**Levels of early-childhood behavioral inhibition predict distinct neurodevelopmental pathways to pediatric anxiety**

**Supplemental Material**

**Methods and Materials**

**Participants**

Participants were drawn from a larger cohort of 291 individuals selected at 4 months of age for a longitudinal study on BI temperament and social-emotional functioning. Of the cohort, individuals taking psychotropic medications or illicit substances, or having contraindications to MRI, were not invited to take part in the current study. All other individuals from the cohort were asked to participate if they were physically healthy, based on medical examination and history; had an IQ>70 (Wechsler, 1999); and did not report acute psychopathology in need of immediate treatment, as determined by structured psychiatric interviews using the Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version (K-SADS-PL; Kaufman et al., 1997). The interviews were conducted by experienced and trained clinicians (Frenkel et al., 2015, Gold et al., 2016). Individuals were offered referrals for treatment if they presented with severe, untreated psychopathology other than an anxiety disorder.

*Diagnoses*. Of the 87 participants who contributed data, four had a diagnosis of current anxiety disorder at age 10 (generalized anxiety disorder [GAD]: 1, social anxiety disorder [SAD]: 1, specific phobia [SP]: 2). At age 13, nine participants had a diagnosis of current anxiety disorder (GAD: 3, SAD: 2, GAD and SAD: 1, SP: 2, NOS: 1).

**Dot-Probe Task**

Each trial started with a 500-ms fixation cross presented in the middle of the screen (Fig. S1). Next, images of two facial expressions from the same actor, taken from the NimStim set (Tottenham et al., 2009), were presented simultaneously for 500 ms. The face images were displayed side by side, and included pairs of angry and neutral (AN) expressions, happy and neutral (HN) expressions, or two neutral expressions. The presentation of these images was followed by a single small arrow probe appearing for 1100 ms at the location of one of the facial expressions. Participants were instructed to indicate the direction of the arrow (up or down) as quickly as possible using a button press. The locations of the emotional faces and probes were counterbalanced throughout the task. On emotion-congruent trials, i.e., angry-congruent (AC) or happy-congruent (HC), the probe replaced the emotional expression; on emotion-incongruent trials, i.e., angry-incongruent (AI) or happy-incongruent (HI), the probe replaced the neutral face.

The task was composed of 48 AC trials, 48 AI trials, 48 HC trials, 48 HI trials, and 96 neutral-neutral (N) trials, evenly distributed between four functional runs (see below). The task was programmed and administered using E-Prime software (Psychology Software Tools, Pittsburgh, PA).

**Imaging data**

*fMRI data preprocessing.* The task consisted of four functional runs, each 4:15 minutes long. In each run, 111 functional image volumes with 47 contiguous interleaved axial slices were obtained with a T2\*-weighted echo-planar sequence (TR = 2300ms; TE = 25ms; flip angle = 500; field of view (FOV) = 240mm; matrix = 96×96; in plane resolution 2.5×2.5×3mm). Functional data were anatomically localized and coregistered to a high-resolution T1-weighted whole-brain volumetric scan, using a high-resolution magnetization prepared gradient echo sequence (MPRAGE; TE = min full; TI = 425ms; flip angle = 70; FOV = 256mm; matrix = 256×256; in plane resolution = 1.0mm).

Preprocessing included slice timing correction, coregistration, and normalization and nonlinear registering of echoplanar data to anatomical scans in Talairach space. Data were smoothed (6 mm full width at half maximum) and resampled to 2.5mm isotropic voxels. Repetition time (TR) pairs with a Euclidean norm motion derivative >2 mm or volumes where >10% of voxels are outliers in terms of signal intensity were censored prior to individual-level analyses (White et al., 2016, White et al., 2017b). To be included in group analyses, participants had to have no more than 20% of TRs censored across conditions. BOLD data were scaled at the voxel-wise time series by their temporal means so that effect estimates could be interpreted as percent signal change relative to the mean.

