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

Supplementary Information

Supplementary Methods

Participants

Participants were enrolled in the UC Davis MIND Institute Autism Phenome Project (APP), a longitudinal study consisting of MRI scanning at four timepoints, enrollment/baseline at 24-42 months of age (Time 1), follow-up imaging at annual intervals for two time points (Time 2 and 3), and at ~9-12 years of age (Time 4). The current study included data from all participants who completed MRI and anxiety assessments at Time 4, Table 1, Supplementary Figure 1. Participants were required to be native English speakers, ambulatory, have no contraindications for MRI, no suspected vision or hearing problems, identified genetic disorders (e.g. Fragile X) or neurological conditions.

At Time 1, ASD diagnosis was confirmed using the Autism Diagnostic Observation Schedule-Generic (ADOS-G) (1) or ADOS-2 (2), the Autism Diagnostic Interview-Revised (ADI-R) (3) and DSM-IV-TR criteria (4). At Time 1, TD children were screened for autism traits using the Social Communication Questionnaire (SCQ) (i.e., scores below 11), were required to fall within two standard deviations on the Mullen Scales of Early Learning (MSEL) (5) and have no first-degree relatives with an ASD diagnosis. The MSEL was not administered to one TD male at Time 1 who was assessed using the Stanford Binet abbreviated battery measure of IQ (6). IQ was assessed at Time 4 using the Differential Ability Scales second edition (DAS-II) (7). Four ASD
participants and one TD participant did not complete DAS assessments at study Time 4. Thus, for all analyses of IQ the latest DQ/IQ measure available for these individuals was assigned. 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.

**Anxiety and Behavioral Assessments**

Anxiety was assessed at Time 4 by research reliable, trained doctoral level psychologists with expertise in autism and anxiety using the Anxiety Disorders Interview Schedule-IV-Parent Interview (ADIS) (8) with the Autism Spectrum Addendum (ADIS-ASA), a semi-structured diagnostic interview designed to differentiate anxiety and autism symptoms and assess a range of problematic anxiety in autistic youth (9). The ADIS includes modules relating to separation, social, specific phobia, and generalized anxiety disorders. In addition to these modules, the ADIS-ASA also assesses forms of anxiety distinctly related to autism, including idiosyncratic fears, fear relating to social confusion, special interest fears, and fears of change. 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. Three TD children had an ADIS CSR>=4 while four autistic children met ADIS or ADIS-ASA criteria for an OCD condition, these participants were excluded and are not described in the current study. Rates of anxiety and anxiety assessment within this cohort have been described in detail previously (10). The current study included 53/71 of the autistic and 43/55 of the TD participants from this previous study.

Behavioral measures were acquired at both Time 1 and Time 4 and utilized for post hoc behavioral analyses. However, not all ASD participants completed all assessments, these included;
the Child Behavior Checklist (CBCL) (Time 1 n=65/71, Time 4=67/71) (11), Repetitive Behavior Scale (RBS) (Time 1 n=57/71, Time 4 n=60/71) (12), Short Sensory Profile (SSP) (Time 1 n=53/71) (13), SSP 2 (Time 4 n=63/71) (14), and Social Responsiveness Scale (SRS) (Time 1 n=61/71, Time 4 n=65/71) (15).

Rates of anxiety and anxiety assessment within this cohort have been described in detail previously (10). The current study included 53/71 of the autistic and 43/52 of the TD participants from this previous study. This study also included 64/71 autistic and 51/55 children from a recent publication detailing sex differences in amygdala volume in relation to psychopathology symptoms in autism (16).

Magnetic Resonance Imaging Acquisition and Region of Interest Approach

All MRI scans were acquired between May 2006 and September 2019 at the UC Davis Imaging Research Center on a 3T Siemens Trio MRI system using an eight-channel head coil. High resolution T1-weighted structural scans were obtained using 3-dimensional magnetization-prepared rapid acquisition gradient-echo (MPRAGE) (T1–T3: TR 2,170 ms, TE 4.86 ms, FOV 256, 192 sagittal slices, 1.0 mm slice thickness, 8:46 acquisition time). This sequence was modified for study Time 4 to shorten scanning duration and increase compliance (T4: TR 2170 ms, TE 3.5 ms, FOV 256 mm, FA 7, 192 sagittal slices, 1.0 mm slice thickness; 5:10 acquisition time). Time 1-3 scans were acquired during natural nocturnal sleep (17). Time 4 scans were acquired while participants were awake using previously described methods (18). Image distortion associated with changes in software over time was controlled for by scanning a calibration phantom (ADNI MAGPHAM, The Phantom Laboratory) at the end of each MRI session and subsequently applying
Distortion corrected, anonymized, and defaced MPRAGE images were uploaded to MRICloud (https://mricloud.org) (19) and segmented into 289 anatomically defined regions using a multi-atlas approach (20) within the fully automated MRICloud T1-Segmentation pipeline version 7A. According to age in years at the time of the scan an age-specific atlas set was used for each participant. For 2- and 3-year-olds, the multi-atlas sets (each comprising 13 pediatric atlases) were optimized using data from the APP cohort at each age group and are available on MRI Cloud (UC Davis 2 Years and UC Davis 3 Years). For older children the appropriate MRI Cloud Pediatric 4-8 Years multi-atlas (based on 10 pediatric atlases) or Pediatric 8-12 Years multi-atlas (based on 28 atlases) was utilized. The whole-brain segmentation output for each participant was downloaded and visually inspected for segmentation quality. This protocol has been used previously and validated against manual tracing of the amygdala (16). Volumes from the left and right amygdala, and left and right hemisphere, were exported for statistical modeling.
Supplementary Figure S1: Longitudinal MRI Sample Structural MRI scans across up to four timepoints are indicated for individual child participants. ASD= autism spectrum disorder, TD= typical development.
**Supplementary Table S1: Hemisphere associations between ASD groups with different anxiety types and TD**

