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Development of Neural Mechanisms Underlying Threat Processing: Associations With Childhood Social Reticence and Adolescent Anxiety

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ABSTRACT

BACKGROUND: Social reticence in early childhood is characterized by shy and anxiously avoidant behavior, and it confers risk for pediatric anxiety disorders later in development. Aberrant threat processing may play a critical role in this association between early reticent behavior and later psychopathology. The goal of this longitudinal study is to characterize developmental trajectories of neural mechanisms underlying threat processing and relate these trajectories to associations between early-childhood social reticence and adolescent anxiety.

METHODS: In this 16-year longitudinal study, social reticence was assessed from 2 to 7 years of age; anxiety symptoms and neural mechanisms during the dot-probe task were assessed at 10, 13, and 16 years of age. The sample included 144 participants: 71 children provided data at age 10 (43 girls, $mean_{age}$ = 10.62), 85 at age 13 (46 girls, $mean_{age}$ = 13.25), and 74 at age 16 (36 girls, $mean_{age}$ = 16.27).

RESULTS: A significant interaction manifested among social reticence, anxiety symptoms, and time, on functional connectivity between the left amygdala and the left dorsolateral prefrontal cortex, voxelwise $p < .001$, clusterwise familywise error $p < .05$. Children with high social reticence showed a negative association between amygdala–dorsolateral prefrontal cortex connectivity and anxiety symptoms with age, compared to children with low social reticence, suggesting distinct neurodevelopmental pathways to anxiety.

CONCLUSIONS: These findings were present across all conditions, suggesting task-general effects in potential threat processing. Additionally, the timing of these neurodevelopmental pathways differed for children with high versus low social reticence, which could affect the timing of effective preventive interventions.

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Early-childhood inhibited behavior is an important risk factor for the development of pediatric anxiety disorders, which typically emerge in adolescence (1,2). However, not all children with inhibited behavior will eventually develop an anxiety disorder. Therefore, discovering factors associated with early behavior and later anxiety is of clinical importance. Previous studies have proposed that threat processing is associated with early-childhood behavior and anxiety (3,4). In this 16-year longitudinal study with three waves of neuroimaging data assessing threat processing in a sample well characterized for early-childhood behavior, we extend this work on neurodevelopmental trajectories of pediatric anxiety disorders.

Early-childhood inhibited behavior has been defined in several ways (5,6). Our laboratory has defined behavioral inhibition as a young child's response to novelty and uncertainty in a laboratory setting (7). The child is observed responding to an unfamiliar adult and to novel objects while the child's caregiver is in the room. Social reticence is defined as shy, anxious, and avoidant behavior observed during interactions with either familiar or unfamiliar same-age peers (8). Social reticence is associated with social competence in middle childhood (9) and is a risk factor for anxiety symptoms (10). While behavioral inhibition predicts social reticence in the current sample, we include children who were not assessed for behavioral inhibition and so focus exclusively on social reticence.

Because not all children with social reticence develop anxiety, it is important to study associations with other risk factors. Neural mechanisms underlying threat processing may affect associations between early-childhood behavior and anxiety (3,4). Moreover, threat bias, the tendency to overly attend to threatening stimuli, relates to anxiety in children and adults (11,12). Previous studies on the neural mechanisms underlying threat bias suggest that amygdala–prefrontal cortex (PFC) connectivity is stable over a 9-week period and that these measures may be more reliable than behavioral bias indices (13). While some studies focus on brain activity (14), most focus on amygdala connectivity during the dot-probe task. For example, children with anxiety disorders showed increased positive amygdala-insula connectivity while maintaining attention on the location of threats, whereas healthy control subjects showed increased positive amygdala-insula

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connectivity while shifting attention away from the location of threats (15). In adults, a history of childhood behavioral inhibition was related to increased negative amygdala–dorsolateral PFC (dlPFC) connectivity while both maintaining and shifting attention from threat-related stimuli (4). Finally, in socially anxious adults, avoidant orientation and slow disengagement during the dot-probe task were related to amygdala–superior temporal sulcus connectivity (16). These studies all focused on amygdala connectivity at one time point, whereas Abend *et al.* (3) included two time points. They showed that behavioral inhibition was related to distinct neurodevelopmental pathways leading to pediatric anxiety symptoms: children with higher levels of behavioral inhibition showed a negative association between amygdala-dlPFC connectivity when maintaining attention to threat and anxiety symptoms with age, whereas children with lower levels of behavioral inhibition showed a positive association with age. The authors concluded that children with higher levels of behavioral inhibition might have an early-emerging deficiency in the capacity to regulate attention capture by threats (3). In that study, threat processing and anxiety symptoms were measured at 10 and 13 years of age. Many anxiety symptoms typically develop in early adolescence (17), and amygdala connectivity with other brain regions continues to develop during adolescence (18,19). Hence, the current study extends these prior findings to late adolescence.

