

# Preschool negative emotionality predicts activity and connectivity of the fusiform face area and amygdala in later childhood

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## Abstract

Negative emotionality (NE) refers to individual differences in the propensity to experience and react with negative emotions and is associated with increased risk of psychological disorder. However, research on the neural bases of NE has focused almost exclusively on amygdala activity during emotional face processing. This study broadened this framework by examining the relationship between observed NE in early childhood and subsequent neural responses to emotional faces in both the amygdala and the fusiform face area (FFA) in a late childhood/early adolescent sample. Measures of NE were obtained from children at age 3 using laboratory observations, and functional magnetic resonance imaging (fMRI) data were collected when these children were between the ages of 9 and 12 while performing a visual stimulus identity matching task with houses and emotional faces as stimuli. Multiple regression analyses revealed that higher NE at age 3 is associated with significantly greater activation in the left amygdala and left FFA but lower functional connectivity between these two regions during the face conditions. These findings suggest that those with higher early NE have subsequent alterations in both activity and connectivity within an extended network during face processing.

**Key words:** negative emotionality; fusiform face area; amygdala; early childhood; temperament

## Introduction

Negative emotionality (NE) refers to individual differences in the propensity to experience and react with negative emotions, such as sadness, anxiety, fear and anger (Costa and McCrae, 1992; Goldberg, 1993). Included in most models of temperament, NE is very similar to the personality trait of neuroticism, and temperamental NE in childhood shows strong continuity with neuroticism in adulthood (as such, the two will not be distinguished in the rest of this report) (Caspi *et al.*, 2005; Clark and Watson, 2008). Elevated levels of NE are evident in many mental disorders, including major depressive disorder (Khan *et al.*, 2005; Kendler *et al.*, 2006; Klein *et al.*, 2011), schizophrenia (Van Os and

Jones, 2001) and anxiety (Malouff *et al.*, 2005) as well as personality disorders (Saulsman and Page, 2004). Identifying the neural correlates of NE, and how they are manifested in development, is critical for understanding how this trait contributes to so many forms of psychopathology (Lahey, 2009; Ormel *et al.*, 2013; Shackman *et al.*, 2016). Despite this, the neural correlates of NE are not well understood within areas involved in visual processing of affective stimuli.

Previous studies that examined the neural correlates of NE and related traits have typically used emotional faces as task stimuli to differentiate neural activity in individuals with high NE from controls (Haas *et al.*, 2007, 2008; Stein *et al.*, 2007;

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Hooker et al., 2008; Chan et al., 2009; Everaerd et al. 2015). The majority of these studies have analyzed only one component of the face processing network, finding that the amygdala is more active in individuals with high NE relative to controls (Haas et al., 2007; Everaerd et al., 2015). These findings of heightened amygdala activity have been reported for both positive and negative emotional faces and have been interpreted as increased salience processing of emotional stimuli (Haas et al., 2007; Stein et al., 2007; Everaerd et al., 2015). However, emotional face processing involves a network comprising multiple visual areas, with a wealth of literature suggesting a close functional relationship between one of these visual areas, the fusiform face area (FFA) and the amygdala during emotional face processing in healthy young adults (Vuilleumier, 2005; Fairhall and Ishai, 2007; Herrington et al., 2011; Di et al., 2017).

Anatomical studies have found that the amygdala receives sensory information from subcortical pathways via the thalamus (Ledoux et al., 1983). Several findings suggest that this input affords the amygdala with early stimulus information which is assessed for salience and can potentially influence downstream sensory processing (i.e. FFA in face processing) (Romanski et al., 1997; Morris et al., 1999; Pasley et al., 2004). Others have proposed that the relationship is bidirectional by showing that FFA also seems to influence amygdala activity during face processing (Herrington et al., 2011). Despite this, only one study, to our knowledge, has reported increased activation of the amygdala and FFA in adults with high NE (Chan et al. 2009). Therefore, based on the close functional relationship between the two regions, incorporating the amygdala as well as the FFA in an analysis of NE may yield a broader understanding of the neural substrates underlying this behavioral reactivity pattern. Indeed, expanding analyses to include multiple components of the face processing network has provided better characterization of autism spectrum disorder symptoms, with activity and connectivity of the amygdala and FFA being associated with gaze fixation and social impairment measures within this population (Dalton et al., 2005; Kleinhans et al., 2008).

As NE is relatively stable across age (Campos et al., 1989; Roberts and DelVecchio, 2000; Dyson et al., 2015), longitudinal studies in youth offer a way of tracing the relationships between the behavioral and neural manifestations of this trait over development. Recently, one such study reported that preschoolers (ages 4–6) with elevated NE had increased amygdala activity during the presentation of sad faces relative to controls, and this amygdala activity was associated with elevated NE levels observed 12 months later (Gaffrey et al., 2016). Additionally, other studies have demonstrated that early behavioral inhibition, which reflects fear of unfamiliar people and situations and overlaps considerably with aspects of NE, predicts subsequent elevated amygdala activity during novelty detection (Schwartz et al., 2003). This study sought to extend this literature by examining the relationship between observed NE in early childhood and subsequent neural responses to faces in both the amygdala and the FFA in a late childhood/early adolescent sample.