| Hemisphere                  | Coef.      | SE       | DF  | t      | p        |
|-----------------------------|------------|----------|-----|--------|----------|
| **Left Hemisphere**         |            |          |     |        |          |
| (Intercept)                 | -16448.4   | 6313.58  | 278 | -2.60524 | 9.68E-03 |
| scan age\(^{0.5}\)          | 11777.44   | 643.3138 | 278 | 18.30745 | 1.14E-49 |
| sex (male reference)        | -44372.7   | 8266.686 | 122 | -5.36765 | 3.86E-07 |
| T1: TD v ASD-no ANX         | -691.757   | 9659.733 | 122 | -0.07161 | 9.43E-01 |
| T1: TD v ASD-DIST ANX       | 6303.78    | 13703.02 | 122 | 0.460029 | 6.46E-01 |
| T1: TD v ASD-DSM ANX        | 3810.999   | 10419.5  | 278 | 0.365757 | 7.15E-01 |
| T1: TD v ASD-DSM & DIST ANX| -4039.43   | 10316.2  | 122 | -0.39156 | 6.96E-01 |
| T1: ASD-no ANX v ASD-DIST ANX | 6995.537   | 14692.73 | 122 | 0.476122 | 6.35E-01 |
| T1: ASD-no ANX v ASD-DSM ANX | 4502.756   | 11660.69 | 278 | 0.386148 | 7.00E-01 |
| T1: ASD-DIST ANX v ASD-DSM ANX | -3347.68   | 11703.91 | 278 | -0.28603 | 7.75E-01 |
| T1: ASD-DIST ANX v ASD-DSM & DIST ANX | -7850.43   | 9773.624 | 279 | -0.80323 | 4.23E-01 |
| T1: ASD-DSM ANX v ASD-DSM & DIST ANX | -905878    | 841487.2 | 122 | -1.07652 | 2.84E-01 |
| T4: TD v ASD-no ANX         | -327771    | 1014972  | 122 | -0.32294 | 7.47E-01 |
| T4: TD v ASD-DIST ANX       | 1618523    | 1337284  | 122 | 1.210306 | 2.29E-01 |
| T4: ASD-no ANX v ASD-DIST ANX | 1615823    | 1337284  | 122 | 1.210306 | 2.29E-01 |
| T4: ASD-no ANX v ASD-DSM ANX | 1261972    | 1127359  | 278 | 1.119405 | 2.64E-01 |
| T4: ASD-DIST ANX v ASD-DSM ANX | -356551    | 1447644  | 278 | -0.2463  | 8.06E-01 |
| T4: ASD-DIST ANX v ASD-DSM & DIST ANX | -1040416   | 1452580  | 278 | -0.71625 | 4.74E-01 |
| T4: ASD-DSM ANX v ASD-DSM & DIST ANX | -683865    | 1269832  | 279 | -0.53855 | 5.91E-01 |
| Age-by-group: TD v ASD-no ANX | 1325.61    | 1125.049 | 278 | 1.178269 | 2.40E-01 |
| Age-by-group: TD v ASD-DIST ANX | -475.855   | 1613.06  | 278 | -0.295   | 7.68E-01 |
| Age-by-group: TD v ASD-DSM ANX | 555.3275   | 1374.59  | 278 | 0.403995 | 6.87E-01 |
| Age-by-group: TD v ASD-DSM & DIST ANX | 1139.278   | 1401.28  | 278 | 0.813027 | 4.17E-01 |
| Age-by-group: ASD-no ANX v ASD-DIST ANX | -1801.46   | 1743.585 | 278 | -1.0332  | 3.02E-01 |
| Age-by-group: ASD-no ANX v ASD-DSM ANX | -770.283   | 1525.597 | 278 | -0.50491 | 6.14E-01 |
| Age-by-group: ASD-no ANX v ASD-DSM & DIST ANX | -186.332   | 1549.751 | 278 | -0.12023 | 9.04E-01 |
| Age-by-group: ASD-DIST ANX v ASD-DSM ANX | 1031.182   | 1914.192 | 278 | 0.538704 | 5.91E-01 |
| Age-by-group: ASD-DIST ANX v ASD-DSM & DIST ANX | 1615.132   | 1933.216 | 278 | 0.835464 | 4.04E-01 |
| Age-by-group: ASD-DSM ANX v ASD-DSM & DIST ANX | 583.9504   | 1753.218 | 279 | 0.333074 | 7.39E-01 |

