Archival Report

Biological Psychiatry

Association of Amygdala Development With Different Forms of Anxiety in Autism Spectrum Disorder

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ABSTRACT

BACKGROUND: The amygdala is widely implicated in both anxiety and autism spectrum disorder. However, no studies have investigated the relationship between co-occurring anxiety and longitudinal amygdala development in autism. Here, the authors characterize amygdala development across childhood in autistic children with and without traditional DSM forms of anxiety and anxieties distinctly related to autism.

METHODS: Longitudinal magnetic resonance imaging scans were acquired at up to four time points for 71 autistic and 55 typically developing (TD) children (~2.5–12 years, 411 time points). Traditional DSM anxiety and anxieties distinctly related to autism were assessed at study time 4 (~8–12 years) using a diagnostic interview tailored to autism: the Anxiety Disorders Interview Schedule-IV with the Autism Spectrum Addendum. Mixed-effects models were used to test group differences at study time 1 (3.18 years) and time 4 (11.36 years) and developmental differences (age-by-group interactions) in right and left amygdala volume between autistic children with and without DSM or autism-distinct anxieties and TD children.

RESULTS: Autistic children with DSM anxiety had significantly larger right amygdala volumes than TD children at both study time 1 (5.10% increase) and time 4 (6.11% increase). Autistic children with autism-distinct anxieties had significantly slower right amygdala growth than TD, autism–no anxiety, and autism–DSM anxiety groups and smaller right amygdala volumes at time 4 than the autism–no anxiety (−8.13% decrease) and autism–DSM anxiety (−12.05% decrease) groups.

CONCLUSIONS: Disparate amygdala volumes and developmental trajectories between DSM and autism-distinct forms of anxiety suggest different biological underpinnings for these common, co-occurring conditions in autism.

https://doi.org/10.1016/j.biopsych.2022.01.016

Symptoms of autism spectrum disorder (ASD or autism) include impaired social interaction and communication and restricted repetitive behaviors (1). It is estimated that 42% to 69% of individuals with autism also meet diagnostic criteria for a clinical anxiety disorder (2,3). Although the amygdala has been widely implicated in both anxiety and autism (4), only three studies have investigated associations between amygdala structure and anxiety within autism (5–7). No studies of autism have investigated the development of the amygdala longitudinally in relation to anxiety or the associations between different forms of anxiety and the amygdala in autism.

Clinical anxiety can manifest in several forms, including generalized anxiety disorder, separation anxiety, specific phobia, and social phobia (henceforth referred to as DSM anxiety) (1). However, distinguishing anxiety from ASD symptoms is challenging (2,8). Recently developed tools recognize classically defined symptoms of anxiety (e.g., anticipatory anxiety, fearful avoidance) that manifest within contexts that are somewhat unique to autism. These symptoms would not be captured by traditional assessments (9). Such autism-distinct anxieties (henceforth referred to as distinct anxiety) include fears related to social confusion (as opposed to fear of negative evaluation, which is required for a DSM diagnosis of social phobia), uncommon phobias (e.g., specific sounds, facial features), excessive worry related to losing access to materials related to circumscribed interests, and fears of change (3).

Research implicates disruption of the amygdala and its network of connections in the emergence of anxiety (4,10). However, studies of amygdala volume in children and adolescents have proved inconsistent, reporting both larger (11–15) and smaller (16–20) volumes being associated with anxiety. Others found no associations (21,22) or associations dependent on sex (23,24) or anxiety type (25). These studies used a range of anxiety assessments including dimensional measures that are nonspecific to the domains of anxiety described in the DSM (e.g., the Child Behavior Checklist [CBCL] and Screen for Child Anxiety Related Emotional

ISSN: 0006-3223

Disorders) (26,27) and grouping individuals across DSM anxiety domains according to diagnostic interviews (e.g., Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version) (28).

Altered amygdala structure and function has also been proposed to underlie social deficits observed in ASD (29–33). Magnetic resonance imaging (MRI) studies of children and adolescents with autism have reported larger (31,33–38), smaller (39,40), and no (41–43) differences in amygdala volumes. In developmental terms, converging lines of evidence using MRI and histological (44) methodologies suggest that the amygdala in autism exhibits an initial volumetric overgrowth during early childhood that is then followed by a slowed trajectory of growth into adulthood.

Despite high rates of anxiety in ASD, only three studies have investigated associations of amygdala structure with anxiety in autism. The first found enlarged right amygdala volumes to be associated with increased scores on the CBCL anxious/depressed subscale in children with autism (~4–15 years) (6). A second recent study found no association between the amygdala and CBCL measures in the Autism Brain Imaging Data Exchange dataset (7). The third compared two groups of autistic children (~7.5–17.5 years) with and without a DSM anxiety diagnosis (5,45) and found that autistic children with clinical DSM anxiety had smaller right amygdala volumes than those without DSM anxiety (5). Thus, two studies indicate a relationship between anxiety and right amygdala volume but, taken together, provide contradictory evidence as to whether amygdala volume is larger, smaller, or unrelated to anxiety in autism. Determining the relationship of amygdala volume with autistic development, with and without co-occurring anxiety, may identify biological correlates specific to these conditions and thus provide a valuable prodromal biological marker of anxiety in autism.

In this study, we characterize and test for anxiety-associated differences in the trajectories of volumetric development of the amygdala across childhood (~2.5–12 years), as well as amygdala volumes during early (~3 years) and late (~11 years) childhood, in autistic children with and without anxiety disorders. We used clinical interviews for both DSM and distinct anxiety to test if these forms of anxiety had different associations with amygdala volume and development in autism. We hypothesize that DSM anxiety will be associated with larger right amygdala volumes and faster development. We further expect that anxieties distinctly related to autism will be associated with effects on the amygdala, but because this has not been previously investigated, we have no a priori prediction as to the nature of this relationship.

METHODS AND MATERIALS

Participants

Participants were enrolled in the UC Davis Medical Investigation of Neurodevelopmental Disorders Institute Autism Phenome Project, a longitudinal study consisting of MRI scanning at four time points, enrollment/baseline at 24 to 42 months of age (time 1), follow-up at annual intervals for two time points (time 2 and 3), and ~9 to 12 years of age (time 4). This study included data from all participants who completed MRI and anxiety assessments at time 4 (Table 1 and Figure S1). At time 1, ASD diagnosis was confirmed using the Autism Diagnostic Observation Schedule (ADOS)–Generic (46) or ADOS-2 (47), the Autism Diagnostic Interview–Revised (48), and DSM-IV-Text Revision criteria (49). At time 1, developmental quotient was assessed using the Mullen Scales of Early Learning (50). IQ was assessed at time 4 using the Differential Ability Scales, second edition (51). Informed consent was obtained from the parent or guardian of each participant. All aspects of the study protocol were approved by the University of California Davis Institutional Review Board. See the Supplement for details.

Table 1. Time 4 Participant Demographics

Measure	ASD, *n* = 71	TD, *n* = 55
Sex, Female/Male, *n*	14/57	21/34
Age, Years, Mean (SD)	11.18 (1.39)	11.34 (0.67)
IQ, Mean (SD)	80.54 (28.77)	111.58 (12.82)
ADOS CSS, Mean (SD)	7.5 (1.96)	–
ADOS Social CSS, Mean (SD)	7.3 (1.73)	–
ADOS Repetitive Restricted Behavior CSS, Mean (SD)	7.8 (1.92)	–
ADIS-DSM Anxiety, *n* (%)	32 (45%)	–
General Anxiety Disorder	12 (17%)	–
Separation Anxiety	7 (10%)	–
Social Phobia	5 (7%)	–
Specific Phobia	27 (38%)	–
ADIS-DIST Anxiety, *n* (%)	28 (39%)	–
Other Social Fear	5 (7%)	–
Atypical Phobia	10 (14%)	–
Special Interest Fear	4 (6%)	–
Fear of Change	15 (21%)	–
Only ADIS-DSM Anxiety, *n* (%)	15 (21%)	–
Only ADIS-DIST Anxiety, *n* (%)	11 (15%)	–
Both DSM and DIST Anxiety, *n* (%)	17 (24%)	–
Neither DSM or DIST Anxiety, *n* (%)	28 (39%)	55 (100%)
Scans per Participant, Mean (SD)	3.08 (0.90)	3.49 (0.74)
Scans at Time 1	67	53
Scans at Time 2	47	45
Scans at Time 3	34	39
Scans at Time 4	71	55
Total Number of Scans	219	192
Number of Participants With Four Scans	32	35
Number of Participants With Three Scans	13	12
Number of Participants With Two Scans	26	8

IQ and ADOS CSSs are given for study time 4.