Here, we used a sample that was previous published (3) and added a late-adolescent (age 16) data point, a time when anxiety often manifests. We focused on social reticence rather than behavioral inhibition, which allowed us to increase our sample size by adding a second, unselected sample to the sample used in the prior work. The goal of this longitudinal study is to characterize the developmental trajectories of neural correlates underlying threat processing and relate these correlates to the association between early-childhood social reticence and adolescent anxiety. Social reticence was assessed repeatedly between 2 and 7 years of age; threat processing and anxiety symptoms were assessed at 10, 13, and 16 years of age. Based on Abend *et al.* (3) in an overlapping sample, we hypothesized distinct neurodevelopmental pathways to pediatric anxiety based on social reticence: children with high social reticence were expected to show a negative association between amygdala-dlPFC connectivity when maintaining attention to threat and anxiety symptoms with age, whereas children with low social reticence were expected to show a positive association with age.

METHODS AND MATERIALS

Participants

Children were recruited from a longitudinal study on behavioral inhibition and early-childhood reticence. The current study includes data from 2 samples: the first sample (n = 291) was selected at 4 months of age based on reactivity to novelty (20); the second sample (n = 384) was recruited from the community at 2 years of age, unselected on the basis of reactivity or other traits (21,22). Both samples were followed until 16 years of age. Data from sample 1 have previously been reported, testing similar research questions in relation to behavioral inhibition but with fewer data points (3). The data from these samples were combined, and sample was included as a covariate in all analyses. Study procedures were approved by the National Institute of Mental Health and University of Maryland-College Park Institutional Review Boards. Informed consent and assent were obtained from parents and children, respectively.

Threat processing was assessed in the magnetic resonance imaging (MRI) scanner at 10, 13, and 16 years of age. Functional MRI (fMRI) data were provided by 183 children for at least one time point. Ten children who provided data at age 10 were excluded from analyses (1 for subthreshold accuracy [below 70%] on the task, 1 for aborting the task, 3 for excessive head motion during the anatomical scan, 2 for excessive head motion during the functional scan [see fMRI individual-level analysis: activation], 2 for missing social reticence scores, and 1 for missing anxiety symptom scores) (see Figures S1 and S2 for an overview per sample). Fifteen children who provided data at age 13 were excluded (2 for subthreshold accuracy, 1 for aborting the task, 1 for technical issues during data collection, and 11 for missing social reticence scores). Sixteen children who provided data at age 16 were excluded (6 for subthreshold accuracy, 2 for excessive head motion during anatomical scan, 1 for aborting the task, 1 for psychotropic medication use, and 6 for missing social reticence scores). The final sample consisted of data provided by 144 participants (Table 1 and Figure S3 for a correlation matrix of the study variables). Nineteen children provided data at all three time points[1] and 48 children provided data at two time points. Linear mixed-effects modeling accounted for missing data in this longitudinal design, resulting in more reliable effect estimates than complete-case analysis (23–25). Four children were Asian (2.78%), 24 were Black or African American (16.67%), 5 were Hispanic or Latino (3.47%), 16 were multiracial (11.11%), and 83 were White (57.64%). The race or ethnicity of 12 participants was unknown (8.33%). fMRI data from 61 children from sample 1 at age 10 and from 64 children from sample 1 at age 13 have been previously published, testing similar research questions on behavioral inhibition (3).

Social Reticence

Social reticence was observed by independent raters during free play, cleanup, and social problem-solving interactions with unfamiliar age- and sex-matched peers at 2, 3, 4, 5, and 7 years of age [see Supplemental Methods and Degnan *et al.* (8,26)]. Children from sample 1 were randomly paired with children from sample 2 for these interactions.[2] All behavior scores were standardized within time point and averaged together to create a social reticence composite (Table S1A, B for the correlations among time points[3]). Social reticence was correlated with behavioral inhibition, r = 0.32, $p < .001$.

[1]Children with fMRI data at all three time points did not differ in social reticence and anxiety symptoms from children with fMRI data at 1 or 2 time points, Fs < 3.23, ps $> .08$.

[2]Dyadic nesting was not taken into account in calculating the composite score because children were paired with different children at all time points, which would average out the effects of partner.

