



Adolescent drinking and brain morphometry: A co-twin control analysis



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ABSTRACT

Developmental changes in structure and functioning are thought to make the adolescent brain particularly sensitive to the negative effects of alcohol. Although alcohol use disorders are relatively rare in adolescence, the initiation of alcohol use, including problematic use, becomes increasingly prevalent during this period. The present study examined associations between normative drinking (alcohol initiation, binge drinking, intoxication) and brain morphometry in a sample of 96 adolescent monozygotic twins. *A priori* regions of interest included 11 subcortical and 20 cortical structures implicated in the existing empirical literature as associated with normative alcohol use in adolescence. In addition, co-twin control analyses were used to disentangle risk for alcohol use from consequences of alcohol exposure on the developing brain. Results indicated significant associations reflecting preexisting vulnerability toward problematic alcohol use, including reduced volume of the amygdala, increased volume of the cerebellum, and reduced cortical volume and thickness in several frontal and temporal regions, including the superior and middle frontal gyri, pars triangularis, and middle and inferior temporal gyri. Results also indicated some associations consistent with a neurotoxic effect of alcohol exposure, including reduced volume of the ventral diencephalon and the middle temporal gyrus.

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1. Introduction

It is commonly thought that the substantial and protracted neurological changes that occur during late childhood, adolescence, and into early adulthood make this a period of heightened vulnerability to the negative effects of alcohol on brain structural and functional development. Developmental changes in gray and white matter reflect increasingly efficient and economical neural activity—white matter volume shows generally linear increases that reflect greater fiber organization and coherence, while gray matter volume shows nonlinear increases then decreases that are thought to reflect the selective synaptic pruning of superfluous neuronal connections, reduction in glial cells, and decreases in myelination (see Bava and Tapert, 2010; Crews et al., 2007; Giedd and Rapoport, 2010). A growing body of literature suggests that these structural and functional changes may make the adolescent brain particularly sensitive to the neurotoxic effects of alcohol. Experimental animal studies (see Spear, 2014), as well as

studies of human adolescents (see Ewing et al., 2014), highlight the deleterious implications of alcohol consumption during this period.

Although alcohol use disorders are relatively rare during adolescence, the initiation of alcohol use and problematic consumption becomes increasingly prevalent in adolescence. By 12th grade, 68% of adolescents report using alcohol, 52% have been drunk, and 22% report binge alcohol use (i.e., 5 or more drinks on the same occasion) in the past 2 weeks (Johnston et al., 2014). Notably, animal research indicates that chronic intermittent alcohol exposure, an analog to binge drinking in humans, has particularly deleterious effects on the adolescent brain (see Crabbe et al., 2011). Thus, there is a need to determine the extent to which normative patterns of alcohol use during adolescence (alcohol initiation, binge drinking, intoxication) affect the developing brain.

A handful of studies has examined gray matter development as a function of subclinical alcohol use in community samples of adolescents (see Ewing et al., 2014; Wilson et al., 2015; see Table 1 for an overview). A series of reports from one research team has examined cross-sectional associations between adolescent alcohol use and brain morphometry. Lisdahl et al. (2013) found that the peak number of drinks during a recent binge was associated with reduced volume of cerebellar gray matter, though there were

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Table 1
Overview of MRI studies of normative alcohol use and brain morphometry in adolescent samples.

Study	Sample	Age range	Sex	Alcohol use	MRI method	Study design	Regions examined	p value	Effect size (<i>d</i>)	
Benegal et al. (2007)	20 HR 21 LR	9–23	0%	Alcohol-naïve, high familial risk	ROI (8)	High-risk family	L prefrontal cortex	1.000	0.00	
							R prefrontal cortex	.579	–0.18	
								L hippocampus ^b	<.001	–1.18
								R hippocampus ^b	<.001	–1.37
								L amygdala^a	.021	–0.75
								R amygdala^a	.003	–0.98
								L caudate	.373	–0.28
								R caudate	.333	–0.31
								L superior frontal gyrus^a	<.001 ^c	–2.11
								R superior frontal gyrus^a	<.001 ^c	–0.96
								L cingulate gyrus^a	<.001 ^c	–1.21
								R cingulate gyrus^a	<.001 ^c	–1.13
								L parahippocampal gyrus^a	<.001 ^c	–1.51
								R parahippocampal gyrus^a	<.001 ^c	–1.21
							L amygdala^a	<.001 ^c	–1.23	
							R amygdala^a	<.001 ^c	–1.20	
							L thalamus^a	<.001 ^c	–1.07	
							R thalamus^a	<.001 ^c	–1.12	
							R cerebellum^a	<.001 ^c	–0.98	
Cheetham et al. (2014)	38 Alc 60 Comp	11–14	52%	Alcohol-related problems	ROI (10)	Longitudinal (4 years) MRI → alcohol	L orbitofrontal cortex	.304	–0.21	
							R orbitofrontal cortex	.204	–0.27	
								L paralimbic anterior cingulate cortex^a	.023	–0.48
								R paralimbic anterior cingulate cortex	.739	–0.07
								L limbic anterior cingulate cortex	.102	0.34
								R limbic anterior cingulate cortex	.828	0.05
								L hippocampus	.294	0.22
								R hippocampus	.601	0.11
								L amygdala	.577	–0.12
								R amygdala	.610	–0.11
Hanson et al. (2010)	15 HR 15 LR	12–14	33%	Alcohol-naïve, high familial risk	ROI (2)	High-risk family	L hippocampus ^d	.237	0.22	
							R hippocampus ^d	1.000	0.00	
Hill et al. (2001)	17 HR 17 LR	15–25	0%	High familial risk	ROI (4)	High-risk family	L hippocampus ^d	.419	–0.28	
							R hippocampus ^d	.727	0.12	
							L amygdala^a	.904	0.04	
							R amygdala^a	.031	0.77	
Hill et al. (2007)	17 HR 16 LR	15–25	0%	High familial risk	ROI (4)	High-risk family	L cerebellum^a	.003	1.15	
							R cerebellum^a	.002	1.21	
Lisdahl et al. (2013)	46 Alc 60 Comp 106 Total	16–19	48%	Binge drinking Max drinks	ROI (4)	Cross-sectional	L cerebellum	.806	–0.05	
							R cerebellum	.204	–0.25	
							L cerebellum^a	.006	–0.58	
							R cerebellum^a	.008	–0.58	
Luciana et al. (2013)	30 Alc 25 Comp	14–19	45%	Alcohol use	Whole-brain SBM	Longitudinal (2 years) Alcohol → MRI	R middle frontal gyrus^a	.006 ^c	–0.97	
Malone et al. (2014)	93 Total	14–18	50%	Alcohol use	ROI (6)	Co-twin control	L lateral orbitofrontal cortex ^b	.011	0.55	
							R lateral orbitofrontal cortex	.134	0.32	
							L medial orbitofrontal cortex	.079	0.37	
							R medial orbitofrontal cortex	.340	0.20	
							L hippocampus	.549	0.12	
							R hippocampus	.909	0.02	
Medina et al. (2007)	16 Alc 21 Comp	15–18	32%	Alcohol use	ROI (2)	Cross-sectional	L hippocampus	.415	–0.27	
							R hippocampus	.387	0.29	
Squeglia et al. (2012)	29 Alc 30 Comp	16–19	49% ^d	Binge drinking	ROI (13)	Cross-sectional	L frontal pole ^d	.489	0.18	
							L pars orbitalis ^d	.827	–0.06	
							L medial orbital frontal gyrus ^d	.295	–0.28	
							L rostral anterior cingulate cortex ^d	.179	–0.35	

