Patterns of Neural Connectivity during an Attention Bias Task Moderate Associations between Early Childhood Temperament and Internalizing Symptoms in Young Adulthood

Supplemental Information

Behavioral Inhibition Composite

At 14 and 24 months, participants were observed in the laboratory for responses to unfamiliar stimuli and coded to provide a measure of behavioral inhibition (BI) (1, 2). The stimuli included exploring an unfamiliar room, the presence of an adult stranger (with and without a clown costume), a novel toy, and crawling through a toy tunnel. At each age, individual scores (e.g., latency to touch a novel toy, latency to vocalize, proximity to mother) were standardized and summed to create a single score (3).

At ages 4 and 7 years, behavioral inhibition was assessed based on children's reactions to three same-sex, same-age, unfamiliar peers. Children were selected for quartets at age 4 based on BI scores at ages 14 and 24 months. Quartet selection on age 7 was based on behavior at age 4. Each quartet therefore included one highly BI child, one child with a low BI score, and two children with scores near the median. At each age the children were led into an unfamiliar laboratory room with numerous attractive toys. Visits were split into several episodes (4), and BI coding focused on two 15-minute free play sessions. Behavior was coded with Rubin's Play Observation Scale (5). Ten-second segments were coded for social participation and the cognitive quality of play. Social reticence (e.g., time spent engaged in unoccupied or on looking behaviors) was used as the index of BI at ages 4 and 7.

At each assessment point, mothers completed questionnaires assessing temperament. At 14 and 24 months, maternal reports of temperament were gathered using the Toddler Behavior Assessment Questionnaire (6, 7), a 111-item measure on which parents were asked to rate the frequency of specific behaviors as they occurred in the past month. For this study, the Social Fearfulness scale, which consists of 19 items measuring inhibition, distress, withdrawal, and shyness, was utilized. Items were averaged, with higher scores indicating greater distress to novel or uncertainty-provoking situations. Mothers completed the Colorado Child Temperament Inventory at 4 and 7 (8). This 30-item measure asked mothers to rate their child with a 5-point Likert scale ranging from 1 (Not at all/Strongly disagree) to 5 (A lot/Strongly agree) on 6 factors pertaining to different dimensions of child temperament. These include emotionality, activity, attention, soothability, sociability, and shyness. The current analysis relied on scores from the shyness scale (9).

To create the overall BI score, individual scores from each assessment wave were standardized and averaged (Cronbach's alpha = 0.83) with higher scores reflecting higher levels of BI (Full cohort sample: Mean = 0.019, SD = 0.60). Table S1 presents scores for each component used in the BI composite score.

In recruiting participants for the current study, cohort members with scores below the median were designated as non-BI while participants above the median were noted as high BI. Participants were recruited in light of the current exclusionary criteria and needed power levels for our planned analyses.

Behavioral Data Analysis

Similar to previous studies, the primary analyses focused on bias to angry-threat faces. Secondary analyses considered happy faces. Bias scores were calculated by subtracting the mean reaction time (RT) in correct congruent trials from the mean RT for correct incongruent trials. Positive values indicate vigilance for the emotion stimuli and negative scores indicate avoidance.

There was an overall bias to allocate attention to threat in the 44 participants, considered together, $t_{(43)} = 2.05$, $p < 0.05$, $d = 0.63$. However, no difference manifested between the BI and non-BI groups, $t_{(42)} = 0.75$, $p = 0.46$, $d = 0.23$, with no significant bias either in the BI, $t_{(20)} = 1.90$, $p = 0.072$, $d = 0.85$ or the non-BI, $t_{(22)} = 0.98$, $p = 0.34$, $d = 0.42$, group. No bias to happy faces emerged in any group (see Table 1). Attention bias scores were not correlated with current internalizing levels (p's > 0.55). The groups did not differ in overall RTs to the happy and angry faces (p's > 0.66).

