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HPA axis genetic variation, pubertal status, and sex interact to predict amygdala and hippocampus responses to negative emotional faces in school-age children

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ABSTRACT

Accumulating evidence suggests a role for stress exposure, particularly during early life, and for variation in genes involved in stress response pathways in neural responsivity to emotional stimuli. Understanding how individual differences in these factors predict differences in emotional responsivity may be important for understanding both normative emotional development and for understanding the mechanisms underlying internalizing disorders, like anxiety and depression, that have often been related to increased amygdala and hippocampus responses to negatively valenced emotional stimuli. The present study examined whether stress exposure and genetic profile scores (10 single nucleotide polymorphisms within four hypothalamic–pituitary–adrenal axis genes: *CRHR1*, *NR3C2*, *NR3C1*, and *FKBP5*) predict individual differences in amygdala and hippocampus responses to fearful vs. neutral faces in school-age children (7–12 year olds; $N = 107$). Experience of more stressful and traumatic life events predicted greater left amygdala responses to negative emotional stimuli. Genetic profile scores interacted with sex and pubertal status to predict amygdala and hippocampus responses. Specifically, genetic profile scores were a stronger predictor of amygdala and hippocampus responses among pubertal vs. prepubertal children where they positively predicted responses to fearful faces among pubertal girls and positively predicted responses to neutral faces among pubertal boys. The current results suggest that genetic and environmental stress-related factors may be important in normative individual differences in responsivity to negative emotional stimuli, a potential mechanism underlying internalizing disorders. Further, sex and pubertal development may be key moderators of the effects of stress-system genetic variation on amygdala and hippocampus responsivity, potentially relating to sex differences in stress-related psychopathology.

Introduction

Stress exposure has been shown to predict elevated threat-related amygdala reactivity across development (Bogdan et al., 2012; Ganzel et al., 2013; Grant et al., 2011; Tottenham et al., 2011). Further, it has been suggested that the timing of stress along the developmental trajectory can greatly alter its influence (Tottenham and Sheridan, 2009), where early life stress can have long lasting and potentially irreversible effects on amygdala function and development (Cohen et al., 2013). Moreover, effects of life stress on amygdala reactivity may be moderated by stress-related genetic variants (e.g., Bogdan et al., 2012; White et al., 2012). Importantly, heightened amygdala and hippocampus response to threat-related stimuli has also been observed in children, adolescents, and adults with depression (e.g. Barch et al., 2012; Beesdo et al., 2009; Bishop et al., 2004; Etkin et al., 2004; Ewbank et al., 2009; Gaffrey et al., 2011; Thomas et al., 2001b; Yang et al., 2010). Amygdala hyperresponsivity is similarly present in unaffected children at risk for depression (based on parental history of depression; Monk et al., 2008), suggesting that these differences may precede the development of psychopathology and that genetic risk and/or early environmental factors may play a key role. Given this and the prominent relationships between stress and depression (e.g. Green et al., 2010; Kessler and Magee, 2009), understanding the relationship between individual differences in stress-related factors and differences in neural responsivity to emotional stimuli can be highly informative both of normative emotional development and of the mechanisms underlying alteration in disorders.

