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Revisiting Resting-State Functional Connectivity of the Amygdala and Subgenual Anterior Cingulate Cortex in Adolescents and Adults With Depression

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ABSTRACT

BACKGROUND: Adolescent depression is a growing public health concern, and neuroimaging offers a promising approach to its pathology. We focused on the functional connectivity of the amygdala and subgenual anterior cingulate cortex (sgACC), which is theoretically important in major depressive disorder (MDD), but empirical evidence has remained inconsistent. This discrepancy is likely due to the limited statistical power of small sample sizes.

METHODS: We rigorously examined sgACC-amygdala connectivity in adolescents and adults with depression using data from the Healthy Brain Network (n = 321; 170 female), the ABCD (Adolescent Brain Cognitive Development) Study (n = 141; 56 female), the Boston Adolescent Neuroimaging of Depression and Anxiety study (n = 108; 75 female), and the REST-meta-MDD project (n = 1436; 880 female). Linear mixed models, Bayesian factor analyses, and meta-analysis were used to assess connectivity.

RESULTS: Our analyses revealed that sgACC-amygdala connectivity in adolescents with MDD was comparable to that in healthy control individuals, whereas adults with recurrent MDD exhibited reduced connectivity. Resampling analysis demonstrated that small sample sizes (i.e., $n < 30$ MDD cases) tend to inflate effects, potentially leading to misinterpretations.

CONCLUSIONS: These findings clarify the state of sgACC-amygdala connectivity in MDD and underscore the importance of refining neurocognitive models separately for adolescents and adults. The study also highlights the necessity for large-scale replication studies to ensure robust and reliable findings.

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Adolescent depression is an important public health concern because it occurs at a critical developmental stage, and its prevalence has increased significantly over the past decade (1). This disorder often remains undiagnosed and untreated, in part because its symptoms can vary from the adult criteria, leading to chronic issues and poor outcomes in adulthood (2,3). Therefore, improving diagnostic and therapeutic strategies is crucial.

Magnetic resonance imaging (MRI) data, such as resting-state functional connectivity (FC), is increasingly being used to explore the brain mechanisms of adolescent depression. This method has the potential to significantly enhance our understanding of mental illnesses in adolescents and contribute to the development of more targeted treatments. MRI studies have shown critical changes in brain regions and neural circuits in adolescents with major depressive disorder (MDD) (4,5). Among key regions that have been implicated in depression, the subgenual anterior cingulate cortex (sgACC) and amygdala have been identified as critical hubs (6,7). The FC between these 2 regions has frequently been reported as altered in depressed populations (8–12), with these alterations being linked to negative affectivity (13), rumination (14), and treatment responsiveness (15). The sgACC is believed to act as a gatekeeper (16), mediating communication between frontal regions and the amygdala. Its dysfunction is thought to contribute to the persistence of depressive symptoms (17). Moreover, a meta-analysis of 24 studies found that alterations in sgACC-amygdala connectivity were most pronounced in studies involving youths (18). This supports the view that sgACC-amygdala connectivity plays a critical role in the pathology of depression, particularly during adolescence.

Despite the insights that have been gained from studying sgACC-amygdala connectivity, the results show significant heterogeneity, particularly for the adolescent cohort. Reports range from hypoconnectivity (11,12) to hyperconnectivity (8,9), and some studies have shown no significant differences (15,19–21). These inconsistencies are often due to small sample sizes in neuroimaging studies, which is a more serious problem in adolescents because data collection is particularly challenging in this population. This problem is further exacerbated by the widely recognized diagnostic heterogeneity in depression, leading to low statistical power and potentially

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misleading conclusions (22–25). Methodological advances and data harmonization across multiple sites have enabled large-scale collaborative neuroimaging projects. These projects, which involve adolescents with psychiatric conditions, aim to enhance our understanding of brain development and mental health, providing increased statistical power and better characterization of diagnostic heterogeneity compared with smaller samples (25).

Here, we leveraged 3 neuroimaging datasets in adolescents, the HBN (Healthy Brain Network) (26), the BANDA (Boston Adolescent Neuroimaging of Depression and Anxiety) study (27), and the ABCD (Adolescent Brain Cognitive Development) Study (28), to examine the FC of sgACC-amygdala in adolescents with MDD. We also analyzed adult MDD data from the REST-meta-MDD (29) project to determine whether the effects are specific to adolescents. We used linear mixed models (LMMs) to test for significant differences. Additionally, we applied Bayesian factor analysis, which not only assesses significant differences but also provides evidence for the absence of differences. Furthermore, we examined how subgroups and sample sizes influenced these results.

METHODS AND MATERIALS

Study Samples

We analyzed 3 adolescent datasets [HBN (26), ABCD (28), BANDA (27)] and 1 adult cohort [REST-meta-MDD (29)]. Demographics and clinical information are detailed in the Supplement, with enrollment protocols and inclusion criteria described in original studies (26,30–32).

Imaging Acquisition and Preprocessing

MRI data acquisition protocols are detailed in the original articles for HBN (26), ABCD (28), BANDA (33), and REST-meta-MDD (30). See the Supplement for more details on preprocessing.

HBN data were preprocessed using the DPARSF (34), closely aligning with the REST-meta-MDD dataset to minimize discrepancies. The REST-meta-MDD dataset only provided preprocessed data, using the same pipeline (30).

The ABCD dataset was preprocessed using an updated version of the Human Connectome Project MRI pipeline (35), incorporating more advanced techniques than the above procedure, such as advanced gradient nonlinearity correction using "topup" (36) and bias field correction using FLIRT and FreeSurfer. Surface-based analysis was used to precisely map the cortical surface while preserving topology. Additionally, the pipeline applied robust noise handling and motion artifact correction through strict motion censoring (37).

