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Interaction between hypothalamic-pituitary-adrenal axis genetic variation and maternal behavior in the prediction of amygdala connectivity in children

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ABSTRACT

High levels of negative, and low levels of positive parenting behaviors can increase the risk of internalizing symptoms in children, but the mechanisms underlying this association are still unclear. One possibility is that parenting behaviors affect the neural correlates of emotion processing in children. Further, genetic variants relevant to the function of the hypothalamic–pituitary–adrenal (HPA) axis are thought to moderate the effect of early experiences on the brain circuits underlying emotion processing, particularly those involving the amygdala. However, no studies have investigated the interactive effect of parenting behaviors and HPA axis-related genes on amygdala activity and connectivity during emotion processing, and in turn internalizing symptoms in children. Participants comprised 80 children (46 females, mean age = 10.0 years) from the community. Observational measures of maternal behavior were collected during mother-child interactions. Children underwent functional magnetic resonance imaging while performing an implicit emotion-processing task, and mothers and children completed measures of child internalizing symptoms. Genetic risk was calculated using an HPA genetic risk score. HPA genetic risk score was indirectly associated with greater child self-reported depressive symptoms via increased amygdala-precuneus connectivity during the emotion-processing task, and interacted with negative maternal parenting behavior to predict increased connectivity between amygdala and superior frontal gyrus, anterior cingulate cortex and parietal cortex. HPA-related genetic variation appears to moderate the effect of negative maternal parenting behavior on the neural underpinnings of emotion processing in children, and may confer risk for depressive symptoms via modulation of amygdala connectivity.

1. Introduction

Internalizing symptoms during childhood increase the risk of developing depression later in life (Kovacs and Devlin, 1998), underscoring the importance of understanding etiological factors to inform preventive efforts. High levels of negative parenting behaviors (e.g. parental over-involvement; aversiveness) and low levels of positive behaviors and warmth have been related to increased levels of internalizing symptoms during childhood (Yap and Jorm, 2015). However, individuals show significant variability in their susceptibility to such environmental factors (Ellis et al., 2011). A possible explanation is that genetic factors may play a role in influencing sensitivity to environmental exposure (i.e.,

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gene-environment (G × E)) (Uher, 2014).

In particular, genes related to the hypothalamic–pituitary–adrenal (HPA) axis are promising candidates for a mechanism that may determine individual differences in sensitivity to stressful family environments. The HPA axis is the key biological stress response system (McEwen, 2007), and dysregulation of this system has been found in depressed patients (Pariante and Lightman, 2008). Previous research has found associations between depression and variations in genes key to HPA axis function, such as the corticotrophin-releasing hormone receptor 1 (CRHR1) (Liu et al., 2006), the glucorticoid receptor (NR3C1) (Zobel et al., 2008) and the FK506 Binding Protein 5 (FKBP5) (Lavebratt et al., 2010). Importantly, several studies have found associations between HPA axis-related genes and depression only when in interaction with childhood adverse experiences, supporting a G × E framework (Appel et al., 2011; Bradley et al., 2008; Zimmermann et al., 2011). However, no research to date investigating HPA axis-related G × E predictors of internalizing symptoms has a) been done in children, and b) investigated parenting as the environmental exposure.

The specific pathways linking G × E and risk for internalizing symptoms are still unclear. One fruitful approach to understanding such mechanistic pathways is to investigate brain function (Insel et al., 2010). Dysfunction of corticolimbic circuits underlying emotion processing, especially those involving the amygdala, have been suggested to represent potential neurobiological mechanism that might underlie the association between G × E effects and depressive/internalizing symptoms (Swartz et al., 2015). The amygdala is intrinsically involved in HPA axis function (Ledoux, 2000) and it has been proposed that HPA axis genetic variation may moderate the effect of the environment on amygdala reactivity and connectivity of related emotional circuits (Bogdan et al., 2016).

Indeed, studies have found that HPA axis-related genes interact with environmental factors to predict amygdala reactivity/connectivity. Two studies adopted a 'cumulative genetic risk' approach, combining 5 and 10 single nucleotide polymorphisms (SNPs), respectively, from four different genes (FKBP5, CRHR1, NR3C2 and NRC31) to create HPA axis genetic 'risk' scores (Iorio et al., 2017; Pagliaccio et al., 2015a). One study found that early life stress was associated with amygdala response to emotional faces only in young adults with higher HPA axis genetic risk (Iorio et al., 2017). The other study found that HPA axis genetic risk interacted with number of stressful events in predicting connectivity between the amygdala and the frontal cortex, caudate, and parahippocampal gyrus, in children (Pagliaccio et al., 2015a). Two further studies focused on a single SNP of the FKBP5 gene: rs1360780 (Holz et al., 2014; White et al., 2012). One of these studies found that individuals homozygous for the 'risk' T-allele, in the context of emotional neglect, showed increased amygdala reactivity to fearful and angry faces, and positive coupling between amygdala and orbitofrontal cortex, compared to young adults heterozygous/homozygous for the non-risk C-allele (Holz et al., 2014). The other study found that emotional neglect in those with the FKBP5 rs1360780 T-allele predicted greater dorsal amygdala reactivity to an emotional faces task (White et al., 2012). Finally, another study investigated the mineralcorticoid receptor (NR3C2) rs5522 polymorphism and found that although the A-allele was associated with amygdala reactivity in the context of emotional neglect, carriers of the G-allele showed the greatest amygdala reactivity (Bogdan et al., 2012).

While evidence to date suggests a moderating role of HPA axis genetic variation in the association between environmental adversity and functioning of emotional circuits, none investigated parenting behaviors, despite the fact that such behaviors are thought to modulate the development of the HPA axis (Caldji et al., 2000), as well as the same emotional circuits implicated in depression (Callaghan and Tottenham, 2015). Focusing on the childhood period is of particular interest given that it is thought to be a sensitive period for parental modulation of child frontoamygdala circuitry (Gee, 2016). Further, none of the aforementioned G × E studies provided evidence that effects on brain function were in turn associated with internalizing symptoms.

