Amygdala Subregion Volumes and Apportionment in Preadolescents — Associations with Age, Sex, and Body Mass Index

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Abstract

Importance: The amygdala, a key limbic structure, plays a critical role in emotional, social, and appetitive behaviors that develop throughout adolescence. Composed of a heterogeneous group of nuclei, questions remain about potential differences in the maturation of its subregions during development.

Objective: To characterize the associations between developmental variables and amygdala subregion volumes during preadolescence.

Design, Setting, and Participants: Cross-sectional Adolescent Brain Cognitive Development℠ (ABCD®) Study data was collected from 3,953 9- and 10-year-old children between September 1, 2016, and October 15, 2018. Data analysis was conducted between June 1, 2023, and July 30, 2024.

Main Outcomes and Measures: Using the *CIT168 Amygdala Atlas*, nine amygdala subregion volumes were quantified from high-quality MRI scans. Linear mixed-effects models were used to examine the effects of age, sex, pubertal stage, and body mass index z-score (BMIz) on subregion volumes and their relative apportionment within the amygdala.

Results: The study population consisted of 3,953 preadolescents (mean [SD] age, 120 [7.41] months; 1,763 [44.6%] female; 57 [1.4%] Asian, 527 [13.3%] Black, 740 [18.7%] Hispanic, 2,279 [57.7%] white, and 350 [8.9%] from other racial/ethnic groups [identified by parents as American Indian/Native American, Alaska Native, Native Hawaiian, Guamanian, Samoan, other Pacific Islander, or other race]). Distinct associations were observed between age, sex, and BMIz and whole amygdala volume, subregion volumes, and subregion apportionment. Pubertal stage was not related to amygdala subregion volumes. Age was associated with near-global expansion of amygdala subregions during this developmental period. Female sex was linked to smaller volumes in most amygdala subregions, with larger relative apportionment in dorsal amygdala subregions and smaller apportionment in the basolateral ventral paralaminar subregion. Higher BMIz was associated with smaller volumes in large laterobasal subregions, with increased relative apportionment in smaller subregions.

Conclusions and Relevance: This cross-sectional study suggests that age, but not pubertal stage, is associated with near-global expansion of the amygdala at ages 9 and 10, while sex and BMIz are linked to distinct changes in amygdala subregions that explain observed differences in total volumes. These findings provide a foundational context for understanding how developmental variables influence amygdala structure in preadolescents, with implications for understanding future risk for brain disorders.

Keywords: amygdala; obesity; sex differences; neuroimaging; puberty; adolescence

Supplemental Information for this study was submitted with this preprint.

Introduction

Adolescence marks a critical period of substantial developmental changes to the amygdala,[1,2] coinciding with the maturation of complex social and emotional behaviors that develop between childhood and adulthood.[3] Although neuroimaging studies typically consider the human amygdala as a homogenous subcortical region, it is comprised of thirteen heterogeneous nuclei — each with distinct cytoarchitecture, connectivity, and functional roles.[4–6] The nuclei of the amygdala are typically divided into three major groups: basolateral, centromedial, and superficial (or "cortical-like") nuclear groups.[6–8]

The basolateral nuclear group (BLN)— containing the lateral (LA), basolateral (BL), basomedial (BM), and paralaminar (PL) nuclei — is the primary afferent layer for processing high-level sensory input in the amygdala and primarily projects to the central (CEN) nucleus.[7,9] The BLN, through its connections from the prefrontal cortex, is important for emotional learning and memory processes, such as fear conditioning.[10] The paralaminar nucleus (PL) is unique in that it also exhibits characteristics similar to cortical-like groups,[6] and it contains late-maturing neurons that migrate and mature during the pubertal age range coinciding with hormonal changes.[11] The central nucleus (CEN), cortical and medial nuclei (CMN), and anterior amygdaloid area (AAA) constitute the centromedial nuclear group and integrate this sensory information to mediate behavioral and autonomic responses.[12] The CEN is the recipient of most intrinsic amygdala projections and, accordingly, the primary output nuclei of the amygdala in a striatum-like system, where it plays a critical role in mediating defensive and appetitive behaviors.[7,13] The CEN also directly projects to the paraventricular nucleus of the hypothalamus where it can modulate cortisol release as an extended component of the hypothalamic-pituitary-adrenal axis.[14] Finally, the superficial nuclear group includes the amygdala transition area (ATA), which includes both the entorhinal cortex and hippocampal boundaries, and the amygdalostriatal transition area (ASTA).[6,8] Functional roles of the superficial nuclear group remain poorly understood compared to the other two nuclear groups; however, two functional MRI (fMRI) studies have linked the region to processing the social relevance of stimuli and to processing emotional valence for auditory stimuli.[15,16]

Structural magnetic resonance imaging (sMRI) studies have shown that total amygdala volumes follow a curvilinear growth trajectory, beginning in late childhood and continuing through adolescence, with peak volumes occurring in mid- to late adolescence.[17,18] Sex differences in total amygdala volumes become more substantial over the duration of adolescence, with females displaying smaller volumes that peak relatively earlier (~14 years) compared to males who undergo a more prolonged growth period (~18-24 years).[17,18] However, age poorly explains variability in amygdala volume compared to other subcortical regions,[18] suggesting substantial inter-individual variability in amygdala development. Other developmental factors, such as pubertal progression[2] and indicators of childhood obesity,[19,20] may also contribute to notable differences in amygdala volume development during childhood and adolescence. Beyond the whole amygdala, assessing developmental heterogeneity among distinct amygdala subregions is crucial for advancing our understanding of human amygdala development and its relationship to risk for psychiatric disorders.[21–24]

The arborization, plasticity, and migration of late-maturing paralaminar neurons to neighboring amygdala nuclei during the pubertal period further indicate adolescence is a critical period of change in amygdala substructure.[11] This is also supported by postmortem findings showing neuron numbers increase in the amygdala in a nucleus-specific manner from childhood through middle adulthood in neurotypical individuals.[25] Moreover, recent advancements of *in vivo* MRI amygdala segmentation methods have enabled the study of structural differences in the development of amygdala subregions in humans. As such, we recently found distinct associations between age and sex for regions of the BLN and CEN volumes[26] and associations of CEN volumes with obesity indicators[20] in childhood and adolescent samples. However, these findings are limited by their wide age ranges and small sample sizes. Together, these findings indicate the need for further research to more accurately characterize the age-related anatomical changes within structurally and functionally distinct amygdala subregions. Additionally, it is important to determine whether other developmental factors (i.e., sex, pubertal

stage, and childhood obesity indicators) influence amygdala subregion volumes and/or apportionment throughout childhood and adolescence.

