; therefore, assessment methods are difficult to standardize yet are being improved on in several new prospective studies in our laboratory and others. The lack of inclusion of a cannabis-only using group can be viewed as a shortcoming; however, inclusion of participants in this group would be challenging given the high rates of alcohol co-use among cannabis users. Another limitation was the use of other drugs by participants in the ALC and ALC+CU groups. Although the contribution of other drugs could certainly affect brain development, there were no consistent patterns among the drugs endorsed by participants, which made it difficult to examine the impact of specific drug effects on brain development.

The present study adds to the growing body of research examining the impact of alcohol and cannabis use on adolescent brain surface area development using a prospective design. Our findings suggest that surface area in the medial orbitofrontal cortex may be a useful intermediate phenotype for exploring the mechanisms underlying the effects of substance use on brain development.

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