This study aimed to investigate the interaction between observed measures of maternal parenting behaviors and HPA axis genetic variation in relation to amygdala reactivity and connectivity during emotion processing, and in turn internalizing symptoms, in a community sample of children. Parenting is particularly important for emotional development during childhood (Callaghan and Tottenham, 2015), and understanding the link between parenting behaviors and internalizing symptoms in this sensitive period is important to understand risk processes for depression. Examining a combination of several SNPs is thought have more predictive power, compared to individual polymorphisms (Bogdan et al., 2016). As such, we adopted a cumulative genetic risk approach similarly to previous studies (Iorio et al., 2017; Pagliaccio et al., 2015a). Given previous findings, we expected that higher negative and lower positive maternal parenting behaviors, in particular in those with higher HPA axis genetic risk, would be associated with increased amygdala reactivity and connectivity with regions involved in emotion processing, including the prefrontal cortex. We also hypothesized that these alterations in amygdala reactivity and connectivity would in turn be associated with higher internalizing symptoms in children (see Fig. 1 for a representation of our model).

2. Materials and methods

The sample consisted of 80 participants taking part in the Family and Childhood Transition Study (FACTS), who were recruited from Melbourne, Australia, as described elsewhere (Simmons et al., 2017). Social disadvantage increases the risk of negative life events and stressors and poses challenges for parenting practices (McLoyd, 1998). Thus, to maximize the variance in parenting behaviors, participants were recruited from metropolitan areas of Melbourne falling within the lower tertile of socioeconomic disadvantage (according to Australian Bureau of Statistics: Canberra, 2013). Of note, the socioeconomic status of participants based on their home address was distributed across the full range (possible scores range from 0 to 100) (see Supplementary Material Fig. S1). The study involved two waves of data collection. During Wave 1, 155 children aged 8–9 years participated in two lab-based interactions with their mothers. Only mothers participated in the study, due to budget constraints. During Wave 2, approximately 18 months later, 125 children completed a magnetic resonance imaging (MRI) brain scan that included a functional task. See Supplementary Material Fig. S2 for a summary of the data collected at each time point. Exclusionary criteria comprised MRI contraindications, history of head trauma or loss of consciousness, history of clinically significant developmental or intellectual disorder and use of psychotropic medication. None of the children had a psychiatric diagnosis from a health professional, as reported by the parents. Five participants did not have parenting data and six children did not provide saliva samples and as such did not have DNA information available. Of the 114 with completed MRI, genetic and parenting data, 34 participants were excluded after performing quality control on the MRI data (see below). The final sample thus included 80 children (46 females; mean age at MRI = 10.00 years, SD = 0.36) with complete MRI, genetic and parenting data.

There were no difference between the sample of 80, and participants excluded from the analysis, except for socioeconomic status (SES; $p = 0.006$), which was lower in the excluded group.

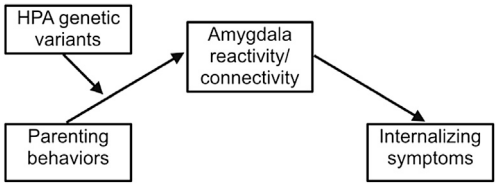

Fig. 1. Model tested in the study.

Ethics approval for this study was granted by the University of Melbourne Human Research Ethics Office. Parental informed written consent and child assent were obtained prior to study participation.

2.1. Parenting behaviors: family interaction task (FIT)

Two 15-min mother-child interactions were performed and video recorded. The first, an Event Planning Interaction (EPI), involved dyads planning two or three pleasant activities to do together, selected from a list in the Pleasant Events Schedule (MacPhillamy and Lewinsohn, 1982). The second, a Problem Solving Interaction (PSI), involved discussing conflictual topics relevant for the dyad, chosen from the Issues Checklist (Prinz et al., 1979). Two trained graduate students independently coded the recorded behaviors during the interactions using a modified version (Richmond et al., 2018) of the Family Interaction Macro-coding System (FIMS) (Holmbeck et al., 2007). Fifty-nine codes were included covering a range of mother and child behaviors that were rated on 5-point Likert scales. A multiple factorial analysis was performed on the FIMS codes for the original Wave 1 sample of 155 to obtain empirically-derived parenting behavior components (Richmond et al., 2018). Four such components were identified: maternal negative behavior (e.g. frequency and intensity of negative affect, anger) during the EPI, maternal negative behavior during the PSI, maternal positive behavior (e.g., frequency and intensity of positive affect, humor, laughter) across both tasks, and maternal communicative behavior (e.g., listening, clarity of thought, providing explanations) across both tasks. See Supplementary Material (section 2) for a list of the codes included in each component, for examples of codes with the corresponding Likert scale, and the correlations between components (in Supplementary Table S1). Factor scores for each of the four components were used in subsequent analyses.

2.2. Saliva collection

Saliva samples used for genetic and cortisol analysis were collected at Wave 1 (see Simmons et al., 2017 for details). For each participant two samples were collected (one on the day prior, and one on the day of the Wave 1 assessment). For each sample, children collected 2.5 ml of saliva in a test tube, via passive drool. Any samples from participants who indicated that samples were provided more than 45 min after waking were excluded.

2.2.1. Cortisol

Samples were frozen at −30 °C, and prior to analysis, defrosted and centrifuged, with the supernatant assayed in duplicate for levels of cortisol using Salimetrics ELISA kits. The two samples were averaged for the analysis. Given that cortisol was positively skewed, to achieve a Gaussian distribution it was log-transformed.