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We have shown previously that a profile score across ten single nucleotide polymorphism (SNPs) on four genes (*CRHR1*, *NR3C2*, *NR3C1*, *FKBP5*) integrally involved in the hypothalamic–pituitary–adrenal (HPA) axis and the experience of early life stress are related to cortisol reactivity and amygdala and hippocampus structure in school-age children (Pagliaccio et al., 2013a). SNPs on these genes of interest have been previously related to increased depression prevalence (e.g. CRHR1: Liu et al., 2006; NR3C2: Kuningas et al., 2007; NR3C1: van West et al., 2005; FKBP5: Lavebratt et al., 2010) and altered cortisol reactivity (e.g. CRHR1: Tyrka et al., 2009; NR3C2: DeRijk et al., 2006; NR3C1: Ising et al., 2008; FKBP5: Menke et al., 2013), as well as other related phenotypes, like suicidality (e.g. CRHR1: Wasserman et al., 2007; FKBP5: Roy et al., 2010) or antidepressant treatment response (e.g. CRHR1: Licinio et al., 2004; FKBP5: Binder et al., 2004). Further details and background are provided in Pagliaccio et al., 2013a. However, effects of sex and pubertal development may be key to understanding the influence of stress on amygdala and hippocampus function in children. For example, sex may moderate cortisol reactivity to acute stressors (e.g. Kirschbaum et al., 1995, 1992) and, as shown in animal studies, also moderates the effects of environmental stress and stress-system genes on the HPA axis and the limbic system (e.g. Bourke et al., 2013; Shors et al., 2001; Zohar and Weinstock, 2011). In addition, stress reactivity has a particularly strong effect on emotion processing during puberty (e.g. Natsuaki et al., 2009) and the brain is particularly sensitive to the effects of environmental stressors during this period (e.g. Holder and Blaustein, 2013). Of note, females tend to begin puberty earlier than males (Carskadon and Acebo, 1993) and sex differences in depression prevalence emerge during this transitional time (e.g. Angold et al., 1998; Angold and Worthman, 1993).

The goal of the current study was to assess whether stress exposure and HPA axis genetic variation predict amygdala and hippocampus responses to negative emotional stimuli in school-age children. We hypothesized that having more HPA axis genetic 'risk' variants, indexed by higher genetic profile scores, and the experience of greater numbers of life stressors, and/or their interaction would predict greater fearful–neutral face activity in the amygdala and hippocampus. In a follow-up analysis, we tested how sex and pubertal status moderated effects of genetic factors, given their moderating roles in stress function and risk for depression. Overall, these analyses aimed to elucidate our understanding of how genetic variation and stress exposure influence individual differences in amygdala and hippocampal responsitivity to emotional stimuli in school-age children. Findings may guide future exploration of whether and how these factors underlie risk for internalizing psychopathology.

Materials and methods

Participants

A subsample of participants ($N = 168$) enrolled in the prospective longitudinal Preschool Depression Study (PDS; total $N = 306$) completed neuroimaging sessions. The PDS is being conducted by the Washington University in St. Louis School of Medicine Early Emotional Development Program (WUSM EEDP) and its broad goal is to explore clinical and neural outcomes relating to preschool-onset depression. The details of the study have been published previously (see, Luby et al., 2009). Briefly, 3- to 5-year old children and their primary caregivers were recruited from the St. Louis metropolitan area. Children and caregivers each completed in-depth clinical interviews annually and children participated in a neuroimaging session at 7–12 years of age. Parental written consent and child assent were obtained prior to study participation and the Institutional Review Board at Washington University approved all experimental procedures.

Of the 134 children who completed the Facial Emotional Processing Task, 12 were excluded due to excessive head motion (see fMRI preprocessing section below). An additional 15 participants who identified as ethnicities other than White or African American were removed from the current analysis to reduce population stratification. (However, the main results are highly consistent when retaining these children in the analysis). This left a final sample of 107 children for the current analyses.

Diagnostic assessments

Trained WUSM EEDP staff conducted up to seven in-person assessments (current subsample M = 4.84, SD = 1.02 assessments) with participants and their parents/guardians from study enrollment through the time of scan. Before children were age 8, a reliable and age-appropriate semi-structured parent-report diagnostic interview was used to assess psychiatric symptoms, the Preschool-Age Psychiatric Assessment (PAPA; Egger et al., 2003). After age 8, the Childhood and Adolescent Psychiatric Assessment (CAPA; Angold and Costello, 2000) was used, which also includes child-report. Interviews were audiotaped, reviewed for reliability, and calibrated for accuracy (Luby et al., 2009). Data from the PAPA/CAPA were used to assess the child's experience of stressful and traumatic life events from birth through the scan session (a full list of events and their frequencies is reported in Supplementary Table 1). The life events factor used in the main analyses represents the sum number of instances of both stressful and traumatic events experienced through the time of scan. We had no a priori method for weighting individual events and counts of stressful versus traumatic events were highly correlated ($r(105) = .436$, $p < 0.001$). Thus, all events were summed equally for the primary results, though an analysis separating stressful and traumatic events is presented in the supplement. PAPA/CAPA data was also used to assess whether children met criteria for relevant psychiatric disorders (Supplementary Table 2 details the number of children meeting criteria for depressive, anxiety, and/or externalizing disorders up to and at the time of scan). Pubertal status at the time of scan was assessed using child self-report on the Tanner Pubertal Staging Questionnaire (Tanner, 1955). As about half of the children were prepubertal (Stage 1 $N = 55$), children in the remaining stages were combined into a "pubertal" group ($N = 51$: Stage 2 $N = 19$, Stage 3 $N = 25$, Stage 4 $N = 7$, Stage 5 $N = 0$).