[3]It should be noted that these correlations are modest and that some of the correlations are lower in the current sample than in the full sample.

Table 1. Overview of the Demographics, Clinical Indices, and Dot-Probe Performance in the Current Sample

Measures	Age 10	Age 13	Age 16
Demographics			
Total, *n* (*n* female)	71 (43 F)	85 (46 F)	74 (36 F)
Sample 1	59 (35 F)	65 (38 F)	43 (24 F)
Sample 2	12 (8 F)	20 (8 F)	31 (12 F)
Age, Years, Mean (SD) [Range]	10.62 (0.46) [9.50 to 11.67]	13.25 (0.76) [12.23 to 15.49]	16.27 (0.68) [12.92 to 17.69]
IQ[a,b], Mean (SD) [Range]	115.17 (11.97) [88 to 138]	116.01 (13.33) [82 to 138]	115.58 (13.99) [77 to 141]
Clinical Indices			
Current Anxiety Disorder, *n*	10	10	3
Social Reticence[a], Mean (SD) [Range]	−0.02 (0.45) [−0.57 to 1.77]	0.03 (0.53) [−0.62 to 2.21]	−0.04 (0.47) [−0.83 to 2.21]
Anxiety Symptoms, Mean (SD) [Range]	17.35 (9.16) [2 to 42]	10.23 (7.17) [0 to 32]	9.15 (7.72) [0 to 29.5]
Social Anxiety, Mean (SD) [Range]	3.95 (2.77) [0 to 12]	3.94 (2.86) [0 to 12]	3.32 (2.87) [0 to 12]
Dot-Probe Performance			
Accuracy, %, Mean (SD) [Range]	89.61% (7.53%) [69.20% to 99.00%]	91.86% (5.78%) [73.80% to 100%]	92.98% (4.40%) [80.40% to 99.40%]
Threat Bias, ms, Mean (SD) [Range]	2.43 (31.99) [−73.42 to 79.25]	6.62 (33.04) [−55.89 to 106.44]	7.61 (21.47) [−48.85 to 96.01]
Happy Bias, ms, Mean (SD) [Range]	7.06 (30.48) [−54.42 to 79.48]	5.60 (30.60) [−68.91 to 89.80]	0.78 (23.29) [−77.73 to 56.93]
ABV Threat, Mean (SD) [Range]	0.05 (0.02) [0.01 to 0.11]	0.05 (0.02) [0.01 to 0.14]	0.06 (0.02) [0.03 to 0.14]
ABV Happy, Mean (SD) [Range]	0.05 (0.02) [0.02 to 0.11]	0.05 (0.02) [0.02 to 0.10]	0.06 (0.02) [0.03 to 0.15]

ABV, attention bias variability; F, female.

[a]IQ and social reticence were measured at one time point. The means seem to change over time, because of the different number of children with usable functional magnetic resonance imaging data at each time point.

[b]IQ scores were missing for 6 participants.

Anxiety Symptoms

Anxiety symptoms were assessed within 6 weeks of each scan using the Screen for Child Anxiety Related Emotional Disorders (SCARED), a reliable 41-item child- and parent-report measure of anxiety symptomology (Figure S4) (27,28). Internal consistency of the SCARED is good for both self-report and parent-report (Cronbach's $\alpha > 0.74$) (27). Participants missing more than 20% of questions would be excluded ($n = 0$); missing data were replaced with the mean of the available items. A total score of 25 or higher has been suggested to indicate clinically significant anxiety (29). Total scores from each parent and youth pair ($r = 0.37$, $p < .001$) were averaged into a mean anxiety score per participant for each time point (3) to mitigate informant differences (30). Anxiety scores were mean centered at each time point.

Dot-Probe Task

Threat-related attention was assessed using the dot-probe task (3,11,15,31). Participants completed the same version of the fMRI dot-probe task at each time point (Figure 1; Supplemental Methods). This task included 5 trial types: angry-congruent (48 trials), angry-incongruent (48 trials), happy-congruent (48 trials), happy-incongruent (48 trials), and neutral-neutral (96 trials). Trials occurred across 4 blocks of 4 minutes and 15 seconds; neural and behavioral responses to each trial type were recorded. The task was programmed and administered using E-Prime (Psychology Software Tools) and included faces from the validated NimStim set (32). In line with prior work, trials were included in analyses only if reaction time (RT) was between 150 and 2000 ms and <2.5 standard deviations from the child's mean RT and if the probe type was correctly identified. Children with mean accuracy below 70% at 1 time point were excluded from that time point ($n = 9$) (3,15).