Rationale

Here, we aimed to characterize total amygdala volumes, amygdala subregion volumes, and relative subregion apportionment in a large, diverse population of preadolescents. To accomplish this, we leveraged the *in vivo CIT168* probabilistic atlas and high-quality 3T MRI data from 3,953 9- and 10-year-olds from the landmark Adolescent Brain Cognitive Development℠ (ABCD®) Study. Based on prior literature,[11,17,18,20,26] we hypothesized age-related increases in amygdala apportionment of BLN regions, sex differences in apportionment of amygdala subregions consistent with prior findings, and obesity-related differences in CEN volumes. Given our previous findings, after accounting for age, we did not expect to find amygdala subregion volumes to relate to pubertal stage at ages 9 and 10 years.

Methods

Study Population and Dataset

Cross-sectional data from the ABCD® Study were obtained from the baseline enrollment visit from the annual 3.0 for (MRI data) and 5.0 data release (all other variables) (http://dx.doi.org/10.15154/8873-zj65). The ABCD Study is the largest longitudinal study of childhood neurodevelopment, enrolling 11,880 children 9 and 10 years of age (mean age = 9.49; 48% female) across 21 sites in the United States between 2016 and 2018, with the aim of following these adolescents for ten years.[27,28] Exclusion criteria for the ABCD Study included lack of English proficiency, severe sensory, neurological, medical or intellectual limitations, and inability to complete an MRI scan.[29] The institutional review board and human research protection programs at the University of California San Diego oversee all experimental and consent procedures with local institutional review board approval at the 21 ABCD sites. Each participant provided written assent to participate in the study, and their legal guardian provided written consent. Given that the *CIT168* amygdala probabilistic atlas was developed and validated on *in vivo* 3T Siemens T1w and T2w data[8] and there are notable between scanner effects within the ABCD study dataset,[30] we chose *a priori* to perform *CIT168* amygdala processing only on participants collected at 13 of the 21 study sites using 3T Siemens scanners (**Supplemental Methods**). After preprocessing the MRI data, we implemented a series of quality control standards and participant selection procedures (**Supplemental Figure 1**). Participants were excluded if their data failed to meet the raw quality control inclusion standards of the ABCD consortium,[31] had incidental neurological findings noted by a radiologist,[32] failed CIT168 segmentation or required contrast to noise quality control metrics, or missing essential covariate data. Lastly, to reduce within-family correlation and meet statistical assumptions for independence, we restricted our sample to one child per family, which was chosen randomly. After excluding participants missing any predictors and randomly selecting one child per family, we had a final analytic sample of 3,953. **Table 1** presents the demographic characteristics of the final analytic sample.

Table 1: Sample Demographics

	Analytic Sample (N=3953)
Age months)	
Mean (SD)	120 (7.41)
Median [Min, Max]	120 [107, 132]
Sex	
Male	2190 (55.4%)
Female	1763 (44.6%)
BMIz	
Mean (SD)	0.359 (1.16)
Median [Min, Max]	0.364 [-5.49, 2.88]
Pubertal Stage	
Pre Puberty	2019 (51.1%)
Early Puberty	962 (24.3%)
Mid Puberty	911 (23.0%)
Late/Post Puberty	61 (1.5%)
Race & Ethnicity	
Non-Hispanic White	2279 (57.7%)
Non-Hispanic Black	527 (13.3%)
Hispanic	740 (18.7%)
Asian	57 (1.4%)
Other [a]	350 (8.9%)
Annual Household Income	
< $50,000 USD	973 (24.6%)
$50,000 to $100,000 USD	1082 (27.4%)
> $100,000 USD	1607 (40.7%)
Don't know/Refuse to answer	291 (7.4%)
Caregiver Education	

Table 1: Sample Demographics

	Analytic Sample (N=3953)
Less than high school diploma	112 (2.8%)
High school diploma/GED	359 (9.1%)
Some college, associate's degree	991 (25.1%)
Bachelor's degree	1083 (27.4%)
Postgraduate degree	1404 (35.5%)
Don't know/Refuse to answer	4 (0.1%)
Handedness	
Right	3165 (80.1%)
Left	277 (7.0%)
Mixed	511 (12.9%)

[a] *"Other" category includes participants who are parent-identified as American Indian/Native American, Alaska Native, Native Hawaiian, Guamanian, Samoan, Other Pacific Islander, or Other Race.*