**Right Amygdala**

| Coef.      | SE       | DF  | t      | p        |
|------------|----------|-----|--------|----------|
| (Intercept)               | 114080.8 | 8784.309| 278 | 12.98688 | 1.84E-03 |
| Scan age^-0.5 | sex (male reference) | T1:TD v ASD-no ANX | T1:TD v ASD-DIST ANX | T1:TD v ASD-DSM ANX | T1: TD v ASD-DSM & DIST ANX | T1: ASD-no ANX v ASD-DIST ANX | T1: ASD-no ANX v ASD-DSM ANX | T1: ASD-DIST ANX v ASD-DSM ANX | T1: ASD-DIST ANX v ASD-DSM & DIST ANX | T1: ASD-DSM ANX v ASD-DSM & DIST ANX | T4:TD v ASD-no ANX | T4:TD v ASD-DIST ANX | T4:TD v ASD-DSM ANX | T4: TD v ASD-DSM & DIST ANX | T4: ASD-no ANX v ASD-DIST ANX | T4: ASD-no ANX v ASD-DSM ANX | T4: ASD-DIST ANX v ASD-DSM ANX | T4: ASD-DIST ANX v ASD-DSM & DIST ANX | T4: ASD-DSM ANX v ASD-DSM & DIST ANX |
|------------|---------------------|-------------------|-------------------|-------------------|------------------|---------------------------|-------------------|---------------------------|---------------------------|---------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| -818889    | -44527.2            | 3045.921          | 5529.317          | 3660.093          | -1209.1          | 2483.395                  | 614.172           | -4255.02                  | -1869.22                  | -4869.19                  | 9305.875       | 4591.785        | 2896.656        | 2192.863         | -4714.09        | -6409.22        | -7113.01        | -1951.67        | -703.792                   |
| 40123.57   | 8043.211            | 9351.008          | 11074.83          | 9849.666          | 9776.294         | 14217.23                 | 11074.83          | 11123.46                 | 14547.24                 | 14840.485                  | 10503.24        | 14844.42        | 11150.78        | 11188.67         | 15900.64        | 12530.97        | 12631.19        | 16419.57        | 9846.646                  |
| -20.4092   | 122                 | 122               | 278               | 278               | 122              | 122                      | 278               | 278                       | 278                       | 279                       | 122             | 122             | 278             | 278             | 122             | 122             | 122             | 278             | 279                       |
| 3.28E-57   | -5.536              | 0.325732          | 0.416766          | 0.371596          | -0.12368         | 0.174675                 | 0.055457          | -0.38253                  | -0.12849                  | -0.5762                  | 0.886            | 0.309327        | 0.259772        | 0.19599          | -0.29647        | -0.51147        | -0.56313         | -0.10372        | -0.07148                  |

**Note:** Regression results for models of left and right hemisphere volume between children autism spectrum disorder with only DSM anxiety (ASD-DSM ANX), only autism distinct anxiety (ASD-DIST ANX), both anxieties (ASD-DSM & DIST ANX), without anxiety (ASD-no ANX), and typical development (TD). Effects of interest are shown for mean age of study Time 1 (T1, 38.11 months/3.18 years) and Time 4 (T4, 136.30 months/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. Coef=coefficient, SE=standard error, DF=degrees of freedom, p=uncorrected p value.
Supplementary Table S2: Amygdala developmental differences between ASD groups with different anxiety types and TD covarying for baseline amygdala volume