The BANDA dataset used the standard Human Connectome Project pipeline (35), with a notable difference compared to the ABCD dataset being the exclusion of the "Preproc" step for further motion correction based on newer methods, such as Power *et al.* (37).

Analyses of sgACC-Amygdala FC in Adolescents With MDD

A region of interest (ROI)-to-ROI analysis was performed using the total sample HBN dataset to examine sgACC-amygdala FC differences between adolescents with MDD and healthy control participants (HCs). Four sgACC ROIs (3-mm-radius spheres; bilateral superior ±5, 34, −4; inferior ±5, 25, −10) (8) and bilateral amygdala ROIs (Automated Anatomical Labeling [AAL] atlas regions 41 and 42) formed the sphere-AAL ROIs. Mean time series were extracted for each ROI, and pairwise Pearson correlations were calculated and converted to *z* scores using Fisher's *r*-to-*z* transformation. To account for site-related variability, CovBat (38) within DPABI was applied to harmonize FCs, including age, sex, IQ, and data quality (mean framewise displacement) as covariates. Group differences were analyzed using an LMM (30), modeling *z*-transformed FC values as the dependent variable, with group and significant demographic differences as fixed effects and site as a random effect. Cohen's *d* was computed to estimate effect size ($d = \frac{T(n_1+n_2)}{\sqrt{df}\sqrt{n_1 n_2}}$). Multiple comparisons were corrected using false discovery rate. Bayesian factors were also calculated to assess the evidence for no group difference using the BayesFactor package (39), with interpretation based on Jeffreys' classification (40).

To reduce biases associated with predefined ROIs, whole-brain seed-to-voxel FC analyses were also performed using the 6 ROIs, with group comparisons conducted via 2-sample *t* tests, adjusting for significant demographic differences as covariates ($p < .05$, uncorrected).

We further examined subgroup differences in sgACC-amygdala FC by comparing 2 subgroups—64 first-episode, drug-naïve (FEDN) adolescents with MDD and 43 adolescents with recurrent MDD—to HCs (see the Supplement for details).

Additionally, several verification analyses were conducted to validate our results: 1) replicating the analysis using Zalesky-AAL ROIs from REST-meta-MDD, 2) applying scrubbing (removal of time points with framewise displacement > 0.5 mm) to control for head motion artifacts, 3) excluding participants with missing IQ data and using matched subsamples to minimize demographic confounders, and 4) restricting to HCs without adverse childhood experiences (ACEs) or parental history of depression, with matched subsamples ensuring comparability.

Analyses of sgACC-Amygdala FC in Adults With MDD

In parallel with the adolescent analysis, we examined sgACC-amygdala FC abnormalities in adults with MDD and HCs using the REST-meta-MDD dataset. Only mean time-series data from 1833 predefined ROIs were available. To be consistent with the adolescent analysis, we selected ROIs using the AAL atlas for the bilateral amygdala (regions 41 and 42) and Zalesky's parcellations for the sgACC regions: region 267 (right superior sgACC), region 581 (bilateral inferior sgACC), and region 686 (left superior sgACC), referred to as Zalesky-AAL ROIs. We applied the same ROI-to-ROI analysis and harmonization procedure as was used for the adolescent dataset. Seed-to-voxel FC analyses were not possible due to dataset limitations.

We also examined subgroup differences in adults with MDD by comparing matched subsamples: 232 FEDN patients versus 232 HCs, 189 patients with recurrent MDD versus 189

HCs, and 119 FEDN patients versus 72 patients with recurrent MDD (no significant demographic differences required matching for this group). LMM and Bayesian factor analysis assessed group differences. The participants in each comparison group were different because they were selected from the same sites as the corresponding patients with MDD. Detailed site and subgroup specifications can be found in Yan *et al.* (30).

Additionally, scrubbing was applied for verification.

Evaluating Reproducibility in 2 Independent Adolescent Cohorts

To test the reproducibility of our HBN results, we conducted parallel analyses with 2 independent adolescent datasets, ABCD and BANDA.

For these CIFTI-format datasets, we used Nibabel (41) to separate cortical and subcortical data. We identified sgACC ROIs using Connectome Workbench's (42) surface-closest-vertex and surface-geodesic-rois commands, creating 3-mm-radius ROIs. After extracting mean time series from sgACC and resampled amygdala ROIs, we analyzed group differences following our previous approach. Because all BANDA participants were scanned at the same site, harmonization was applied only to the ABCD dataset.

Meta-Analytic Synthesis of the Results From 3 Adolescent Cohorts

We conducted Bayesian model-averaged meta-analysis (JASP) (43) across our 3 datasets to enhance statistical power. Using Cohen's *d* and standard errors from demographically matched samples, we evaluated evidence for null and alternative hypotheses with default priors (44). We report averaged estimates with confidence intervals and Bayes factors for both hypotheses and heterogeneity.

Sampling Variability

We examined the distribution of group differences across increasing sample sizes by randomly resampling participants from the MDD and HC groups (equal numbers from each) with replacement in the HBN dataset. We performed 1000 resamplings at each of 27 sample sizes (10–142 participants/group, limited by MDD $n = 142$). Group differences were assessed using LMM, with sampling variability shown by Cohen's *d* standard deviations.

We selected 1000 resamplings with $n = 30$ for each group (total sample size of $n = 60$) to illustrate how sampling variability influences group differences in a typically used sample size in the field. We ranked effect sizes and chose 2 extreme examples (most positive and most negative) for each ROI-to-ROI FC to highlight variability.