CIT168 Amygdala Atlas: Segmentation and Quality Control

First, minimally processed T1w and T2w images collected using the harmonized Siemens 3T ABCD imaging protocol were downloaded (http://dx.doi.org/10.15154/1520591), and the Human Connectome Project (HCP) minimal preprocessing pipeline[33] was implemented to perform brain extraction, bias correction, alignment, and registration to the MNI 152 template from the FMRIB Software Library (FSL) version 6. Next, the *CIT168 Atlas*[8] was implemented to obtain participant-level probabilistic atlas estimates of left and right total amygdala volumes and nine bilateral amygdala subregions. Note that the *CIT168 Atlas* groups subregions of the BL nucleus based on visibility in the MRI templates used for labeling. The dorsal and intermediate subdivisions of the BL (BLD and BLI, respectively) are combined as the BLDI label, and the ventral and paralaminar regions are combined as the BLVPL label. B-spline bivariate symmetric normalization (SyN) diffeomorphic registration algorithm from Advanced Normalization Tools[34] (ANTs) version 2.2.0 was adapted for image registration of T1w and T2w participant images to the *CIT168 Atlas* (**Figure 1**); then, probabilistic atlas labels were mapped to individual space using inverse warping. Probabilistic volumes were calculated using *fslstats* from FSL version 5.0.7 to extract the total volume of voxels included in an ROI and the mean probability of those voxels, and then multiplying those two values to yield a probabilistic volume for an ROI. Based on prior work,[26,35] only images with a contrast-to-noise ratio (CNR) of 1.0 or higher were included in our final analyses. We implemented a stringent approach by requiring all four CNRs to be > 1.0, including both the left and right hemispheres for both the T1w and T2 images. Amygdala relative volume fractions (RVFs) for each subregion were calculated by dividing the probabilistic volume of a subregion by the total probabilistic amygdala volume for a given hemisphere. Violin plots of subregion volumes are in **Supplemental Figure 2**.

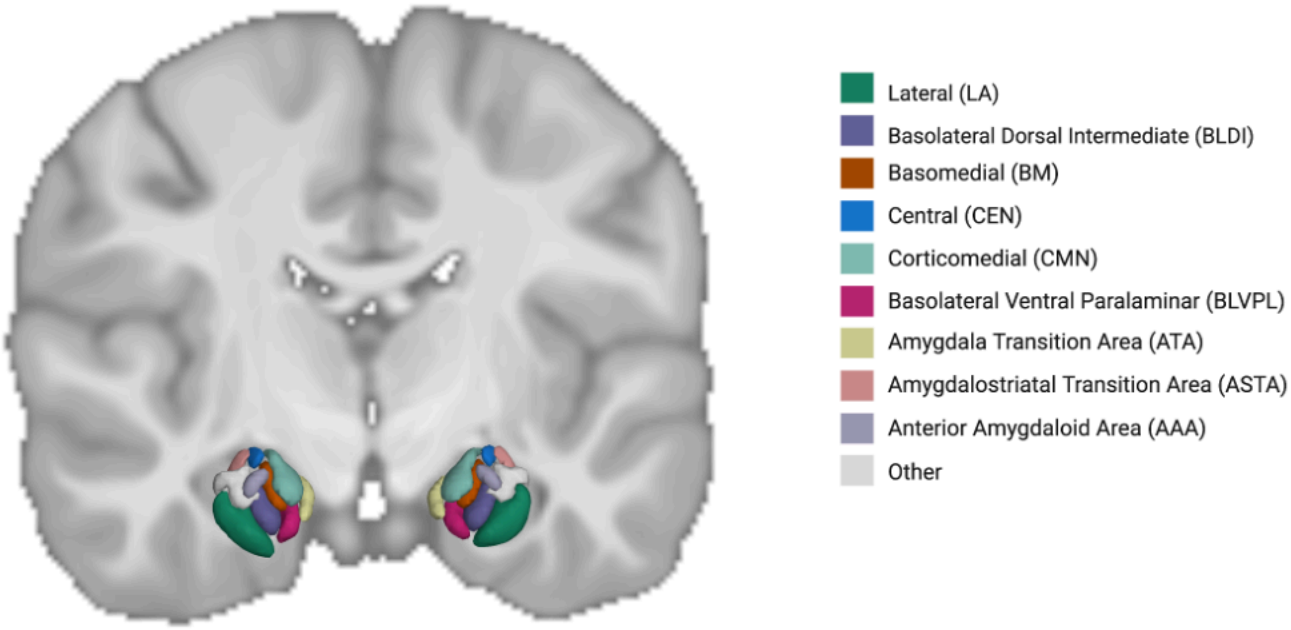

Figure 1. Probabilistic amygdala nuclei segmentation in a representative subject with the *CIT168 Atlas*. Structural T1w and probabilistic estimates of 9 subregions (threshold set at 0.5 for visualization) are shown in the coronal plane.

Developmental Variables and Covariates

We included the child's age (in *months*), sex at birth (*Male* or *Female*), pubertal stage, and body mass index z-scores (BMIz) as primary predictors of interest based on our hypotheses. We utilized the caregiver report Pubertal Development Scale, which was converted to a corresponding Tanner stage (*Pre-Puberty*, *Early Puberty*, *Mid-Puberty*, *Late Puberty*, and *Post-Puberty*).[36,37] Due to the small number of participants in the latter two categories during this age, we collapsed these two groups into a single category (*Late/Post-Puberty*) for analysis.

Body mass index (BMI) was calculated in kg/m^2 using average standing height and weight, measured by a trained research assistant two to three times at the visit. BMIz for the sex and age of each participant was calculated using the CDC's United States Growth Charts.[38] Participants wearing a non-removable prosthetic/cast or identified as having an extreme value by CDC cutoffs[38] were excluded from the study.

We also included additional demographic and socioeconomic variables based on prior identified relationships with total amygdala volumes,[39,40] including within the ABCD Study, during this age range that might act as potential confounders in our analyses, including race/ethnicity (*Non-Hispanic White*, *Non-Hispanic Black*, *Hispanic*, *Asian/Other*), average household income (*≥$100K USD*, *$50k to <$100K USD*, *<$50K* USD, or *Don't Know/Refuse to Answer*), and highest household education (*Post-Graduate Degree*, *Bachelor's Degree*, *Some College*, *High School Diploma/GED*, *less than High School Diploma*, or *Don't Know/Refuse to Answer*). We also included precision variables related to MRI collection, including handedness (*right*, *left*, or *mixed*) and the ABCD collection site to account for scanner-related differences. To account for differences in the total volume of the right/left amygdala related to total brain size, we also included intracranial volume (ICV) as a covariate in our volume-based analyses.