The absence of a between-group difference in the current study for the behavioral measure of attention bias influences our interpretations of the brain-imaging data. Some prior imaging studies with the dot-probe task (10, 11) have emphasized the importance of comparing participants who perform similarly, in terms of behavioral data. This is often done in imaging studies to eliminate between-group differences in behavior and examine only neural differences in the context of comparable task performance. Other imaging studies have emphasized the importance of capturing between-group differences in behavior when comparing neural responses to the task (12). This is because such between-group differences in behavior demonstrate the relevance of the specific task to the phenotype being examined. It is important to note that prior behavioral reports have found relations between BI and performance on the dot-probe task in adolescence (13). These studies relied on larger samples ($n = 138$) which may have had sufficient power to detect the BI-attention bias link. Alternately, our lack of a group effect may reflect the longer time lag between BI assessment and completing the dot-probe task in young adulthood. Finally, our data may reflect previous work suggesting that the context of testing may shape the behavioral manifestations of attention biases (12, 14).

fMRI Activation Analysis

Datasets from individual subject analyses were converted to Talairach space and group-level analyses were performed using a two-sample *t*-test with AFNI's 3dttest comparing BI and non-BI participants. Angry congruent and incongruent correct trials were combined to create one singular condition (angry) containing 48 events; the same was done for the happy/neutral trials. The 48 neutral/neutral events served as a contrast.

The principal effect of interest was the neural response of BI individuals to angry relative to neutral faces, in line with the focus on threat-processing in the attention bias literature (7). Angry faces are considered particularly ecologically-valid stimuli for examining the processes leading to the emergence of anxiety. However, we also completed parallel analyses with happy-neutral trials, motivated by two concerns. First, these analyses served to test the specificity of our findings linked to threat processing. Second, recent work suggests that a history of BI is also associated with unique patterns of neural responses to positive incentive cues (4-6). The current analyses allowed us to contrast responses to threat and positive emotion

Significant activations in voxel-level maps at the group level were defined as exceeding a brain-wide voxel-level threshold of $p < 0.005$ and a subsequent cluster correction based on Monte Carlo simulations to generate a corrected cluster threshold, $p < 0.05$. Cluster corrections were applied regionally to the three a priori regions of interest (ROIs), ventrolateral prefrontal cortex, dorsolateral prefrontal cortex (dlPFC), insula, as well as to the whole-brain–defined by an intersection mask of all participants using volume correction to generate a corrected *p*-value of 0.05 using AlphaSim (AFNI). Selection of ventrolateral prefrontal cortex and dlPFC ROIs was based on prior research using the dot-probe task (12, 19). For the insula, prior psychophysiological interaction (PPI) analyses on the dot-probe task have found significant amygdala-insula connectivity (10). Considerable evidence implicates the insula in mood disorders, and anatomical studies delineate strong dlPFC-insula connections (20, 21). Thus, the insula ROI also afforded the opportunity to test hypotheses on mediation of functional connectivity between the amygdala and dlPFC, two regions that are weakly anatomically connected with each other but strongly connected to the insula, via Granger causality analysis (described in the main text).

fMRI Activation Results

Analyses focused on the contrast of correct responses to angry trials relative to neutral trials. No between-group differences surpassed statistical thresholds in an angry-versus-neutral contrast. This included the amygdala and three frontal ROIs, at ROI-derived thresholds, as well as in other regions, at the more stringent whole-brain-corrected threshold.

Significant amygdala activation ($p < 0.01$) was evident in the sample as a whole, both for angry (right: x, y, z = 22, 0, -10, $t_{(43)} = 1.45$; left: -22, -1, -10, $t_{(43)} = 1.11$) and neutral (right: 18, -1, -10, $t_{(43)} = 1.21$; left: -21, 1, -10, $t_{(43)} = 0.15$), relative to the baseline. However, amygdala activation in the sample as a whole did not emerge in the angry-versus-neutral contrast (Figure S1).