2.2.2. Genotyping

DNA was recovered from the stabilized saliva samples. Ten single nucleotide polymorphisms (SNPs) in four genes (Table 1) and 60 unlinked ancestry informative markers (AIMs; Supplementary Table S2) were genotyped with Agena Bioscience MassARRAY on a Compact Spectromer by the Australian Genome Research Facility. Seventy-seven participants had predominantly European (CEU) ancestry and 3 had Asian (CHB) ancestry (calculated based on the 60 SNPs).

The SNPs included in the HPA genetic risk score were selected on the basis of literature highlighting their relevance for stress and depression, as well as modulation of corticolimbic neural circuits (Bogdan et al., 2016). The HPA genetic risk score has been found to predict cortisol levels in children (Pagliaccio et al., 2014). For each HPA SNP, a risk allele was nominated based on previous literature (following Pagliaccio et al., 2014) (see Table 1). An unweighted score was calculated for each individual based on the number of risk alleles they possessed, with a total score ranging from 0 to 10.

Minor allele frequencies in the sample and p-values from tests for Hardy-Weinberg equilibrium (HWE) are shown in Table 1. There was no significant deviation from HWE.

2.3. MRI

Neuroimaging data were acquired on a 3 T S TIM Trio scanner (Siemens, Erlangen, Germany) at the Murdoch Children's Research Institute in Melbourne, Australia. Participants lay supine in a 32-channel head coil. Prior to the scan, participants underwent a MRI mock session where they looked at pictures of the machine, received information about the procedure and practiced staying still in a life-size replica of an MRI scanner (Simmons et al., 2017).

2.3.1. MRI parameters

Structural T1-weighted images were acquired as follows: MPRAGE MoCo, repetition time = 2530 msec; echo time1 = 1.74 msec, echo time2 = 3.6 msec, echo time3 = 5.46 msec, echo time4 = 7.32 msec; flip angle = 7°, field of view = 256 × 256 mm^2, producing 176 contiguous slices with 1.0 mm^3 voxel dimensions. Functional scan parameters included 136 whole-brain T2*- weighted echo-planar images (TR = 3000 ms, TE = 35 ms, flip angle = 85°) within a field of view of 216 × 216 mm^2, with a voxel size of 3 mm^3. Forty interleaved slices were acquired.

2.3.2. Affective faces fMRI task

Participants underwent an implicit version of a widely used emotional face-matching task for children (Hariri et al., 2000). The presented faces were from the NimStim Set of Facial Expressions (Tottenham et al., 2009). Participants were instructed to either match the gender of one of two faces (presented at bottom of screen) to a target face

Table 1
Single nucleotide polymorphisms included in the HPA genetic risk score.

Gene	SNP	Alleles	MAF	HWE p	Reliability[a]	Scoring[b]
CRHR1	rs4792887	C > T	0.07	0.09	100%	TT = 1,TC = 0.5,CC = 0
CRHR1	rs110402	C > T	0.50	0.80	100%	TT = 1,CT = 0,CC = 0
CRHR1	rs242941	G > T	0.26	0.82	99%	AA = 1,CA = 1,CC = 0
CRHR1	rs242939	A > G	0.04	0.07	99%	CC = 1,CT = 1,TT = 0
CRHR1	rs1876828	G > A	0.21	0.54	100%	CC = 1,TC = 1,TT = 0
NR3C2	rs5522	A > G	0.09	0.93	99%	GG = 1,AG = 1,AA = 0
NR3C1	rs41423247	G > C	0.35	0.21	100%	GG = 1,CG = 1,CC = 0
NR3C1	rs10482605	T > C	0.16	0.08	99%	AA = 1,AG = 0,GG = 0
NR3C1	rs10052957	G > A	0.30	0.76	100%	AA = 1,GA = 0,GG = 0
FKBP5	rs1360780	C > T	0.29	0.46	100%	TT = 1,CT = 1,CC = 0

Alleles = Alleles present in current sample (major > minor).
MAF = Minor allele frequency for current sample.
HWE = Hardy-Weinberg equilibrium.
[a] Calculated by re-genotyping a random 10% of genotyped participants.
[b] According to (Pagliaccio et al., 2014), whereby 1 = highest risk, 0.5 = moderate risk, 0 = least risk.

above (face condition), or match shapes in a similar fashion (control condition). This implicit version of the task was used because it has been shown to robustly activate the amygdala (e.g., Fu et al., 2008; Tao et al., 2012). Each block consisted of six consecutive trials containing angry or fearful faces, or circular shapes. Three 2400 ms blocks for each emotional face condition (6 stimuli of 4000 ms each) and six 24000 ms blocks (6 stimuli of 4000 ms each) for the control condition were presented. A fixation cross (rest condition - 10s) separated each block. The total length of the task was approximately 7 min. Two versions of the task were administered in a counterbalanced manner; one displaying angry faces first and one displaying fearful faces first. The order of the block was as follows: rest, shapes, rest, faces.

2.3.3. Image preprocessing

The images were processed using CONN functional connectivity toolbox v17 (Whitfield-gabrieli & Nieto-castanon, 2012). The experimental design included the onset and the duration for the three conditions: rest, shapes and emotional faces (angry and fearful faces combined). First, the images were pre-processed following the default MNI pipeline implemented in CONN, which includes: functional realignment and unwarping, slice-timing correction, structural segmentation and normalization, functional normalization, outlier detection (Artifact Detection Toolbox (www.nitrc.org/projects/artifact_detect/), and smoothing (using a kernel of 8 mm). All spatial preprocessing steps were implemented using SPM12 (Wellcome Department of Imaging Neuroscience, London, UK; www.fil.ion.ucl.ac.uk/spm). Second, we employed a denoising step, which applies linear regression and band-pass filtering in order to remove unwanted motion, physiological, and other artifactual effects from the BOLD signal. In this step we included the following parameters: 1) the top five principal components of white matter and cerebrospinal fluid (CSF) tissue classes (based on signal decomposition of tissue signals from structural segmentation masks with principal components analysis, implemented using aCompCor (Behzadi et al., 2007)), 2) 'scrubbing' parameters (as many regressors as identified as 'invalid scans' for each participant based on the combination of a threshold of framewise displacement > 0.90 mm and a global BOLD signal changes > 5 z-score), 3) realignment parameters (12 regressors: 6 motion parameters + 6 first-order temporal derivatives), and 4) task regressors, following recommendations (Whitfield-gabrieli & Nieto-castanon, 2012). In this final step the images were also band passed filtered (Hz 0.008–0.09).