Behavioral analyses included two indices of attentional processes: attention bias scores and attention bias variability (ABV) (3,11,33). Threat bias scores were computed by subtracting the mean RT in threat-congruent trials from the mean RT in threat-incongruent trials, with positive scores (i.e., faster responses to threat-congruent trials) reflecting attention bias toward threat (15,34). Happy bias scores were computed with the same procedure for happy trials. Both angry and happy stimuli were considered to test for bias specificity to threat.

ABV measures fluctuations in attention allocation and has been suggested to reflect impairments in attentional control with relation to anxiety (3,35). Using a moving-window algorithm, threat bias scores were calculated (as described above) for 10 successive angry-neutral trials. To control for associations between mean and variance, the standard deviation of these scores was calculated and then divided by the participant's overall mean RT (34,36). The same procedure was applied to happy-neutral trials to calculate happy ABV scores. Table S2 shows the intraclass correlations across time points for the behavioral measures.

Behavioral Data Analysis

To test associations among social reticence, anxiety, and attention bias at all time points, we ran two linear mixed-effects models in R (*nlme* package) (37) with attention bias or ABV scores as the dependent variable. Anxiety symptoms (SCARED scores), social reticence, task condition (threat and happy), and time (age 10, 13, and 16 years) were included as independent variables; sample (1 or 2) and sex (male or female) were included as covariates; subject was modeled as random effect. All statistical tests were two sided; significance threshold was set to $\alpha < 0.05$. Follow-up paired *t* tests were

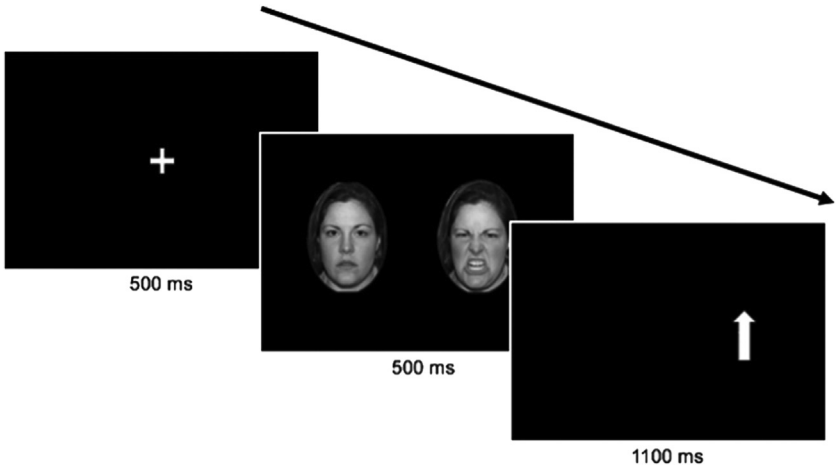

Figure 1. Overview of the dot-probe task. Reproduced with permission from Harrewijn *et al.* (53).

conducted for significant effects using the phia (38) and emmeans (39) packages.

fMRI Individual-Level Analysis

fMRI data were collected during the dot-probe task on two identical 3T MR750 General Electric scanners (Waukesha) with identical 32-channel head coils and scan parameters. fMRI data were analyzed with AFNI version 20.2 (40,41). Data preprocessing included the following steps: removing the first 4 pre–steady-state volumes, despiking, slice timing correction, aligning functional scans to structural scans, nonlinear registration to standard Talairach space (TT_N27 template), volume registration, spatial smoothing with a 6 mm (full width at half maximum) Gaussian kernel, and scaling (allowing interpretation of effect estimates values as percent signal change relative to the mean). We used generalized psychophysiological interaction (gPPI) (42) analysis to assess task-specific differences in amygdala functional connectivity. We used FreeSurfer (43) to extract subject-specific amygdala seeds. Then, we ran a whole-brain individual-level general linear model including correct responses in the 5 conditions, incorrect responses, 6 motion regressors (displacement in x, y, and z axes, rotational movement of roll, pitch, and yaw), time series in the amygdala seed, and the PPI terms (products of the detrended and demeaned seed and the 5 task condition regressors). gPPI analysis was run for left and right amygdala separately.

fMRI Group-Level Analysis (gPPI)