Table 1 (Continued)

Study	Sample	Age range	Sex	Alcohol use	MRI method	Study design	Regions examined	p value	Effect size (<i>d</i>)
Squeglia et al. (2014)	20 Alc 20 Comp	12–17	38%	Heavy drinking	ROI (98)	Longitudinal (3 years)	R pars triangularis^a	.024	–0.75
							R rostral anterior cingulate cortex^a	.008	–0.89
				MRI → Alcohol	R caudal anterior cingulate cortex^a	.028	–0.72		
					L isthmus cingulate^a	.028	–0.72		
				ROI (98)	Alcohol → MRI	L middle temporal gyrus^a	.031	–0.68	
					L inferior temporal gyrus^a	.012	–0.84		
					L caudate^a	.026	–0.74		
				L ventral diencephalon^a	.011	–0.87			
					Brain stem^a	.030	–0.72		

Notes: Sample includes the number of participants in the high-risk (HR) and low-risk (LR) groups, the drinking (Alc) and comparison (Comp) groups, or the total sample (Total). Age range is at baseline for longitudinal studies. Sex is percentage female. Alcohol use includes high familial risk, or alcohol use, binge drinking, or alcohol-related problems. Magnetic resonance imaging (MRI) method includes region-of-interest (ROI) analysis (with the number of ROIs examined presented in parentheses) and whole-brain voxel-based morphometry (VBM) or surface-based morphometry (SBM). Study design includes high-risk family, cross-sectional, and longitudinal study designs; for longitudinal studies, the length of follow-up is presented in parentheses and the timing of assessment of MRI and alcohol use is indicated by →. Regions examined includes subcortical and cortical regions. Effect sizes are indexed by Cohen's *ds*, with negative effect sizes indicating reduced volume of subcortical and cortical regions or reduced cortical thickness for the high-risk or drinking groups relative to the comparison groups (by convention, 0.20 indicates a small effect size, 0.40 indicates a moderate effect size, and 0.80 indicates a large effect size); effect sizes and *p* values were provided in the study or were calculated using given statistics.

^a Region examined as an ROI in the present report based on the results of this study, also noted in bold.

^b ROI previously examined in the present sample (Malone et al., 2014) and, thus, not examined in the present report.

^c *p* value corrected for multiple comparisons.

^d Significant alcohol group x sex interactions, *p* < .05, reported.

no group differences in cerebellar volumes between binge drinking versus nondrinking adolescents. Medina et al. (2007) found no differences between alcohol-using versus nondrinking adolescents in hippocampal volumes. Squeglia et al. (2012) also found no differences between binge drinking adolescents versus nondrinking adolescents in cortical thickness of any frontal regions examined, though they did report interactions between binge drinking and participant sex in 4 frontal regions.