| Left Amygdala                      | Coef.  | SE    | DF    | t     | p     |
|------------------------------------|--------|-------|-------|-------|-------|
| Age-by-group: TD v ASD-no ANX      | 7.22   | 5.43  | 270.00 | 1.33  | 0.185 |
| Age-by-group: TD v ASD-DIST ANX    | 0.00   | 7.75  | 270.00 | 0.00  | 1.00  |
| *Age-by-group: TD v ASD-DSM ANX    | 15.64  | 7.08  | 270.00 | 2.21  | 0.028 |
| Age-by-group: TD v ASD-DSM & DIST ANX | 1.72   | 6.51  | 270.00 | 0.26  | 0.791 |
| Age-by-group: ASD-no ANX v ASD-DIST ANX | -7.22  | 8.41  | 270.00 | -0.86 | 0.391 |
| Age-by-group: ASD-no ANX v ASD-DSM ANX | 8.43   | 7.79  | 270.00 | 1.08  | 0.281 |
| Age-by-group: ASD-no ANX v ASD-DSM & DIST ANX | -5.49  | 7.29  | 270.00 | -0.75 | 0.452 |
| Age-by-group: ASD-DIST ANX v ASD-DSM ANX | 15.65  | 9.56  | 270.00 | 1.64  | 0.103 |
| Age-by-group: ASD-DIST ANX v ASD-DSM & DIST ANX | 1.73   | 9.15  | 270.00 | 0.19  | 0.850 |
| Age-by-group: ASD-DSM ANX v ASD-DSM & DIST ANX | -13.92 | 8.56  | 272.00 | -1.63 | 0.105 |
| Age-by-group-by-baseline: TD v ASD-no ANX | 0.02   | 0.03  | 270.00 | 0.58  | 0.563 |
| Age-by-group-by-baseline: TD v ASD-DIST ANX | -0.01  | 0.07  | 270.00 | -0.09 | 0.927 |
| Age-by-group-by-baseline: TD v ASD-DSM ANX | -0.03  | 0.04  | 270.00 | -0.80 | 0.425 |
| Age-by-group-by-baseline: TD v ASD-DSM & DIST ANX | 0.03   | 0.05  | 270.00 | 0.63  | 0.529 |
| Age-by-group-by-baseline: ASD-no ANX v ASD-DIST ANX | -0.02  | 0.07  | 270.00 | -0.33 | 0.738 |
| Age-by-group-by-baseline: ASD-no ANX v ASD-DSM ANX | -0.05  | 0.04  | 270.00 | -1.17 | 0.243 |
| Age-by-group-by-baseline: ASD-no ANX v ASD-DSM & DIST ANX | 0.01   | 0.05  | 270.00 | 0.24  | 0.810 |
| Age-by-group-by-baseline: ASD-DIST ANX v ASD-DSM ANX | -0.02  | 0.08  | 270.00 | -0.31 | 0.754 |
| Age-by-group-by-baseline: ASD-DIST ANX v ASD-DSM & DIST ANX | 0.04   | 0.08  | 270.00 | 0.45  | 0.654 |
| Age-by-group-by-baseline: ASD-DSM ANX v ASD-DSM & DIST ANX | 0.06   | 0.06  | 272.00 | 1.10  | 0.272 |

| Right Amygdala                     | Coef.  | SE    | DF    | t     | p     |
|------------------------------------|--------|-------|-------|-------|-------|
| Age-by-group: TD v ASD-no ANX      | 53.03  | 396.21| 270.00| 0.13  | 0.894 |
| *Age-by-group: TD v ASD-DIST ANX   | 1343.09| 541.23| 270.00| 2.48  | 0.014 |
| Age-by-group: TD v ASD-DSM ANX     | -717.72| 533.65| 270.00| -1.34 | 0.180 |
| Age-by-group: TD v ASD-DSM & DIST ANX | -312.56| 455.05| 270.00| -0.69 | 0.493 |
| *Age-by-group: ASD-no ANX v ASD-DIST ANX | 1290.06| 590.42| 270.00| 2.18  | 0.030 |
| Age-by-group: ASD-no ANX v ASD-DSM ANX | -770.74| 583.51| 270.00| -1.32 | 0.188 |
| Age-by-group: ASD-no ANX v ASD-DSM & DIST ANX | -365.59| 512.71| 270.00| -0.71 | 0.476 |
| *Age-by-group: ASD-DIST ANX v ASD-DSM ANX | -2060.81| 690.01| 270.00| -2.99 | 0.003 |
| *Age-by-group: ASD-DIST ANX v ASD-DSM & DIST ANX | -1655.65| 631.00| 270.00| -2.62 | 0.009 |
| Age-by-group: ASD-DSM ANX v ASD-DSM & DIST ANX | 405.16| 620.49| 272.00| 0.65  | 0.514 |
| *Age-by-group-by-baseline: TD v ASD-no ANX | -5.48 | 2.45 | 270.00 | -2.24 | 0.026 |
| Age-by-group-by-baseline: TD v ASD-DIST ANX | 0.61 | 4.36 | 270.00 | 0.14 | 0.889 |
| Age-by-group-by-baseline: TD v ASD-DSM ANX | 1.36 | 2.77 | 270.00 | 0.49 | 0.624 |
| Age-by-group-by-baseline: TD v ASD-DSM & DIST ANX | -3.20 | 3.19 | 270.00 | -1.00 | 0.316 |
| Age-by-group-by-baseline: ASD-no ANX v ASD-DIST ANX | 6.09 | 4.66 | 270.00 | 1.31 | 0.193 |
| *Age-by-group-by-baseline: ASD-no ANX v ASD-DSM ANX | 6.84 | 3.23 | 270.00 | 2.12 | 0.035 |
| Age-by-group-by-baseline: ASD-no ANX v ASD-DSM & DIST ANX | 2.28 | 3.60 | 270.00 | 0.63 | 0.526 |
| Age-by-group-by-baseline: ASD-DIST ANX v ASD-DSM ANX | 0.75 | 4.84 | 270.00 | 0.15 | 0.877 |
| Age-by-group-by-baseline: ASD-DIST ANX v ASD-DSM & DIST ANX | -3.81 | 5.10 | 270.00 | -0.75 | 0.456 |
| Age-by-group-by-baseline: ASD-DSM ANX v ASD-DSM & DIST ANX | -4.56 | 3.81 | 272.00 | -1.20 | 0.233 |