To test associations between social reticence, anxiety, and task-specific functional connectivity at all time points, we ran a linear mixed-effects model using AFNI's 3dLMEr (24) with the output from the individual-level gPPI as the dependent variable. Anxiety symptoms (total SCARED scores), social reticence, task condition (angry-congruent, angry-incongruent, happy-congruent, happy-incongruent, and neutral-neutral), and time (age 10, 13, and 16 years)[4] were included as independent variables; recruitment source (sample 1 or 2) and sex (female or male) were included as covariates; subject was modeled as random effect. AFNI's 3dClustSim (44), which assumes a non-Gaussian autocorrelation smoothing function (44) in light of Eklund *et al.* (45), was run on all data and showed a minimum size of 45 contiguous voxels (Nearest Neighbor = 2) for a voxelwise threshold of $p < .001$ (two sided) and a clusterwise $\alpha < 0.05$, based on 10,000 Monte-Carlo simulations. For significant effects, we extracted individual-level betas for post hoc analysis using linear mixed-effects models in R (nlme package) (37).

[4]Time point (instead of actual age) was included because we do not assume similar age effects across the whole range (e.g., between 10 and 13 years and 13 and 16 years).

RESULTS

Behavioral Analyses

No significant main or interaction effects manifested for social reticence, anxiety, task condition, or time on attention bias scores, $p > .10$ (Table S3). There was a significant main effect of time on ABV scores, with children showing greater ABV at age 16 versus at age 10, $b = 0.01$, $p < .001$ and at age 16 versus at age 13, $b = 0.01$, $p < .001$ (Figure 2). There were no other significant main or interaction effects, $ps > .19$ (Table S4).

Task-Specific Functional Connectivity

Left Amygdala. No brain regions showed a significant 4-way interaction among social reticence, time point, anxiety symptoms, and condition in left amygdala functional connectivity. However, there was a significant 3-way interaction among social reticence, anxiety symptoms, and time with the left dlPFC (coordinates: 46.2, −11.2, 38.8; $k = 62$) and the right occipital cortex (coordinates: −43.8, 58.8, 31.2; $k = 53$) (voxelwise threshold of $p < .001$ and a clusterwise familywise error $p < .05$). Results for the lower-order interactions and main effects are provided in Table S5.

To decompose these interaction effects, follow-up analyses were conducted separately for children with high versus low levels of social reticence (median split) (Figure 3A; see Figure S5 for Johnson-Neyman plots). For children with high social reticence, simple slope analysis indicated that anxiety and amygdala-dlPFC connectivity were positively associated

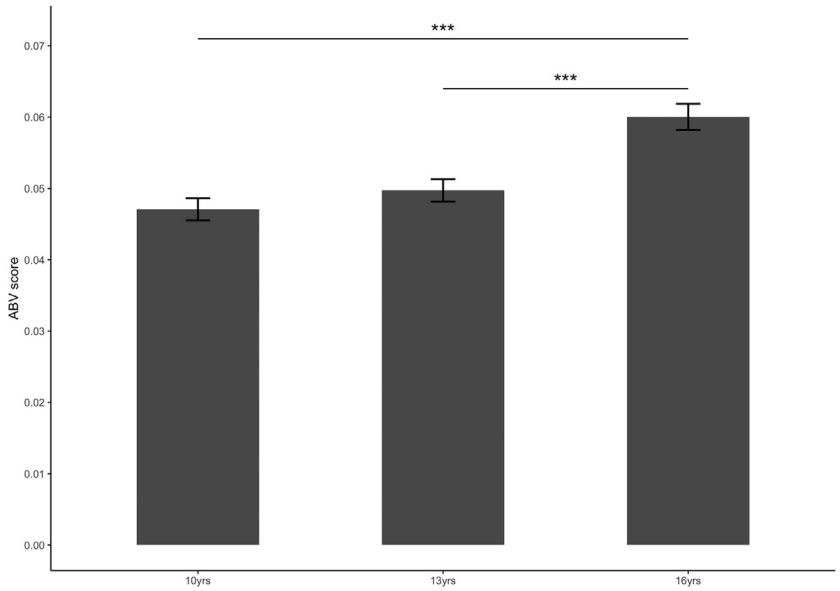

Figure 2. Effect of time on attention bias variability (ABV), averaged across angry-neutral and happy-neutral trials. ***$p < .001$.