ADIS-DIST, Anxiety Disorders Interview Schedule–Autism Spectrum Addendum autism-distinct anxieties; ADIS-DSM, Anxiety Disorders Interview Schedule–DSM Anxieties; ADOS, Autism Diagnostic Observation Schedule; ASD, autism spectrum disorder; CSS, calibrated severity score; DIST, distinct; TD, typically developing.

Anxiety Assessment

Anxiety was assessed at time 4 using the Anxiety Disorders Interview Schedule-IV-Parent Interview (ADIS) (45) with the

Autism Spectrum Addendum (ASA), a semistructured diagnostic interview designed to differentiate between anxiety and autism symptoms (9). ADIS includes modules relating to separation, social, specific phobia, and generalized anxiety disorders (DSM anxiety). In addition, the ADIS-ASA assesses forms of anxiety distinctly related to autism, including idiosyncratic fears, fear relating to social confusion, special interest fears, and fears of change (distinct anxiety). For each module, a clinical severity rating (CSR) ranging from 0 (no interference) to 8 (severe interference) is prescribed, with 4 being the cutoff for clinical determination of significant interference. Rates of anxiety and assessment within this cohort have been described in detail previously (3). Additional measures were acquired at both time 1 and time 4 including the CBCL (27), Repetitive Behavior Scale (RBS) (52), Short Sensory Profile (SSP) (53), SSP 2 (54), and Social Responsiveness Scale (SRS) (55). See the Supplement for details.

MRI Acquisition and Region of Interest Approach

All T1-weighted structural MRI scans were acquired at the UC Davis Imaging Research Center on a 3T Siemens Trio (Siemens Corp.). Time 1 to time 3 scans were acquired during natural nocturnal sleep (56). Time 4 scans were acquired while participants were awake using previously described methods (57). Distortion-corrected, anonymized, and defaced images were uploaded to MRICloud (https://mricloud.org) (58) and segmented into 289 anatomically defined regions using a multiatlas approach (59). Volumes from the left and right amygdala and hemispheres were exported for statistical modeling. See the Supplement for details.

Statistical Modeling

Linear mixed-effects modeling was performed using R (version 3.6; R Core Team, 2019). We first compared differences in amygdala volumes and development associated with anxiety between autistic children with (ADIS-DSM CSR $\geq$ 4) and without DSM anxiety and with (ADIS-ASA distinct anxiety CSR $\geq$ 4) and without distinct anxiety. Models included all autistic individuals with categorical factors for DSM and distinct anxiety; sex, age in months, and hemispheric volume as covariates; and individual as a random effect with age as a random slope. Here, overlapping anxieties are accounted for by separate DSM and distinct categorical variables.

A secondary analysis was conducted to compare amygdala volumes and development between five groups of interest: ASD with 1) only DSM anxiety, 2) only distinct anxiety, 3) both DSM and distinct anxiety, and 4) no clinical anxiety and 5) typically developing (TD) children without anxiety. Effects of interest for all analyses were mean group differences at the average age between study time 1 (38.11 mo/3.18 years) and time 4 (136.30 mo/11.36 years) and differences in developmental trajectories between groups (age-by-group interactions). Age was modeled by selecting from a range of polynomial terms (-3, -2, -1, -0.5, 0.5, 1, 2, 3) that returned the lowest log likelihood (left = 0.5, right = -0.5) (60). Analyses were repeated separately for the left and right amygdala. Results for each analysis were corrected for using false discovery rate (FDR) (61) within each hemisphere.

RESULTS

Demographics and Anxiety

Children with autism and TD children did not significantly differ ($p > .05$, two-tailed t test) for age of scan at time 4 and age of ADIS-ASA assessment. Compared with the TD sample, children with autism had significantly lower overall IQ scores ($p < .001$) and fewer longitudinal time points per individual ($p = .008$). Compared with the TD sample, the ASD sample also contained a significantly higher proportion of males ($\chi^2_1 = 4.39$, $p = .03$).

Within the autism sample, 61% (43/71) of participants met diagnostic criteria for at least one form of clinical anxiety, with 45% (32/71) meeting criteria for one or more DSM anxieties, 39% (28/71) meeting criteria for one or more distinct anxieties, and 24% (16/71) meeting criteria for both a DSM and distinct anxiety. In total, 39% (28/71) of children with autism did not reach clinical thresholds for any anxiety type (Table 1). No significant differences (analysis of variance [ANOVA] $p > .05$) were found between ASD groups with 1) only DSM anxiety, 2) only distinct anxiety, 3) both DSM and distinct anxiety, and 4) no clinical anxiety in terms of time 4 scan age, number of MRI time points, IQ, or ADOS calibrated severity score. No significant differences were found for the male-to-female ratio between the four ASD groups ($\chi^2_1 = 5.10$, $p = .16$).

Differences in Amygdala Volumes Associated With Clinical DSM Anxieties and Autism-Distinct Anxieties

Compared with autistic children without DSM anxieties, those with DSM anxieties (CSR $\geq$ 4) had larger right amygdala volumes at time 4 (4.94% mean increase, $p = .017$, FDR $p = .038$); no statistically significant differences were observed in the left amygdala. Compared with autistic children without distinct anxiety, those with distinct anxiety (CSR $\geq$ 4) showed smaller right and left amygdala volumes at both time 1 (right: -4.02% mean decrease, $p = .019$, FDR $p = .38$; left: -5.54%, $p = .006$, FDR $p = .018$), and time 4 (right: -4.91% mean decrease, $p = .001$, FDR $p = .038$; left: -7.14%, $p = .002$, FDR $p = .012$). Autistic children with autism-distinct anxiety also showed a statistical trend ($p = .049$, FDR $p = .074$) of slower right amygdala development than autistic children without distinct anxiety (Figure 1 and Table 2).

Differences Between Autistic Children With Only DSM Anxiety, Only Distinct Anxiety, Both DSM and Distinct Anxieties, and No Clinical Anxiety and TD Children

The above analyses indicate differential associations of amygdala volume between autistic children with DSM (increased volume) and distinct (decreased volume) anxieties. Given the overlap within participants of DSM and distinct anxiety diagnoses and to investigate the relationship of amygdala volume with TD children, we conducted a secondary analysis of amygdala volume and development between five groups of interest: 1) autistic children with only DSM anxieties (ASD-DSM) ($n = 16$), 2) autistic children with only distinct anxieties (ASD-distinct) ($n = 11$), 3) autistic children with both DSM and distinct anxieties (ASD-both anxieties) ($n = 16$), 4)

autistic children with no clinical anxiety (ASD-no anxiety) (n = 28), and 5) TD children without clinical anxiety (n = 55) (Figure 2, Table 3, and Table S3).

The ASD-DSM group had significantly larger right amygdala volumes at both study time 1 (5.10% mean increase, p = .008, FDR, p = .038) and time 4 (6.11% mean increase, p = .009, FDR, p = .038) compared with the TD group. No differences in amygdala developmental trajectories between the ASD-DSM group and ASD-no anxiety or TD groups were observed. The ASD-distinct group was found to have a significantly altered developmental trajectory of the right amygdala marked by slower growth compared with the TD (p = .009, FDR p = .038), ASD-no anxiety (p = .009, FDR p = .038), and ASD-DSM (p = .006, FDR p = .038) groups. Slower right amygdala development in the ASD-distinct group resulted in significantly smaller right amygdala volume at time 4 (11.36 years) compared with the ASD-no anxiety (−8.13% mean decrease, p = .004, FDR p = .038) and ASD-DSM (−12.05% mean decrease, $p < .001$, FDR p = .010) groups. No results for the left amygdala reached statistical significance after FDR correction.

Effects of IQ and Autism Severity on Anxiety Group Differences

No significant differences in IQ or ADOS calibrated severity score measures were found between the four autism groups (ANOVA $p > .05$); inclusion of these variables within models did not change statistically significant differences between the groups, and they were not significantly associated with amygdala volumes. Compared with TD children, children with autism showed nonsignificant increases in left (t_{125} = 1.85, p = .066) and right (t_{125} = 1.76, p = .079) hemispheric volumes. Using the same five-group mixed-effect model structure, no significant differences between groups in either left or right hemisphere volumes were observed at time 1 or time 4 or developmentally (i.e., age-by-group interactions), suggesting that our findings are not due to global hemispheric effects (Table S1). No significant age-by-group-by-baseline amygdala volume effects between the distinct anxiety and other groups were found (Table S2).