*Analyses*. All analyses relied on an event-related design. Individual-level general linear models (GLM) included regressors for correct trials across the five task conditions (AC, AI, HC, HI, N), incorrect trials, and for baseline drift and motion (i.e., rotational movement of roll, pitch, yaw, and motion displacement in the x, y, and z axes). To identify task-specific differences in functional connectivity, we used generalized psychophysiological interaction (gPPI; McLaren et al., 2012). gPPI models differences in BOLD connectivity between a “seed” region and other brain regions as a function of task condition. Here, we used the right and left amygdala, anatomically defined by the AFNI Talairach Daemon atlas (White et al., 2017b, Hardee et al., 2013), as seeds. A separate individual-level GLM was created for each seed, with the PPI terms for the task conditions calculated as the products of detrended and demeaned seed and trial condition regressors.

**Behavioral data analysis**

Prior to all behavioral analyses, data from the dot-probe task were first cleaned according to common procedures (e.g., Britton et al., 2014, Naim et al., 2015). Trials were included in analyses only if RT was between 150 and 2000ms and less than 2.5 standard deviations away from the participant’s mean RT, and if the probe type was correctly identified. Participants with mean accuracy rates <70% were excluded from all analyses (e.g., White et al., 2017b).

*Attention bias scores*. Two types of attention bias scores were computed by subtracting the mean RT in congruent trials from the mean RT in incongruent trials separately for each emotion expression (White et al., 2017b, Badura-Brack et al., 2015). Threat bias scores were computed using trials featuring angry faces, with positive threat bias scores reflecting a bias toward threat; happy bias scores were computed using trials featuring happy faces, with positive happy bias scores reflecting a bias toward positive stimuli.

*Attention bias variability (ABV) scores*. ABV scores to threat and to positive stimuli were calculated by applying a moving-window algorithm, in accord with previous studies (Badura-Brack et al., 2015, Naim et al., 2015). Specifically, attention bias scores were computed separately for all successive 10 AN trials and all successive 10 HN trials (using the same method for calculating bias scores detailed above), creating two series of attention bias scores (one for threat bias scores and one for happy bias scores). The standard deviation of each series was calculated, and was then divided by the participant’s mean overall RT to control for associations between mean and variance. This resulted in one ABV score for threat-related stimuli and one ABV score for positive-related stimuli, per participants.

**Results**

**Imaging Analyses**

*Auxiliary analyses*. A set of additional auxiliary analyses tested the omnibus Group × Anxiety × Condition × Time interaction effect. To verify the validity of the LME model used in the primary analysis (Matta et al., 2017), we tested the omnibus effect within the sub-sample of participants who provided data at both time-points (n=38). This analysis replicated the results found in the full sample, yielding a Group × Anxiety × Condition × Time interaction effect in left DLPFC (LPI peak coordinates: [-16,34,39], 540 mm3). This cluster was contained within that obtained in the primary analysis but was smaller. One would expect a smaller cluster due to lower power associated with a smaller sample. In a second analysis, for completeness, we repeated the primary analysis using BI as a continuous variable. This analysis yielded a Group × Anxiety × Condition × Time interaction effect in two smaller, nearby left DLPFC clusters (peak coordinates: [-16,36,39] and [-16,54,34], both 687 mm3), with partial overlap with the cluster obtained in the primary analysis. In a third analysis, we included sex as a nuisance variable in the design. This analysis also yielded the Group × Anxiety × Condition × Time interaction effect in a left DLPFC cluster (LPI peak coordinates: [-16,34,39], 593 mm3). This cluster was contained within, but smaller than, the cluster reported in the primary analysis. Furthermore, to verify that sex did not moderate the primary effect of interest, we examined the Group × Anxiety × Condition × Time × Sex interaction effect. No significant clusters emerged for this effect, in line with data from other studies, which found no evidence that sex moderates aspects of attention bias examined in the current study (Abend et al., 2018, White et al., 2017b, White et al., 2017a). In a fourth set of analyses, we aimed to verify that brain function did not differ between participants who provided data at one time-point vs. those who provided data at both time-points. These analyses contrasted left amygdala and right amygdala connectivity, as well as basic functional activation, as a function of number of visits, separately for each time-point. These analyses revealed no significant clusters as a function of this group assignment.

*Right amygdala connectivity: lower-order effects*. The Group × Anxiety × Time interaction in the DLPFC cluster was not significant for the AI condition, *F*(1,32)<0.01, *p*>0.99; the HC condition, *F*(1,32)=2.44, *p*=0.13; the HI condition, *F*(1,32)=0.03, *p*=0.87; and the N condition, *F*(1,32)=0.11, *p*=0.73. Since these interaction effects were not significant, they were not further decomposed.