Study designs that offer greater causal inference than cross-sectional studies include longitudinal studies that prospectively assess alcohol use and brain morphometry over time and high-risk family studies that compare adolescents at high and low familial risk for problematic alcohol use (see Table 1). Longitudinal studies provide evidence of brain deviations that precede alcohol initiation, as well as effects of alcohol exposure on the developing brain. Cheetham et al. (2014) examined brain volume in a sample of substance-naïve preadolescents and found that reduced volume of the left paralimbic anterior cingulate cortex predicted alcohol-related problems, suggesting this deviation may reflect premorbid risk for problematic alcohol use. Squeglia et al. (2014) examined brain volume in a sample of minimally substance-using adolescents and found reduced volume at baseline in the rostral and caudal anterior cingulate cortex, isthmus cingulate, and pars triangularis, consistent with preexisting risk; moreover, initiation of heavy alcohol use predicted reduced volume in the ventral diencephalon, middle and inferior temporal gyrus, caudate, and brain stem, none of which showed group differences at baseline, consistent with an exposure effect of alcohol on cortical development. Luciana et al. (2013) examined cortical thickness using whole-brain surface-based morphometry in a sample of adolescents who were substance-naïve at baseline and found that initiating alcohol use predicted reduced cortical thickness in the middle frontal gyrus; no group differences were found at baseline in this region.

High-risk family studies provide evidence of premorbid brain deviations. Benegal et al. (2007) examined brain volume using whole-brain voxel-based morphometry in a sample of adolescents at high and low familial risk for problematic alcohol use and found the high-risk group had reduced volume in the superior frontal gyrus, parahippocampal gyrus, amygdala, cingulate gyrus, thalamus, and cerebellum, suggesting preexisting vulnerability for

problematic alcohol use. Hill et al. (2001) also found reduced volume of the left amygdala in a sample of high-risk adolescents and young adults, consistent with preexisting vulnerability, but found no group differences in hippocampal volume. Moreover, and in contrast to Benegal et al. (2007) and Lisdahl et al. (2013), Hill et al. (2007) found increased cerebellar volume in the high-risk group. Hanson et al. (2010) found no group differences in hippocampal volumes, though they did report interactions between familial risk and right/left hippocampal asymmetry. In an innovative extension of the high-risk family study design, the co-twin control study design, Malone et al. (2014) examined brain volume in a sample of adolescent twin pairs that varied in their levels of alcohol use and found that drinking was associated with reduced volume in the left lateral orbitofrontal cortex; a co-twin difference analysis indicated this likely reflected a preexisting vulnerability toward alcohol use.

An integral component of the scientific method is the confirmation of effects in independent samples by independent research teams. Although most of the studies reviewed here took steps to avoid Type I error, including selecting a priori regions of interest and correcting for multiple comparisons, the many regions typically examined, the relatively small sample sizes, and the relatively few significant effects warrant further research to help clarify inconsistencies and verify positive findings. Examination of the studies in Table 1 reveals remarkably little overlap in the regions examined and, with some notable exceptions, few positive findings reported by more than one study. Determining the effects of normative alcohol use on the developing adolescent brain requires the independent confirmation of published findings using a study design that maximizes causal inference in a sample size with sufficient power to detect previously reported effect sizes.

We attempted to confirm each of the previously reported positive findings of deviations in volume of subcortical and cortical regions, as well as cortical thickness, in a community sample of 96 adolescent monozygotic (MZ) twins who underwent a comprehensive neuroimaging assessment. As reviewed above, in a previous report on the present sample of adolescent twins we examined motivated decision making in relation to adolescent drinking with a secondary focus on brain volume in regions selected a priori as being relevant to performance on the decision-making task used: the orbitofrontal cortex and the hippocampus. We found reduced

volume of the orbitofrontal cortex in alcohol-using adolescents, but nonsignificant effects for hippocampal volume (Malone et al., 2014). In the present report, we expanded upon Malone et al. (2014) by examining all regions previously found to be associated with adolescent alcohol use, selecting a priori regions of interest each of the subcortical and cortical structures identified in the existing empirical literature as showing deviations among alcohol-using adolescents (see Table 1 for an overview). Whereas Malone et al. (2014) used a cross-sectional study design, the present report used longitudinal data from two study waves to maximize power.

In addition to examining associations between drinking and brain morphometry, we applied the MZ co-twin control study design to help disentangle preexisting risk for problematic alcohol use from alcohol exposure effects. Adolescents who initiate alcohol use early and who misuse alcohol differ from those who do not in a variety of ways. The co-twin control design controls for factors confounded with alcohol exposure and the outcome of interest that twins share, and, thus, provides a more appropriate measure of exposure effects than is possible with singletons. Specifically, co-twin control analyses decompose an individual's level of exposure into two orthogonal components: that which is shared with the co-twin, and that which differs from the co-twin (or, strictly speaking, the deviation of each individual twin from the respective twin mean, which is equivalent to the twin difference). The first, between-twin pair component, captures the mean exposure effect, which is fully confounded with all shared factors that predispose toward alcohol use in the first place. It is exactly analogous to what is measured in studies of singletons. The second, within-twin pair component, represents effects of alcohol exposure unconfounded by these shared influences. Significant within-twin pair effects, thus, permit stronger inferences about the causal influence of alcohol exposure on the observed outcome. Significant between-twin pair effects, particularly in the absence of within-twin pair effects, suggest that preexisting vulnerabilities shared by twins accounts for the observed outcome. Notably, the co-twin control approach accounts for *all* genetic and shared environmental confounders, both measured and unmeasured.

2. Methods

2.1. Participants and procedures

The sample consisted of 96 MZ twin participants in the AdBrain study, one of several population-based studies at the Minnesota Center for Twin and Family Research (MCTFR). Participant recruitment, inclusion/exclusion criteria, and twin and parental informed assent and consent procedures are described in detail in Malone et al. (2014) and are only briefly reviewed here. Adolescents completed a comprehensive, multimodal assessment that included interview, questionnaire, laboratory, and neuroimaging assessments at baseline (Wave 1) and at a one-year follow-up (Wave 2); twins ranged in age from 13.97 to 16.82 years at the baseline assessment, and 50% were female. Mothers also completed assessments at both waves. (See Table 2 for an overview of sample characteristics.)