Post Hoc Behavioral Analyses

To determine if differences in behaviors associated with autism could explain amygdala differences between the DSM and distinct anxiety groups, we performed exploratory post hoc analyses to investigate differences between the ASD-DSM, ASD-distinct, ASD-both anxieties, and ASD-no anxiety groups in measures including the CBCL subscales (27), RBS (52), SSP (time 1) and SSP 2 (time 4) (53,54), and SRS (55). At time 4, the ASD-DSM and ASD-both anxieties groups had significantly higher CBCL anxious depressed and DSM anxiety problems t scores compared with the ASD-distinct and ASD-no anxiety groups (ANOVA, $p < .05$; Tukey honestly significant difference, $p < .05$). These results indicate concordance between CBCL and ADIS assessments of DSM anxiety. The ASD-DSM group also showed significantly higher CBCL internalizing behavior scores compared with the ASD-distinct and ASD-no anxiety groups (ANOVA, $p < .05$; Tukey honestly significant difference, $p < .05$). In addition, at time 4, autism groups with anxiety (of any kind) had higher measures of CBCL thought problems, total SSP 2, and total RBS scores than the ASD-no anxiety group; however, Tukey honestly significant difference tests showed these elevated scores to be significant for only the ASD-both anxieties group (CBCL thought problems and RBS) or the ASD-both and ASD-DSM groups (SSP 2) compared with the ASD-no anxiety group (ANOVA, $p < .05$; Tukey honestly significant difference, $p < .01$). These findings indicate that autistic children with DSM and/or distinct anxiety experience elevated levels of sensory sensitivities, unusual thought processes, and repetitive and restricted behaviors—all potential indications of elevated distress in anxious children with autism (62). The ASD-both anxieties group also had significantly higher time 4 SRS scores than the ASD-no anxiety group (ANOVA, p = .02; Tukey honestly significant difference, $p < .01$). No significant

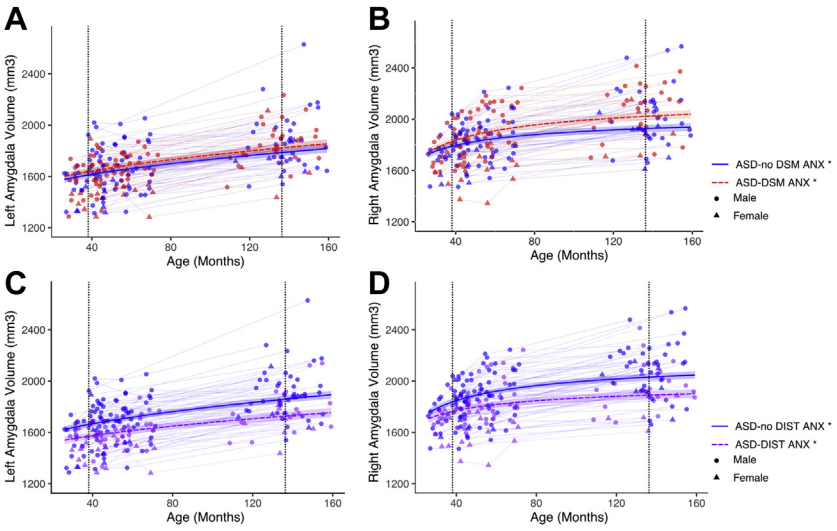

Figure 1. Associations between amygdala volume and DSM and autism-distinct anxieties. Longitudinal development of **(A)** left and **(B)** right amygdala volumes are plotted for autistic children with and without DSM anxiety (ASD-DSM ANX and ASD-no DSM ANX, respectively). Compared with autistic children without DSM anxiety, those with DSM anxiety showed larger right amygdala volumes at both time 1 (5.87% larger, p = .005, false discovery rate–adjusted p = .048) and time 4 (6.15% larger, p = .005, false discovery rate–adjusted p = .048). Compared with autistic children without autism-distinct anxieties (ASD-no DIST ANX), those with distinct anxieties (ASD-DIST ANX) showed statistical trends ($p < .05$, false discovery rate–adjusted $p > .05$) of **(C)** smaller left amygdala volumes at both time 1 (−5.44% smaller, p = .024) and time 4 (−6.75% smaller, p = .011) and **(D)** right amygdala volumes at time 4 (−5.75% mean decrease, p = .020) compared with ASD-no DIST ANX. Vertical dotted lines indicate mean ages at study time 1 (38.11 mo/3.18 years) and time 4 (136.30 mo/11.36 years). Note that here, some individuals within the DSM/DIST ANX groups have duel DSM and distinct anxiety diagnoses; in addition, individuals within the no DSM group may have distinct clinical severity rating scores ≥4 (and vice versa for the no DIST group). These overlapping anxieties are accounted for by modeling separate DSM and distinct categorical variables.

Table 2. Amygdala Associations With Autism-Distinct and DSM Anxiety in Autism

Measure	Coef.	SE	*df*	*t*	*p*	FDR *p*	% Diff. (95% CI)
Left Amygdala							
Intercept	1649.86	22.99	144.00	71.77	<.001	–	–
Scan age^0.5	35.03	5.22	144.00	6.71	<.001	–	–
Sex (male reference)	−47.14	40.28	67.00	−1.17	.246	–	–
Hemisphere	0.00	0.00	144.00	4.64	<.001	–	–
T1[a]: ASD-no DSM vs. ASD-DSM	−92.05	32.31	67.00	−2.85	.006	.018	5.54 (−0.03 to 10.82)
T1: ASD-no DIST vs. ASD-DIST	26.69	31.18	67.00	0.86	.395	.474	−1.66 (3.86 to −7.48)
T4[a]: ASD-no DSM vs. ASD-DSM	−132.67	40.60	67.00	−3.27	.002	.012	7.14 (0.91 to 13.02)
T4: ASD-no DIST vs. ASD-DIST	36.18	39.54	67.00	0.91	.363	.474	−2.03 (4.25 to −8.70)
Age-by-group: ASD-no DIST vs. ASD-DIST	−7.38	6.02	144.00	−1.23	.222	.444	–
Age-by-group: ASD-no DSM vs. ASD-DSM	1.72	5.90	144.00	0.29	.771	.771	–
Right Amygdala							
Intercept	1811.58	22.13	144.00	81.88	<.001	–	–
Scan age^−0.5	−2171.38	379.38	144.00	−5.72	<.001	–	–
Sex (male reference)	−19.84	39.23	67.00	−0.51	.615	–	–
Hemisphere	0.00	0.00	144.00	5.89	<.001	–	–
T1[a]: ASD-no DSM vs. ASD-DSM	−74.47	31.08	67.00	−2.40	.019	.038	4.02 (−0.92 to 8.73)
T1: ASD-no DIST vs. ASD-DIST	37.26	29.96	67.00	1.24	.218	.218	−2.07 (2.80 to −7.19)
T4[a]: ASD-no DSM vs. ASD-DSM	−140.37	40.03	67.00	−3.51	.001	.006	6.91 (1.33 to 12.22)
T4[a]: ASD-no DIST vs. ASD-DIST	95.25	39.02	67.00	2.44	.017	.038	−4.94 (0.86 to −11.09)
Age-by-group[b]: ASD-no DIST vs. ASD-DIST	863.31	434.14	144.00	1.99	.049	.074	–
Age-by-group: ASD-no DSM vs. ASD-DSM	−759.75	425.12	144.00	−1.79	.076	.091	–

Regression table for models of left and right amygdala volume between children with ASD with and without DSM anxiety (ASD-DSM, ASD-no DSM) and with and without distinct anxiety (ASD-DIST, ASD-no DIST). Effects of interest are shown for mean age of T1 (38.11 mo/3.18 years) and T4 (136.30 mo/11.36 years) as well as developmental differences (age-by-group interactions). Note that some individuals within the DSM/DIST anxiety groups have dual DSM and distinct anxiety diagnoses; in addition, individuals within the no DSM group may have distinct CSR scores ≥4 (and vice versa for the no DIST group). These overlapping anxieties are accounted for by modeling separate DSM and distinct categorical variables. Reference group is always indicated first.