**Note**: Developmental differences (age-by-group) and three-way interactions with baseline amygdala volume (age-by-group-by-baseline) regression results for models of left and right amygdala volume, between children autism spectrum disorder with only DSM anxiety (ASD-DSM ANX), only autism distinct anxiety (ASD-DIST ANX), both anxieties (ASD-DSM & DIST ANX), without anxiety (ASD-no ANX), and typical development (TD). Reference group is always indicated first. Regressions were repeated switching reference group to provide all group comparisons. Coef=coefficient, SE=standard error, DF=degrees of freedom, p=uncorrected p value, *effects of interest p<0.05.
## Supplementary Table S3: Autism anxiety group behavioral and demographic summary

| Group          | ASD no ANX | ASD DSM | ASD DIST | ASD BOTH |
|---------------|------------|---------|----------|----------|
| n            | 28         | 16      | 11       | 16       |
| Male / Female| 23 / 5     | 15 / 1  | 9 / 2    | 10 / 6   |
| Timepoint     | T1         | T4      | T1       | T4       | T1     | T4     |
| DQ / IQ       | 70.09 (19.73) | 79.88 (26.36) | 78.6 (19.82) | 92.4 (32.56) | 68.66 (19.89) | 74.6 (26.48) | 74.22 (15.10) | 82.06 (28.13) |
| ADOS CSS      | 7.46 (1.89) | 7.21 (2.25) | 7.81 (1.86) | 7.75 (1.77) | 7.54 (1.29) | 8.36 (1.68) | 6.84 (1.31) | 7.25 (1.73) |
| ADOS SA       | 7.00 (1.78) | 6.75 (2.01) | 6.93 (1.43) | 7.37 (1.14) | 7.27 (1.48) | 8.27 (1.42) | 6.31 (1.13) | 7.56 (1.67) |
| ADOS RRB      | 8.46 (1.50) | 8.10 (2.00) | 8.68 (1.30) | 8.12 (2.41) | 8.36 (1.50) | 8.27 (1.42) | 8.18 (1.79) | 6.93 (1.28) |
| SRS Total     | 69.39 (10.78) | 69.68 (15.08) | 68.66 (8.53) | 74.33 (12.06) | 72.45 (10.93) | 78.20 (10.89) | 73.20 (8.68) | 82.06 (6.94) |
| CBCL total    | 61.23 (9.64) | 57.65 (11.96) | 65.08 (8.53) | 63.75 (9.42) | 65.00 (6.04) | 62.00 (6.59) | 63.18 (11.52) | 64.20 (6.13) |
| CBCL external | 59.92 (9.70) | 51.81 (10.28) | 62.85 (7.50) | 54.87 (10.85) | 60.45 (5.92) | 56.60 (6.83) | 60.75 (13.94) | 53.73 (9.16) |
| CBCL internal | 59.61 (9.26) | 56.65 (11.18) | 62.84 (7.31) | 66.13 (8.29) | 64.00 (5.72) | 54.18 (9.32) | 61.25 (9.44) | 63.80 (8.34) |
| CBCL DSM ODD  | 58.11 (9.06) | 56.25 (7.12) | 59.14 (8.75) | 56.5 (8.09) | 57.54 (6.03) | 56.45 (6.00) | 59.56 (10.05) | 55.53 (5.43) |
| CBCL DSM ADHD | 60.30 (7.71) | 60.69 (7.93) | 61.21 (7.30) | 61.62 (9.33) | 60.00 (8.39) | 60.45 (7.09) | 60.37 (7.95) | 62.6 (7.57) |
| CBCL DSM anxiety | 54.96 (6.17) | 55.11 (5.63) | 54.64 (6.80) | 64.06 (8.75) | 54.27 (4.05) | 55.00 (4.38) | 52.75 (6.35) | 62.66 (8.91) |
| CBCL DSM affective | 62.34 (9.04) | - | 60.07 (7.74) | - | 63.81 (7.53) | - | 62.43 (8.66) | - |
| CBCL DSM depressive | - | 60.03 (8.51) | - | 65.33 (8.06) | - | 60.63 (8.80) | - | 64.20 (8.83) |