RESULTS

Sample Composition

There were 142 adolescents with MDD and 179 HCs in the HBN dataset, 718 adults with MDD and 718 HCs in the REST-meta-MDD dataset, 77 adolescents with MDD and 64 HCs in the ABCD dataset, and 54 adolescents with MDD and 54 HCs in the BANDA dataset. In the HBN dataset, we used the total sample because only a subsample could achieve demographic balance after matching procedure. Results were verified using a well-matched subsample of 70 adolescents with MDD and 70 HCs (see Table S4). Demographically matched samples were used for all other datasets. See the Supplement for details.

Independent *t* tests (age, IQ) and a χ^2 test (sex) were performed. Adolescents with MDD in the HBN dataset were significantly older ($t_{319} = 12.3$, $p < .001$), had lower mean IQ ($t_{319} = -2.0$, $p = .04$), and had a lower percentage of females ($\chi^2_1 = 4.9$, $p = .03$). No significant differences were found in the other datasets. Detailed demographics are presented in Table 1.

Intact sgACC-Amygdala FC in Adolescents With MDD

Using the large-scale HBN dataset, we examined sgACC-amygdala FC in adolescent depression. Following ROI definitions from previous findings of altered connectivity (8), we compared 142 adolescents with MDD with 179 HCs. LMM analysis revealed no significant FC differences between groups across all ROI pairs (maximum $t_{315} = 1.45$, minimum $p = .15$) (Figure 1A and Table S1). We also conducted Bayesian factor analysis to quantify evidence for the null hypothesis (45). Results showed moderate evidence for intact sgACC-amygdala connectivity in adolescents with MDD across most edges, with only 1 edge showing anecdotal evidence (Table S1).

Whole-brain seed-to-voxel analysis revealed no significant clusters in either sgACC or amygdala regions, even at a lenient threshold ($p < .05$, uncorrected) (Figure 1B).

We also examined sgACC-amygdala FC in MDD subgroups, comparing 64 FEDN adolescents with MDD and 43 adolescents with recurrent MDD to HCs. LMMs showed no significant differences, and Bayesian analysis provided anecdotal to moderate evidence for the null hypothesis (Table S1).

Verification analyses included 1) using Zalesky-AAL ROIs as in the REST-meta-MDD dataset, with no significant differences after correcting for multiple comparisons (Figure S1 and Table S2); 2) applying scrubbing for head motion control, with no significant effects (Table S3); 3) excluding participants with missing IQ data and using a well-matched sample, still with no significant results (Tables S4 and S5); and 4) comparing demographically matched participants with MDD to HCs without ACEs or parental depression history, also showing no significant differences (Tables S6 and S7).

Reduced sgACC-Amygdala FC in Adults With MDD

We examined adults with MDD using the REST-meta-MDD dataset and compared 718 adults with MDD to 718 HCs. Unlike in adolescents, a significant decrease in FC was observed between the inferior sgACC and right amygdala ($t_{1434} = -2.66$, $p = .008$) (Figure 2 and Table S8).

In subgroup analyses, we compared 232 FEDN adults with MDD to 232 HCs, 189 adults with recurrent MDD to 189 HCs, and 119 FEDN adults with MDD to 72 adults with recurrent MDD (Table S9). FC between the inferior sgACC and right

Table 1. Demographic Characteristics of Study Participants

	HBN			REST-Meta-MDD			ABCD			BANDA		
	MDD, n = 142	HC, n = 179	p	MDD, n = 718	HC, n = 718	p	MDD, n = 77	HC, n = 64	p	MDD, n = 54	HC, n = 54	p
Sex, Female	85	85	.03	448	432	.39	30	26	.84	41	34	.14
Age, Years	15.2 (2.9)	10.9 (3.2)	<.01[a]	33.8 (11.4)	34.5 (13.2)	.32	10.0 (0.6)	10.0 (0.6)	.65	15.6 (0.8)	15.3 (0.8)	.08
IQ[b]	104.1 (15.3)	107.4 (13.6)	.04				93.6 (18.1)	91.7 (13.8)	.48	113.5 (15.1)	116.5 (14.1)	.29
Education, Years				12.9 (2.8)	13.1 (3.1)	.27						

Data are presented as n or mean (SD).

ABCD, Adolescent Brain Cognitive Development; BANDA, Boston Adolescent Neuroimaging of Depression and Anxiety; HBN, Healthy Brain Network; HC, healthy control participant; MDD, major depressive disorder.

[a]False discovery rate–corrected $q < .05$.

[b]IQ was assessed using Full Scale IQ in the HBN and BANDA datasets and the age-corrected composite score from the NIH Toolbox Cognition Battery in the ABCD dataset.

amygdala was significantly reduced in adults with recurrent MDD ($t_{376} = -2.74$, $p = .006$) but not in FEDN adults with MDD ($t_{462} = 0.69$, $p = .49$). Direct comparisons also showed reduced FC in recurrent MDD ($t_{189} = 2.9$, $p = .004$), suggesting that recurrent MDD primarily contributes to decreased sgACC-amygdala FC (Table S8).

We also applied scrubbing for aggressive head motion control, and the results remained consistent (Table S8).