% Diff, percent difference; ASD, autism spectrum disorder; ASD-DIST, autism spectrum disorder with distinct anxiety; ASD-DSM, autism spectrum disorder with DSM anxiety; ASD-no DIST, autism spectrum disorder without distinct anxiety; ASD-no DSM, autism spectrum disorder without DSM anxiety; Coef., coefficient; CSR, clinical severity rating; FDR *p*, false discovery rate–adjusted *p* value; T1, time 1; T4, time 4.

[a]Effects of interest FDR $p < .05$.

[b]Effects of interest $p < .05$.

differences between groups in CBCL subscales, total SRS, RBS, or SSP were observed at time 1 (Tables S3–S6).

DISCUSSION

The primary aim of this study was to characterize amygdala volume and development across childhood in relation to different types of anxiety in children with autism. We examined two categories of problematic anxiety in ASD: traditional DSM anxieties and anxieties distinct to autism contexts (2,3). Initial analyses comparing autistic children with and without these anxieties revealed DSM anxiety to be associated with enlarged amygdala volumes and distinct anxieties to be associated with smaller amygdala volumes. A second analysis comparing TD

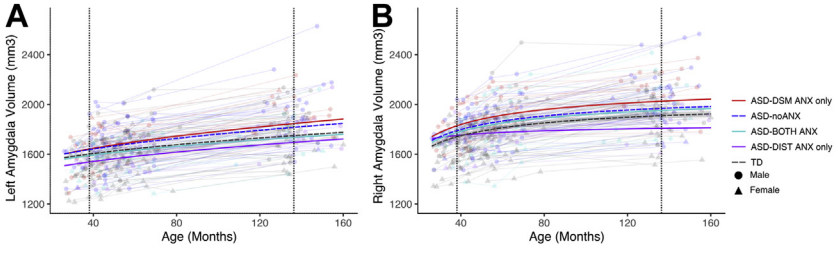

Figure 2. Associations between amygdala volume and different forms of anxiety in autism spectrum disorder (ASD). Longitudinal development of **(A)** left and **(B)** right amygdala volumes are plotted for autistic children with only DSM anxiety (ASD-DSM ANX), with only autism-distinct anxiety (ASD-DIST ANX), with both DSM and distinct anxieties (ASD-BOTH ANX) and without anxiety (ASD-no ANX) and typically developing (TD) children without anxiety. Vertical dotted lines indicate mean ages at study time 1 (38.11 mo/3.18 years) and time 4 (136.30 mo/11.36 years). ASD-DSM ANX was found to have significantly larger right amygdala volumes at both study time 1 (4.59% larger, $p = .012$, false discovery rate [FDR]–adjusted $p = .044$) and time 4 (5.19% larger, $p = .01$, FDR-adjusted $p = .044$) compared with TD children. ASD-DIST ANX had a significantly altered developmental trajectory of the right amygdala marked by slower growth compared with the TD ($p = .009$, FDR-adjusted $p = .044$), ASD-no ANX ($p = .009$, FDR-adjusted $p = .044$), and ASD-DSM ANX ($p = .007$, FDR-adjusted $p = .044$) groups, which resulted in significantly smaller right amygdala volumes at time 4 compared with the ASD-no ANX (−8.85% smaller, $p = .004$, FDR-adjusted $p = .044$), ASD-DSM (−11.04% smaller, $p < .001$, FDR-adjusted $p = .013$), and ASD-both anxieties (−8.26% smaller, $p = .011$, FDR-adjusted $p = .044$) groups.

Table 3. Amygdala Associations Between ASD Groups With Different Anxiety Types and TD Group

Measure	Coef.	SE	*df*	*t*	*p*	FDR *p*	% Diff. (95% CI)
Left Amygdala							
Intercept	1625.44	19.27	277	84.33	<.001	–	–
Scan age^0.5	26.95	3.63	277	7.42	<.001	–	–
Sex (male reference)	−83.57	26.05	122	−3.21	.002	–	–
Hemisphere	0.00	0.00	277	7.29	<.001	–	–
T1: TD vs. ASD-no ANX	35.58	29.37	122	1.21	.228	.474	−2.22 (2.85 to −7.52)
T1: TD vs. ASD-DIST ANX	−63.53	41.79	122	−1.52	.131	.393	3.97 (−2.94 to 10.59)
T1: TD vs. ASD-DSM ANX	40.29	34.77	277	1.16	.248	.474	−2.52 (3.31 to −8.60)
T1: TD vs. ASD-DSM and DIST ANX	−12.22	33.21	277	−0.37	.713	.832	0.76 (−5.07 to 6.35)
T1[a]: ASD-no ANX vs. ASD-DIST ANX	−99.11	44.87	122	−2.21	.029	.164	6.05 (−1.52 to 13.20)
T1: ASD-no ANX vs. ASD-DSM ANX	4.71	38.10	277	0.12	.902	.933	−0.29 (6.13 to −7.10)
T1: ASD-no ANX vs. ASD-DSM and DIST ANX	−47.80	37.49	277	−1.28	.203	.469	2.92 (−3.62 to 9.09)
T1[a]: ASD-DIST ANX vs. ASD-DSM ANX	103.82	48.40	277	2.15	.033	.164	−6.75 (1.96 to −16.36)
T1: ASD-DIST ANX vs. ASD-DSM and DIST ANX	51.31	47.85	277	1.07	.285	.474	−3.34 (5.05 to −12.58)
T1: ASD-DSM ANX vs. ASD-DSM and DIST ANX	−52.51	39.31	278	−1.34	.183	.457	3.20 (−4.11 to 9.99)
T4: TD vs. ASD-no ANX	66.48	36.17	122	1.84	.069	.294	−3.80 (2.00 to −9.89)
T4: TD vs. ASD-DIST ANX	−55.01	51.06	122	−1.08	.283	.474	3.14 (−4.70 to 10.61)
T4[a]: TD vs. ASD-DSM ANX	96.46	44.39	277	2.17	.031	.164	−5.51 (1.34 to −12.71)
T4: TD vs. ASD-DSM and DIST ANX	−15.00	41.96	277	−0.36	.721	.832	0.86 (−5.91 to 7.30)
T4[a]: ASD-no ANX vs. ASD-DIST ANX	−121.49	54.75	122	−2.22	.028	.164	6.69 (−1.71 to 14.56)
T4: ASD-no ANX vs. ASD-DSM ANX	29.98	48.39	277	0.62	.536	.699	−1.65 (5.69 to −9.49)
T4: ASD-no ANX vs. ASD-DSM and DIST ANX	−81.48	46.76	277	−1.74	.083	.310	4.49 (−2.89 to 11.40)
T4[a]: ASD-DIST ANX vs. ASD-DSM ANX	151.47	60.34	277	2.51	.013	.164	−8.94 (1.02 to −17.97)
T4: ASD-DIST ANX vs. ASD-DSM and DIST ANX	40.01	59.05	277	0.68	.499	.699	−2.36 (7.01 to −12.81)
T4[a]: ASD-DSM ANX vs. ASD-DSM and DIST ANX	−111.46	51.43	278	−2.17	.031	.164	6.04 (−2.21 to 13.62)
Age-by-group: TD vs. ASD-no ANX	5.61	5.22	277	1.08	.283	.474	–
Age-by-group: TD vs. ASD-DIST ANX	1.55	7.45	277	0.21	.835	.895	–
Age-by-group: TD vs. ASD-DSM ANX	10.21	6.51	277	1.57	.118	.393	–
Age-by-group: TD vs. ASD-DSM and DIST ANX	−0.51	6.30	277	−0.08	.936	.936	–
Age-by-group: ASD-no ANX vs. ASD-DIST ANX	−4.07	8.05	277	−0.51	.614	.767	–
Age-by-group: ASD-no ANX vs. ASD-DSM ANX	4.59	7.18	277	0.64	.523	.699	–
Age-by-group: ASD-no ANX vs. ASD-DSM and DIST ANX	−6.12	6.99	277	−0.88	.382	.573	–
Age-by-group: ASD-DIST ANX vs. ASD-DSM ANX	8.66	8.94	277	0.97	.333	.526	–
Age-by-group: ASD-DIST ANX vs. ASD-DSM and DIST ANX	−2.05	8.79	277	−0.23	.815	.895	–
Age-by-group: ASD-DSM ANX vs. ASD-DSM and DIST ANX	−10.72	8.01	278	−1.34	.182	.457	–
Right Amygdala							
Intercept	1763.83	18.38	277	95.96	<.001	–	–
Scan age^−0.5	−2219.73	273.45	277	−8.12	<.001	–	–
Sex (male reference)	−83.95	25.18	122	−3.33	.001	–	–
Hemisphere	0.00	0.00	277	8.10	<.001	–	–
T1: TD vs. ASD-no ANX	49.78	27.90	122	1.78	.077	.210	−2.86 (1.66 to −7.56)
T1: TD vs. ASD-DIST ANX	11.06	39.92	122	0.28	.782	.835	−0.64 (5.34 to −6.85)
T1[b]: TD vs. ASD-DSM ANX	88.78	33.17	277	2.68	.008	.038	−5.10 (0.13 to −10.54)
T1: TD vs. ASD-DSM and DIST ANX	34.09	31.79	277	1.07	.284	.474	−1.96 (3.11 to −7.22)
T1: ASD-no ANX vs. ASD-DIST ANX	−38.71	42.90	122	−0.90	.369	.553	2.16 (−4.57 to 8.55)
T1: ASD-no ANX vs. ASD-DSM ANX	39.00	36.36	277	1.07	.284	.474	−2.18 (3.52 to −8.18)
T1: ASD-no ANX vs. ASD-DSM and DIST ANX	−15.68	35.96	277	−0.44	.663	.796	0.88 (−4.93 to 6.40)
T1: ASD-DIST ANX vs. ASD-DSM ANX	77.72	46.33	277	1.68	.095	.236	−4.44 (2.88 to −12.38)
T1: ASD-DIST ANX vs. ASD-DSM and DIST ANX	23.03	45.94	277	0.50	.616	.796	−1.31 (5.77 to −9.01)
T1: ASD-DSM ANX vs. ASD-DSM and DIST ANX	−54.68	38.51	278	−1.42	.157	.313	2.99 (−3.32 to 8.92)
T4: TD vs. ASD-no ANX	58.92	35.88	122	1.64	.103	.238	−3.09 (2.19 to −8.61)
T4[a]: TD vs. ASD-DIST ANX	−101.14	50.72	122	−1.99	.048	.145	5.30 (−1.78 to 12.06)
T4[b]: TD vs. ASD-DSM ANX	116.74	44.11	277	2.65	.009	.038	−6.11 (0.15 to −12.66)