Furthermore, to determine whether our findings were specific for NE, we also examined the relationship between positive emotionality (PE) in early childhood and subsequent neural responses to faces. PE is closely related to the personality trait of extraversion and refers to individual differences in the propensity to experience and react with positive moods, to be interested in and engaged with the environment and to seek out social interactions (Watson and Clark, 1997; Depue and Collins, 1999; Lucas et al., 2000). As studies have found a positive

relationship between amygdala activity and both PE and NE during processing of happy faces (Canli et al., 2001; Stein et al., 2007), we attempted to control for PE effects to determine the unique contribution of NE to FFA and amygdala activations during face processing. Additionally, due to the robust association between NE and depressive symptoms, we also controlled for children's current depressive symptoms as assessed by the parental report on Children's depression inventory 2 (CDI-2) (Kovacs, 2004).

We hypothesized that youth with high NE would exhibit greater amygdala and FFA activation relative to those with lower NE during face processing, controlling for age, gender, CDI and PE. This is based on the findings of increased activity of the FFA (Chan et al., 2009) and amygdala during face processing in adults with heightened NE (Haas et al., 2007, 2008; Stein et al., 2007; Hooker et al., 2008). As the relationship between NE and activity of the FFA and amygdala has been found for negative, neutral, as well as positive face stimuli (Haas et al., 2007; Stein et al., 2007; Chan et al., 2009), we first examined the relationship between early NE and subsequent neural activity using a composite face model including all three types face stimuli (neutral, happy and sad) and then examined whether the effect is associated with specific stimuli. Furthermore, given the relatively well-known functional connectivity between FFA and amygdala (Morris et al., 1999; Pasley et al., 2004; Herrington et al., 2011), we applied psychophysiological interaction (PPI) analyses to test the hypothesis that functional connectivity between the FFA and amygdala during face processing would also be altered in children with high, relative to low, NE.

## Materials and methods

### Subjects

This study was part of a larger longitudinal study assessing the relationship between temperament in early childhood and later psychological health (see Olino et al., 2010 for details). Five hundred and fifty-nine children were recruited for the study and completed laboratory observations of temperament at age 3 [mean ( $M$ ) = 3.44,  $s.d.$  = 0.25]. Participants were recruited from the community utilizing commercial mailing lists, screened for major medical conditions and developmental disorders and required to have at least one English speaking biological parent. Seventy-nine participants, with oversampling of children with elevated NE at age 3, were recruited for an functional magnetic resonance imaging (fMRI) study when they were 9- to 12-years old. The mean NE scores of this fMRI subsample ( $M$  = 0.16,  $s.d.$  = 0.45) were significantly higher [ $t(114) = 2.88$ ,  $P < 0.05$ ] than that of the entire sample ( $M$  = 0.00,  $s.d.$  = 0.55).

After screening for excessive head motion during fMRI (67% of usable data, see preprocessing protocol later), a total of 62 participants (30 female; age:  $M$  = 10.64,  $s.d.$  = 0.90) remained in the reported analysis. Their NE scores (all:  $M$  = 0.17,  $s.d.$  = 0.47; males:  $M$  = 0.19,  $s.d.$  = 0.49; females:  $M$  = 0.15  $s.d.$  = 0.46) were similar to the 17 who were removed from analysis due to motion ( $M$  = 0.09,  $s.d.$  = 0.34), [ $t(34) = 0.79$ ,  $P > 0.05$ ]. Individual differences in average head motion during fMRI also did not significantly correlate with NE values across subjects ( $r = 0.18$ ,  $P > 0.10$ ). The majority (83%) of this subsample was Caucasian. Due to computer error, behavioral data from only 44 participants were retained for the face task during fMRI. Therefore, the main statistical models included fMRI data from all 62 participants while additional analyses were conducted to replicate the main findings with the subsample of 44 participants

with behavioral data, adjusting for accuracy and reaction time effects.

### Early temperament assessment

Temperament was assessed when children were 3-years old, using the Laboratory Temperament Assessment Battery (LAB-TAB) (Gagne et al. 2011). The LAB-TAB is a series of laboratory tasks that elicit temperament-relevant emotional responses. For example, in ‘snack delay’ a child was instructed to wait for the experimenter to ring a bell before eating a snack, while in ‘impossibly perfect green circles’ the experimenter asked the child to draw a circle, during which the experimenter responded by mildly criticizing the child’s work and asking the child to draw another circle (for greater detail see Olino et al., 2010; Dyson et al., 2015). Raters coded videotapes of the Lab-TAB, rating discrete emotions (e.g. sadness, anger, fear and positive affect) as expressed by facial, vocal and bodily indicators. These indicators were rated on an intensity scale of 1–3, with 1 being the least intense. Within each task, these ratings were summed for bodily, facial and vocal channels, and the sums were averaged across tasks. These average scores were then standardized, with the standardized scores then being averaged to form the composite NE and PE scores (Olino et al., 2010; Dyson et al. 2015). There were no significant correlations between NE ( $M=0.17$ ,  $s.d.=0.47$ ) and PE ( $M=-0.08$ ,  $s.d.=0.71$ ) scores ( $r=-0.01$ ,  $P>0.5$ ). Additionally, these measures have shown good interrater reliability values within this cohort (see Dyson et al. 2015 for details).