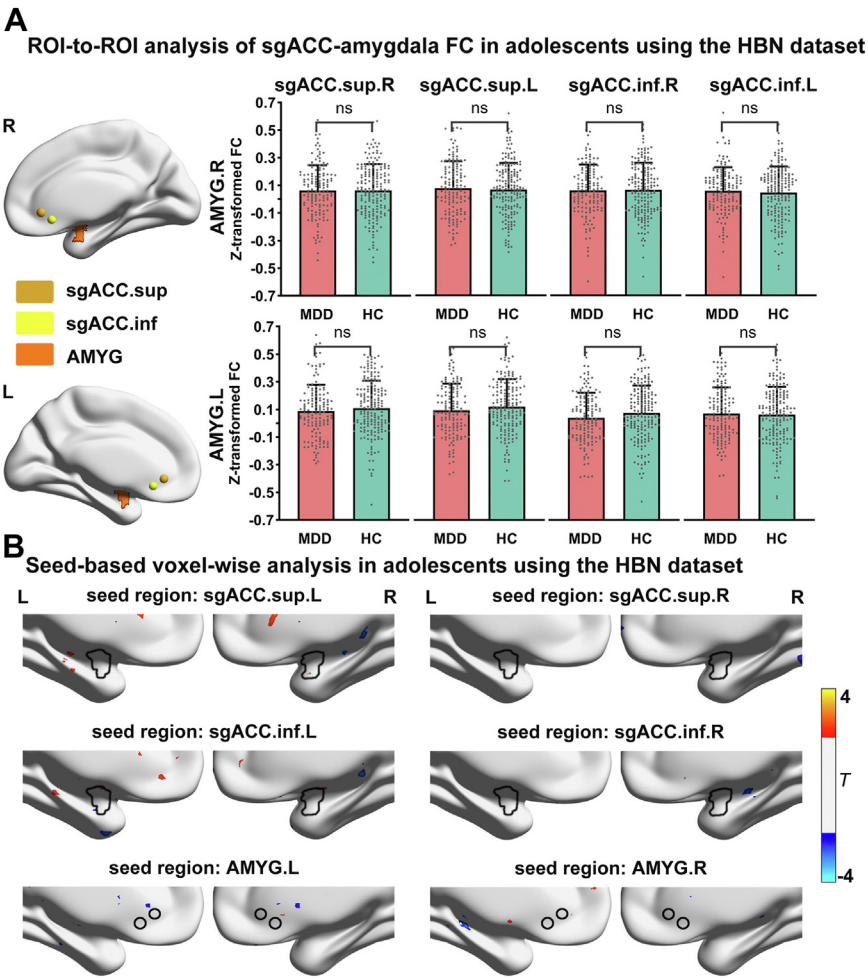

Figure 1. Comparison of subgenual anterior cingulate cortex (sgACC)–amygdala (AMYG) functional connectivity (FC) between adolescents with major depressive disorder (MDD) and healthy control participants (HCs) in the HBN (Healthy Brain Network) dataset. **(A)** Region of interest (ROI)-to-ROI analysis using a linear mixed model to assess abnormalities in adolescents with MDD. Brain maps show the selected sgACC and amygdala ROIs (sphere-AAL ROIs; see the Methods and Materials for details). Bar charts show mean z-transformed FC values with error bars representing standard deviations, and scatter plots below each bar graph show the distribution of individual data points. **(B)** Voxelwise FC analysis using predefined ROIs as seeds (4 in the sgACC and 2 in the amygdala). Brain maps show regions with detected connectivity differences between adolescents with MDD and HCs under an uncorrected $p < .05$ threshold. Contours outline the target regions for each seed for reference. ns indicates false discovery rate–corrected $q > .05$. AAL, Automated Anatomical Labeling; inf, inferior; L, left; ns, not significant; R, right; sup, superior.

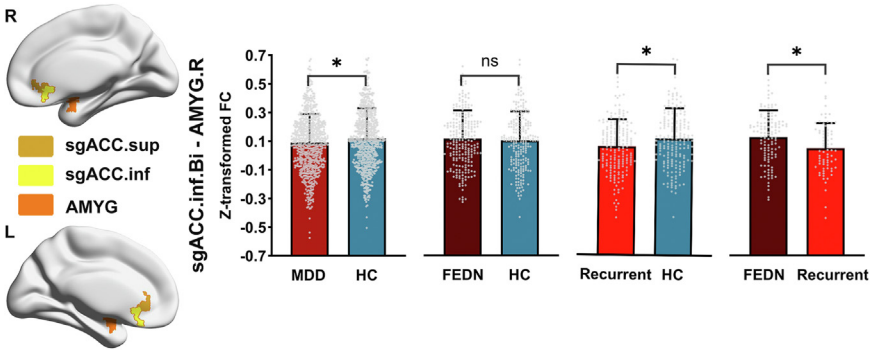

Figure 2. Comparison of subgenual anterior cingulate cortex (sgACC)–amygdala (AMYG) functional connectivity (FC) between adults with major depressive disorder (MDD) and healthy control participants (HCs) in the REST-meta-MDD dataset. Region of interest (ROI)-to-ROI analysis using a linear mixed model to assess abnormalities in adults with MDD and subgroups. Brain maps display the predefined sgACC and amygdala ROIs (Zalesky-AAL ROIs). Bar plots show mean *z*-transformed bilateral inferior sgACC–right amygdala FC across participants, with error bars representing standard deviations. Scatter plots below each bar plot show the distribution of individual data points. *false discovery rate–corrected $q < .05$. No other comparisons showed significant differences after FDR correction, so only the significant inferior sgACC–right amygdala FC is depicted in the bar plots. AAL, Automated Anatomical Labeling; Bi, bilateral; FEDN, first-episode, drug-naïve; inf, inferior; L, left; ns, not significant; R, right; sup, superior.

Reproducibility in 2 Independent Adolescent Cohorts

Given the inherent challenges of collecting adolescent neuroimaging data (46), we sought to validate our findings beyond the HBN dataset's sample size limitations. We analyzed 2 independent datasets—ABCD and BANDA—using surface-based cortical methods (47) because validation across different populations and methodologies provides robust evidence for reproducibility (48).

In the ABCD dataset (77 adolescents with MDD, 64 HCs) and the BANDA dataset (54 adolescents with MDD, 54 HCs), no significant sgACC-amygdala FC differences were found after correcting for multiple comparisons (Figure 3 and Table S10). Although a few edges showed significant differences without correction, these trends were inconsistent across datasets, indicating poor reproducibility. Thus, these differences are likely incidental and do not reflect true FC patterns.