Statistical Analyses

Analyses were conducted using the R statistical software Version 4.3.1 (R Core Team, 2023). For Linear Mixed Effect (LME) models, we used *lme4* package version 1.1-35.5.[41] Model diagnostics were assessed using *performance* package version 0.12.2.[42] Age was centered at the youngest enrollment age, 108 months. To aid in the interpretability of parameter estimates and make fixed effect coefficients within regions directly comparable, we standardized each MRI outcome variable (i.e., total amygdala volumes, amygdala subregions volumes, relative proportions of amygdala subregion, ICV) using our analytical sample of 3,953 participants. Standardized parameter estimates are interpreted as the corresponding increase/decrease in standard deviations of the respective MRI outcome variable.

Our first LME models measured the effect of our primary developmental variables (i.e., age, sex, pubertal stage, and BMIz) on hemispheric amygdala total volume while controlling for effects of our covariates (see **Developmental Variables and Covariates**) and random effect of ABCD site (**Supplemental Methods**). A p-value less than 0.05 was used as our threshold of significance. Next, the same LME structure was used with amygdala subregion volumes as the outcome (**Supplemental Methods**). Lastly, we used LME models to investigate how the primary developmental variables were associated with amygdala subregion apportionment using subregion RVF as the outcome while again controlling for effects of covariates and random effects of the ABCD site (**Supplemental Methods**). Given that the RVF accounts for total hemispheric amygdala volume, these models did not include ICV. To account for multiple comparisons across subregion analyses, false discovery rate (FDR) correction was separately performed for each model set (i.e., volumes, proportions), and an FDR-corrected p-value (p-FDR) less than 0.05 was used as our threshold for detecting significant effects. To quantify the overall significance of pubertal stage, we used a Type III ANOVA with Satterthwaite's methods to obtain an F-value and associated p-value for the pubertal stage variable in each of our LME models.

Results

The primary analytic sample was slightly older (by approximately one month), had a higher proportion of male participants, a slightly lower BMI z-score, and was more likely to be non-Hispanic white and from higher socioeconomic backgrounds, as indicated by household income and parental education, compared to the larger ABCD study sample (**Supplemental Table 1**).

Age Effects

Age (in months) was positively associated with both total left amygdala [β = 0.006, 95% CI: 0.003 – 0.009, p < 0.001] and right amygdala volumes [β = 0.006, 95% CI: 0.003 – 0.009, p < 0.001] (**Supplemental Table 2**). Age was also positively associated with volumes in all amygdala subregions, except for the left AAA and CMN (**Figure 1 and Supplemental Table 2**). Age was not associated with differences in subregion apportionment except for the left AAA, which displayed a significant decrease in RVF (**Figure 1** and **Supplemental Table 3**).