### Child depressive inventory

Parent ratings of child’s current depressive symptoms were assessed prior to scanning via the 17 item CDI-2 (Kovacs, 2004). There were no significant correlations between NE and CDI ( $M=7.23$ ,  $s.d.=4.69$ ) scores ( $r=-0.07$ ,  $P>0.5$ ). Additionally, this measure has demonstrated good reliability (Saylor et al., 1984).

### Visual stimulus identity matching task during fMRI

Each participant performed one task run, which included three iterations of four kinds of task blocks in one of the two pseudo-random orders. These task blocks (16s each) were interleaved with fixation blocks (14s each). The four different task conditions were defined by the type of visual stimuli presented in each block: neutral, sad and happy faces and houses. At the beginning of each task block, there was a 1s warning period, followed by four trials. Each trial begun with a fixation cross that lasted 600ms followed by the simultaneous presentation of two visual images for 3000ms. Participants made button presses to indicate whether or not the two images were identical or different within that time period. One-half of the trials showed matched images and one-half non-match. The task lasted a total of 6.2min.

### Image data acquisition, preprocessing and analysis

All anatomical and functional images were acquired utilizing a Siemen Trio 3 T scanner. For each child, the scanning session began by the acquisition of T1-weighted high resolution structural images using the following MPRAGE sequence: slices = 176, slice thickness = 1 mm, TR = 2400 ms, TE = 3.16 ms, flip angle = 8°, matrix size = 256 × 256, FOV = 256 × 256, voxel resolution = 1 × 1 × 1 mm<sup>3</sup>. After that, a set of inplane T2-weighted structural images were collected in the axial plane, aligned to the AC-PC

with the following parameters: slices = 37, slice thickness = 3.5mm, TR = 6450 ms, TE = 88 ms, flip angle = 120°, matrix size = 256 × 256, FOV = 256 × 256, voxel resolution = 1 × 1 × 3.5 mm<sup>3</sup>. T2\*-weighted axial-oblique functional images were acquired using the following EPI sequence: 37 interleaved axial slices, slice thickness = 3.5 mm, TR = 2000 ms, TE = 30 ms, flip angle = 90°, matrix size = 64 × 64, FOV = 224 × 224 mm, voxel resolution = 3.5 × 3.5 × 3.5 mm<sup>3</sup> and 186 volumes were collected.

All images were processed using SPM8 (<http://www.fil.ion.ucl.ac.uk/spm8/>). Prior to analysis, images were corrected for slice timing, realigned to the middle scan and from this realignment six parameter rigid body transformations were calculated. Structural images were coregistered with the mean functional image, segmented and then normalized to a standard space utilizing the MNI template. Functional images were then normalized utilizing the same parameters as the structural normalization. Finally, the functional images were smoothed using a 6mm FWHM Gaussian kernel.

Univariate analysis was conducted using the general linear model (GLM). Each of the four stimulus conditions (neutral face, happy face, sad face and house) was modeled in the GLM as a regressor using a boxcar function convolved with the canonical hemodynamic response function. The six motion parameters, the Euclidean norm calculated from these parameter and volumes with high frame-to-frame motion [composite motion  $\geq 0.5$  of Euclidean norm and/or mean global signal ( $Z \geq 3.0$ )] were entered into the model as covariates of no interest. Beta values were estimated for each voxel for the regressors, and these estimated parameters were used to generate the contrasts of interest. The average Euclidean norm moved was 0.156, after removing frames that exceeded 0.5.

### Region of interest (ROI) definition

ROIs were initially defined in the group face > house contrast ( $P < 0.001$  uncorrected). The group ROI masks were spheres with a radius of 9mm and centered at the peak coordinates of the activation clusters in the left and right FFA. For the left and right amygdala, the clusters fell within and beyond the bounds of the anatomical region. Given this, and that previous studies of amygdala have utilized the anatomical template (Pezawas et al., 2005; Iidaka et al., 2006), all the following analyses were conducted with the anatomical amygdala from the SPM anatomy toolbox version 2.0 (Eickhoff et al., 2005). Additionally, we defined left and right parahippocampal place area (PPA) using the group house > face contrast to serve as a control region in subsequent analyses.