| CBCL DSM somatic | - | 55.38 (9.85) | - | 60.26 (7.17) | - | 55.09 (6.39) | - | 57.33 (9.49) |
| CBCL DSM conduct | - | 54.65 (5.94) | - | 57.87 (7.97) | - | 57.63 (6.50) | - | 54.66 (6.38) |
| CBCL aggressive behavior | 59.88 (9.51) | 55.85 (7.38) | 62.21 (9.26) | 57.31 (7.54) | 59.27 (5.62) | 57.63 (5.78) | 62.31 (12.61) | 57.00 (6.53) |
| CBCL attention problems | 63.03 (8.00) | 64.48 (11.39) | 62.71 (7.59) | 67.12 (12.59) | 63.81 (8.36) | 63.27 (10.00) | 64.00 (10.19) | 68.66 (6.17) |
| CBCL sleep problems | 56.88 (7.67) | - | 55.71 (8.09) | - | 59.00 (6.43) | - | 57.87 (7.37) | - |
| CBCL withdrawn | 71.50 (11.69) | 60.88 (8.42) | 69.92 (10.70) | 64.50 (8.44) | 76.36 (10.31) | 58.90 (10.55) | 72.12 (10.83) | 62.33 (7.81) |
| CBCL somatic complaints | 57.73 (7.12) | 57.34 (8.24) | 61.07 (6.52) | 61.80 (7.18) | 60.90 (6.74) | 56.27 (5.90) | 60.37 (6.87) | 58.93 (8.45) |
| CBCL anxious depressed | 53.26 (4.79) | 55.55 (7.49) | 53.50 (3.15) | 64.53 (9.02) | 53.00 (3.13) | 53.45 (3.90) | 52.43 (3.94) | 63.60 (10.02) |
| CBCL emotional reactivity | 56.26 (7.12) | - | 60.69 (7.64) | - | 58.27 (6.38) | - | 59.81 (11.02) | - |
| CBCL social problems | - | 59.61 (7.71) | - | 63.86 (9.35) | - | 59.80 (5.45) | - | 59.86 (5.80) |
| CBCL thought problems | - | 62.70 (9.63) | - | 69.00 (6.16) | - | 70.40 (5.60) | - | 70.00 (6.86) |
| CBCL defiance | - | 53.61 (4.83) | - | 56.37 (6.56) | - | 55.80 (5.86) | - | 53.26 (4.16) |
| RBS                    | T1  | T2  | T3  | T4  | T5  | T6  | T7  | T8  |
|------------------------|-----|-----|-----|-----|-----|-----|-----|-----|
|                        | 25.1 (15.93) | 16.72 (13.69) | 31.33 (13.62) | 31.78 (22.93) | 27.63 (14.24) | 38.18 (22.48) | 25.83 (14.42) | 33.75 (23.33) |
| SSP / SSP2 total       | 142.10 (19.75) | 45.88 (12.55) | 138.25 (16.65) | 58.33 (16.54) | 134.1 (16.13) | 53.6 (9.19) | 140.58 (19.86) | 62.92 (7.30) |

Note: Behavioral and demographics summary of different autism anxiety subgroups, autism with no anxiety (ASD no ANX), autism with only DSM anxiety (ASD DSM), autism with only distinct anxiety (ASD DIST), and autism with both DSM and distinct anxiety (ASD BOTH). Values given as mean (standard deviation). n=number of participants, T1= study Time 1, T4= study Time 4, DQ=Mullen scales of early learning developmental quotient at T1, IQ= Differential Ability Scales II IQ at T4, ADOS=Autism Diagnostic Observation Schedule, CCS=calibrated severity scores, RRB=repetitive restricted behavior, SA=social affect, SRS=Social Responsiveness Scale, CBCL=Child Behavior Check List subscales, RBS=Repetitive Behavior Scale, SSP Short Sensory Profile.
## Supplementary Table S4: Time 1 between group post hoc behavioral analyses (ANOVA)