Table 3. Continued

Measure	Coef.	SE	*df*	*t*	*p*	FDR *p*	% Diff. (95% CI)
T4: TD vs. ASD-DSM and DIST ANX	42.66	41.50	277	1.03	.305	.481	−2.23 (3.68 to −8.42)
T4[b]: ASD-no ANX vs. ASD-DIST ANX	−160.07	54.42	122	−2.94	.004	.038	8.13 (0.47 to 15.34)
T4: ASD-no ANX vs. ASD-DSM ANX	57.82	48.13	277	1.20	.231	.433	−2.94 (3.87 to −10.17)
T4: ASD-no ANX vs. ASD-DSM and DIST ANX	−16.26	46.34	277	−0.35	.726	.835	0.83 (−6.02 to 7.28)
T4[b]: ASD-DIST ANX vs. ASD-DSM ANX	217.88	60.02	277	3.63	<.001	.010	−12.05 (−2.58 to −20.36)
T4[b]: ASD-DIST ANX vs. ASD-DSM and DIST ANX	143.80	58.60	277	2.45	.015	.053	−7.95 (1.05 to −17.92)
T4: ASD-DSM ANX vs. ASD-DSM and DIST ANX	−74.08	51.22	278	−1.45	.149	.313	3.66 (−3.85 to 10.61)
Age-by-group: TD vs. ASD-no ANX	−119.80	386.05	277	−0.31	.757	.835	–
Age-by-group[b]: TD vs. ASD-DIST ANX	1469.87	557.89	277	2.63	.009	.038	–
Age-by-group: TD vs. ASD-DSM ANX	−366.28	475.37	277	−0.77	.442	.631	–
Age-by-group: TD vs. ASD-DSM and DIST ANX	−112.22	459.27	277	−0.24	.807	.835	–
Age-by-group[b]: ASD-no ANX vs. ASD-DIST ANX	1589.67	603.15	277	2.64	.009	.038	–
Age-by-group: ASD-no ANX vs. ASD-DSM ANX	−246.48	527.62	277	−0.47	.641	.796	–
Age-by-group: ASD-no ANX vs. ASD-DSM and DIST ANX	7.58	513.01	277	0.01	.988	.988	–
Age-by-group[b]: ASD-DIST ANX vs. ASD-DSM ANX	−1836.15	663.74	277	−2.77	.006	.038	–
Age-by-group[a]: ASD-DIST ANX vs. ASD-DSM and DIST ANX	−1582.09	652.28	277	−2.43	.016	.053	–
Age-by-group: ASD-DSM ANX vs. ASD-DSM and DIST ANX	254.07	583.68	278	0.44	.664	.796	–

Regression results for models of left and right amygdala volume between children with autism spectrum disorder with only DSM anxiety (ASD-DSM ANX), only autism distinct anxiety (ASD-DIST ANX), both anxieties (ASD-DSM and DIST ANX), without anxiety (ASD-no ANX), and TD. Effects of interest are shown for mean age of T1 (38.11 mo/3.18 years) and T4 (136.30 mo/11.36 years) as well as developmental differences (age-by-group interactions). Reference group is always indicated first. Regressions were repeated switching reference group to provide all group comparisons.

% Diff., percent difference; ANX, anxiety; ASD, autism spectrum disorder; ASD-DIST ANX, autism spectrum disorder with distinct anxiety; ASD-DSM ANX, autism spectrum disorder with DSM anxiety; Coef., coefficient; FDR *p*, false discovery rate–adjusted *p* value; T1, time 1, T4, time 4; TD, typically developing.

[a]Effects of interest $p < .05$.

[b]Effects of interest FDR $p < .05$.

children and four subgroups of children with autism (only DSM anxiety, only distinct anxiety, both anxieties, and no anxiety) found DSM anxiety to be associated with larger right amygdala volumes compared with TD children at both ∼3 (time 1) and ∼11 (time 4) years of age but no differences in developmental trajectory. Autistic children with distinct anxiety had slower development of the right amygdala from the ages of ∼3 to 11 compared with TD and other autistic children and had smaller right amygdala volumes at ∼11 years of age compared with other autistic children. These results support an association, albeit a complex one, between amygdala volume, ASD, and co-occurring anxiety. Our results also identify a novel association between the development of amygdala volume and anxieties distinctly related to autism.

The only three previous studies of amygdala volumes and anxiety in ASD report conflicting results, finding both larger (6) and smaller (5) right amygdala to be related to anxiety or finding no associations (7). These results support traditional DSM anxieties being associated with larger amygdala volumes. Autism-distinct anxieties are estimated to occur at relatively high frequencies in children with autism (3) and have not been previously accounted for in imaging research. Accordingly, associations of smaller amygdala volumes in autistic children with these distinct anxieties may partially explain inconsistent findings in ASD.

We found that autistic children with anxiety have elevated sensory sensitivities (SSP), RBS, and thought problems (CBCL), which may be indicative of elevated levels of distress. We also noted that autistic children with DSM anxiety had higher CBCL internalizing behavior scores than those with distinct anxiety or no anxieties. However, no differences in CBCL internalizing behaviors were observed between the distinct anxiety and no anxiety groups. Despite finding no differences in measures of core autism features (e.g., ADOS calibrated severity score) between children with and without distinct anxieties, we hypothesize that a latent variable related to the autism phenotype contributes to both autism-distinct anxieties and smaller amygdala volumes. Others have reported smaller amygdala volumes in ASD to be associated with decreased joint attention, eye fixation, and emotional face processing speeds (31,42). Emergence of smaller right amygdala volumes due to slower development is indicative of a brain-behavior relationship between amygdala development and onset of distinct anxieties. Replication and future studies are needed to further examine the phenomenology of distinct anxieties and the ways in which their behavioral and neurobiological profiles relate to autism and vary from those of DSM anxiety.