at 10 years of age (b = 0.02; 95% CI, 0.0004–0.03), not associated at 13 years of age (b = −0.01; 95% CI, −0.03 to 0.002), and negatively associated at 16 years of age (b = −0.2; 95% CI, −0.03 to −0.002). These associations were different between time points 10 and 13 and 10 and 16, $b_{10–13yrs}$ = 0.03, $b_{10–16yrs}$ = 0.03, χ^2_1s > 11.63, ps < .001, but not between time points 13 and 16, $b_{13–16yrs}$ = 0.004, χ^2_1 = 0.18, p = .67. A different trend emerged for children with low social reticence, whereby anxiety and amygdala-dlPFC connectivity were not associated at 10 years of age (b = −0.002; 95% CI, −0.02 to 0.01), 13 years of age (b = −0.007; 95% CI, −0.02 to 0.01), and 16 years of age (b = 0.01; 95% CI, −0.003 to 0.03). These associations were different between time points 13 and 16, $b_{13–16yrs}$ = −0.02, χ^2_1 = 4.83, p = .03, but not between time points 10 and 13 or 10 and 16, $b_{10–13yrs}$ = 0.004, $b_{10–16yrs}$ = −0.01, χ^2_1s < 3.04, ps > .08.

Follow-up analyses for amygdala–occipital cortex connectivity (Figure 3B; Figure S6 for Johnson-Neyman plots) indicated that for children with high social reticence, the relationship between anxiety and functional connectivity was positive at 10 years of age (b = 0.01; 95% CI, 0.001–0.02), negative at 13 years of age (b = −0.01; 95% CI, −0.02 to −0.0003) and not associated at 16 years of age (b = −0.01; 95% CI, −0.02 to 0.01). These associations were different between time points 10 and 13 and 10 and 16, $b_{10–13yrs}$ = 0.03, $b_{10–16yrs}$ = 0.02, χ^2_1s > 6.75, ps < .01, but not between time points 13 and 16, $b_{13–16yrs}$ = −0.01, χ^2_1 = 0.57, p = .45. In contrast, for children with low social reticence, the relationship between anxiety and functional connectivity was not significant at 10 years of age (b = −0.01; 95% CI, −0.02 to 0.002), 13 years of age (b = −0.01; 95% CI, −0.02 to 0.004), and 16 years of age (b = 0.01; 95% CI, −0.002 to 0.02). These associations were different between time points 10 and 16 and 13 and 16, $b_{10–16yrs}$ = −0.02, $b_{13–16yrs}$ = −0.02, χ^2_1s > 6.36, ps < .05, but not between time points 10 and 13, $b_{10–13yrs}$ = −0.002, χ^2_1 = 0.10, p = .75.

Right Amygdala. No brain regions showed significant 4-way interactions among social reticence, time point, anxiety symptoms, and condition in functional connectivity with the right amygdala. Unlike for the left amygdala, no 3-way interaction occurred among social reticence, anxiety symptoms, and time point for the dlPFC. There was a 3-way interaction in the cerebellum, middle occipital cortex, superior temporal gyrus, and thalamus, but these will not be discussed here because we hypothesized findings in the dlPFC. Results for the lower-order interactions and main effects are shown in Table S6.

DISCUSSION

The goal of this study was to characterize the developmental trajectories of neural mechanisms underlying threat processing and relate these trajectories to associations between early-childhood social reticence and adolescent anxiety. We continued the study by Abend *et al.* (3) by including an adolescent time point and a second unselected sample and examining social reticence rather than behavioral inhibition. Attention bias and ABV were not associated with social reticence and anxiety symptoms. Children with high social reticence showed a negative association between amygdala-dlPFC connectivity and anxiety symptoms with age, compared to children with low social reticence. However, this amygdala-dlPFC connectivity was in response to potential threat processing in general and not specific to threat bias.

Differential amygdala-PFC connectivity has been found in studies examining threat processing and anxiety (3,4,15). Amygdala-PFC connectivity supports emotion regulation and develops in adolescence (18). The negative association between the amygdala and the dlPFC might suggest less efficient emotion regulation during threat processing. Here, we show that the combination of high social reticence in early childhood and strong negative amygdala-PFC connectivity at 13 and 16