Of the 114 participants whose imaging data was pre-processed, twenty-three were excluded from subsequent analysis due to having $\geq$20% invalid volumes. An additional 11 participants were excluded due to maximal amplitude of translational or rotational displacements (x, y, z) > 3 mm and 3°, respectively. This approach was adopted to account for different types of participants' head motion that can introduce artifacts in the data (Power et al., 2012). Thus, the final sample included in all analyses presented below comprised 80 children.

2.3.4. Models

Initial models included just the main effects of HPA axis score and parenting. The effects of parenting on amygdala activity and connectivity are reported elsewhere (Pozzi et al., 2019). When examining the main effect of HPA axis risk score, the parenting behavior scores were entered in the regression model (one parenting behavior score per model). Subsequent models included the G × E interaction term. These models also included the main effects of the HPA axis risk score and the parenting behavior score. HPA axis genetic risk score and parenting behavior variables were mean centered prior to creating interaction terms, and these mean centered variables were included in the models. A winsorizing procedure was used to replace extreme parenting behavior scores (>3 SD of the sample mean) before mean-centering. This involved 3 scores for maternal negative behavior during the EPI, 2 scores for maternal positive behavior and 1 score for maternal communicative behavior. Results are reported with winsorized values, however note that running the analyses with the original values did not change the results.

We included age as a covariate of no interest in all analyses (region of interest [ROI] and gPPI), in addition to ancestry, sex, maternal depressive symptoms at Wave 2 and socioeconomic status (SES). Pubertal (i.e., Tanner) stage (assessed via Pubertal Development Scale (PDS) (Petersen et al., 1988) was considered as a covariate, however was not included because the majority of participants were only Tanner stage 1 (49%) or 2 (34%). Ancestry (based on genetic analysis) was included as dichotomous variable (European, Asian). Note that re-running the analysis without the participants with Asian ancestry ($N = 3$) did not change the results substantively.

It has been suggested that studies should include in analyses, along with relevant covariates, all covariate-by-gene and covariate-by-environment interactions (Keller, 2014). As such, we ran additional analyses including these interaction terms (see Supplementary Material, section 3). Of note, this resulted in 21 predictors per model, and given the relatively small sample size, these models likely suffer from overfitting and low power.

2.3.5. Region of interest (ROI) analysis

The pre-processed and denoised contrasted images (emotional faces > shapes) resulting from the preprocessing pipeline implemented in CONN (Whitfield-gabrieli & Nieto-castanon, 2012) were entered into second level *t*-test models in SPM12 (Wellcome Department of Imaging Neuroscience, London, UK; www.fil.ion.ucl.ac.uk/spm). Given our a priori hypotheses we performed ROI analyses for left and right the amygdala. We applied an initial voxel-level $p < 0.001$ threshold and then small volume corrections with pFWE<0.05 within the left and right amygdala masks, created from the Automated Anatomical Labeling (AAL) atlas implemented in the Pickatlas toolbox (Maldjian et al., 2003).

2.3.6. Generalized psychophysiological interaction (gPPI) analysis

Generalized psychophysiological interaction (gPPI) analysis was employed using left and right amygdala seed regions. gPPI analysis were conducted in CONN (Whitfield-gabrieli & Nieto-castanon, 2012). Amygdala masks were derived from FSL Harvard-Oxford Atlas maximum likelihood subcortical atlas using a probability threshold of 25%. First level gPPI analyses were run to compute the effect of the interaction between the seed region BOLD timeseries for each participant (for the emotional faces > shapes contrast) and the PPI interaction term (created by multiplying the extracted time-series by the experimental condition emotional faces > shapes) on whole-brain voxel-wise BOLD timeseries.

Second-level analyses were then performed to investigate associations between left and right amygdala connectivity and GxE (HPA axis risk score in interaction with each parenting behavior).

At the second level (within CONN) result maps were thresholded at the cluster level using $p < 0.006$ Family Wise Error (FWE) correction, with a voxel-level threshold of $p < 0.001$ using Gaussian Random-field theory. The critical α for cluster correction was pFWE<0.006 based on a Bonferroni adjustment for the number of tests (i.e., 2 seeds x 4 parenting components = 8 tests).

2.4. Questionnaire measures

Children and parents completed questionnaire measures of child internalizing symptoms on the same day as the fMRI (i.e., at Wave 2). Children completed the Children's Depression Inventory 2 (CDI-2) (Kovacs, 1992) and the Spence Children Anxiety Scale (SCAS) (Spence, 1998), while the mothers completed the Child Behavior Checklist for Ages 6–18 (CBCL) (Achenbach, 2001). Note that while mothers completed the entire CBCL, only the internalizing scale scores (derived from the sum of the Anxious/Depressed, Withdrawn, and Somatic Complaints subscales) are used here given the aims of the study. Maternal depressive symptoms were assessed with the Centre for Epidemiologic Studies Depression Scale (CESD) (Radloff, 1977). One child did not complete the CDI-2 and three mothers did not complete the CBCL. A

winsorizing procedure was used to replace extreme values (>3 SD of the sample mean). This involved one participant each for the CDI-2, SCAS and CBCL Internalizing subscale.