2.2. Measures

2.2.1. Alcohol and other substance use

Alcohol and other substance use and abuse was assessed at Waves 1 and 2 using a version of the Substance Abuse Module (SAM; Robins et al., 1987) of the Composite International Diagnostic Interview (CIDI; Robins et al., 1988). The MCTFR-expanded version of the SAM assesses drinking frequency, quantity, misuse, and density of alcohol and other substance use. We computed an

Table 2
Sample characteristics.

	Wave 1	Wave 2
Age	15.50 (0.9)	16.40 (0.9)
Ever used alcohol	20(21%)	33(34%)
Drink index	1.22 (2.89)	1.95 (3.44)
Binge drinking	8(8%)	10(10%)
Number of times intoxicated	4.46 (31.99)	5.52 (32.99)
Maximum number of drinks in 24 h	0.86 (2.91)	1.46 (3.54)
Ever used nicotine	14(15%)	25(26%)
Ever used marijuana	9(9%)	14(15%)
Ever used other drugs	2(2%)	5(5%)

Note: $N = 96$ at Wave 1 and Wave 2. Mean (SD) or n (%).

alcohol index by summing responses on four items assessing alcohol use in the preceding 12 months (frequency of drinking, number of drinks typically consumed per occasion, maximum number of drinks consumed in a single 24-hour period, and the number of times intoxicated); because responses to each question were skewed and sparse, they were transformed into ordinal measures (with five to six categories per item) prior to summing. Comprehensive data on the validity of the alcohol index are provided in McGue et al. (2014). Possible scores on the alcohol index ranged from 0 to a maximum of 23. Internal consistency of the alcohol index was good at both waves (Cronbach's alphas were .91 and .95 at Waves 1 and 2, respectively); intraclass correlation coefficients (ICCs), corrected for age and sex, indicated a moderate level of twin similarity at both waves (ICCs were .76 and .54 at Waves 1 and 2, respectively), indicating an increase in twin differences, which accompanied an increase in the initiation of alcohol use. There were no significant sex differences in any of the alcohol or other substance use variables at either Wave 1 or 2 (all $ps > .050$). (See Table 2 for descriptive statistics for alcohol and other substance use at Waves 1 and 2.)

2.3. Neuroimaging

2.3.1. Image acquisition

Imaging data were collected on the same Siemens 3T Tim Trio scanner (Siemens Medical Systems, Erlangen, Germany) at both Wave 1 and Wave 2 using a 12-channel array head coil at the University of Minnesota's Center for Magnetic Resonance Research (CMRR). Three-dimensional T1-weighted anatomical images were acquired in the sagittal plane using a magnetization prepared rapid gradient echo (MP-RAGE) sequence (TR = 2530 ms, TE = 3.65 ms, flip angle 7°, matrix size = 256 × 256 with a FOV of 256, 240 sagittal slices with 1-mm isomorphic voxels).

2.3.2. Image processing

All images were reviewed for quality prior to subsequent processing. Imaging data were available for 94 participants at Wave 1 and 94 participants at Wave 2 (data for one participant were lost due to equipment failure and excessive motion artifact was evident in one participant's scan at Wave 1; one mother declined to allow her twins to be scanned at Wave 2). Imaging data were processed using the standard FreeSurfer pipeline (Fischl et al., 2002, 2004; <http://surfer.nmr.mhg.harvard.edu>), which includes removal of non-brain tissue using a hybrid procedure combining watershed and surface deformation (Segonne et al., 2004), automated Talairach transformation; segmentation of subcortical structures (Fischl et al., 2002); intensity normalization (Sled et al., 1998); tessellation of the boundaries between gray matter and cerebrospinal fluid (CSF) on one side and white matter on the other (Dale et al., 1999; Dale and Sereno, 1993; Fischl and Dale, 2000); automated correction of topological errors; and deforming the gray and white matter and CSF and white matter, respectively. The

resulting surface cortical models are subsequently inflated (Fischl et al., 1999a), registered to a spherical atlas by means of a procedure that uses individual folding patterns in order to match cortical geometry across participants (Fischl et al., 1999b), and parcellated into regions of interest based on gyral and sulcal structure (Desikan et al., 2006; Fischl et al., 2004).

Each step of the pipeline was reviewed for accuracy, and modifications were made as necessary using FreeSurfer tools (e.g., correcting skull-stripping errors and dura matter included in the pial surface, preventing extension of the cerebellum into non-brain areas, correcting FreeSurfer's white matter volume in several scans to include areas incorrectly omitted). As described above, we selected several regions of interest based on positive findings in the existing empirical literature. These included volume of the amygdala, thalamus, caudate, cerebellum, ventral diencephalon, and brainstem, as well as volume and thickness of the superior and middle frontal gyrus, pars triangularis, anterior cingulate cortex and rostral and caudal anterior cingulate cortex, isthmus cingulate, middle and inferior temporal gyrus, and parahippocampal gyrus; we did not consider the two regions previously examined in the present sample (i.e., the hippocampus, orbitofrontal cortex; Malone et al., 2014). Although effects were sometimes reported for only one hemisphere in previous studies, we considered both left and right hemispheres in the present analyses.