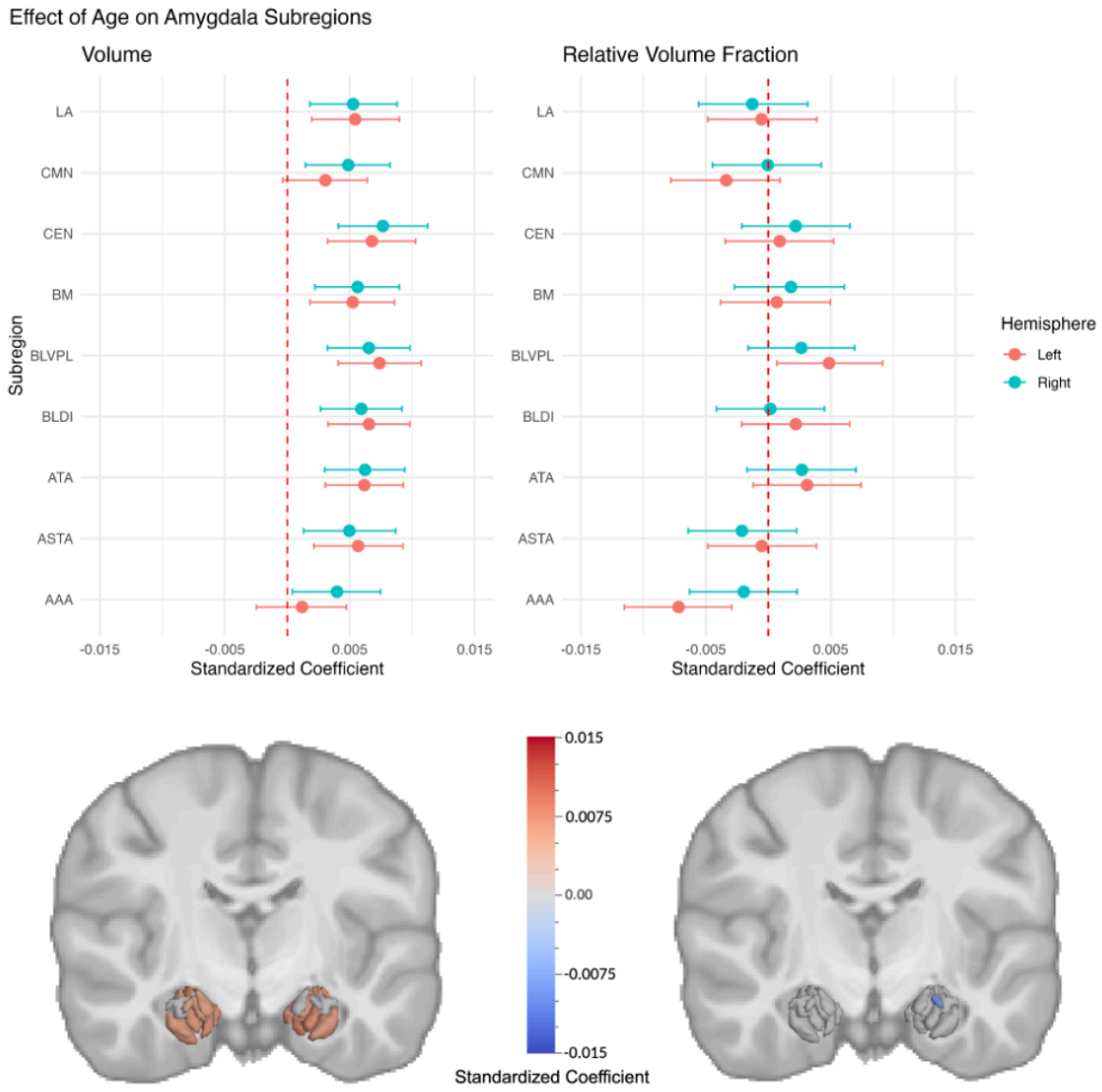

Figure 1. Age Effects on Amygdala Subregions. Standardized beta coefficients with 95% confidence intervals (CIs) for age effects on subregion volumes (left panel) and RVFs (right panel) by hemisphere. CIs intersecting the dashed line indicate null effects. Subregion beta coefficients that remained significant after FDR correction are heat mapped onto the amygdala in the bottom panel of the figure.

Sex Effects

Female preadolescents had smaller total left [β = -0.233, 95% CI: -0.289 – -0.177, p < 0.001] and right amygdala volumes [β = -0.168, 95% CI: -0.224 – -0.112, p < 0.001] as compared to male preadolescents (**Supplemental Table 4**). This sex difference was observed for most amygdala subregions, except for the bilateral CMN, right CEN, and right BM (**Figure 2**, **Supplemental Table 4**). Sex differences were also observed in the apportionment of amygdala subregions (**Figure 2**, **Supplemental Table 5**). In both hemispheres, females exhibited larger amygdala RVFs in the CMN, CEN, and ASTA, and smaller RVFs in the BLVPL. Females were found to have larger amygdala RVFs in the right BM, but smaller RVFs in the right LA and left ATA.

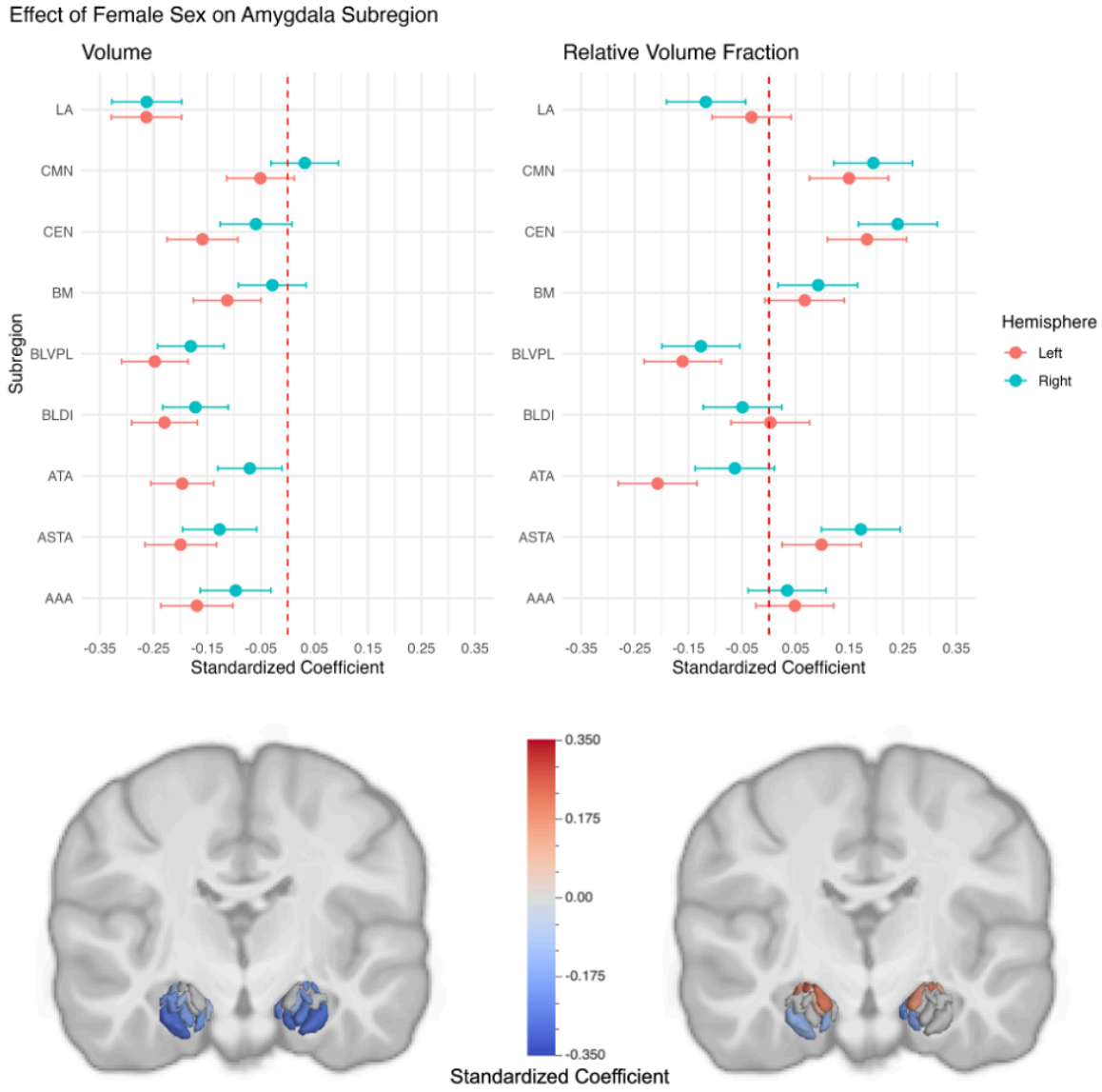

Figure 2. Sex Effects on Amygdala Subregions. Standardized beta coefficients with 95% CIs for female sex effects on subregion volumes (left panel) and RVFs (right panel) by hemisphere. CIs intersecting the dashed line indicate null effects. Subregion beta coefficients that remained significant after FDR correction are visualized on the amygdala in the bottom panel of the figure.