For each individual, outlier voxels were defined as those with activation more than 2s.d. above the average activation within the group ROI mask. These outlier voxels were first removed, and the top 100 voxels with highest beta values during the face blocks were selected and defined as an individual’s ROI. The left FFA consisted of only 90 voxels as some participants did not have 100 voxels after outlier removal. This process was done for the left and right FFA, PPA and anatomical amygdala. These individual ROIs were used in the multiple regression and PPI analyses when extracting betas from whole-brain maps.

### Multiple regression analyses

Multiple regression models tested NE as a predictor variable for brain activation and functional connectivity while controlling for age, gender, PE, CDI and average head motion. Specifically, the models were applied to examine the relationship between NE at

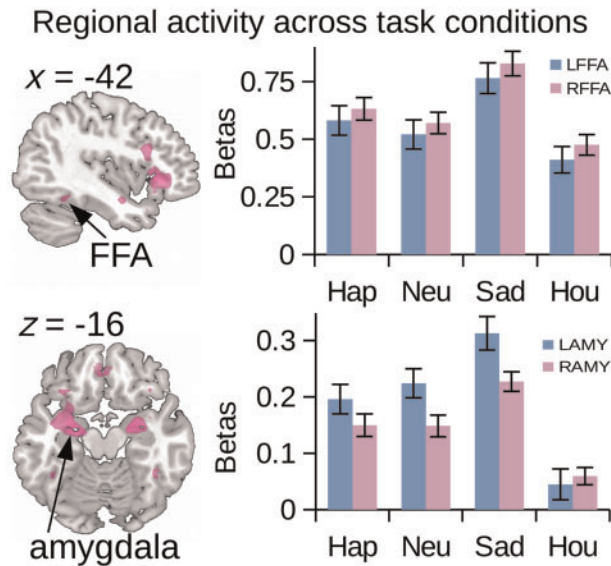


Fig. 1. Face-related activation clusters detected in the face > house group contrast. The maps are thresholded at  $P < 0.001$ . See Table 1 for peak coordinates and abbreviations. Bar graphs display activation in the amygdala and FFA for each face and house stimulus type: happy (Hap), neutral (Neu), Sad, and House (Hou).

age 3 and activity in the FFA and amygdala during the face conditions at ages 9–12. Models were first applied to the composite face condition including all three facial expressions, with the specific happy, sad and neutral conditions assessed using *post hoc* tests. All ROI-based analyses were conducted on beta values extracted from the composite and specific face > house contrasts. As our a priori hypothesis involved four ROIs, amygdala and FFA in the two hemispheres, we used an initial significance threshold of  $P < 0.05/4 = 0.01$  to assess significant NE effects while *post hoc* analyses of specific face types (happy, sad and neutral) were held at a significance threshold of  $P < 0.05$ .

In addition, whole-brain multiple regression analysis was conducted with models predicting neural activity using NE values while controlling for age, gender, PE, CDI and average head motion. The whole-brain analysis was conducted to determine if NE bore a relationship to face-related activity in brain regions that were outside of the bounds of the ROIs. Effects of NE were evaluated at a voxel-wise threshold of  $P < 0.001$ , while multiple comparison correction was performed on a cluster-wise level, the threshold for which was estimated by 3dClustSim (<http://afni.nimh.nih.gov>) (Cox et al., 2017). Based on the results of the simulation, a cluster threshold of 29 voxels was set for whole-brain analysis. All results from whole-brain multiple regression analyses subsequently described met these statistical criteria unless otherwise noted.

Similarly, a second set of multiple regression analyses was performed on the subset of 44 individuals with behavioral data, while controlling for accuracy and reaction time. This model was used to determine the unique contribution of NE to face-related activation while controlling for the behavioral measurements from the face task. Model significance was assessed using the whole-brain and ROI analyses at the same corrected thresholds as earlier.

### PPI analysis

To determine whether early NE scores influenced the functional connectivity between FFA and amygdala, we applied PPI

analysis (implemented through the generalized PPI toolbox; McLaren et al., 2012) with the left and right FFA as seed regions. FFA seeds were defined individually using each participant's face > baseline contrast thresholded at  $P < 0.001$  and peak coordinate that fell within the group FFA mask. Final seed regions for each individual were defined by removing outlier voxels that were more than 2 s.d. above the mean activation within the ROI mask, and including the top 50 activated voxels. Individual PPI maps were created for each participant, which consisted of beta values corresponding to the extent a voxel's signal could be predicted from the interaction term of the face blocks and activity of the FFA. FFA functional connectivity (beta values from PPI) was extracted from the left and right amygdala. These values were then used as the dependent variable in multiple regression models to test for NE effects in predicting functional connectivity while controlling for age, gender, PE, CDI and average head motion. As our a priori hypothesis involved four specific regions, amygdala and FFA in the two hemispheres, an initial significance threshold of  $P < 0.05/4 = 0.01$  was used to determine significant effects while *post hoc* analyses of specific face types (happy, sad, neutral) were held at a significance threshold of  $P < 0.05$ . Additionally, for comparison purposes, we calculated PPI values using the left and right PPA as seed regions and examined the relationship of NE to connectivity between these regions and the FFA and amygdala ROIs.