| measure               | df | Sum Sq | Mean Sq | F-value | P-value |
|-----------------------|----|--------|---------|---------|---------|
| ADOS CSS              | 3  | 7.44   | 2.482   | 0.867   | 0.463   |
| ADOS RRB              | 3  | 2.08   | 0.6933  | 0.295   | 0.829   |
| ADOS SA               | 3  | 7.3    | 2.434   | 1.029   | 0.386   |
| SRS total             | 3  | 217    | 72.18   | 0.733   | 0.536   |
| CBCL total            | 3  | 178    | 59.26   | 0.659   | 0.58    |
| CBCL external         | 3  | 81     | 26.85   | 0.266   | 0.85    |
| CBCL internal         | 3  | 185    | 61.56   | 0.858   | 0.468   |
| CBCL DSM ODD          | 3  | 37     | 12.42   | 0.159   | 0.924   |
| CBCL DSM ADHD         | 3  | 11     | 3.67    | 0.06    | 0.98    |
| CBCL DSM anxiety      | 3  | 15.4   | 5.13    | 0.139   | 0.936   |
| CBCL DSM affective    | 3  | 93     | 31.13   | 0.434   | 0.729   |
| CBCL aggressive behavior | 3  | 112   | 37.29   | 0.386   | 0.763   |
| CBCL attention problems | 3  | 17   | 5.77    | 0.079   | 0.971   |
| CBCL sleep problems   | 3  | 76     | 25.46   | 0.451   | 0.717   |
| CBCL withdrawn        | 3  | 271    | 90.26   | 0.734   | 0.536   |
| CBCL somatic complaints | 3  | 149.6 | 49.87   | 1.052   | 0.376   |
| CBCL anxious depressed | 3  | 10.1  | 3.358   | 0.204   | 0.893   |
| CBCL emotional reactivity | 3  | 218   | 72.72   | 1.073   | 0.367   |
| RBS                   | 3  | 267    | 88.84   | 0.527   | 0.665   |
| SSP total             | 3  | 453    | 150.9   | 0.441   | 0.725   |

**Note:** Four group ANOVA results for behavioral measures (measure) between autistic children with only DSM anxiety, autistic children with only autism distinct anxiety, autistic children with both DSM and distinct anxieties, and autistic children with no anxiety. Autism Diagnostic Observation Schedule (ADOS), calibrated severity scores (CSS), repetitive restricted behavior (RRB), social affect (SA), Social Responsiveness Scale (SRS), Child Behavior Check List (CBCL), Repetitive Behavior Scale (RBS), Short Sensory Profile (SSP).
## Supplementary Table S5: Time 4 between group post hoc behavioral analyses (ANOVA)

| measure                  | df | Sum Sq | Mean Sq | F-value | P-value |
|--------------------------|----|--------|---------|---------|---------|
| ADOS CCS                 | 3  | 12.46  | 4.153   | 1.082   | 0.3630  |
| ADOS RRB                 | 3  | 20.06  | 6.688   | 2.345   | 0.0807  |
| ADOS SA                  | 3  | 18.31  | 6.104   | 1.693   | 0.1770  |
| *SRS total              | 3  | 1565   | 521.8   | 3.444   | 0.0221  |
| CBCL total               | 3  | 508    | 169.42  | 1.864   | 0.1460  |
| CBCL external           | 3  | 199    | 66.43   | 0.697   | 0.5570  |
| *CBCL internal          | 3  | 1444   | 481.4   | 5.11    | 0.0032  |
| CBCL DSM ODD            | 3  | 8.9    | 2.97    | 0.063   | 0.9790  |
| CBCL DSM ADHD           | 3  | 45     | 14.83   | 0.227   | 0.8770  |
| *CBCL DSM anxiety       | 3  | 1194   | 397.9   | 7.882   | 0.0001  |
| CBCL aggressive behavior| 3  | 36     | 12.13   | 0.246   | 0.8640  |
| CBCL attention problems | 3  | 265    | 88.33   | 0.792   | 0.5030  |
| CBCL withdrawn          | 3  | 235    | 78.29   | 1.042   | 0.3800  |
| CBCL somatic complaints | 3  | 255    | 85.11   | 1.422   | 0.2450  |
| *CBCL anxious depressed | 3  | 1435   | 478.4   | 7.362   | 0.0003  |
| CBCL social problems    | 3  | 199    | 66.48   | 1.194   | 0.3190  |
| *CBCL thought problems  | 3  | 804    | 267.9   | 4.314   | 0.0079  |
| CBCL defiance           | 3  | 116.3  | 38.76   | 1.37    | 0.2600  |
| CBCL DSM depressive     | 3  | 360    | 120.15  | 1.65    | 0.1870  |
| CBCL DSM somatic        | 3  | 268    | 89.49   | 1.169   | 0.3290  |
| CBCL DSM conduct        | 3  | 158.8  | 52.92   | 1.196   | 0.3180  |
| *RBS                    | 3  | 3673   | 1224    | 4.067   | 0.0110  |
| *SSP2 total             | 3  | 2956   | 985.3   | 6.448   | 0.0008  |