Pubertal Stage Effects

Pubertal stage was not significantly related to total left [$F(3, 3926.5) = 0.246$, $p = 0.86$] or right amygdala volumes [$F(3, 3920.5) = 1.876$, $p = 0.13$] (**Supplemental Table 6**). Moreover, pubertal stage was unrelated to subregion volumes or RVFs (**Supplemental Tables 6-7**).

BMIz Effects

BMIz was also negatively associated with both total left [β = -0.035, 95% CI: -0.055 – -0.015, p = 0.001] and total right amygdala volumes [β = -0.024, 95% CI: -0.044 – -0.004, p = 0.02] (**Supplemental Table 8**). In both hemispheres, BMIz was also found to be negatively associated with LA and BLDI volumes (**Figure 3, Supplemental Table 8**). For apportionment, BMIz was associated with larger RVFs of the bilateral CMN, bilateral ATA, and right BM and was associated with smaller RVFs for bilateral LA and right BLDI (**Figure 3, Supplemental Table 9**).

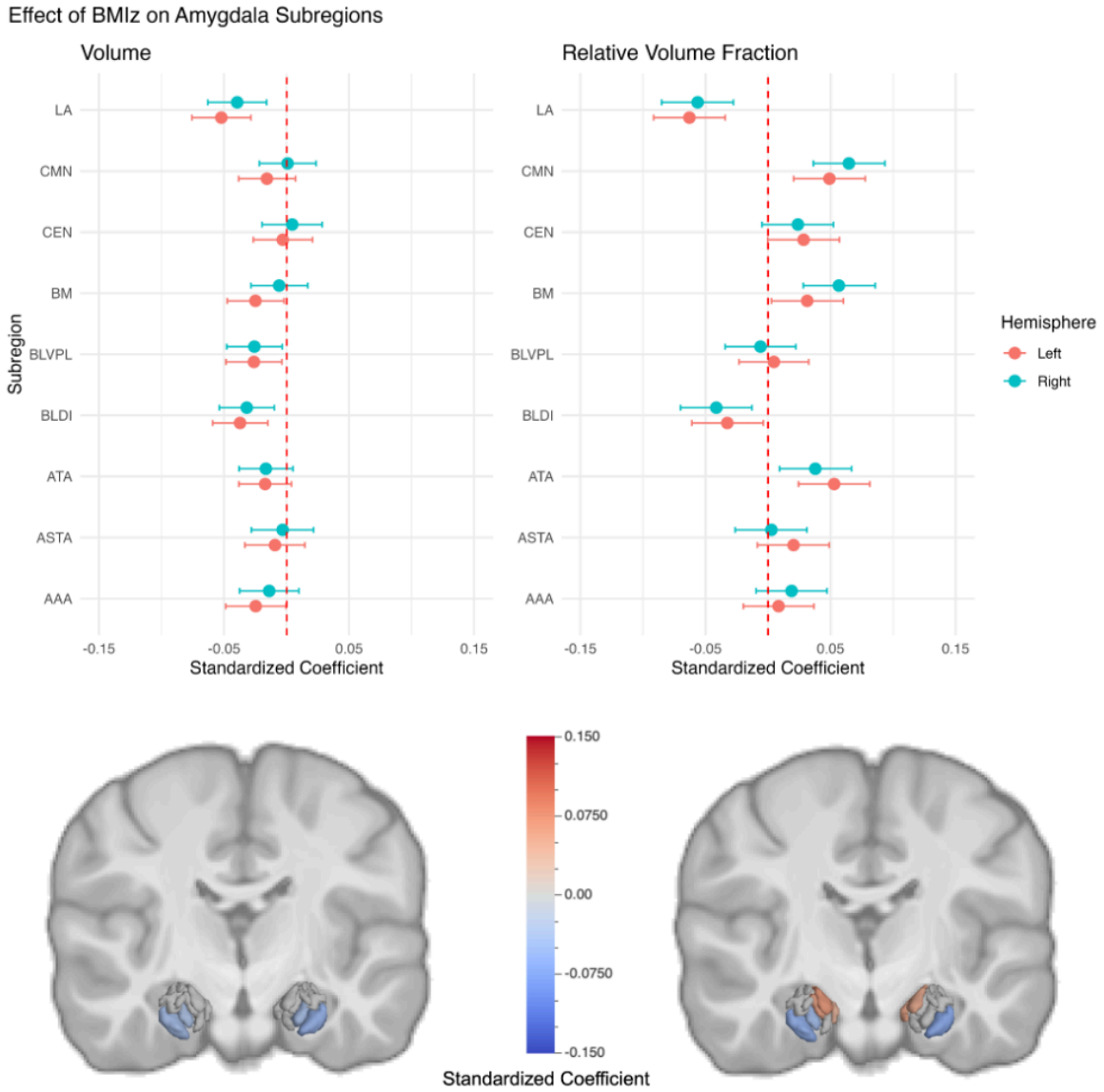

Figure 3. BMIz Effects on Amygdala Subregions. Standardized beta coefficients with 95% CIs for BMIz on subregion volumes (left panel) and RVFs (right panel) by hemisphere. CIs intersecting the dashed line indicate null effects. Subregion beta coefficients that remained significant after FDR correction are visualized on the amygdala in the bottom panel of the figure.