In addition to the significant Group × Anxiety × Condition × Time interaction effect identified in the DLPFC cluster, additional significant clusters emerged for the Group × Anxiety × Condition interaction effect (Table 2), reflecting time-invariant effects of BI level, anxiety symptoms, and task condition on right amygdala functional connectivity. These clusters were within the left and right superior frontal gyrus (5671 mm3 and2656 mm3, respectively; Fig. S3A) and right dorsomedial PFC (1359 mm3;Fig. S3B). These clusters revealed a similar pattern of results. Primarily, in the HC condition, the low BI group showed a negative association between connectivity and anxiety symptoms, whereas the high BI group showed a positive association; the group differences between these associations for these clusters were significant (*p*s<0.001).

**Tables**

**Table S1.** Results of activation analyses. Presented are all significant clusters that emerged for all effects tested within the linear mixed-effects model.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Peak Talairach Coordinates | Cluster Size (mm3; k) | Peak Location | Brodmann Area |
|  | x | y | z |
| Group x Anxiety x Time |
|  | -1 | 1 | 46 | 1171; 75 | L supplementary motor area | L BA6 |
| Anxiety x Time |
|  | 46 | 11 | -1 | 2265; 145 | R insula | R BA13 |
| Group x Time |  |  |  |  |  |  |
|  | -39 | 21 | 26 | 3046; 195 | L inferior frontal gyrus | L BA9 |
|  | 41 | 26 | 19 | 2875; 184 | R inferior frontal gyrus | R BA46 |
| Anxiety |  |  |  |  |  |  |
|  | -51 | 29 | 1 | 2875; 184 | L inferior frontal gyrus | L BA45 |
| Time |  |  |  |  |  |  |
|  | -44 | 24 | 9 | 6312; 404 | L inferior frontal gyrus | L BA13 |
|  | 54 | 21 | -4 | 5359; 343 | R inferior frontal gyrus | R BA47 |
|  | -34 | 11 | 11 | 3687; 236 | L insula | L BA13 |
|  | 4 | 19 | 31 | 2875; 184 | R mid-cingulate gyrus | R BA32 |
|  | 29 | 24 | -1 | 2234; 143 | R insula | R BA13 |
|  | 24 | 51 | -6 | 2125; 136 | R superior frontal gyrus | R BA10 |

***Note***: L = left; R = right; BA = Brodmann area; k = cluster size (number of contiguous voxels). Initial voxel-wise threshold was *p*=0.005; cluster size threshold was 734 mm3 (k=56; reflecting a family-wise error rate of α=0.05).

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**Figures**

**Figure S1.** Progression of a trial in the dot-probe task.

**Figure S2.** Decomposition of higher-order interaction effects into lower-order effects (top to bottom). Arrows originate at the condition by which each effect is decomposed. Grayed boxes indicate that the effect immediately below it was significant for that condition/sample. β indicates beta estimate for the task condition in the functional connectivity analysis.Anxiety was assessed the Screen for Child Anxiety Related Disorders.

*Note*: AC = angry-congruent, AI = angry-incongruent, HC = happy-congruent, HI = happy-incongruent, N = neutral, BI = behavioral inhibition.

**Figure S3.** Associations between anxiety symptoms severity and functional connectivity between the right amygdala and (A) left and right superior frontal gyrus, and (B) right dorsomedial prefrontal cortex, per task condition (AC, AI, HC, HI, N) and group (Low BI, High BI). Each bar represents the slope estimate between SCARED scores and generalized psychophysiological interaction beta estimates for the relevant task condition. Asterisks within bars indicate slope estimates significantly different than 0; asterisks between bars indicate significant differences between slope estimates. Error bars indicate standard error of the slope estimate.

*Note*: SCARED = Screen for Child Anxiety Related Disorders, AC = angry-congruent, AI = angry-incongruent, HC = happy-congruent, HI = happy-incongruent, N = neutral, BI = behavioral inhibition; +, *p*<0.10, \*, *p*<0.05, \*\*, *p*<0.01, \*\*\*, *p*<0.001.