For the initial activation analyses, no group differences were found for happy trials. The associated PPI analyses also showed modest results, which are noted in the main text.

Moderated-Mediation Analysis

The potential role of neural connectivity in the relation between early temperament and internalizing problems in young adulthood was evaluated using a moderated mediation model based on the work of Preacher, Rucker and Hayes (22). This allowed us to examine whether the relations in the current study takes the form of mediation, whereby BI affects a continuous measure of internalizing symptoms via its effect on neural connectivity during the task, or moderation, whereby BI and connectivity patterns interact to shape levels of internalizing symptoms.

The standard approach (23) for testing mediation requires three linear models to estimate 1) the relation between behavioral inhibition and internalizing (parameter c), 2) the relation between neural connectivity and internalizing (parameter b), 3) the relation between behavioral inhibition and neural connectivity (parameter a), and finally, 4) the residualized effect between behavioral inhibition and internalizing (parameter c´). All paths must be significantly different from zero for mediation to be possible. In a moderated mediation model, one can see if neural connectivity moderates the relation between behavioral inhibition and internalizing (parameter ab). In this instance, moderation is present when the relation between behavioral inhibition and internalizing varies as a function of neural connectivity. As the model uses a single outcome measure, we ran the analyses separately for each PPI connectivity pathway, amygdala-dlPFC and amygdala-insula. See Figures S2 and S3 for a graphical presentation of the analyses presented in the main text.

Table S1. Individual standardized scores for the behavioral and maternal-report measures used to create the behavioral inhibition (BI) composite. Means are presented for the overall sample (*n* = 44) and separately for the BI and non-BI groups. Standard deviations (±) are presented in parentheses.

	Full Sample	**BI**	**NON-BI**
Sample size	44	21	23
14mo Social Fear	0.227 (1.124)	0.872 (1.071)	-0.465 (0.691)
24mo Social Fear	0.223 (1.064)	1.017 (0.854)	-0.510 (0.666)
4yr Shyness	0.162 (1.028)	0.656 (1.024)	-0.382 (0.666)
7yr Shyness	0.005 (0.880)	0.620 (0.550)	-0.500 (0.596)
14mo Inhibition	0.046 (1.028)	0.191 (0.963)	-0.249 (0.894)
24mo Inhibition	0.055 (0.921)	0.416 (0.770)	-0.395 (0.716)
4yr Social Reticence	0.201 (1.436)	0.699 (1.487)	-0.512 (0.567)
7yr Social Reticence	-0.005 (1.447)	-0.002 (1.095)	-0.355 (0.584)
BI Score	0.061 (0.733)	0.614 (0.716)	-0.429 (0.244)