We found that autistic children with DSM anxiety had the largest amygdala volumes compared with other groups, with significantly larger right amygdala volumes than TD children at both ∼3 and ∼11 years of age. It is important to emphasize that amygdala enlargement in autistic children with DSM anxiety was already present at 3 years of age, before clinical anxiety is typically diagnosed. This and the finding that there were no differences in the trajectory of amygdala development between ∼2.5 and 12 years would suggest that the process responsible for enlarged amygdala related to DSM anxiety

occurred either prenatally or during an early postnatal period. This indicates that enlarged amygdala may be a potential prodromal marker of anxiety in autism. Amygdala enlargement also predated elevated CBCL measures of anxiety in the ASD-DSM group; however, this may be attributable to low sensitivity of the CBCL within a sample of children with autism who were likely to be developmentally below the cognitive start age of the CBCL (1.5 years) at study enrollment (3).

Autistic children without anxiety also showed statistical trends toward having larger amygdala volumes than TD children, while the group of autistic children with both DSM and distinct anxieties had marginally smaller amygdala volumes than autistic children without anxiety. Opposing associations of amygdala volume with DSM (larger) and distinct (smaller) anxieties is also supported by autistic children with both forms of anxiety having amygdala volumes between those of the DSM and distinct anxiety groups. Further studies with larger samples will be needed to confirm these trends. While the underlying mechanisms contributing to smaller and larger amygdala volumes are unclear, larger volumes could be a product of atypical amygdala neurogenesis, which has been noted in ASD (44), while both biological mechanisms (e.g., excitotoxicity) (63) and environmental factors (e.g., poorer quality social interaction) (64) could contribute to stunted amygdala development. The process of untangling these various factors may be difficult because we suspect that neurophenotypic differences between autistic individuals with and without anxiety arise from complex interactions between biological and environmental variables that likely differ on an individual basis.

Significant differences in amygdala volume between autistic individuals with and without autism-distinct anxiety were found bilaterally. However, effects between TD and different autism anxiety groups (DSM only, distinct only, both DSM and distinct, and no anxiety) were only significant within the right hemisphere. This is consistent with the two previous studies that have reported relationships between anxiety and amygdala volume in autism (5,6). However, positive associations between both left and right amygdala activation and anxiety have been reported in autism (65,66). Meta-analyses have reported predominately leftward lateralization of amygdala activation in response to various emotional processing tasks, which has been postulated to result from increased right amygdala habituation (67,68). Indeed, emotional processing functional imaging studies suggest the right amygdala to be more engaged in rapid processing of potentially threatening stimuli and the left in prolonged stimulus evaluation (67,69,70). Thus, while the amygdala is likely to be affected bilaterally in autistic children with anxiety, a failure of the right amygdala to habituate to anxiolytic stimuli may contribute to these lateralized findings.

Consistent with the Research Domain Criteria framework (30,66), previous findings suggest that effects of DSM forms of anxiety on the amygdala may cut across diagnostic boundaries. For example, functional MRI studies report increased amygdala activation in response to facial processing tasks and resting-state amygdala-prefrontal decoupling to be related to anxiety both in ASD (65,66,71,72) and other (4,10,73,74) populations. Furthermore, a recent longitudinal study found positive associations of amygdala volume with anxiety levels from 4 to 18 years of age in TD children (13). This study was limited by a lack of a control group comprising nonautistic individuals with clinically significant DSM anxiety. However, we would hypothesize such amygdala enlargement in nonautistic anxious individuals based on our findings of larger amygdala volumes being associated with DSM anxieties in ASD. In this view, divergent findings of smaller amygdala volumes associated with distinct anxieties may indicate that these manifestations of anxiety are not only distinctly related to autism but also distinct from traditional DSM classification of anxieties. However, given that distinct anxieties are intrinsically linked to autism and less commonly reach clinical levels in individuals without autism, direct comparison of distinct anxieties between autistic and nonautistic groups will be challenging.

This study benefited from a longitudinal design that allowed for the characterization of amygdala growth across early to midchildhood as well as clinician-based assessments of both traditional DSM and autism-distinct anxieties. However, it is important to note limitations. The individual anxiety groups had relatively small sample sizes. Both DSM and distinct anxieties incorporate multiple anxiety subtypes that may, in themselves, have different developmental effects on amygdala volume (25); our sample sizes precluded investigating differences between individual forms of anxiety. Complicating this further are multiple co-occurring anxiety diagnoses. For example, of the 32 children with autism in our sample with a DSM anxiety diagnosis, 37.5% (n = 12) met ADIS criteria for two or more DSM anxiety diagnoses. We assessed autism-distinct anxiety using the ADIS-ASA, a diagnostic clinical interview. However, future studies could use autism-specific parental and patient anxiety reports, which may provide more dimensional assessments of anxiety and be easier to widely implement across large samples (75,76). We focused solely on the amygdala due to the structure's broad implications in both anxiety and ASD. However, the amygdala is highly interconnected with other brain regions and thus investigating a broader neural network critical for social and emotional processing in a multimodal manner would undoubtedly be informative. Higher-resolution imaging would afford the ability to investigate individual contributions of the 13 subnuclei, which are exceedingly challenging to segment given the standard $\sim$1-mm^3 resolution of current structural MRI scans. Finally, consistent with previous findings in TD (77) and autism (37,78), we found a significant effect of sex, with males having larger amygdala volumes than females. This study included an insufficient number of females with autism to investigate sex-by-anxiety interactions in amygdala volumes, which is critically important given findings by our group indicating sex-specific relationships of amygdala volumes with psychopathology (79) and in the amygdala resting-state connectome (80) in larger samples at younger ages.

In conclusion, this study aimed to investigate the effect of different forms of anxiety on amygdala volume and development in children with autism. Traditional DSM forms of anxiety were found to be associated with larger right amygdala volumes while anxieties distinctly related to autism were associated with smaller right amygdala volumes and slower right amygdala development. While additional studies are needed to clarify amygdala-anxiety relationships, considering previous findings, these results support DSM anxiety having common

effects on amygdala volume across diagnostic classifications. Opposing amygdala volumetric relationships between autism-distinct anxieties compared with DSM anxieties suggest that these autism-related anxiety presentations may be associated with a distinct syndrome of anxiety closely related to the autistic phenotype. Collectively, these results indicate that the amygdala is an important brain region for future efforts to identify and stratify those individuals with autism who endure debilitating co-occurring anxiety.

ACKNOWLEDGMENTS AND DISCLOSURES

This research was supported by an Autism Center of Excellence grant awarded by the National Institute of Child Health and Development (Grant No. P50 HD093079 [to DGA, CWN, MS, SJR]), the National Institute of Mental Health (Grant Nos. R01MH104438 [to CWN], R01MH103284 [to MS], and R01MH103371 [to DGA]), the Medical Investigation of Neurodevelopmental Disorders (MIND) Institute Intellectual and Developmental Disabilities Research Center (Grant No. P50 HD103526), the MIND Institute Autism Research Training Program (Grant No. T32MH073124 [to DSA]), and a Medical Research Council eMedLab Medical Bioinformatics Career Development Fellowship (Grant No. MR/L016311/1 [to AA]).

We thank the families and children who participated in the Autism Phenome Project study and all members of the research study staff.

DGA is on the scientific advisory board for Stemina Biomarker Discovery and Axial Biotherapeutics. CMK receives royalties for an edited book on anxiety and autism published by Academic Press. In addition, she has received honoraria and consulting fees for training other researchers on the Autism Spectrum Addendum. All other authors report no biomedical financial interests or potential conflicts of interest.

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Received Jul 28, 2021; revised Jan 18, 2022; accepted Jan 22, 2022.

Supplementary material cited in this article is available online at https://doi.org/10.1016/j.biopsych.2022.01.016.