## Results

### Behavioral results

For the 44 participants who had behavioral data for the stimulus identity matching task, they were on average more accurate at houses ( $M = 88\%$ ,  $s.d. = 11\%$ ) than faces ( $M = 75\%$ ,  $s.d. = 20\%$ ),  $t(43) = -6.23$ ,  $P < 0.001$ , which was comprised of neutral faces ( $M = 78\%$ ,  $s.d. = 18\%$ ), happy faces ( $M = 75\%$ ,  $s.d. = 23\%$ ) and sad faces ( $M = 73\%$ ,  $s.d. = 24\%$ ). Reaction time data displayed a similar pattern, with participants performing marginally faster during the house condition ( $M = 1371.90$  ms,  $s.d. = 231.73$  ms) than the face conditions ( $M = 1418.47$  ms,  $s.d. = 206.92$  ms),  $t(43) = 1.99$ ,  $P = 0.053$ , which was comprised of neutral ( $M = 1444.9$  ms,  $s.d. = 290.61$  ms), happy ( $M = 1423.4$  ms,  $s.d. = 279.49$  ms) and sad faces ( $M = 1484$  ms,  $s.d. = 224.54$  ms). Participants' accuracy and response time were not significantly correlated with NE ( $-0.21 < r < 0.28$ ,  $P > 0.05$ ).

### Face-related brain activations

At the group level, direct contrasts of face and house conditions revealed activation of a subset of the typical nodes of the face processing network (Figure 1), including the bilateral FFA, superior temporal sulcus (STS) and amygdala ( $P < 0.001$ ), with the bilateral amygdala and right STS activation clusters reaching cluster corrected significance threshold ( $k \geq 29$ ). The peak coordinates of these clusters are shown in Table 1. Responses of bilateral FFA and amygdala in each stimulus condition are shown in Figure 1 (right).

### Multiple regression—ROIs

We first examined the a priori hypothesis regarding the relationship between individual differences in NE at age 3 and activity in the face-related ROIs in late childhood/early adolescence (Figure 2). In the multiple regression model predicting face-related activation in the left FFA, NE was a significant factor,  $t(56) = 3.93$ ,  $P < 0.001$ . More specifically, NE was significant in

**Table 1.** Suprathreshold activation clusters in the face > house group contrast of the stimulus matching task ( $P < 0.001$ )

	X	Y	Z	Peak t-value	Cluster size
L. amygdala	-21	-9	-15	9	282
R. amygdala	21	-9	-12	7.1	200
L. anterior temporal face patch	-48	6	-30	3.92	8
R. anterior temporal face patch	42	9	-30	5.3	(part of the R. amygdala cluster)
L. FFA	-42	-48	-18	4.55	12
R. FFA	42	-45	-15	5.7	13
L. superior temporal sulcus	-48	-36	-3	3.68	30
R. superior temporal sulcus	60	-42	6	4.88	186

L, left; R, right.

the models predicting the left FFA activation in response to the neutral [ $t(56) = 4.02$ ,  $P < 0.001$ ], happy [ $t(56) = 4.68$ ,  $P < 0.001$ ] and sad [ $t(56) = 2.15$ ,  $P < 0.05$ ] face conditions. Additionally, the relationship between NE and left amygdala activation during the neutral face condition was trending toward significance, with  $t(56) = 2.15$  and  $P = 0.05$ . No other ROIs showed face-related activations that were significantly predicted by NE ( $P > 0.06$ ). Models including CDI and PE revealed no significant correlation between PE and FFA or amygdala activation ( $P > 0.5$ ), while CDI was positively associated with face-related activity in the right amygdala [ $t(56) = 2.36$ ,  $P < 0.05$ ].

In the model that included reaction time and accuracy, NE was a significant predictor for face-related activations in the left FFA but not after correcting for multiple comparisons [ $t(40) = 2.37$ ,  $P = 0.02$ ]. NE was not a significant factor for any other ROIs in these models that included the task performance variables ( $P > 0.5$ ). To determine whether adding reaction time or accuracy values affected the relationship between NE and activation in the FFA and amygdala, we tested a model with only NE in this subsample. NE still failed to reach the corrected significance threshold ( $P = 0.02$ ) in these latter models.

### Multiple regression—whole-brain

To further examine whether voxels beyond the a priori ROIs showed face-related activation in association with NE across subjects, we conducted a whole-brain multiple regression analysis. Higher NE at age 3 was positively correlated with greater left FFA and left amygdala activation during the face condition relative to the house condition (Figure 3, Table 2). The left FFA cluster fell within the 9 mm group defined ROI for left FFA while the left amygdala fell within the anatomical ROI for left amygdala. Potential confounding factors including age, gender, PE, CDI and head motion were controlled in this analysis. Additionally, activation clusters in the right precentral gyrus and right inferior prefrontal gyrus were also significantly associated with NE (see Table 2 for coordinates).