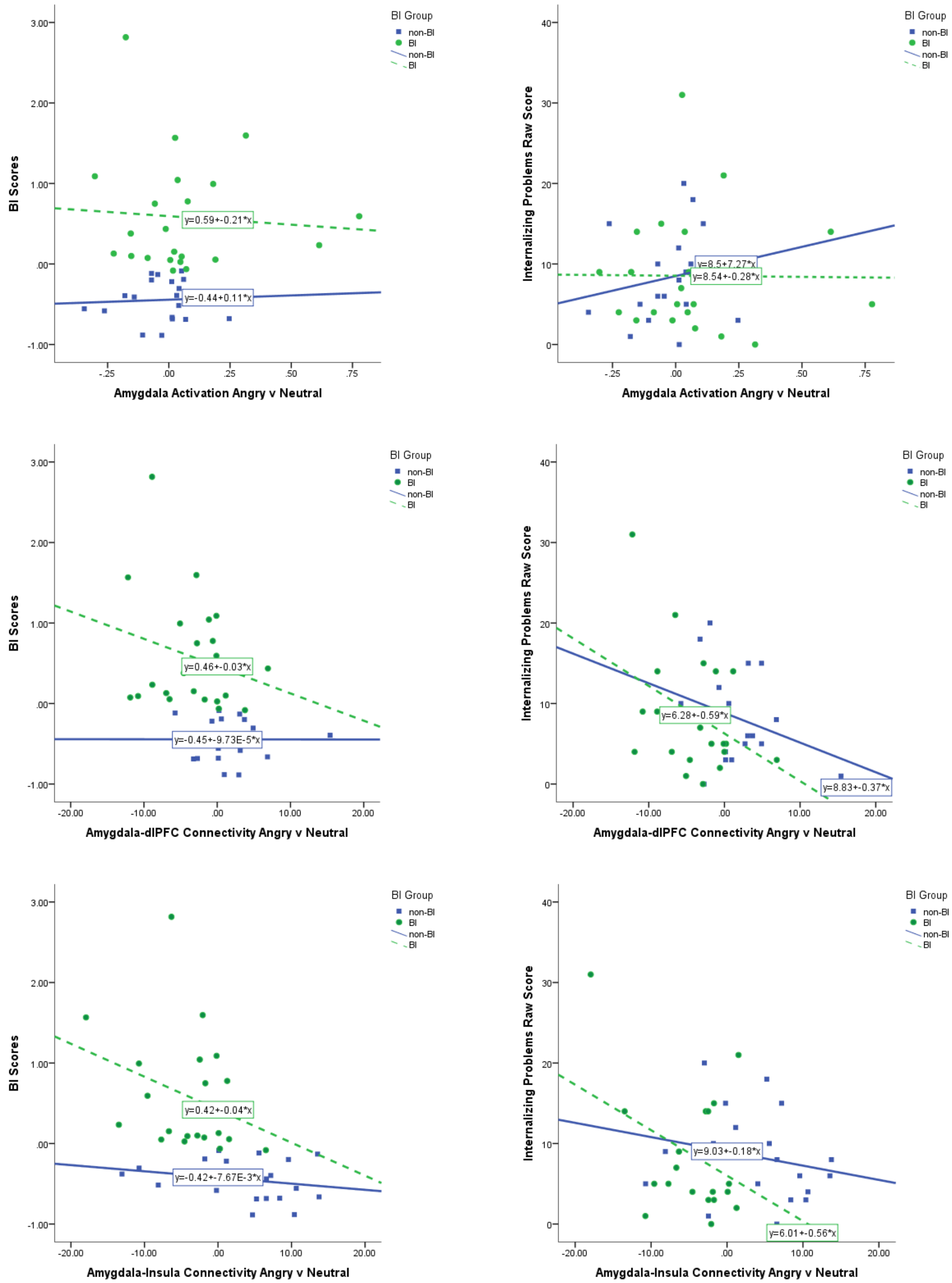

Figure S1. Scatterplots reflecting the relations between neural activity during the attention-bias task and our central measures of BI and internalizing problems. The top row of scatterplots presents activation levels for the angry versus neutral contrast on the x-axis, with BI scores presented on the left and internalizing scores on the right. The second row of scatterplots presents connectivity levels for the angry versus neutral comparison for the amygdala-dlPFC PPI analysis on the x-axis, with BI scores presented on the left and internalizing scores on the right. The third row of scatterplots presents connectivity levels for the angry versus neutral comparison for the amygdala-insula PPI analysis on the x-axis, with BI scores presented on the left and internalizing scores on the right. BI, behavioral inhibition; dlPFC, dorsolateral prefrontal cortex; PPI, psychophysiological interaction.