REFERENCES

1. American Psychiatric Association (2013): Diagnostic and Statistical Manual of Mental Disorders, 5th Edition Washington, DC: American Psychiatric Publishing.
2. Kerns CM, Kendall PC (2012): The presentation and classification of anxiety in autism spectrum disorder. Clin Psychol Sci Pract 19:323–347.
3. Kerns CM, Winder-Patel B, Iosif AM, Nordahl CW, Heath B, Solomon M, Amaral DG (2021): Clinically significant anxiety in children with autism spectrum disorder and varied intellectual functioning [published correction appears online ahead of print, J Clin Child Adolesc Psychol, Mar 27, 2020]. J Clin Child Adolesc Psychol 50:780–795.
4. Kalin NH (2017): Mechanisms underlying the early risk to develop anxiety and depression: A translational approach. Eur Neuropsychopharmacol 27:543–553.
5. Herrington JD, Maddox BB, Kerns CM, Rump K, Worley JA, Bush JC, *et al.* (2017): Amygdala volume differences in autism spectrum disorder are related to anxiety. J Autism Dev Disord 47:3682–3691.
6. Juranek J, Filipek PA, Berenji GR, Modahl C, Osann K, Spence MA (2006): Association between amygdala volume and anxiety level: Magnetic resonance imaging (MRI) study in autistic children. J Child Neurol 21:1051–1058.
7. Yarger HA, Nordahl CW, Redcay E (2021): Examining associations between amygdala volumes and anxiety symptoms in autism spectrum disorder [published online ahead of print Oct 21]. Biol Psychiatry Cogn Neurosci Neuroimaging.
8. Wood JJ, Gadow KD (2010): Exploring the nature and function of anxiety in youth with autism spectrum disorders. Clin Psychol Sci Pract 17:281–292.
9. Kerns CM, Renno P, Kendall PC, Wood JJ, Storch EA (2017): Anxiety Disorders Interview Schedule–Autism Addendum: Reliability and validity in children with autism spectrum disorder [published correction appears in J Clin Child Adolesc Psychol 2020 49:iv]. J Clin Child Adolesc Psychol 46:88–100.
10. Duvarci S, Pare D (2014): Amygdala microcircuits controlling learned fear. Neuron 82:966–980.
11. De Bellis MD, Casey BJ, Dahl RE, Birmaher B, Williamson DE, Thomas KM, *et al.* (2000): A pilot study of amygdala volumes in pediatric generalized anxiety disorder. Biol Psychiatry 48:51–57.
12. De Bellis MD, Keshavan MS, Shifflett H, Iyengar S, Dahl RE, Axelson DA, *et al.* (2002): Superior temporal gyrus volumes in pediatric generalized anxiety disorder. Biol Psychiatry 51:553–562.
13. Albaugh MD, Nguyen TV, Ducharme S, Collins DL, Botteron KN, D'Alberto N, *et al.* (2017): Age-related volumetric change of limbic structures and subclinical anxious/depressed symptomatology in typically developing children and adolescents. Biol Psychol 124:133–140.
14. Qin S, Young CB, Duan X, Chen T, Supekar K, Menon V (2014): Amygdala subregional structure and intrinsic functional connectivity predicts individual differences in anxiety during early childhood. Biol Psychiatry 75:892–900.
15. Tottenham N, Hare TA, Quinn BT, McCarry TW, Nurse M, Gilhooly T, *et al.* (2010): Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. Dev Sci 13:46–61.
16. Warnell KR, Pecukonis M, Redcay E (2018): Developmental relations between amygdala volume and anxiety traits: Effects of informant, sex, and age. Dev Psychopathol 30:1503–1515.
17. Milham MP, Nugent AC, Drevets WC, Dickstein DP, Leibenluft E, Ernst M, *et al.* (2005): Selective reduction in amygdala volume in pediatric anxiety disorders: A voxel-based morphometry investigation. Biol Psychiatry 57:961–966.
18. Mueller SC, Aouidad A, Gorodetsky E, Goldman D, Pine DS, Ernst M (2013): Gray matter volume in adolescent anxiety: An impact of the brain-derived neurotrophic factor Val(66)Met polymorphism? [published correction appears in J Am Acad Child Adolesc Psychiatry 2013; 52:329]. J Am Acad Child Adolesc Psychiatry 52:184–195.
19. Strawn JR, Hamm L, Fitzgerald DA, Fitzgerald KD, Monk CS, Phan KL (2015): Neurostructural abnormalities in pediatric anxiety disorders. J Anxiety Disord 32:81–88.
20. Strawn JR, Wehry AM, Chu WJ, Adler CM, Eliassen JC, Cerullo MA, *et al.* (2013): Neuroanatomic abnormalities in adolescents with generalized anxiety disorder: A voxel-based morphometry study. Depress Anxiety 30:842–848.
21. Gold AL, Steuber ER, White LK, Pacheco J, Sachs JF, Pagliaccio D, *et al.* (2017): Cortical thickness and subcortical gray matter volume in pediatric anxiety disorders. Neuropsychopharmacology 42:2423–2433.
22. Merz EC, Tottenham N, Noble KG (2018): Socioeconomic status, amygdala volume, and internalizing symptoms in children and adolescents. J Clin Child Adolesc Psychol 47:312–323.