Imaging data from Waves 1 and 2 were processed using the FreeSurfer longitudinal pipeline, which creates an unbiased template and image for a given participant using data from multiple time points (Reuter and Fischl, 2011) using a robust registration (Reuter et al., 2010). Subsequent image processing steps (skull stripping, Talairach transformation; atlas registration, spherical maps, and parcellations) use common information from this template. Using the longitudinal pipeline increases power to detect potentially subtle inter-individual differences by using additional information in the form of multiple scans to produce more robust and reliable measurements of subcortical and cortical structures (Reuter et al., 2012). We used FreeSurfer version 5.3, which is compatible with data processed initially with version 5.1 of the standard pipeline because it accommodates individuals with a single scan, minimizing bias due to attrition or problematic data.

2.4. Statistical analyses

2.4.1. Linear mixed models

We conducted a series of linear mixed models (LMMs) to examine associations between the alcohol index and volume in subcortical and cortical regions, as well as cortical thickness, in the selected regions of interest at the two waves. The alcohol index was treated as a time-varying covariate. LMM analyses were conducted in R version 3.1.2 (R Core Team, 2014) using lmer from the lme4 package (Bates et al., 2014). Random intercepts were included at both the individual twin level and the twin pair level to account for within-twin (repeated measures) and within-twin pair (resemblance between twins in a twin pair) correlations, respectively. All models included participant age and sex as covariates, and analyses of volume also included total brain volume (FreeSurfer's "BrainSeg-NotVent," the sum of the volume of all brain structures, including cerebellum).

2.4.2. Co-twin control analyses

We conducted co-twin control analyses for all significant associations between the alcohol index and volume in subcortical and cortical regions, and cortical thickness. These analyses decompose exposure effects into between-twin pair and within-twin pair effects. The between-twin pair effect is represented by the twin-pair mean. Although, by definition, this represents mean-level alcohol exposure for the twin pair, it is completely confounded

with the genetic and shared environmental vulnerability toward problematic alcohol use shared by MZ twins, and a significant between-pair effect is consistent with a preexisting vulnerability. The within-twin pair effect consists of each individual twin's deviation from the twin pair's mean-level exposure effect, so that a significant within-twin pair effect reflects effects of alcohol exposure unconfounded by shared genetic and environmental risk. Co-twin control analyses were also conducted using lmer, specifying random intercepts at the individual and twin pair levels to account for within-twin and within-twin pair correlations, respectively. We examined residuals and the distribution of random effects for outliers; there were no extremely outlying observations for any analysis, nor was any participant consistently an outlier.

3. Results

3.1. Volume of subcortical structures

Results of LMMs and co-twin control analyses examining associations between drinking and volume of subcortical structures and the cerebellum are presented in Table 3. Drinking was associated with reduced volume of the right amygdala, but with increased volume of the left cerebellum; co-twin control analyses indicated that the between-twin pair effect was significant for both regions, suggesting that deviations in amygdala and cerebellar volume primarily reflected preexisting vulnerability for problematic alcohol use. Drinking was also associated with reduced volume of the left ventral diencephalon, and follow-up co-twin control analyses indicated that the within-twin pair effect was significant, consistent with an exposure effect of alcohol on this region. There were no significant associations with volume of the other subcortical structures examined.

3.2. Cortical volume

Results of LMMs and co-twin control analyses examining associations between drinking and cortical volume are presented in Table 4. Drinking was associated with reduced volume of the right middle frontal gyrus, the right pars triangularis, the left and right middle temporal gyri, and the left and right inferior temporal gyri. Co-twin control analyses indicated that the between-twin pair effect was significant for all regions except the right inferior temporal gyrus, which approached significance ($p = .066$), suggesting that deviations in cortical volume in these regions primarily reflected preexisting vulnerability for problematic alcohol use. In addition, the within-twin pair effect was significant for the left middle temporal gyrus, consistent with an effect of alcohol exposure on this region.

3.3. Cortical thickness

Results of LMMs and co-twin control analyses examining associations between drinking and cortical thickness are presented in Table 5. Drinking was associated with reduced thickness of the right superior frontal gyrus, the right middle frontal gyrus, the right pars triangularis, and the left and right middle temporal gyri. Co-twin control analyses indicated that the between-twin pair effect was significant for all regions, consistent with preexisting vulnerability for problematic alcohol use.

4. Discussion

A growing body of evidence indicates that even normative alcohol use in adolescence is associated with brain deviations. However, although studies have considered several brain regions, few

Table 3
Associations between drinking and volume of subcortical structures.

Region	Co-twin control								
	Linear mixed models			Between-twin pair effect			Within-twin pair effect		
	Beta (SE)	p value	Effect size (d)	Beta (SE)	p value	Effect size (d)	Beta (SE)	p value	Effect size (d)
L amygdala	−0.003 (0.003)	.261	−0.20	–	–	–	–	–	–
R amygdala	−0.010 (0.003)	<.001	−0.61	−0.016 (0.004)	<.001	−0.76	−0.002 (0.004)	.642	−0.09
L thalamus	−0.001 (0.013)	.929	−0.02	–	–	–	–	–	–
R thalamus	−0.004 (0.011)	.724	−0.06	–	–	–	–	–	–
L caudate	0.003 (0.004)	.934	0.02	–	–	–	–	–	–
R caudate	<0.001 (0.005)	.979	0.01	–	–	–	–	–	–
L cerebellum	0.085 (0.040)	.035	0.42	0.098 (0.054)	.047	0.37	0.070 (0.059)	.235	0.24
R cerebellum	0.047 (0.040)	.235	0.23	–	–	–	–	–	–
L ventral diencephalon	−0.015 (0.007)	.021	−0.39	−0.007 (0.009)	.434	−0.14	−0.024 (0.010)	.012	−0.45
R ventral diencephalon	−0.006 (0.006)	.314	−0.18	–	–	–	–	–	–
Brainstem	−0.006 (0.015)	.691	−0.07	–	–	–	–	–	–