Meta-Analysis of 3 Adolescent Datasets

To summarize the findings from the 3 adolescent datasets and enhance statistical power, we conducted a Bayesian model-averaged meta-analysis, providing evidence for both null and alternative hypotheses.

Across all 8 edges, moderate evidence supporting the null hypothesis was found, with Bayes factor values ranging from 3.61 to 8.39 (Figure 4). Similarly, all credible intervals of the model-averaged posterior distribution for μ crossed 0. Overall, the evidence for the null hypothesis was stronger, suggesting that sgACC-amygdala connectivity remained intact in adolescents with MDD.

Bayes factors for heterogeneity (BF_{fr}) across edges ranged from 0.76 to 2.49, with most edges (6/8) showing anecdotal evidence for fixed effects, suggesting limited evidence of substantial heterogeneity across datasets.

Reliable Group Comparison Requires Large Samples

Previous studies with smaller samples of adolescents have reported significantly reduced (11,12) or elevated (8,9) sgACC-amygdala FC, whereas our results showed that sgACC-amygdala FC was intact in adolescents with MDD. To address this discrepancy, we simulated the effects of independent studies by varying sample sizes to titrate group differences in the HBN dataset (Figure 5A) (n = 10–142 for each group). When the sample size was relatively small, the standard deviation of the effect size was large, suggesting that effects can be greatly inflated by chance. As the sample size increased, the standard deviation across resampling decreased.

To highlight the influence of sampling variability on detecting effects in typical sample sizes (n = 30 for each group, n = 60 total), we ranked the Cohen's ds and selected 2 extreme examples for each sgACC-amygdala FC. The results showed that 2 independent subsamples could reach opposite conclusions based on sampling variability alone, as shown in Figure 5B.

DISCUSSION

In this study, we examined sgACC-amygdala connectivity in MDD across 3 large-scale adolescent cohorts and 1 adult cohort. Our results suggest distinct patterns of sgACC-amygdala FC in adolescents and adults. Specifically, adolescents with MDD had similar sgACC-amygdala FC to HCs, a consistent finding in both the FEDN and recurrent subgroups. In contrast, adults with MDD showed a decline in sgACC-amygdala FC, with the reduction being most evident in adults with recurrent MDD. Furthermore, our resampling analysis demonstrated how sample size variability can lead to the contradictory results that have often been observed in the MDD literature, thus highlighting the need for large, well-characterized cohorts for reliable psychiatric neuroimaging research.

The prevailing opinion in the field suggests that the abnormal sgACC-amygdala connectivity is closely linked to depressive states (17,49). Our results suggest that this may not apply to adolescents with MDD. Compared with adults, the top-down control of the prefrontal cortex over the limbic system, particularly the amygdala, is reduced during adolescence. This is due to the slower development of the prefrontal cortex and its weaker connections with the amygdala (50), which results in poor emotional regulation. Over time, as the prefrontal cortex matures and these connections strengthen, the ability to regulate emotions improves. Because the frontoamygdala connectivity in adolescence is already immature and weak, even if the depression can dampen sgACC-amygdala connectivity as has been shown in adults with MDD, its effect size