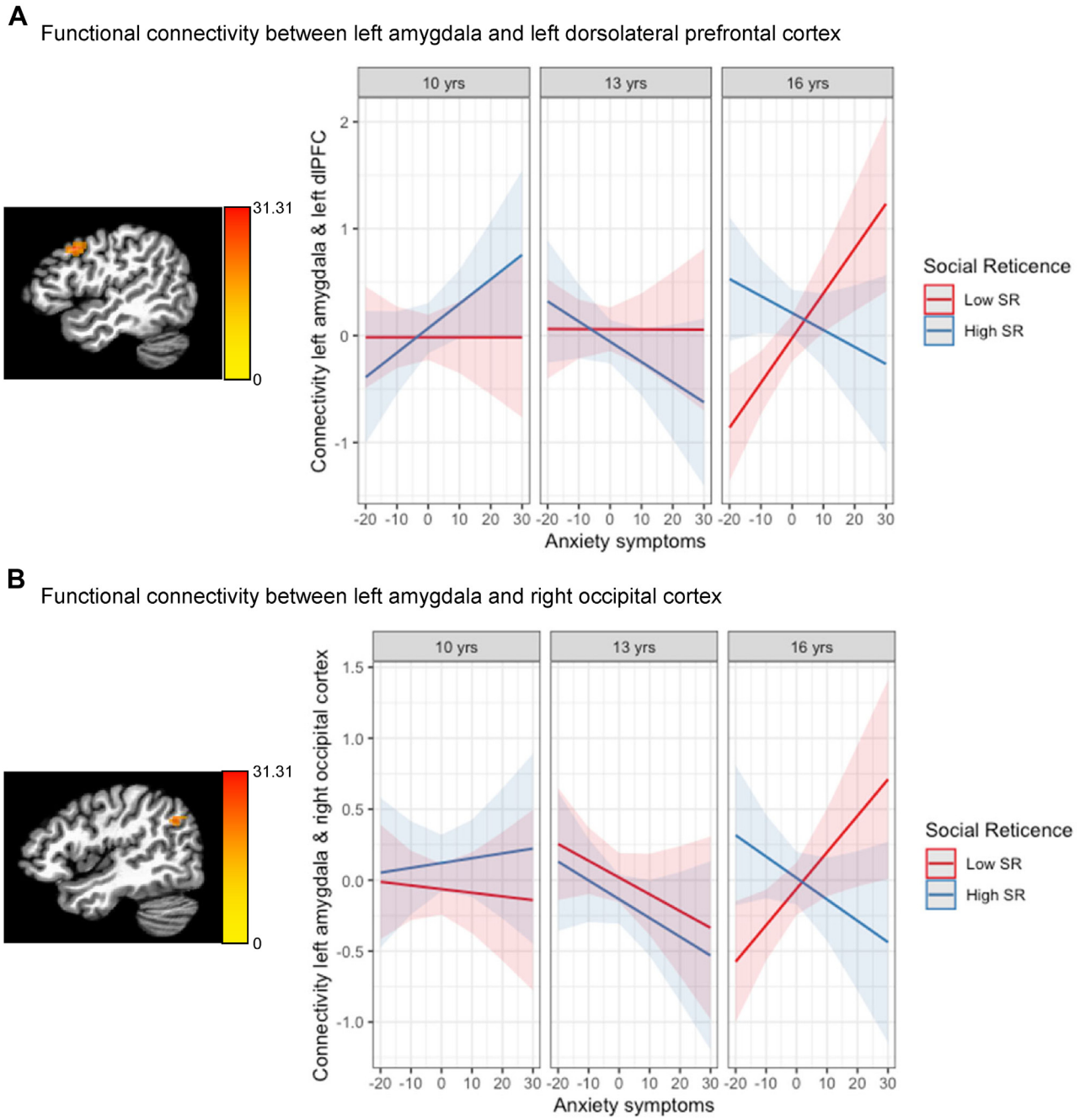

Figure 3. Three-way interaction among social reticence (SR), anxiety symptoms, and time point on functional connectivity of the left amygdala with left dorsolateral prefrontal cortex (dlPFC) **(A)** and right occipital cortex **(B)**. SR was dichotomized (by median split) for follow-up analyses.

years of age increases risk for adolescent anxiety symptoms. Understanding relationships between socially reticent behavior and brain function could inform targeted prevention. This study also found that overall response to potential threats, rather than responses to threats in association with attention demands, relates to anxiety symptoms. This could suggest that future studies attempting to identify risk might focus on overall levels of potential threat responsivity.

Similar neurodevelopmental pathways were reported by Abend *et al.* (3) in an overlapping sample. Extending these past findings into late adolescence builds on findings showing risk for anxiety disorders to change past 13 years of age (17), a time when amygdala connectivity also changes (18,19). We showed that the timing of neurodevelopmental pathways might differ for children with high versus low early social reticence. Children with high social reticence showed an early

change (from 10 to 13 years of age) from a negative to a positive association between amygdala-dlPFC connectivity and anxiety; in contrast, children with low social reticence showed a later change (from 13 to 16 years of age) in the association between amygdala-dlPFC connectivity and anxiety. Because high social reticence places children at risk for anxiety, the current findings could inform timing of preventive interventions in these children. More specifically, preventive interventions might work better when started before this early change at 13 years of age.