Notes: Results of linear mixed models (LMMs) and co-twin control analyses. LMMs examined associations between the drink index and volume of subcortical structures; models included random intercepts at the individual and twin-pair levels to account for within-individual and within-twin pair correlations, and all models included participant age and sex, and total brain volume, as covariates. Significant associations were followed up using co-twin control analyses that decomposed effects into between-twin pair (preexisting vulnerability shared by twins) and within-twin pair (effects of alcohol exposure). *p* values and effect sizes, indexed as Cohen's *d*s (0.20 indicates a small effect size, 0.40 indicates a moderate effect size, and 0.80 indicates a large effect size), were calculated using *t* statistics (*dfs* range from 103 to 143). Significant regions are noted in **bold**.

regions have been examined in multiple studies and there has been little confirmation of positive findings by independent research teams in independent samples. In the present report, we identified all regions previously found to be associated with normative alcohol use in adolescence and/or familial risk of alcohol problems and attempted to confirm positive findings in a sample of adolescent twins. We found evidence of deviations in volume of subcortical and cortical structures, as well as cortical thickness, in a number of regions. Moreover, our co-twin control analyses allowed us to determine whether deviations were due to between-twin pair effects, reflecting shared genetic and environmental influences that confer risk for alcohol use, or within-twin pair effects, reflecting differences in drinking within the twin pair and suggesting the deviation is accountable by alcohol exposure.

We examined volume of the amygdala, thalamus, caudate, cerebellum, ventral diencephalon, and the brainstem; we reported null results for the hippocampus in a previous examination in the present sample (Malone et al., 2014). Although we failed to confirm earlier positive findings for the thalamus, caudate, and brainstem, we did find significant associations between drinking and reduced volume of the right amygdala, increased volume of the left cerebellum, and reduced volume of the left ventral diencephalon. Reduced amygdala volume has been reported in adults with alcohol use disorders (Wrase et al., 2008), as well as adolescents and young adults at high familial risk for problematic alcohol use (Benegal et al., 2007; Hill et al., 2001). Our co-twin control analysis suggests that the reduced right amygdala volume observed in our sample reflects preexisting vulnerability toward problematic alcohol use, rather than an alcohol exposure effect.

Table 4
Associations between drinking and cortical volume.

Region	Co-twin control								
	Linear mixed models			Between-twin pair effect			Within-twin pair effect		
	Beta (SE)	p value	Effect size (d)	Beta (SE)	p value	Effect size (d)	Beta (SE)	p value	Effect size (d)
L superior frontal gyrus	−0.012 (0.020)	.556	−0.14	–	–	–	–	–	–
R superior frontal gyrus	−0.039 (0.027)	.149	−0.30	–	–	–	–	–	–
L middle frontal gyrus	−0.054 (0.031)	.083	−0.35	–	–	–	–	–	–
R middle frontal gyrus	−0.088 (0.038)	.022	−0.44	−0.151 (0.050)	.003	−0.58	−0.004 (0.057)	.950	−0.01
L pars triangularis	−0.005 (0.005)	.316	−0.23	–	–	–	–	–	–
R pars triangularis	−0.017 (0.006)	.006	−0.67	−0.024 (0.008)	.006	−0.69	−0.010 (0.009)	.289	−0.26
L anterior cingulate cortex	−0.009 (0.006)	.139	−0.30	–	–	–	–	–	–
R anterior cingulate cortex	−0.002 (0.006)	.804	−0.05	–	–	–	–	–	–
L rostral anterior cingulate cortex	−0.007 (0.005)	.161	−0.28	–	–	–	–	–	–
R rostral anterior cingulate cortex	−0.002 (0.005)	.670	−0.09	–	–	–	–	–	–
L caudal anterior cingulate cortex	−0.003 (0.003)	.330	−0.20	–	–	–	–	–	–
R caudal anterior cingulate cortex	−0.001 (0.003)	.817	−0.05	–	–	–	–	–	–
L isthmus cingulate	0.005 (0.003)	.140	0.30	–	–	–	–	–	–
R isthmus cingulate	0.001 (0.003)	.681	0.09	–	–	–	–	–	–
L middle temporal gyrus	−0.039 (0.011)	<.001	−0.75	−0.045 (0.014)	.002	−0.64	−0.032 (0.016)	.049	−0.42
R middle temporal gyrus	−0.042 (0.011)	<.001	−0.76	−0.053 (0.015)	<.001	−0.71	−0.029 (0.017)	.100	−0.35
L inferior temporal gyrus	−0.044 (0.017)	.012	−0.53	−0.059 (0.023)	.012	−0.52	−0.025 (0.026)	.329	−0.21
R inferior temporal gyrus	−0.029 (0.013)	.025	−0.49	−0.031 (0.017)	.066	−0.39	−0.026 (0.019)	.183	−0.30
L parahippocampal gyrus	−0.003 (0.005)	.510	−0.13	–	–	–	–	–	–
R parahippocampal gyrus	0.001 (0.004)	.809	−0.05	–	–	–	–	–	–

Notes: Results of linear mixed models (LMMs) and co-twin control analyses. LMMs examined associations between the drink index and cortical volume; models included random intercepts at the individual and twin-pair levels to account for within-individual and within-twin pair correlations, and all models included participant age and sex, and total brain volume, as covariates. Significant associations were followed up using co-twin control analyses that decomposed effects into between-twin pair (preexisting vulnerability shared by twins) and within-twin pair (effects of alcohol exposure). *p* values and effect sizes, indexed as Cohen's *d*s (0.20 indicates a small effect size, 0.40 indicates a moderate effect size, and 0.80 indicates a large effect size), were calculated using *t* statistics (*dfs* range from 71 to 111). Significant effects are noted in **bold**.