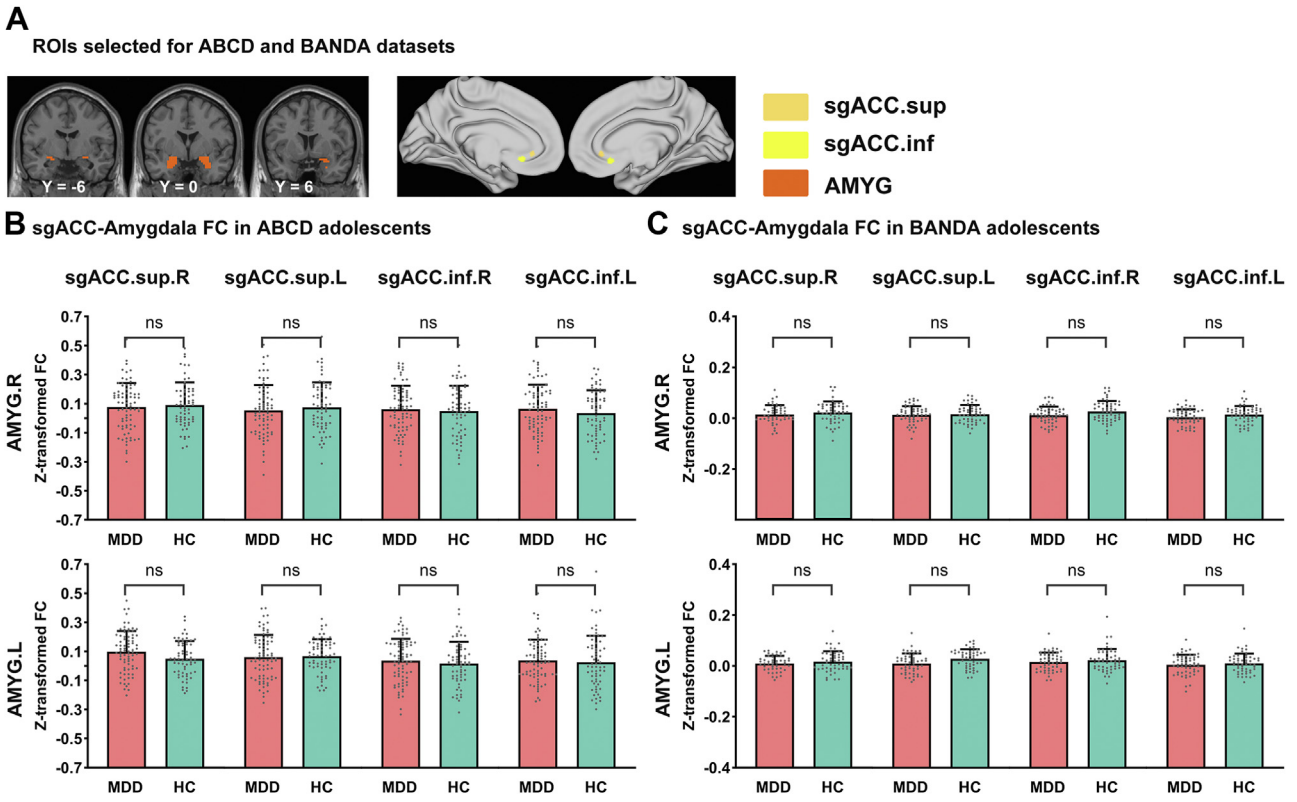

Figure 3. Comparison of subgenual anterior cingulate cortex (sgACC)–amygdala (AMYG) functional connectivity (FC) between adolescents with major depressive disorder (MDD) and healthy control participants (HCs) in the ABCD (Adolescent Brain Cognitive Development) and BANDA (Boston Adolescent Neuroimaging of Depression and Anxiety) datasets. **(A)** The selected sgACC and amygdala regions of interest (ROIs). **(B, C)** ROI-to-ROI analysis was performed in the **(B)** ABCD dataset and the **(C)** BANDA dataset. Bar plots show the averaged *z*-transformed sgACC-amygdala FC across participants, with error bars representing standard deviations. Scatter plots below each bar plot show the distribution of individual data points. inf, inferior; L, left; ns, not significant; R, right; sup, superior.

would be too small to be significantly detected. The immaturity and weakness of frontoamygdala connectivity in adolescence may explain the absence of detectable sgACC-amygdala FC abnormalities in adolescents with MDD. This indicates that sgACC-amygdala connectivity may provide limited insights when studying adolescents with MDD. However, in adults with MDD, we observed a significant reduction. This was only found in patients with recurrent MDD, not in the FEDN subgroup. Previous studies have reported reductions (51,52) as well as nonsignificant changes in sgACC-amygdala FC (53,54). Our results suggest that the impaired connectivity findings may be due to recurrent episodes and may serve as a specific condition marker for this subgroup. This finding highlights the necessity of distinguishing the heterogeneity within the broad category of depression, particularly emphasizing the importance of understanding the distinct neurobiological pathways in subgroups such as recurrent MDD. This differentiation could provide new insights into the specific neural mechanisms that underlie these subtypes. Taken together, our results indicate that adolescents with MDD may not share the same neurocognitive underpinnings as adults. This highlights the need for a transformative neurocognitive model tailored to adolescents. Consistent with this, depression typically begins during adolescence (55) and differs significantly in symptoms, genetic architecture, and treatment responses (56,57). Recognizing the unique developmental trajectory of depression can lead to more effective interventions and a better understanding of the disorder's progression from adolescence to adulthood.

Our resampling analysis highlights that small sample sizes in psychiatric neuroimaging can lead to misleading conclusions. A recent neuroimaging meta-analysis of 99 experiments on unipolar depression demonstrated a lack of significant convergence (58). Marek *et al.* (23) showed that replicating brain-behavior associations in mental health requires thousands of individuals. Together, these studies and our results argue for prioritizing the replication and rebuilding of robust neurocognitive models using large-scale datasets rather than designing new paradigms with limited samples. Furthermore, simply increasing the sample size and focusing on the shared characteristics of MDD is not enough. It is important to recognize that depression is a heterogeneous disorder that includes a wide range of subtypes with different characteristics (59). For example, a recent large-scale MDD study (N = 1801) found that even the best machine learning algorithms only achieved a diagnostic classification accuracy of 62% (60). This suggests that no individual-level biomarkers for MDD were detectable, probably due to the inherent heterogeneity.