Maternal depressive symptoms at Wave 2 and SES (measured with the neighborhood derived Socio-Economic Indexes for Areas Index of Relative Socio-Economic Disadvantage scale, SEIFA (Australian Bureau of Statistics: Canberra, 2013)) were not correlated with any parenting components (p's > 0.05) but were included in all the models to rule out their possible confounding effects. One participant was missing SES data and two were missing maternal depressive symptoms data at Wave 2. A single imputation procedure with the Expectation-Maximization algorithm in SPSS was used to account for these missing data.

2.5. Interaction interpretation and mediation analysis

To interpret significant G × E interaction effects, ROI values (first eigenvariate of the time-series across all voxels within a 6 mm sphere around the cluster peak) and connectivity values (average beta values within each significant cluster) were extracted for plotting and simple slopes analysis in Mplus (version 7.2). The extracted values were entered as dependent variables in linear regression models, while HPA axis score, parenting behavior and their interaction were entered as independent variables. Simple slopes were evaluated at ±1 SD of the mean of the HPA genetic risk score. Johnson-Neyman interval plots were also produced to investigate the region of significance. A maximum likelihood estimator was used for all analyses, with all inferential tests based on bootstrapped (10,000 resamples) standard errors and associated p-values. To investigate the potential relevance of G or G × E -related reactivity/connectivity for child functioning, the total scores from the CDI-2, SCAS and CBCL/6–18 Internalizing subscale were regressed on to the extracted values within each significant cluster from the ROI and gPPI analyses. Mediation analyses were conducted to test the theoretical indirect association between G or G × E and symptoms, via amygdala reactivity and/or connectivity. An indirect effect was considered significant if 95% bias-corrected confidence intervals (CI) from bootstrapped analyses (10,000 resamples) did not contain zero.

3. Results

3.1. Demographic characteristics

See Table 2 for demographics and descriptive information for the sample. See Supplementary Table S1 for correlation between variables.

3.2. HPA axis function

There was no association between HPA genetic risk score and morning cortisol at Wave 1 (p > 0.05).

3.3. Region of interest (ROI) analysis

There was no association between HPA axis genetic score and left or right amygdala activity in response to angry and fearful faces, and no association between HPA axis genetic score in interaction with any of the four parenting behaviors and left or right amygdala activity.

3.4. gPPI

Table 3 presents a summary of the results from the connectivity analysis.

HPA axis genetic risk score was positively associated with connectivity between left amygdala and right precuneus (Fig. 2). The interaction between maternal negative behavior during the PSI and HPA genetic risk score was associated with connectivity between the left amygdala and right superior frontal gyrus (Fig. 3). Probing the interaction revealed that maternal negative behavior, when coupled with greater genetic risk score, was associated with greater connectivity between left amygdala and right superior frontal gyrus (b = 0.157, SE = 0.045, p = 0.001, 95% CI 0.074, 0.256) and lower connectivity when coupled with lower risk score (b = −0.171, SE = 0.051, p = 0.001, 95% CI -0.261, −0.063). Johnson-Neyman interval analysis showed that the interaction was significant for values of the HPA genetic score below 2.91 and above 4.88 (see Fig. 4).

The interaction between HPA axis genetic risk score and maternal negative behavior during the EPI was associated with connectivity between the left amygdala and right parietal operculum cortex, right post-central gyrus and right cingulate cortex (See Supplementary Material Fig. S3, S4, S5). Probing the interactions revealed that maternal negative behavior, when coupled with greater genetic risk score, was associated with greater connectivity between left amygdala and right post-central gyrus (b = 0.189, SE = 0.061, p = 0.002, CI 0.085, 0.338) and, when coupled with lower genetic risk score, lower connectivity between left amygdala and right parietal operculum cortex (b = −0.273, SE = 0.080, p = 0.001, CI -0.409, −0.079), right anterior cingulate cortex (b = −0.327, SE = 0.077, p < 0.001, CI -0.467, −0.162) and right post-central gyrus (b = −0.176, SE = 0.071, p = 0.014, CI -0.335, −0.059). Johnson-Neyman analysis showed that the interaction was significant for values of the HPA genetic score below 2.79 and above 4.77 (see Supplementary Material Fig. S6). No significant associations were found for the right amygdala or for any other parenting behavior.

3.5. Association with child functioning

Connectivity between the amygdala and precuneus (positively associated with HPA axis genetic risk score) was positively associated with CDI-2 scores (b = 0.267, SE = 0.113, p = 0.018) (Fig. 2). Mediation

Table 2
Sample characteristics.

	Mean ± SD	Range
Age	10.00 ± 0.36	9.44–10.82
SES	44.94 ± 25.537	1–95
Child SCAS Total W2	24.325 ± 14.612	1–78
Child CDI Total W2	6.532 ± 5.641	0–25
Child CBCL Internalizing W2	5.61 ± 4.71	0–26
Child HPA genetic risk score	4.044 ± 1.05	2–6.5
Cortisol W1	0.39 ± 0.4	0.12–3.64
Mother CESD W2	9.447 ± 9.788	0–47
Maternal negative behavior during the EPI	−0.086435 ± 0.753162	−1.052358–2.392291
Maternal negative behavior during the PSI	0.005204 ± 0.945512	−1.371198–2.457341
Maternal communicative behavior	0.234538 ± 0.810269	−4.094929–2.014284
Maternal positive behavior	0.014515 ± 0.951280	−1.859561–3.102349

EPI = Event Planning Interaction.
PSI = Problem Solving Interaction.
HPA = hypothalamic–pituitary–adrenal axis.
CDI= Children's Depression Inventory.
SCAS= Spence Children's Anxiety Scale.
CBCL Internalizing = CBCL Anxious and Depressed subscale + Withdrawn/Depressed subscale + Somatic Complaints subscale.
CESD= Centre for Epidemiologic Studies Depression Scale.
SES= Socioeconomic status measured with the SEIFA Index of Relative Socio-Economic Disadvantage (IRSD) scale, expressed in percentile. Note that a low score indicates greater disadvantage in general while a high a score a lack of disadvantage.
W2 = Wave 2.
Note that factor scores (Richmond et al., 2018) from each of the four components were used in analyses and displayed in the table (referred to as 'maternal negative behavior during the EPI', 'maternal negative behavior during the PSI', 'maternal positive behavior' and 'maternal communicative behavior').