Table 5
Associations between drinking and cortical thickness.

Region	Co-twin control								
	Linear mixed models			Between-twin pair effect			Within-twin pair effect		
	Beta (SE)	p value	Effect size (d)	Beta (SE)	p value	Effect size (d)	Beta (SE)	p value	Effect size (d)
L superior frontal gyrus	–0.001 (0.003)	.784	–0.04	–	–	–	–	–	–
R superior frontal gyrus	–0.007 (0.003)	.027	–0.34	–0.010 (0.004)	.024	–0.40	–0.004 (0.005)	.445	–0.13
L middle frontal gyrus	–0.005 (0.003)	.085	–0.26	–	–	–	–	–	–
R middle frontal gyrus	–0.007 (0.003)	.031	–0.33	–0.010 (0.004)	.020	–0.44	–0.003 (0.005)	.578	–0.10
L pars triangularis	–0.007 (0.004)	.068	–0.28	–	–	–	–	–	–
R pars triangularis	–0.011 (0.004)	.006	–0.47	–0.013 (0.005)	.006	–0.49	–0.008 (0.006)	.210	–0.22
L anterior cingulate cortex	–0.005 (0.003)	.116	–0.27	–	–	–	–	–	–
R anterior cingulate cortex	–0.006 (0.004)	.157	–0.23	–	–	–	–	–	–
L rostral anterior cingulate cortex	–0.003 (0.004)	.446	–0.13	–	–	–	–	–	–
R rostral anterior cingulate cortex	–0.008 (0.005)	.164	–0.22	–	–	–	–	–	–
L caudal anterior cingulate cortex	–0.003 (0.004)	.456	–0.14	–	–	–	–	–	–
R caudal anterior cingulate cortex	–0.002 (0.004)	.704	–0.07	–	–	–	–	–	–
L isthmus cingulate	–0.003 (0.003)	.440	–0.14	–	–	–	–	–	–
R isthmus cingulate	–0.002 (0.003)	.577	–0.10	–	–	–	–	–	–
L middle temporal gyrus	–0.007 (0.003)	.016	–0.40	–0.011 (0.004)	.007	–0.48	–0.002 (0.005)	.620	–0.09
R middle temporal gyrus	–0.009 (0.003)	.005	–0.46	–0.012 (0.004)	.004	–0.50	–0.005 (0.005)	.326	–0.17
L inferior temporal gyrus	–0.006 (0.004)	.115	–0.25	–	–	–	–	–	–
R inferior temporal gyrus	–0.005 (0.003)	.160	–0.22	–	–	–	–	–	–
L parahippocampal gyrus	–0.002 (0.005)	.653	–0.08	–	–	–	–	–	–
R parahippocampal gyrus	–0.002 (0.004)	.563	–0.10	–	–	–	–	–	–

Notes: Results of linear mixed models (LMMs) and co-twin control analyses. LMMs examined associations between the drink index and cortical thickness; models included random intercepts at the individual and twin-pair levels to account for within-individual and within-twin pair correlations, and all models included participant age and sex as covariates. Significant associations were followed up using co-twin control analyses that decomposed effects into between-twin pair (preexisting vulnerability shared by twins) and within-twin pair (effects of alcohol exposure). *p* values and effect sizes, indexed as Cohen's *d*s (0.20 indicates a small effect size, 0.40 indicates a moderate effect size, and 0.80 indicates a large effect size), were calculated using *t* statistics (*d*s range from 116 to 179). Significant effects are noted in **bold**.

Previous studies of cerebellar volume have been less consistent. One study of adolescents at high familial risk for problematic alcohol use found increased cerebellar volume (Hill et al., 2007), while another high-risk family study found reduced cerebellar volume (Benegal et al., 2007); a cross-sectional study found an association between the maximum number of drinks consumed during a binge and reduced cerebellar volume, but no differences in cerebellar volumes between binge-drinking and nondrinking adolescents (Lisdahl et al., 2013). Our co-twin control analysis suggests that increased left cerebellar volume primarily reflects preexisting vulnerability toward problematic alcohol use, consistent with results reported by Hill et al. (2007), but not Benegal et al. (2007). However, the within-pair effect reflecting exposure was clearly not zero, suggesting that there may be exposure effects, the magnitude of which requires a larger sample to detect. Additional research is needed to resolve these inconsistencies. Further research will also help to link findings of increased cerebellar volume in samples of normative drinking adolescents, as well as high-risk but substance-naïve adolescents, with evidence from samples of adults with alcohol use disorders, which indicate that the neurotoxic effects of heavy, chronic alcohol exposure result in reduced cerebellar volume (Chanraud et al., 2007; Mechtcheriakov et al., 2007). We also found that drinking was associated with reduced volume of the left ventral diencephalon, and follow-up co-twin control analyses suggest this reflects an effect of alcohol exposure. These results are consistent with Squeglia et al.'s (2014) finding that heavy alcohol use predicted reduced volume of the ventral diencephalon. The ventral diencephalon in FreeSurfer comprises a collection of different structures, which complicates determining what the causal effects of alcohol exposure might be. Future research that confirms this positive finding in causally informative study designs will help to clarify the role of alcohol exposure for this region.