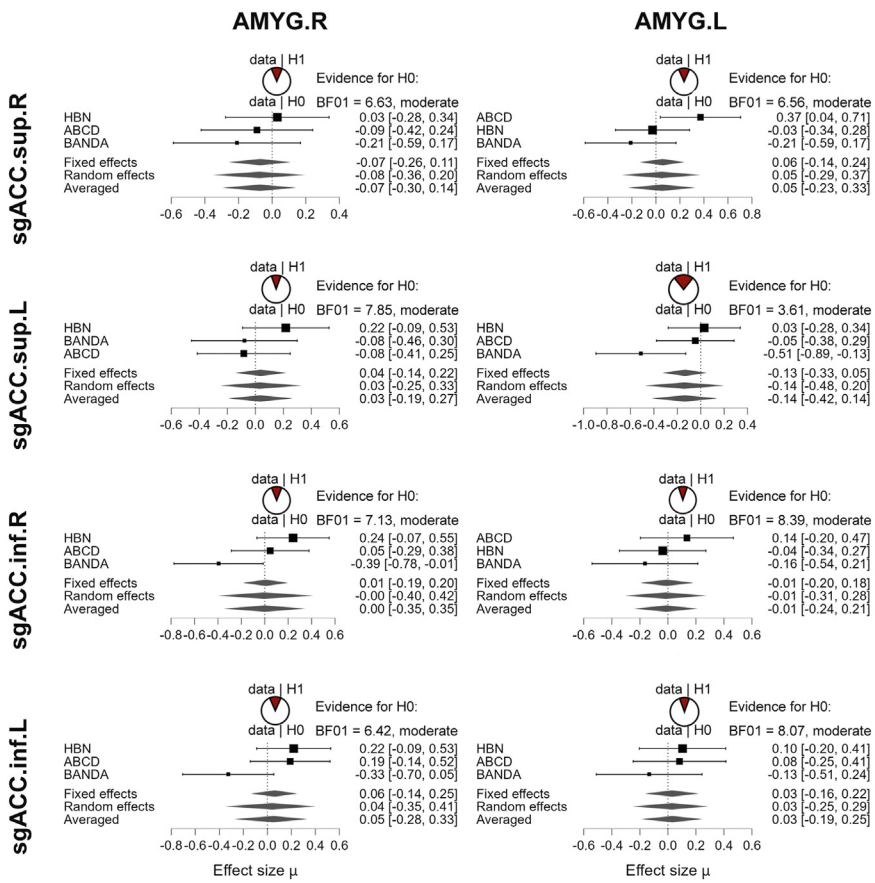

Figure 4. Meta-analysis of 3 adolescent datasets. The forest plot displays the observed effect sizes together with their corresponding confidence intervals for each study. The bottom section of the plot presents the meta-analytic estimates for fixed, random, and averaged models. The averaged estimate is calculated as a weighted mean of the fixed- and random-effect estimates, providing a more comprehensive result by balancing these models. The pie chart illustrates the Bayes factors (BFs). ABCD, Adolescent Brain Cognitive Development; AMYG, amygdala; BANDA, Boston Adolescent Neuroimaging of Depression and Anxiety; HBN, Healthy Brain Network; inf, inferior; L, left; R, right; sgACC, subgenual anterior cingulate cortex; sup, superior.

Therefore, research needs to identify reliable subtypes with large datasets to advance clinical translation and truly understand the heterogeneity within MDD. Our study has begun with a preliminary examination of the episodes. Future research should focus on improving clinical information in large databases to deepen our understanding of the underlying mechanisms behind episodes and refine the classification of subtypes.

Selecting an appropriate preprocessing pipeline for neuroimaging studies presents a significant challenge because different preprocessing choices can substantially influence group-level findings (61). Recent methodological studies have shown that preprocessing methods show only moderate agreement with each other (62), and no single approach provides perfect control of artifacts (61). Newer techniques like ICA-AROMA (independent component analysis–automatic removal of motion artifacts) demonstrate performance comparable to traditional approaches such as head motion parameter regression (63). When fields lack consensus on standard methods and accessible ground truths, reproducibility can be more of an ideal than a reality (62). To address these methodological concerns, we evaluated the robustness of our findings using multiple preprocessing approaches in the adolescent datasets. This included both comparing different types of pipelines (interpipeline) and testing different steps within pipeline (intrapipeline). Our results remained consistent across these variations. For the adult dataset, data-sharing policies limited our validation to within-pipeline comparisons only. In the future, we recommend that researchers routinely evaluate results across different preprocessing strategies and encourage data repositories to provide multiple preprocessing variants to enable such comprehensive methodological validation.

This study has several limitations. Despite utilizing 3 adolescent neuroimaging datasets, the total sample size is still less than half of that of the adult dataset. Based on observed effect sizes in all adult MDD (0.14) and recurrent MDD (0.28), a sample size of 632 for all MDD or 159 for recurrent MDD in each group would provide over 80% power at an alpha level of .05. It is possible that similar effects exist in the adolescent population, but our sample size may be too small to detect them. Despite our efforts to test reproducibility across multiple independent datasets, further replication will require larger datasets as they become available.

Family history of psychopathology and early-life adversity in HCs could be important confounders that obscure group differences. We attempted to address this by selecting HBN control participants without a parental history of depression and with ACE scores below 4, but the resulting sample was small, and no significant differences were found, despite an effect size of 0.61 in 1 comparison (13 recurrent MDD vs. 13 HC; $t = 1.5$, $p = .15$). Future large-scale studies with stricter inclusion criteria for HCs are needed to explore this issue further. Our study also suggests that the type of depressive episode significantly contributes to