Table 3
Summary of the significant results.

Effect	Seed	Clusters (x, y, z)	Region	Size	Cluster p-FWE	Peak p-FWE	T
HPA*							
	L amy	+10–48 + 8	R Precuneus	277	0.004615	0.117623	4.99
Maternal neg. PSI x HPA							
	L amy	+8 + 12 +64	R Sup Frontal	441	0.000225	0.104876	5.03
Maternal neg. EPI x HPA							
	L amy	+50–28 + 28	R Parietal Operc	413	0.000325	0.003572	6.03
		+38–10 + 28	R Post-central	388	0.000506	0.203518	4.81
		+00 + 28 +28	R Ant Cingulate	373	0.000664	0.570199	4.38

EPI = Event Planning Interaction.
PSI = Problem Solving Interaction.
HPA = hypothalamic–pituitary–adrenal axis genetic risk score.
Amy = amygdala.
L = left; R = right.
Note: Results were thresholded at the cluster level using a $p < 0.006$ Family Wise Error (FWE) correction, with a voxel level threshold of $p < 0.001$.
* The effect for HPA genetic risk score was significant at $p_{FWE} < 0.006$ for 3 of 4 regression models, and was significant at $p_{FWE} = 0.01$ for the model that included maternal negative behavior during the EPI. Note that the results are presented here from the model that included maternal negative behavior during the PSI.

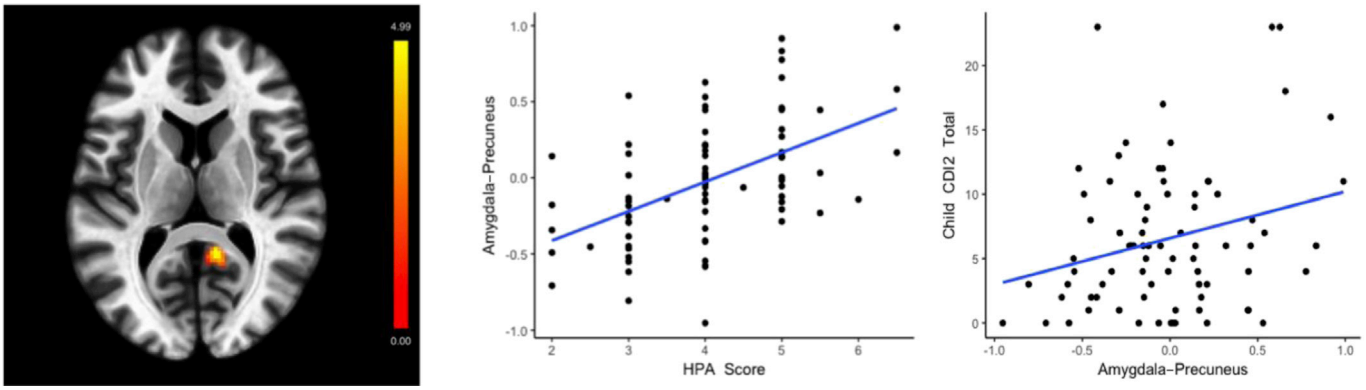

Fig. 2. Association between HPA axis genetic risk score, left amygdala-right precuneus connectivity, and child depressive symptoms. Significant voxels identified in the precuneus (left), direction of the association (middle), and link between connectivity and child depressive symptoms (right). Note that the scatterplot depicted in middle panel of the figure is for display purposes only, as the connectivity values were based on their already significant association with HPA score.

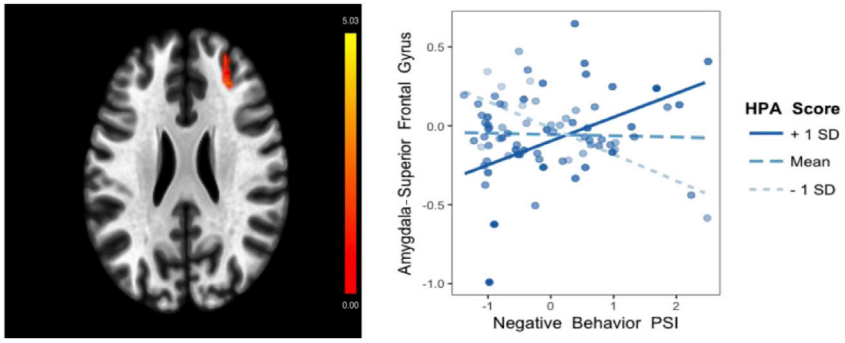

Fig. 3. Association of the interaction between maternal negative behavior during the PSI and HPA axis genetic risk score, with left amygdala-right superior frontal gyrus connectivity. Significant voxels identified in the superior frontal gyrus (on the left) and plot of the interaction (on the right). For display purpose, non mean-centered scores are shown, but mean-centered variables were used in analyses.

analysis showed a significant indirect effect of HPA axis genetic risk score on CDI-2 scores, such that greater genetic risk score predicted greater symptoms through increased connectivity between left amygdala and right precuneus (indirect effect = 0.752, SE = 0.413; 95% CI: 0.072, 1.723). Genetic risk score did not directly predict CDI-2 scores (total effect = 0.621, SE = 0.630; 95% CI: −0.535, 1.938).