Discussion

Our findings indicate that age, sex, and BMIz are associated with variations in amygdala subregion volumes and apportionment in a large, diverse sample of 9- and 10-year-old preadolescents. Age was associated with increases in the volume of nearly all subregions (~89%), even within this narrow two-year age range; yet age did not explain differences in apportionment of amygdala subregions. These findings suggest that the amygdala may undergo a near-global (i.e., non-specific) expansion of its subregion volumes during this period of preadolescence. In contrast, once accounting for overall smaller total amygdala volume differences in females, notable sex differences in amygdala subregion apportionment patterns were seen, with larger relative CMN, CEN, ASTA, and BM and smaller relative BLVPL, LA, and ATA volumes in females as compared to male preadolescents. Similarly, childhood weight status was negatively associated with smaller total amygdala volumes, albeit this effect was primarily driven by two of the large basolateral subregions (i.e., LA and BLDI), with relative increases observed in the CMN, ATA, and BM. Lastly, pubertal stage displayed no statistically significant associations with amygdala volumes or apportionment during this age range, which contrasts with theorized relationships about the influence of pubertal progression on amygdala substructure.[2,11] However, this may be due to (1) an insufficient time window to capture the full effects of puberty, (2) the parent-report Tanner Staging method lacking granular resolution, and/or (3) a mismatch between pubertal brain changes and the physical changes assessed by the current method of Tanner Staging. Building upon animal,[43–45] postmortem,[25] and prior MRI studies,[26,35,46] our findings lend support to the growing evidence that notable differences exist in the structural development of distinct amygdala nuclei and subregions across childhood and adolescence, influenced by age, sex, and indicators of childhood obesity.

Amygdala subregions and their relative apportionment within the amygdala evolve with age during development in a sex-specific manner.[26] Studying the amygdala as a single, unified structure obscures important individual variability in anatomical changes across its structurally and functionally distinct subregions during childhood and adolescence. The paralaminar nucleus, included as part of the BLVPL subregion in the *CIT168 Atlas*, contains neurons that continue to mature and migrate into adulthood.[47–50] Notably, many of these quiescent excitatory neurons initiate changes to their transcriptional profiles during adolescence, indicating their maturation.[51] Some of these recently-matured neurons migrate to neighboring nuclei, which may contribute to the reconfiguration of amygdalae substructure or cortical connectivity.[11] However, the repository of immature paralaminar neurons that remain into adulthood may act as a reservoir of neuroplasticity for the amygdalar-hippocampal interface.[51] Supporting this, post-mortem studies have demonstrated increased mature neurons in specific BLN subregions from childhood to middle adulthood.[25] Consistent with other MRI studies,[26,52] our findings suggest that age, but not pubertal development, is associated with these observed amygdalae changes, with a broadly similar scale of increases across subregion volumes within this narrow age range. Age-related increases from 9 to 10 years likely reflect the period before the peak (or plateau) of total amygdala growth, which occurs uniformly across subregions during preadolescence. In contrast, prior work observed non-linear volume decreases within distinct subregions from ages 10 to 17 years, occurring in a sex-specific manner and leading to notable differences in amygdala apportionment.[26] Moreover, the amygdala undergoes specific age-related changes in the cellular microstructure of subregions, with increasing neural density of BLN subregions (i.e., LA, BLDI, BLVPL).[46] Future longitudinal study during later developmental periods is warranted to better probe the heterogeneity in amygdalar subregion growth patterns following the 'peak' period during adolescence and subsequent refinement.

Total amygdala volumes, assessed using sMRI, are larger in males than in females, with differences in developmental trajectories observed across adolescence.[17,18] In developing the *CIT168 Atlas* in young adults, Tyszka and Pauli[8] noted that sex differences in total amygdala and subregion volumes disappeared after normalizing for ICV or amygdala relative volume fraction. However, in 10- to 17-year-olds, a significant effect of sex was observed for the absolute volumes of the LA, BLDI, BM, CMN, AAA, and total amygdala, with males having larger volumes even after accounting for differences in ICV. In this study, we confirm the emergence of these sex differences as early as ~10 years of age for the majority of the prior identified subregions (i.e., LA, BLDI, AAA, total amygdala) and introduce novel findings in four additional amygdala subregions (i.e., ASTA, ATA,

BLVPL, and CEN). Additionally, sex differences in amygdala apportionment patterns for 9- and 10-year-olds observed in the current study were largely consistent with the findings utilizing a broader study of ages 10 to 17 years — females have smaller relative volumes of the BLVPL and larger relative volumes of the CEN and ASTA. Notably, we also observed a novel finding of larger relative volumes in the CMN for female preadolescents. These three relatively larger subregions in females (i.e., CEN, CMN, ASTA) belong to two distinct nuclear groups but are adjacent in the dorsal region of the amygdala, near the stria terminalis—a major efferent pathway of the amygdala that projects to the hypothalamus, modulating the output of hypothalamic nuclei both directly and indirectly.[53] Our novel findings regarding sex differences in amygdala substructure may be attributed to the increased statistical power provided by our larger sample size, or they may reflect that sex differences in amygdala subregion volumes vary across developmental stages. Interestingly, Campbell et al.[26] observed that the age-related changes in the apportionment of amygdala subregions in males result in a greater similarity of amygdala apportionment to that of females by age 17 as compared to the differences observed at age 10. Recently, GWAS studies have also revealed that genetic loci associated with amygdala whole and subregion volumes overlap with genetic risk factors for common brain disorders.[54,55] Consequently, our findings on sex differences in the amygdalar subregion volumes provide an interesting basis for future research to explore whether the observed amygdala apportionment findings in male and female adolescents play a role in the notable sex differences in the prevalence and timing of the onset of brain disorders that emerge later in adolescence and adulthood.