We further examined whether a specific face condition was driving these effects. In general, we found a similar positive relationship between NE and activity in the left amygdala, left FFA and right inferior frontal gyrus during the neutral face condition (though the left amygdala with a cluster size of 21 did not reach the cluster threshold of 29). This effect was also found in the left FFA and right precentral gyrus for the happy face condition (see Table 2 for coordinates).

In the subsample with accuracy and reaction time data, NE as a predictor yielded similar results for the left FFA and left amygdala, albeit neither area reached the cluster significance threshold. To determine whether including reaction time or accuracy values in the regression model affected the relationship

between NE and FFA/amygdala activation, we tested a model with only NE in this subsample. In this latter model, amygdala activation but not FFA was above the significance threshold. CDI and PE did not show a significant association with FFA or amygdala activation.

### PPI—interaction between face processing and FFA functional connectivity

We further examined whether the functional connectivity between FFA and the amygdala was associated with individual differences in NE. With the left FFA as the seed region, the multiple regression model revealed that the PPI beta estimates for the left amygdala during the face versus house contrast were predicted by NE [ $t(56) = -2.438$ ,  $P = 0.01$ ] (Figure 4). More specifically, NE was a significant predictor for FFA-amygdala coupling during the neutral [ $t(56) = -2.78$ ,  $P < 0.01$ ] and sad [ $t(56) = -2.39$ ,  $P < 0.05$ ] face conditions. However, NE was not a significant predictor for connectivity between left FFA and right amygdala during the face conditions ( $P > 0.2$ ). With the right FFA as the seed region NE was not a significant predictor for connectivity between FFA and left or right amygdala ( $P > 0.2$ ). We also confirmed that similar effects were not evident with the left and right PPA as seed regions. Further analysis also indicated that CDI did not predict connectivity patterns between FFA and amygdala ( $P > 0.5$ ), while PE predicted increased connectivity between the left FFA and right amygdala [ $t(56) = 2.93$ ,  $P < 0.01$ ] during face processing (data not shown).

## Discussion

We examined the relationship between an observational measure of NE in 3-year-old children and their brain activation and functional connectivity to faces measured in late childhood/early adolescence. Our data supported our initial predication that high NE would be associated with greater amygdala and FFA activity during face processing. Our initial hypothesis that amygdala and FFA activity during face processing would bear positive relationships to early measures of NE was confirmed, while the connectivity between these regions was indeed altered for those with high NE in that higher NE was associated with lower levels of connectivity between the FFA and amygdala during face processing. These findings suggest that young children with high NE may develop along a different trajectory in terms of subsequent FFA and amygdala activity and connectivity relative to their low NE peers. The implications of these altered neural patterns are discussed below.