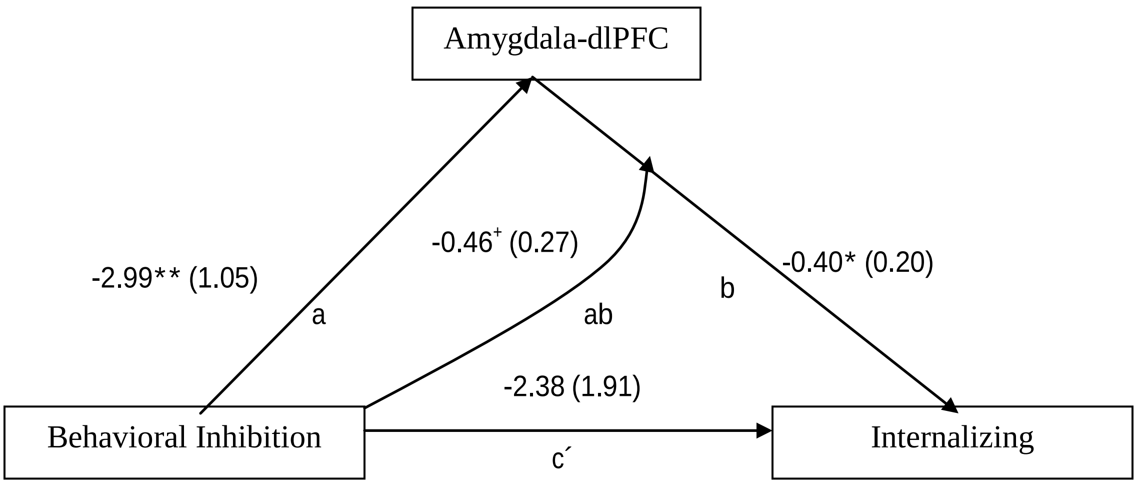

Figure S2. Path results for the moderated mediation model involving the amygdala-dlPFC PPI connectivity, BI, and Internalizing levels. Noted are the effect coefficients with standard errors in parentheses. BI, behavioral inhibition; dlPFC, dorsolateral prefrontal cortex; PPI, psychophysiological interaction. *$p < 0.01$, *$p < 0.05$, $^{+}p < 0.10$

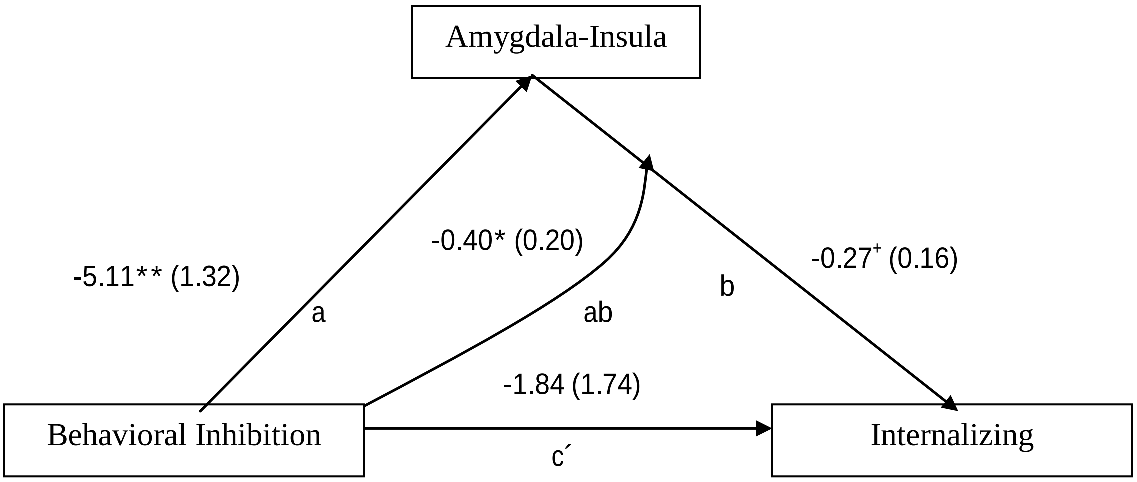

Figure S3. Path results for the moderated mediation model involving the amygdala-insula PPI connectivity, BI, and Internalizing levels. Noted are the effect coefficients with standard errors in parentheses. BI, behavioral inhibition; PPI, psychophysiological interaction. $^{**}p < 0.01$, $^{*}p < 0.05$, $^{+}p < 0.10$.

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