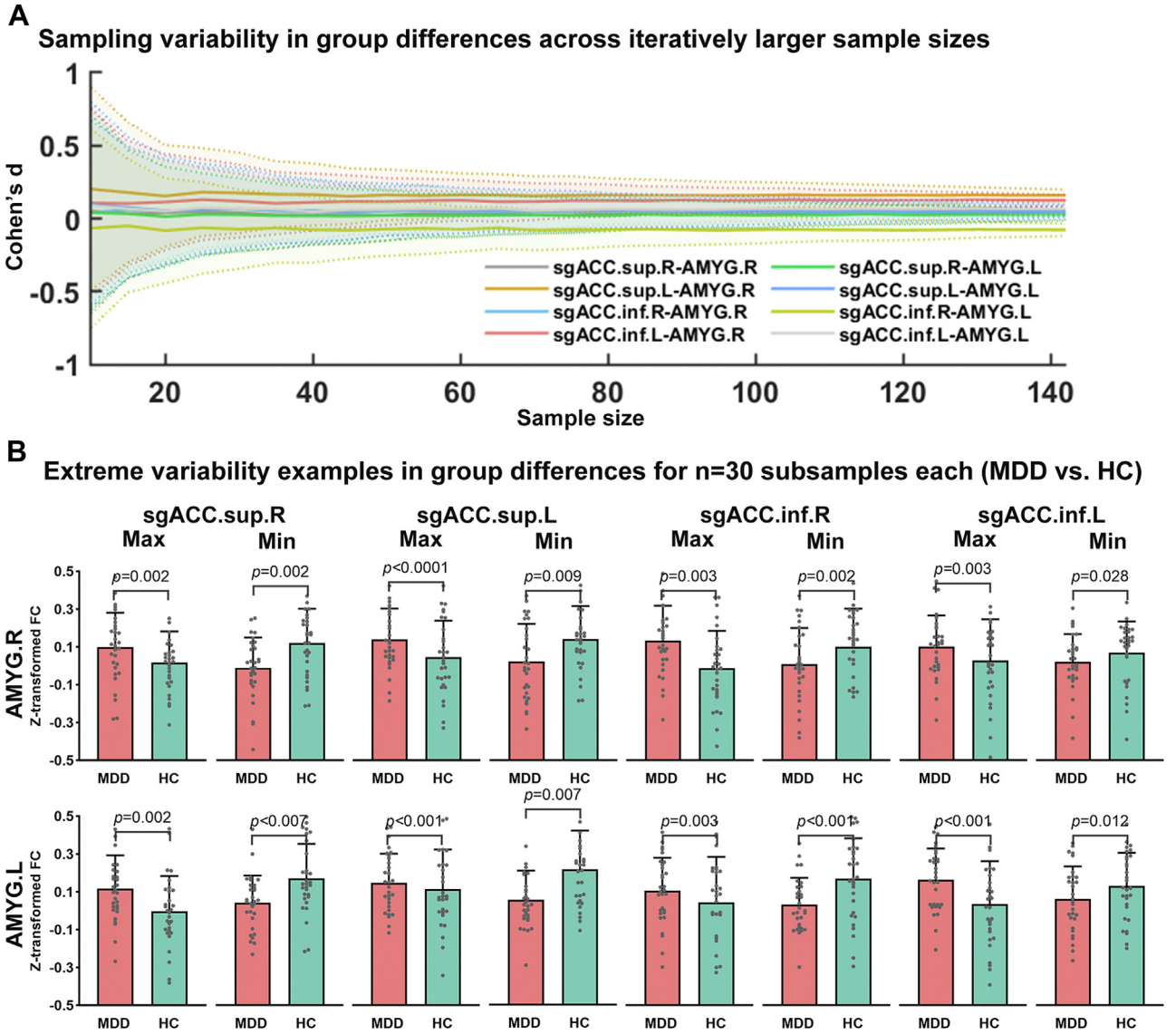

Figure 5. Sampling variability of group differences in subgenual anterior cingulate cortex (sgACC)–amygdala (AMYG) functional connectivity (FC) between adolescents with major depressive disorder (MDD) and healthy control participants (HCs). **(A)** Sampling variability assessed through 1000 resamples for each sample size across 27 bins, with increments of 5 participants per bin. The plot displays the distributions of Cohen's *d* for each ROI pair, which were calculated using linear mixed models. Solid lines denote the mean Cohen's *d* across 1000 resamples, while dashed lines indicate the standard deviation across these subsamples. **(B)** Illustration of variability with 2 extreme examples at $n = 30$ each for the MDD and HC groups. Each example shows extreme group differences where one group exhibits the most positive Cohen's *d* and the other group exhibits the most negative Cohen's *d* across resamples. *p* Values are derived from linear mixed models. inf, inferior; L, left; R, right; sup, superior.

the heterogeneity in brain connectivity seen in adults with MDD. However, the specific characteristics of these episodes and their relationship with sgACC-amygdala FC require more detailed investigation. There are likely other factors that contribute to MDD heterogeneity that need exploration. For example, evidence suggests that MDD associated with ACE differs in incidence and pathology compared with non-ACE MDD (64,65). In HBN, we identified only 8 valid ACE MDD cases, making it difficult to examine this subtype. Future large-scale functional MRI studies with rich and diverse subtype information are crucial for a thorough investigation.

Another limitation of our study concerns the age composition of our samples. While we aimed to maximize sample sizes given the scarcity of adolescent MDD neuroimaging data, this led to some age range considerations that need to be addressed. Although the World Health Organization defines adolescence as ages 10 to 19 years, our ABCD sample included participants ages 9 to 10 years, representing the transition between preadolescence and early adolescence. To address this limitation, we conducted a supplementary analysis in which we excluded participants younger than 10 years, which yielded consistent results (maximum $t = 1.49$, minimum $p = .14$). Similarly, while

most HBN participants fell within the conventional adolescent age range, some exceeded this range. To maintain clear developmental boundaries with our adult dataset while preserving adequate statistical power, we restricted our HBN analyses to participants ages 10 to 18 years. These age-restricted analyses remained consistent (116 MDD vs. 96 HCs, maximum $t = 1.24$, minimum $p = .22$). Although our findings appear robust across these age ranges, future research with larger samples should examine more specific developmental periods within adolescence to validate these patterns.

Finally, we acknowledge that a complete understanding of depression's neural circuitry requires examining connectivity between the amygdala and multiple prefrontal regions, particularly the dorsolateral prefrontal cortex, which plays a crucial role in cognitive control (66). While the dorsolateral prefrontal cortex–amygdala pathway likely represents another important circuit in depression alongside sgACC-amygdala connectivity (17), our study specifically focused on resolving inconsistencies in the literature regarding sgACC-amygdala connectivity. This targeted approach allowed us to conduct an in-depth analysis using large datasets and rigorous statistical methods. Future research should systematically investigate connectivity patterns between other prefrontal regions and the amygdala, as well as potential interactions among these circuits, to build a more comprehensive model of depression's neural mechanisms and inform targeted interventions.

Conclusions

The current study used multiple large-scale neuroimaging data to investigate the FC between sgACC and amygdala in MDD. We found different patterns across age groups: while adults with recurrent MDD showed reduced connectivity, adolescents with MDD showed similar levels of connectivity as HCs. This disparity suggests that mechanisms that are well documented in adults may not be uniformly applicable to younger populations, thus highlighting the need to refine neurocognitive models for adolescents and adults separately. Furthermore, our resampling analysis highlights the importance of large sample sizes to avoid inflated effects and potential misinterpretations in neuroimaging studies. These results emphasize the need for robust replication studies, which are critical to validating the results and providing a solid foundation for future research.

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ARTICLE INFORMATION

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