23. van der Plas EAA, Boes AD, Wemmie JA, Tranel D, Nopoulos P (2010): Amygdala volume correlates positively with fearfulness in normal healthy girls. Soc Cogn Affect Neurosci 5:424–431.
24. McQueeny T, Padula CB, Price J, Medina KL, Logan P, Tapert SF (2011): Gender effects on amygdala morphometry in adolescent marijuana users. Behav Brain Res 224:128–134.
25. Suor JH, Jimmy J, Monk CS, Phan KL, Burkhouse KL (2020): Parsing differences in amygdala volume among individuals with and without social and generalized anxiety disorders across the lifespan. J Psychiatr Res 128:83–89.
26. Muris P, Merckelbach H, Mayer B, van Brakel A, Thissen S, Moulaert V, Gadet B (1998): The Screen for Child Anxiety Related Emotional Disorders (SCARED) and traditional childhood anxiety measures. J Behav Ther Exp Psychiatry 29:327–339.
27. Achenbach TM (1999): The Child Behavior Checklist and related instruments. In: Maruish ME, editor. The Use of Psychological Testing for Treatment Planning and Outcomes Assessment, 2nd ed. Mahwah, NJ: Lawrence Erlbaum Associates Publishers. 429–466.
28. Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, *et al.* (1997): Schedule for Affective Disorders and Schizophrenia for School-age Children-Present and Lifetime Version (K-SADS-PL): Initial reliability and validity data. J Am Acad Child Adolesc Psychiatry 36:980–988.
29. Müller RA, Fishman I (2018): Brain connectivity and neuroimaging of social networks in autism. Trends Cogn Sci 22:1103–1116.
30. Hennessey T, Andari E, Rainnie DG (2018): RDoC-based categorization of amygdala functions and its implications in autism. Neurosci Biobehav Rev 90:115–129.
31. Mosconi MW, Cody-Hazlett H, Poe MD, Gerig G, Gimpel-Smith R, Piven J (2009): Longitudinal study of amygdala volume and joint attention in 2- to 4-year-old children with autism. Arch Gen Psychiatry 66:509–516.
32. Munson J, Dawson G, Abbott R, Faja S, Webb SJ, Friedman SD, *et al.* (2006): Amygdalar volume and behavioral development in autism. Arch Gen Psychiatry 63:686–693.
33. Schumann CM, Barnes CC, Lord C, Courchesne E (2009): Amygdala enlargement in toddlers with autism related to severity of social and communication impairments. Biol Psychiatry 66:942–949.
34. Sparks BF, Friedman SD, Shaw DW, Aylward EH, Echelard D, Artru AA, *et al.* (2002): Brain structural abnormalities in young children with autism spectrum disorder. Neurology 59:184–192.
35. Groen W, Teluij M, Buitelaar J, Tendolkar I (2010): Amygdala and hippocampus enlargement during adolescence in autism. J Am Acad Child Adolesc Psychiatry 49:552–560.
36. Kim JE, Lyoo IK, Estes AM, Renshaw PF, Shaw DW, Friedman SD, *et al.* (2010): Laterobasal amygdalar enlargement in 6- to 7-year-old children with autism spectrum disorder. Arch Gen Psychiatry 67:1187–1197.
37. Li G, Chen MH, Li G, Wu D, Lian C, Sun Q, *et al.* (2019): A longitudinal MRI study of amygdala and hippocampal subfields for infants with risk of autism. In: Zhang D, Zhou L, Jie B, Liu M, editors. Graph Learning in Medical Imaging: First International Workshop, GLMI 2019, Held in Conjunction with MICCAI 2019, Shenzen, China, October 17, 2019, Proceedings. Cham, Switzerland: Springer International Publishing, 164–171.
38. Nordahl CW, Scholz R, Yang X, Buonocore MH, Simon T, Rogers S, Amaral DG (2012): Increased rate of amygdala growth in children aged 2 to 4 years with autism spectrum disorders: A longitudinal study. Arch Gen Psychiatry 69:53–61.
39. Aylward EH, Minshew NJ, Goldstein G, Honeycutt NA, Augustine AM, Yates KO, *et al.* (1999): MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. Neurology 53:2145–2150.
40. van Rooij D, Anagnostou E, Arango C, Auzias G, Behrmann M, Busatto GF, *et al.* (2018): Cortical and subcortical brain morphometry differences between patients with autism spectrum disorder and healthy individuals across the lifespan: Results from the ENIGMA ASD working group. Am J Psychiatry 175:359–369.
41. Barnea-Goraly N, Frazier TW, Piacenza L, Minshew NJ, Keshavan MS, Reiss AL, Hardan AY (2014): A preliminary longitudinal volumetric MRI study of amygdala and hippocampal volumes in autism. Prog Neuropsychopharmacol Biol Psychiatry 48:124–128.
42. Nacewicz BM, Dalton KM, Johnstone T, Long MT, McAuliff EM, Oakes TR, *et al.* (2006): Amygdala volume and nonverbal social impairment in adolescent and adult males with autism. Arch Gen Psychiatry 63:1417–1428.
43. Haar S, Berman S, Behrmann M, Dinstein I (2016): Anatomical abnormalities in autism? Cereb Cortex 26:1440–1452.
44. Avino TA, Barger N, Vargas MV, Carlson EL, Amaral DG, Bauman MD, Schumann CM (2018): Neuron numbers increase in the human amygdala from birth to adulthood, but not in autism. Proc Natl Acad Sci U S A 115:3710–3715.
45. Albano AM, Silverman WK (1996): The Anxiety Disorders Interview Schedule for Children for DSM-IV: Clinical Manual (Child and Parent Versions). Oxford: Oxford University Press.
46. Lord C, Risi S, Lambrecht L, Cook EH Jr, Leventhal BL, DiLavore PC, *et al.* (2000): The autism diagnostic observation schedule—generic: A standard measure of social and communication deficits associated with the spectrum of autism. J Autism Dev Disord 30:205–223.
47. Lord C, Rutter M, DiLavore PC, Risi S, Gotham K, Bishop S (2012): Autism Diagnostic Observation Schedule, 2nd Edition (ADOS-2) Torrence, CA: Western Psychological Services.
48. Lord C, Rutter M, Le Couteur A (1994): Autism Diagnostic Interview-Revised: A revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord 24:659–685.
49. American Psychiatric Association (1994): Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Washington, DC: American Psychiatric Publishing.
50. Mullen EM (1995): Mullen Scales of Early Learning. Circle Pines, MN: American Guidance Service.
51. Elliott C (2007): Differential Ability Scales, Second ed. San Antonio, TX: Harcourt Assessment.
52. Bodfish JW, Symons FW, Lewis MH (1999): The Repetitive Behavior Scale. Chapel Hill, NC: Western Carolina Center Research Reports.
53. Dunn W (1999): Sensory Profile. San Antonio, TX: Psychological Corporation.
54. Dunn W (2014): Sensory Profile 2. Bloomington, MN: Pearson.
55. Constantino J (2012): Social Responsiveness Scale, 2nd Edition (SRS-2) Torrance, CA: Western Psychological Services.
56. Nordahl CW, Simon TJ, Zierhut C, Solomon M, Rogers SJ, Amaral DG (2008): Brief report: Methods for acquiring structural MRI data in very young children with autism without the use of sedation. J Autism Dev Disord 38:1581–1590.
57. Nordahl CW, Mello M, Shen AM, Shen MD, Vismara LA, Li D, *et al.* (2016): Methods for acquiring MRI data in children with autism spectrum disorder and intellectual impairment without the use of sedation. J Neurodev Disord 8:20.
58. Mori S, Wu D, Ceritoglu C, Li Y, Kolasny A, Vaillant MA, *et al.* (2016): MRICloud: Delivering high-throughput MRI neuroinformatics as cloud-based software as a service. Comput Sci Eng 18:21–35.
59. Wang H, Suh JW, Das SR, Pluta JB, Craige C, Yushkevich PA (2013): Multi-atlas segmentation with joint label fusion. IEEE Trans Pattern Anal Mach Intell 35:611–623.
60. Long J, Ryoo J (2010): Using fractional polynomials to model non-linear trends in longitudinal data. Br J Math Stat Psychol 63:177–203.
61. Benjamini Y, Hochberg Y (1995): Controlling the false discovery rate: A practical and powerful approach to multiple testing. J R Stat Soc B 57:289–300.
62. Cashin A, Yorke J (2018): The relationship between anxiety, external structure, behavioral history and becoming locked into restricted and repetitive behaviors in autism spectrum disorder. Issues Ment Health Nurs 39:533–537.
63. Siegle GJ, Konecky RO, Thase ME, Carter CS (2003): Relationships between amygdala volume and activity during emotional information processing tasks in depressed and never-depressed individuals: An fMRI investigation. Ann N Y Acad Sci 985:481–484.

64. Sallet J, Mars RB, Noonan MP, Andersson JL, O'Reilly JX, Jbabdi S, *et al.* (2011): Social network size affects neural circuits in macaques. Science 334:697–700.
65. Herrington JD, Miller JS, Pandey J, Schultz RT (2016): Anxiety and social deficits have distinct relationships with amygdala function in autism spectrum disorder. Soc Cogn Affect Neurosci 11:907–914.
66. Herrington JD, Maddox BB, McVey AJ, Franklin ME, Yerys BE, Miller JS, Schultz RT (2017): Negative valence in autism spectrum disorder: The relationship between amygdala activity, selective attention, and co-occurring anxiety. Biol Psychiatry Cogn Neurosci Neuroimaging 2:510–517.
67. Wright CI, Fischer H, Whalen PJ, McInerney SC, Shin LM, Rauch SL (2001): Differential prefrontal cortex and amygdala habituation to repeatedly presented emotional stimuli. Neuroreport 12:379–383.
68. Sergerie K, Chochol C, Armony JL (2008): The role of the amygdala in emotional processing: A quantitative meta-analysis of functional neuroimaging studies. Neurosci Biobehav Rev 32:811–830.
69. Hung Y, Smith ML, Bayle DJ, Mills T, Cheyne D, Taylor MJ (2010): Unattended emotional faces elicit early lateralized amygdala–frontal and fusiform activations. Neuroimage 50:727–733.
70. Morris JS, Öhman A, Dolan RJ (1998): Conscious and unconscious emotional learning in the human amygdala. Nature 393:467–470.
71. Hall GBC, Doyle KAR, Goldberg J, West D, Szatmari P (2010): Amygdala engagement in response to subthreshold presentations of anxious face stimuli in adults with autism spectrum disorders: Preliminary insights. PLoS One 5:e10804.
72. Bartolotti J, Sweeney JA, Mosconi MW (2020): Functional brain abnormalities associated with comorbid anxiety in autism spectrum disorder. Dev Psychopathol 32:1273–1286.
73. Etkin A, Wager TD (2007): Functional neuroimaging of anxiety: A meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. Am J Psychiatry 164:1476–1488.
74. Bishop SJ, Duncan J, Lawrence AD (2004): State anxiety modulation of the amygdala response to unattended threat-related stimuli. J Neurosci 24:10364–10368.
75. Scahill L, Lecavalier L, Schultz RT, Evans AN, Maddox B, Pritchett J, *et al.* (2019): Development of the Parent-Rated Anxiety Scale for youth with autism spectrum disorder. J Am Acad Child Adolesc Psychiatry 58:887–896.e2.
76. Rodgers J, Wigham S, McConachie H, Freeston M, Honey E, Parr JR (2016): Development of the anxiety scale for children with autism spectrum disorder (ASC-ASD). Autism Res 9:1205–1215.
77. Ruigrok ANV, Salimi-Khorshidi G, Lai MC, Baron-Cohen S, Lombardo MV, Tait RJ, Suckling J (2014): A meta-analysis of sex differences in human brain structure. Neurosci Biobehav Rev 39:34–50.
78. Zhang W, Groen W, Mennes M, Greven C, Buitelaar J, Rommelse N (2018): Revisiting subcortical brain volume correlates of autism in the ABIDE dataset: Effects of age and sex. Psychol Med 48:654–668.
79. Nordahl CW, Iosif AM, Young GS, Hechtman A, Heath B, Lee JK, *et al.* (2020): High psychopathology subgroup in young children with autism: Associations with biological sex and amygdala volume. J Am Acad Child Adolesc Psychiatry 59:1353–1363.e2.
80. Lee JK, Amaral DG, Solomon M, Rogers SJ, Ozonoff S, Nordahl CW (2020): Sex differences in the amygdala resting-state connectome of children with autism spectrum disorder. Biol Psychiatry Cogn Neurosci Neuroimaging 5:320–329.