Beyond amygdala-dlPFC connectivity, other findings manifested for amygdala–occipital cortex connectivity. This has not been reported previously because previous studies have focused exclusively on connectivity between the amygdala and the PFC and/or the insula (3,4,15,16). Future studies reporting whole-brain findings are necessary to reveal whether these effects are chance findings or more stable patterns in the dot-probe task.

The direction of this connectivity (positive vs. negative correlation) differs between studies. Moreover, other studies [including Abend *et al.* (3) in an overlapping sample] have found connectivity with the right, instead of the left, amygdala. However, it should be noted that the literature on laterality of the amygdala in emotion processing is unclear (46,47). Finally, the associations between amygdala-PFC connectivity, behavior, and anxiety are complex. Some have found significant amygdala-PFC connectivity only when maintaining attention to threat [in an overlapping sample (3)] (15), whereas others have found this across all task conditions (S.P. Haller *et al*., unpublished data, May 2022). More broadly, reliability in fMRI paradigms is lower for contrasts involving conditions with similar demands (e.g., angry vs. happy faces) than for contrasts involving distinct conditions (e.g., faces vs. objects) (48). We found amygdala-dlPFC connectivity across task conditions (i.e., no 4-way interaction), possibly reflecting individual differences in potential threat processing generally, rather than specific aspects of threat processing. Such a task-general effect is in line with the absence of behavioral findings in our study and with findings on differential amygdala-PFC connectivity during resting state in children with anxiety disorders (49). These task-general effects can be studied in two different ways in task-based fMRI. First, functional connectivity can be computed independently of task events. This task-general connectivity is more reflective of individual differences in behavior than functional connectivity during resting state (50,51) but has not yet been applied to the dot-probe task. Second, the similarity between task-general connectivity and resting-state connectivity is also a measure of task-general effects and is related to task performance (52). In the dot-probe task, task performance is related to the similarity between task-general and resting-state functional connectivity (53). These studies suggest that measures other than task-specific connectivity may be more predictive of individual differences in the dot-probe task.

The dot-probe task rightfully has been criticized due to low reliability for the behaviors it engages (13,54–56), consistent with lack of behavioral effects in the current study. Moreover, the findings with ABV as reported in Abend *et al.* (3) in an overlapping sample were not found here. The association between behavioral inhibition, anxiety, and fluctuations in attention allocation was found at 13 and not at 10 years of age. The lack of findings with ABV in the current study could suggest that these associations are less sensitive to developmental effects beyond age 13, which would explain why there was no interaction between social reticence, anxiety, and time. Even though other measures during the dot-probe task, such as computational (16,57) and neuroimaging (13) measures, are more reliable, it is problematic to relate these other measures to unreliable behavioral measures. One possible solution is to relate neuroimaging findings to real-life measures of threat processing. For example, decreased amygdala-dlPFC connectivity during the dot-probe task was associated with using more distraction after negative events in real life (58). Moreover, increased amygdala–anterior PFC connectivity while receiving social feedback was associated with a real-life attention bias toward a potentially critical judge (59). These studies are examples of how neuroimaging findings could be related to real-life processes to enhance ecologic validity.

To our knowledge, this was the first study to include three waves of longitudinal neuroimaging data in a sample well characterized for social reticence, continuing previous research (3) by focusing on social reticence, including data from late adolescence, and adding a second unselected sample. A few limitations should be noted. First, children were recruited from two samples, one group was selected at 4 months of age based on reactivity to novelty and one unselected group was recruited from the community at 2 years of age. However, children from both samples only differed in age, and we accounted for sample in all analyses. Second, attrition from inclusion at 4 months or 2 years of age to inclusion in the MRI scans was high. Third, this was not a clinical sample, so anxiety symptoms were relatively low. Fourth, adult faces were used in the dot-probe task. It is important to study whether these effects are similar when adolescent faces are used. Fifth, puberty was assessed, but the analyses would have been underpowered if puberty were included. It should be noted that puberty could also be related to the change from negative to positive connectivity-anxiety association from 10 to 13 years of age. Sixth, it is not possible to estimate effect sizes in linear mixed-effects models with fMRI data. It should be noted that effect sizes in fMRI research in general are typically no larger than medium. This might limit the actionable conclusions drawn from this study, so these findings should be replicated in a larger, more diverse sample.

To conclude, we found distinct neurodevelopmental pathways to pediatric anxiety based on social reticence: children with high social reticence showed a negative association between amygdala-dlPFC connectivity and anxiety symptoms with age compared to children with low social reticence. These patterns of functional connectivity were present across all task conditions, early in children with high social reticence, and later in children with low social reticence.

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