**Note:** Four group ANOVA results for behavioral measures (measure) between autistic children with only DSM anxiety, autistic children with only autism distinct anxiety, autistic children with both DSM and distinct anxieties, and autistic children with no anxiety. Autism Diagnostic Observation Schedule (ADOS), calibrated severity scores (CSS), repetitive restricted behavior (RRB), social affect (SA), Social Responsiveness Scale (SRS), Child Behavior Check List (CBCL), Repetitive Behavior Scale (RBS), Short Sensory Profile (SSP) 2. * indicates p<0.05.
### Supplementary Table S6: Time 4 between group post hoc behavioral analyses (Tukey Honest)

| measure          | group        | diff       | lwr        | upr        | p       |
|------------------|--------------|------------|------------|------------|---------|
| SRS total        | DIST-BOTH   | -3.866667 | -17.13885  | 9.405521   | 0.867876|
|                  | DSM-BOTH    | -7.733333 | -19.60434  | 4.137673   | 0.3220066|
|                  | *noANX-BOTH | -12.386667 | -23.00442  | -1.768916  | 0.0159182|
|                  | DSM-DIST    | -3.866667 | -17.13885  | 9.405521   | 0.867876|
|                  | noANX-DIST  | -8.52      | -20.68416  | 3.644161   | 0.2604331|
|                  | noANX-DSM   | -4.653333 | -15.27108  | 5.964417   | 0.6555905|
| CBCL internal    | DIST-BOTH   | -9.618182 | -19.786293 | 0.5499295  | 0.0702363|
|                  | DSM-BOTH    | 2.333333  | -7.019969  | 11.6866354 | 0.9122238|
|                  | *noANX-BOTH | -7.146154 | -15.451452 | 1.1591441  | 0.1159625|
|                  | *DSM-DIST   | 11.951515 | 1.783404   | 22.1196265 | 0.0148827|
|                  | noANX-DIST  | 2.472028  | -6.741234  | 11.6852903 | 0.893538 |
|                  | *noANX-DSM  | -9.479487 | -17.784785 | -1.1741893 | 0.0190589|
| CBCL DSM anxiety | *DIST-BOTH  | -7.666667 | -15.102978 | -0.2303552 | 0.0408333|
|                  | DSM-BOTH    | 1.3958333 | -5.336849  | 8.1285153  | 0.947126 |
|                  | *noANX-BOTH | -7.555556 | -13.588232 | -1.5228795 | 0.0082906|
|                  | *DSM-DIST   | 9.0625    | 1.725164   | 16.3998362 | 0.0094725|
|                  | noANX-DIST  | 0.1111111 | -6.589686  | 6.8119079  | 0.9999697|
|                  | *noANX-DSM  | -8.9513889| -14.86163  | -3.0411474 | 0.0009477|
| CBCL anxious depressed | *DIST-BOTH | -10.145455 | -18.586246 | -1.704663  | 0.0122003|
|                  | DSM-BOTH    | 0.9333333 | -6.831065  | 8.697732   | 0.9886818|
|                  | *noANX-BOTH | -8.0444444| -14.892001 | -1.196888  | 0.0149274|
|                  | *DSM-DIST   | 11.0787879| 2.637997   | 19.519579  | 0.0051836|
|                  | noANX-DIST  | 2.1010101 | -5.304915  | 9.706935   | 0.8852547|
|                  | *noANX-DSM  | -8.9777778| -15.825334 | -2.130222  | 0.0052434|
| CBCL thought problems | DIST-BOTH | 0.4        | -8.093397  | 8.89339682 | 0.9993055|
|                  | DSM-BOTH    | -1        | -8.731191  | 6.73119081 | 0.9861697|
|                  | *noANX-BOTH | -7.296296 | -13.995978 | -0.5966145 | 0.027589 |
|                  | DSM-DIST    | -1.4      | -10.013877 | 7.21387655 | 0.9732698|
|                  | noANX-DIST  | -7.696296 | -15.397809 | 0.00521613 | 0.0502183|
|                  | noANX-DSM   | -6.296296 | -13.148071 | 0.55547832 | 0.0826491|
| RBS              | DIST-BOTH   | 2.902098  | -15.91798  | 21.7221752 | 0.9767909|
|                  | DSM-BOTH    | -2.104396 | -19.79856  | 15.5897659 | 0.9890677|
|                  | noANX-BOTH  | -15.825175| -31.89588  | 0.2455256  | 0.0550953|
|                  | DSM-DIST    | -5.006494 | -23.51594  | 13.5029535 | 0.890248 |
|                  | *noANX-DIST | -18.727273| -35.69146  | -1.7630843 | 0.025005 |
|                  | noANX-DSM   | -13.720779| -29.42657  | 1.9850074  | 0.1071999|
| SSP total        | DIST-BOTH   | -9.323077 | -23.06931  | 4.423156   | 0.2868705|
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