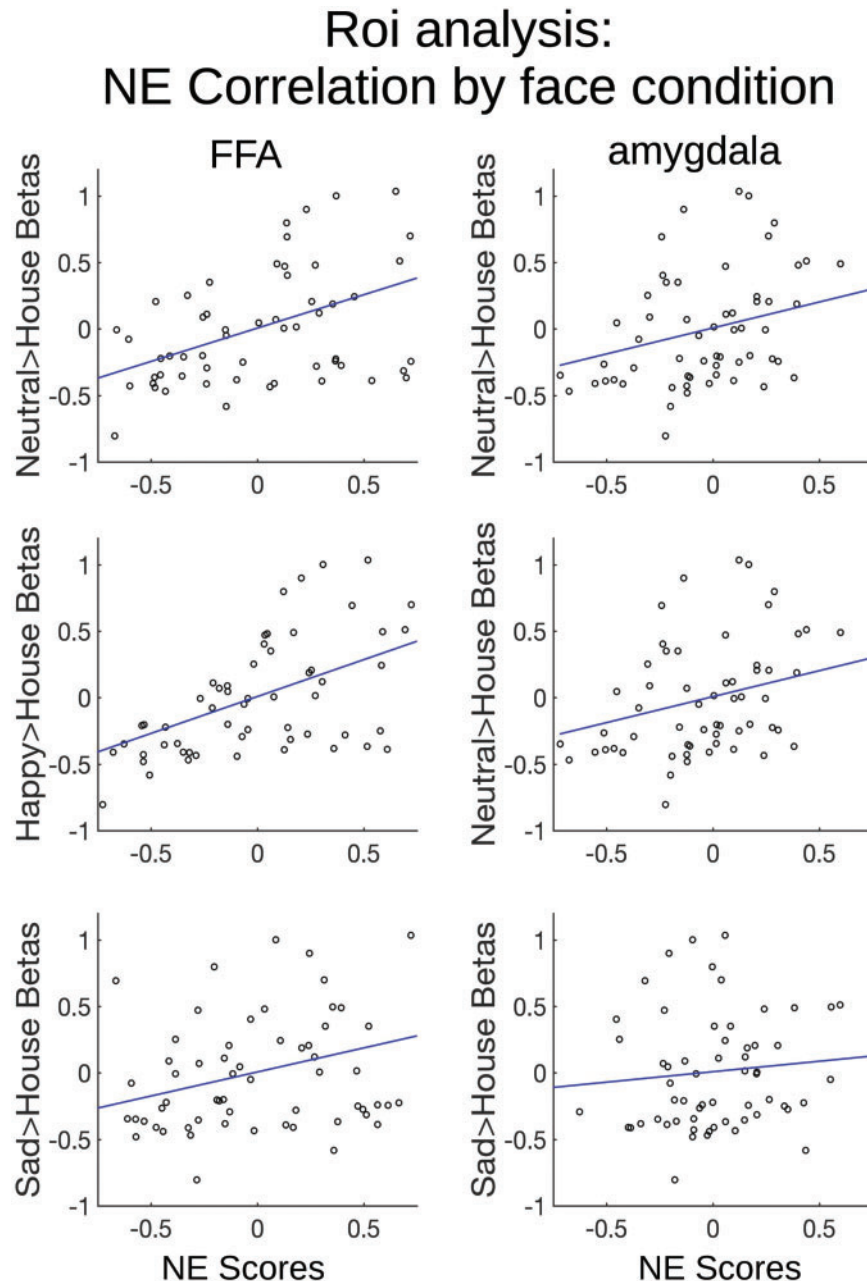


Fig. 2. Scatter plots displaying across-subject partial correlations between NE and beta values extracted from the left FFA ( $r$ 's = .53, .47, .27) and amygdala ( $r$ 's = .19, .25, .04) ROIs during the happy, neutral and sad face conditions respectively.

### Early NE and amygdala/FFA activity in later childhood/early adolescence

We found that children with elevated NE scores measured at age 3 subsequently had greater amygdala and FFA activity in response to happy, sad and neutral faces, which is consistent with several other studies conducted with adults in which high NE was associated with heightened amygdala activity during face processing relative to controls (Haas *et al.*, 2007; Stein *et al.*, 2007). Our results also corroborate with the one study in the literature that reported a similar relationship between high NE and FFA activity in an adult sample (Chan *et al.*, 2009). We thus extended these previous findings by demonstrating that a relationship between high NE and heightened activity in both the amygdala and FFA is present in youth.

Our findings further corroborate the only study, to our knowledge, that investigated the longitudinal relationship between NE and neural correlates of face processing in children. Gaffrey *et al.* (2016) examined a preschool sample and found that amygdala activity in response to sad faces was associated with negative affect observed 1 year later. Our findings complement this report in that we found the converse relationship between early NE and amygdala activity up to 9 years later, and extend this relationship to a core face processing region, the FFA. Taken together, these findings suggest that the increased amygdala and FFA activity found in adults with high NE are also evident in high NE children. Future studies involving multiple time-point assessments of NE are necessary to further substantiate this claim.

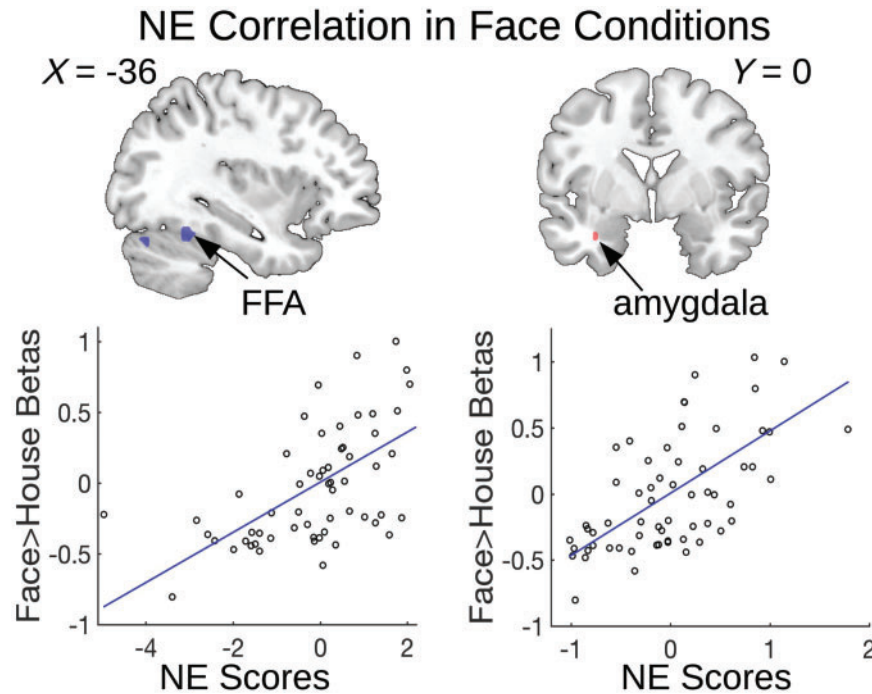


Fig. 3. Association between face-related activations and NE from the whole-brain analysis. The top image shows left FFA and left amygdala activations that were associated with greater NE values (at  $P < 0.001$ ,  $k \geq 29$ ). Scatter plots in the bottom show the partial correlation between NE and the beta values extracted from these two clusters for visualization.