We also considered cortical volume and thickness in regions previously found to be associated with normative drinking in adolescence, including the superior and middle frontal gyri, the pars triangularis, the anterior cingulate cortex, the rostral and caudal

anterior cingulate cortex, the isthmus cingulate, the middle and inferior temporal gyri, and the parahippocampal gyrus. Results were generally consistent for cortical volume and thickness. We found significant associations between drinking and reduced cortical volume and thickness in several frontal and temporal regions, including the superior and middle frontal gyri, pars triangularis, and middle and inferior temporal gyri. The adolescent brain undergoes marked developmental changes, including increases, then decreases in gray matter volume and cortical thickness, which are thought to reflect increasingly efficient processing (Bava and Tapert, 2010; Crews et al., 2007; Giedd and Rapoport, 2010). Notably, our co-twin control analyses suggest that reduced cortical volume and thickness primarily reflect preexisting vulnerability toward alcohol use, rather than an alcohol exposure effect. Our finding of associations between drinking and reduced cortical volume and thickness in frontal and temporal regions may reflect delayed or stunted growth of gray matter, as opposed to more rapid pruning. Our co-twin control analyses did suggest that reduced cortical thickness in the left middle temporal gyrus reflects an alcohol exposure effect. It is possible that increasing or more problematic alcohol use may interfere with cortical development in these regions. Luciana et al. (2013) found larger-than-expected reductions in cortical thickness in the right middle frontal gyrus following alcohol initiation in adolescence. We did not find a significant within-pair effect for the middle frontal gyrus, but it is possible that the greater alcohol use reported in Luciana et al.'s (2013) slightly older adolescent sample increased the effects of alcohol exposure on this region.

Most research on associations between alcohol use and the developing adolescent brain has focused on chronic, heavy use in samples of adolescents with alcohol use disorders. However, sub-clinical levels of drinking also warrant investigation. Alcohol use, including binge drinking, becomes increasingly prevalent during the adolescent years, and it is important to determine the implications of these normative patterns of drinking on the adolescent brain. Our community sample of adolescents reported relatively

low levels of alcohol use on average, with only about one third having initiated alcohol use. Nonetheless, the sample did show variability on the alcohol index, and a subset of adolescents evidenced problematic alcohol use by Wave 2 (e.g., multiple instances of binge drinking and intoxications). Our results indicated that even these normative levels of drinking are associated with brain deviations. Associations between adolescent alcohol use and brain deviations are often interpreted as reflecting a neurotoxic effect of alcohol exposure on the brain. However, most study designs are unable to account for confounding factors that predispose individuals to begin drinking in the first place. The results of our co-twin control study design suggested that most of the brain deviations associated with even the relatively low levels of drinking in our sample reflected preexisting vulnerability toward problematic alcohol use, rather than an effect of alcohol exposure.

Although the present report focuses on alcohol use, it is notable that a subset of participants also reported using other substances, including nicotine and marijuana. We conceptualize problematic alcohol use as indexing a liability toward a broader spectrum of externalizing problems, including the use of psychoactive substances, in general, rather than a specific substance, in particular (see Hicks et al., 2004; Kendler et al., 2003a,b; Krueger et al., 2002; Vrieze et al., 2012). Thus, we would expect that the brain deviations we report as reflecting preexisting vulnerability toward problematic alcohol use likely also reflect vulnerability toward other substance use, as well as other externalizing problems, such as antisocial behavior. Preliminary support comes from cross-sectional studies examining associations between brain deviations and externalizing problems. For example, Benegal et al. (2007) found that reduced amygdala volume was associated with higher externalizing symptoms, and Ameis et al. (2014) found that reduced medial temporal cortical thickness was associated with higher externalizing symptoms. Future research using causally informative designs, including longitudinal, high-risk family, and co-twin control study designs, will help to clarify the extent to which these brain deviations reflect preexisting risk.

The present study has a number of strengths. We conducted a comprehensive review of the existing literature in order to identify regions of interest that have previously shown associations with adolescent alcohol use, and we attempted to confirm these positive findings in a community sample of normatively drinking adolescents with sufficient power to detect the moderate-to-large effects reported in previous studies. Moreover, our sample of adolescent MZ twins allowed us to conduct co-twin control analyses that help to differentiate brain deviations that likely reflect preexisting vulnerability for the development of problematic alcohol use from the neurotoxic effects of alcohol consumption. However the present study also has important limitations. Although our population-based sample was appropriate for the focus of this study on normative, rather than clinical, alcohol use, the majority of adolescents had not yet initiated drinking, reducing variability in the alcohol index. It is possible that additional and/or stronger associations with brain deviations would have been found had our sample shown greater or more problematic alcohol use.

5. Conclusions

Even normative alcohol use in adolescence is associated with brain deviations. Some deviations, including reduced volume of the amygdala, increased volume of the cerebellum, and reduced cortical volume and thickness in frontal and temporal regions reflect preexisting deviations that may confer risk for the drinking initiation and the development of problematic alcohol use, while others, including reduced volume of the ventral diencephalon and the middle temporal gyrus, appear to reflect the neurotoxic effects of alcohol consumption on the developing adolescent brain.

Conflict of interest statement

The authors declare that they have no conflicts of interest surrounding this investigation.

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