Previous studies have reported mixed findings regarding the relationship between childhood obesity and total amygdala volumes.[19,56,57] In our study, higher BMIz was associated with smaller total amygdala volumes, primarily driven by differences in BLN subregions (i.e., LA and BLDI), the two largest measured subregions. After accounting for the overall smaller amygdala volumes, higher BMIz was linked to decreased relative volumes of the LA and BLDI, and increased relative volumes of the CMN, ATA, and BM. These findings contrast with earlier studies that found no relationship between BMIz and amygdala subregions in 405 adolescents aged 10 to 17 years[26] and a relationship between CEN volumes and waist-to-height ratio (WHtR) in 71 youth aged 8 to 22 years.[20] The reasons for the lack of replication of CEN volume effects in our study remain unclear, though it is possible that sample differences influenced the previous findings. While rodent models suggest a significant role for the CEN in regulating homeostatic and cue-mediated eating behaviors,[58,59] distinct populations of basolateral amygdalar principal cells both mediate and suppress appetitive behaviors outputted by the CEN.[60] Future research is needed to explore whether the observed effects of BMIz relate to higher-order disruption in the eating behavior circuity of the amygdala.[60] Our work builds upon prior evidence of a relationship between childhood obesity and global amygdala volumes to suggest that pediatric weight status is associated with specific subregional differences in amygdala volumes and apportionment. Elucidating the mechanisms linking weight status to amygdala substructure could provide critical insights into the neural underpinnings of disordered eating patterns (e.g., emotional eating behaviors) and inform targeted interventions.

Future Directions and Limitations

It is important to acknowledge both the strengths and limitations of this study. Our study underscores the utility of the *CIT168 Atlas,* a probabilistic atlas of amygdala subregions, in studying neurodevelopment in pediatric populations, contributing to a more refined understanding of amygdala substructure development. Importantly, the *in vivo* segmentation template approach uses joint high accuracy diffeomorphic registration of T1- and T2-weighted structural images to display reliable extraction of major amygdala nuclei and subregions on individual subjects and has been validated against manual-tracing and cross-referencing to four histological sources.[8] Moreover, the current study used a rigorous approach limited to high-quality 3T images collected on scanners from a single manufacturer (Siemens) with appropriate contrast-to-noise ratios. However, in doing so, the final sample did not represent the larger ABCD study cohort. Moreover, our sample included participants from only 13 of the 21 ABCD study sites, as the *CIT168* was developed and validated using 3T Siemens scanner data. Thus, further research is needed to assess the fidelity of *CIT168 Atlas* registrations to data acquired on MRI machines from other manufacturers. Additionally, our study focuses on a single wave of data from this large longitudinal cohort, specifically when participants are 9- to 10-years-old. Implementing the atlas in future data

waves will be crucial for gaining a deeper understanding of how age, sex, and body mass influence the developmental trajectories of amygdala subregions throughout adolescence.

Conclusions

We uncovered distinct associations between amygdala total volumes, subregion volumes, and subregion relative volumes with age, sex, and BMIz, but not with pubertal stage, in nearly 4,000 preadolescents ages 9 to 10 years. Age was associated with a near-global growth of the amygdala, without changes to apportionment. Female sex was related to smaller total amygdala volumes and smaller volumes in most subregions; however, when controlling for total amygdala volume, female sex was associated with larger relative volume in dorsal subregions. Our childhood obesity metric (i.e., BMIz) was related to smaller total amygdalae, primarily driven by decreases in the volume of two BLN subregions (i.e., LA and BLDI), which led to greater relative volumes in smaller subregions. This research contributes to the growing evidence that distinct neurodevelopmental patterns exist among heterogenous amygdala nuclei and subregions across childhood and adolescence, with potential relevance to socioemotional and physical health.

Acknowledgments

A special thanks to the participants and families of the ABCD Study. The research described in this article was supported by the National Institutes of Health [NIEHS R01ES032295, R01ES031074] and EPA grants [RD 83587201, RD 83544101].

Data used in the preparation of this article were obtained from the Adolescent Brain Cognitive Development (ABCD) Study (https://abcdstudy.org), held in the NIMH Data Archive (NDA). This is a multisite, longitudinal study designed to recruit more than 10,000 children aged 9-10 and follow them over 10 years into early adulthood. The ABCD Study is supported by the National Institutes of Health Grants [U01DA041022, U01DA041028, U01DA041048, U01DA041089, U01DA041106, U01DA041117, U01DA041120, U01DA041134, U01DA041148, U01DA041156, U01DA041174, U24DA041123, U24DA041147]. A full list of supporters is available at https://abcdstudy.org/nih-collaborators. A listing of participating sites and a complete listing of the study investigators can be found at https://abcdstudy.org/principal-investigators.html. ABCD consortium investigators designed and implemented the study and/or provided data but did not necessarily participate in the analysis or writing of this report. This manuscript reflects the views of the authors and may not reflect the opinions or views of the NIH or ABCD consortium investigators. The ABCD data repository grows and changes over time.

We would like to acknowledge ABCD Consortium staff for collecting data. We would also like to acknowledge J. Max Landau and Jade Li for their assistance in performing a manual visual quality control of a sample of image segmentations. Alethea de Jesus contributed to data cleaning and formatting of ABCD tabulated data prior to data analyses.

Competing Interests

The authors declare no competing interests.

Author Contributions

LNO, JMT, and MMH conceived and designed the project. ABCD Consortium staff acquired data. LNO, CT, and JM completed the preprocessing and processing of MRI data. LNO, HA, and MMH completed statistical analyses. LNO and MMH drafted the manuscript, and all study authors reviewed and edited the manuscript before submission.

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