There were no other significant associations between amygdala and precuneus connectivity and SCAS or CBCL Internalizing subscale scores, or between amygdala connectivity for any other significant cluster and CDI-2, SCAS or CBCL Internalizing subscale scores.

4. Discussion

In this study, we investigated the association between HPA-related genetic variation, maternal parenting behaviors and amygdala activity and connectivity during implicit emotion processing in a community sample of children. Our analysis revealed three main findings: first, higher HPA genetic risk score was associated with greater amygdala-precuneus connectivity, which in turn was associated with greater child self-reported levels of depressive symptoms. Second, the interaction between HPA axis genetic risk score and maternal negative behavior was

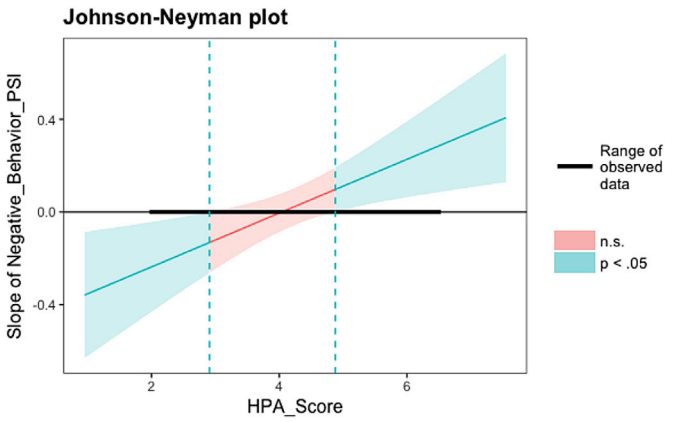

Fig. 4. Johnson-Neyman plot. The association of the interaction between maternal negative behavior during the PSI and HPA axis genetic risk score was significant for values of the HPA genetic score below 2.91 and above 4.88 (note that very similar results were found for maternal negative behavior during the EPI, see Supplementary Material Fig. S6). Note: the y-axis is the conditional slope of the predictor.

associated with connectivity between the amygdala and the superior frontal gyrus, parietal operculum cortex, post-central gyrus and anterior cingulate cortex. Finally, HPA axis genetic risk neither alone nor in interaction with maternal parenting behavior was associated with amygdala reactivity.

The lack of effect on amygdala reactivity is not in line with some previous studies that have found an association between HPA-related genetic variation in interaction with early stress or emotional neglect, and heightened amygdala reactivity (Bogdan et al., 2012; Iorio et al., 2017; White et al., 2012). However, it is in line with the idea that dysregulation of the HPA axis may particularly impair the functioning of corticolimbic *circuits* (Bogdan et al., 2016).

Increased connectivity (or reduced negative connectivity) between the amygdala and precuneus has been found in children with depression (Luking et al., 2012). The precuneus is thought to be involved in self-referential processing (Cavanna and Trimble, 2006), and altered precuneus connectivity in individuals exposed to early adversity has been hypothesized to reflect increased self-centered mental imagery (Teicher et al., 2014). In our study, the association between increased HPA-related genetic variation and increased amygdala-precuneus connectivity may reflect greater sensitivity to negative environmental stimuli (i.e., negative emotional faces), and potentially greater ascription of the relevance of negative emotional faces to the self. This pattern of increased connectivity was associated with greater levels of depressive symptoms, consistent with the idea that biased interpretation of emotional stimuli, particularly faces, may be one of the mechanisms underlying depression risk (Joormann and Gotlib, 2006) (consistent with the specificity of our findings for depressive but not anxiety symptoms). Our result is also in line with other studies that have found an association between HPA genetic variation and increased amygdala connectivity with areas involved in emotion processing, such as orbitofrontal cortex and post-central gyrus (Holz et al., 2014; Pagliaccio et al., 2015a), and provides evidence that HPA genetic variants may confer risk for depressive symptoms via altering affective brain function.

HPA axis genetic variation moderated the effect of negative maternal parenting behavior on connectivity between the amygdala and the superior frontal gyrus, anterior cingulate cortex, post-central gyrus and parietal operculum cortex. The anterior cingulate cortex and the superior frontal gyrus are involved in monitoring and cognitive control of emotional states (Ochsner et al., 2004). The post-central gyrus and the parietal operculum cortex (i.e., somatosensory cortex) are thought to play a role in the recognition of emotions (Adolphs, 2002). There is consistent evidence that early life stress is associated with neurobiological changes in the brain circuits underlying emotion processing and emotion regulation (Teicher et al., 2016), particularly amygdala-prefrontal cortex connectivity (Jedd et al., 2015). Indeed, we previously found that maternal negative behavior during the problem solving interaction was associated with increased amygdala-superior parietal cortex connectivity, particularly in boys (Pozzi et al., 2019, In Press). Similarly, it has been proposed that adverse parenting influences the development of the fronto-amygdala circuitry (Callaghan and Tottenham, 2015). Existing studies have provided evidence that such environmental impact on emotional neurocircuitry may be exacerbated in those with HPA genetic risk (Holz et al., 2014; Iorio et al., 2017; Pagliaccio et al., 2015a; White et al., 2012). We provide the first evidence that HPA genetic risk may moderate the effect of normative variation in negative maternal parenting behaviors on amygdala connectivity.