Importantly, our NE results remained significant even with CDI being controlled for. CDI is potentially a critical confounding variable as in a different subsample of our cohort NE observed at age 3 was related to later depressive symptomatology after a stressful event (Kopala-Sibley et al., 2016). Of note, CDI as a factor was a significant predictor of similar patterns for increased activity in the left FFA during face processing, though the effect was weaker relative to NE. These results offer preliminary evidence that current depressive symptomatology and early NE may independently predict similar neural patterns during face processing, though further research is needed. It is worth to mention that the somewhat weaker relationship between NE and neural activation found for the subsample with behavioral data for the face task was likely due to the smaller sample size rather than the impact of behavioral performance on the relationship of NE with FFA/amygdala activity as this relationship was similar even when NE was the sole predictor in the statistical models.

### Implications of FFA and amygdala connectivity and early temperament

Interestingly, FFA and amygdala showed increased activity but decreased functional connectivity during face processing, suggesting that NE may be associated with a lack of integration between regions in the face network. As previous studies have demonstrated that processing of emotional faces is generally associated with increased connectivity between these regions (Fairhall and Ishai, 2007), our findings suggest that children with high NE at an early age may develop weak coupling between these regions during face processing. In contrast, early high PE related to increased connectivity between FFA and

Table 2. Clusters in face > house group contrast showing positive association with NE scores, and in contrasts of each specific face condition in which these associations maintain ( $P < 0.001$ ,  $k \geq 29$ )

	X	Y	Z	Peak t-value	Cluster size
Face					
L. amygdala	-39	-9	-27	4.70	33
L. FFA	-39	-48	-21	4.34	67
R. precentral gyrus	39	-12	51	4.14	29
R. ventral prefrontal cortex	30	39	-3	3.69	29
Neutral					
L. FFA	-39	-48	-21	4.36	93
R. ventral prefrontal cortex	27	39	0	4.55	35
Happy					
L. FFA	-39	-51	-21	5.07	54
R. precentral gyrus	33	3	57	4.95	195

Note: No suprathreshold clusters were found for the sad face condition.

amygdala. Taken together, these findings suggest a potential disassociation of the impact of early NE and PE on amygdala-FFA connectivity at a later age and highlight the importance of studying functional connectivity between the FFA and amygdala as a neural correlate of early temperament and risk of psychopathology.

### Limitations and future directions

Although we used a longitudinal design, data on NE and neural processing of faces were each collected at only one time point. This limits our ability to draw inferences regarding trajectories

## NE and FFA - amygdala functional connectivity

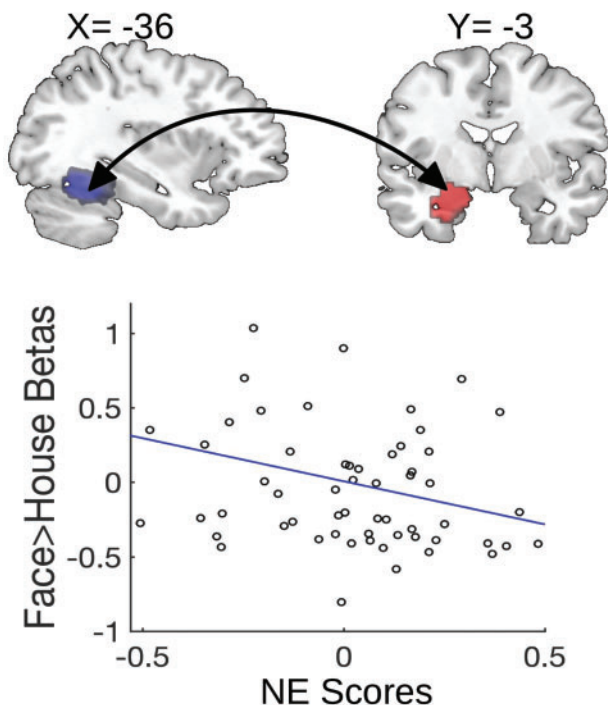


Fig. 4. Relationship between FFA-amygdala functional connectivity and NE. Images display the average left FFA and left amygdala mask across all individuals. Scatter plots show partial correlations of the beta values extracted for the face-condition PPI with NE scores.

of temperament and elevated FFA and amygdala activity and lowered functional connectivity. Future studies should examine NE and fMRI at multiple time points throughout development. Our findings highlight that future studies should include the broader face processing network, particularly the FFA, in conjunction with the amygdala, and that the connectivity between these regions should also be assessed to better characterize the trajectory of face processing as related to individual differences in NE. This would further explicate how early NE relates to both salience and basic visual face processing, as well how such a relationship plays a role in the elevated risk for various psychopathologies associated with NE.

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