Interestingly, we found that greater maternal negative behavior, when coupled with lower genetic risk score, was associated with lower amygdala connectivity with prefrontal regions involved in emotion processing and emotion regulation. Previous research has shown that amygdala-prefrontal cortex connectivity shifts from positive to negative during the transition from childhood to adolescence (Gee et al., 2013). This timing may be accelerated in the absence of maternal care, to promote resilience and coping in the short term (Gee et al., 2013). Indeed, negative amygdala-prefrontal coupling is thought to reflect the adoption of (mature) top-down regulatory strategies (Gee, 2016). In contrast, increased amygdala connectivity with the prefrontal cortex has been considered a sign of upregulated response of regions involved in cognitive control (Jedd et al., 2015). In our study, the lack of association with symptoms limits our ability to establish whether decreased amygdala connectivity with cortical regions represents an adaptive outcome. However, given that negative amygdala-prefrontal coupling is thought to be associated to greater emotion regulation, we speculate that lower HPA genetic 'risk' may buffer the effect of negative maternal parenting behavior on amygdala connectivity and emotional reactivity.

Of note, our results were lateralized, whereby we found significant associations only for left amygdala connectivity. It has been hypothesized that the left amygdala may have a more prominent role in processing arousal (Gläscher and Adolphs, 2003), but the exact significance in the context of negative maternal parenting remains to be explored.

Maternal negative behaviors during the EPI (positive context) and during the PSI (negative context) were associated with different regions of connectivity. It has been suggested that the context of maternal negative behavior might be particularly important in determining the significance of parental behavior for children, more so than the valence of the behavior alone (Schwartz et al., 2012). Specifically, negative behavior that is out of context (i.e., not congruent with the task demands, as in the EPI) has been shown to be particularly important in predicting adolescent depression (Schwartz et al., 2012). However, our findings suggest that negative behaviors in both congruent and incongruent contexts are important in influencing child emotion processing at the neural level.

Of further note, we found effects of HPA genetic variants only in interaction with negative maternal parenting behavior, but not positive or communicative behaviors. It may be that other genes that we did not investigate in this study may influence the effect of these other types of maternal behaviors on the brain (e.g. Little et al., 2015).

In our study we did find support for an association between HPA genetic risk score and cortisol. Of note, one previous study (Pagliaccio et al., 2014) found an association with stress-induced cortisol levels (i.e., changes in cortisol after a stressful task). We collected only one sample in the morning (across two days). It is thus possible that HPA genetic risk may be associated with HPA-axis reactivity and not basal activity. However, further research is required to confirm an association between the HPA genetic risk score and HPA axis function.

This work is not without limitations. The study was not designed to test genetic-environment correlations (Jaffee and Price, 2007). As such, we cannot determine whether parenting behavior is influenced by the

parents' own genetic make up, which are also inherited by the children. Studies with adoptive and biological relatives would help to disentangle genetic and environmental effects. The sample size, although consistent with previous fMRI studies (Fani et al., 2014; Pagliaccio et al., 2015b; Tozzi et al., 2015), for a genetic variation study is small. Moreover, the small sample size in interaction analysis may increase the risk of Type I error, especially when the nature of the interaction appears to be a cross-over (Dick et al., 2015). Including all covariate-by-gene and covariate-by-environment interactions in our models (Keller, 2014) resulted in non-significant findings when using stringent correction for multiple comparisons. However, we showed that it is likely that the inclusion of such a high number of predictors (21) within a relatively small sample size resulted in a loss of power, rather than Type I error. Controlling for each covariate-by-gene and covariate-by-environment interaction term, as pointed out by Keller (2014), is a desirable practice. However, when the study includes several covariates and a small sample, this practice is difficult to implement. Others have included only an exiguous number of covariates, such as ancestry and sex (Pagliaccio et al., 2015a,b), but the confounders we included in this study seem all equally relevant and necessary for the study. Nevertheless, this should be acknowledged as a limitation. Considering these limitations, especially the small sample size, our results should be considered exploratory and replication is required before any strong conclusions can be made. Given that paucity of studies on parenting, genetic risk and emotion processing, we believe that our study provides an important contribution that we hope will stimulate future work in the area. The HPA axis genetic risk score was unweighted such that it assumes additive effects between SNPs, which is unlikely. However, it is considered a valid alternative (rational) approach to polygenic risk scores weighted from GWAS (Bogdan et al., 2016) (which required large discovery samples). The link between HPA genetic variants included in this study and HPA function is still to be clarified. While one previous study found an association between the HPA genetic risk score and stress-induced cortisol levels in children (Pagliaccio et al., 2014), we did not find an association with morning cortisol in our sample. As such, our hypothesis that the HPA genetic risk score may influence the responsivity of the system is speculative. We included ancestry as a covariate of no interest in our analysis, but we did not assess cultural variables, which are likely to influence parenting style and its interpretation. As we did not perform a psychiatric interview, we cannot exclude the possibility that some of the children may have had a psychiatric diagnosis (not reported by parents) that may have influenced the results. As we did not include measures of childhood maltreatment, we cannot rule out that the some of children included in the study may have been exposed to abuse or neglect. The MRI was acquired 18 months after the parenting interaction tasks, and as such we cannot rule out the possibility that maternal parenting behaviors may have changed over time. Only mothers were included in the study due to budget constrain, however fathers also play an important role in child emotional development (Sheeber et al., 2007). As such, in this study we do not know whether our results are specific to mothers or what the contribution of fathers may be. In the fMRI task we did not include a range of emotional faces (such as positive and sad faces). As such, we cannot establish if our findings are specific to angry and fearful stimuli. Finally, some results (e.g., involving the parietal cortex) were not easily interpretable based on existing literature, and as such future research is needed to support a more in depth interpretation of our results.

To conclude, in this study we demonstrated that genetic variation of the HPA axis directly, and in interaction with maternal negative parenting behavior, was associated with amygdala connectivity with regions involved in self-referential processing and emotion regulation, and may confer risk for depressive symptoms via an effect on these circuits.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroimage.2019.05.013.

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