Genetic profile scores (GPS)

Extensive details on the rationale, methods, and limitations of our HPA axis genetic profile score (GPS) creation in this sample have been published previously (Pagliaccio et al., 2013a). Briefly, previous work has documented the utility of additively combining genetic variants to

Table 1
Demographic and brain activity variables.

	Mean	Std	Minimum	Maximum
Genetic profile scores	4.466	1.312	1.0	7.5
Stressful and traumatic life events	17.776	10.418	4	50
Age at scan (months)	123.930	15.186	83	153
Fearful–neutral face activity				
Left amygdala	0.029	0.227	−0.620	0.570
Right amygdala	0.034	0.260	−0.820	0.710
Left hippocampus	0.020	0.156	−0.330	0.450
Right hippocampus	0.001	0.157	−0.440	0.370
	Counts for each subgroup			
Sex	Female = 54, male = 53			
Ethnicity	White = 67, African American = 40			
Pubertal status	Prepubertal = 55, Pubertal = 52			

Demographic and brain activity variables: mean, standard deviation (std), minimum, and maximum values are presented for predictors of interest and age at scan as well as for outcomes of interest (fearful–neutral face magnitude estimates in the left and right amygdala and hippocampus. Counts of participants by sex, ethnicity and pubertal status are also presented.

Table 2
Follow-up regressions testing genetic profile scores (GPS) × puberty × sex interactions predicting fearful–neutral face activity.

	Left amygdala			Right amygdala			Left hippocampus			Right hippocampus		
	b	*β*	*p*	*b*	*β*	*p*	*b*	*β*	*p*	*b*	*β*	*p*
Constant	0.044		0.092	0.043		0.157	0.031		0.083	0.017		0.352
Ethnicity	−0.058	−0.249	0.276	0.005	0.026	0.939	0.002	0.011	0.959	−0.041	0.011	0.263
Sex	0.039	0.152	0.370	0.053	0.180	0.308	0.045	0.261	0.135	0.001	0.261	0.969
GPS	−0.011	−0.063	0.603	0.022	0.112	0.370	0.007	0.061	0.615	0.002	0.061	0.881
GPS × ethnicity	−0.016	−0.091	0.702	−0.023	−0.117	0.630	0.002	0.019	0.934	−0.002	0.019	0.945
GPS × sex	0.058	0.337	0.090	0.070	0.353	0.083	**0.052**	**0.437**	**0.028**	**0.050**	**0.437**	**0.037**
Pubertal status	−0.002	−0.029	0.966	0.003	0.000	0.950	0.027	0.173	0.381	0.008	0.173	0.787
GPS × puberty	0.055	0.316	0.168	0.041	0.205	0.380	−0.001	−0.005	0.982	−0.015	−0.005	0.571
Puberty × sex	0.166	0.661	0.063	**0.228**	**0.813**	**0.030**	**0.148**	**0.888**	**0.017**	0.093	0.888	0.129
Ethnicity × puberty	0.143	0.630	0.169	0.130	0.499	0.286	0.060	0.382	0.400	−0.001	0.382	0.991
GPS × puberty × sex	**0.194**	**1.124**	**0.006**	**0.193**	**0.972**	**0.021**	**0.102**	**0.855**	**0.036**	**0.143**	**0.855**	**0.004**
Model R^2	0.161			0.123			**0.168**			**0.172**		
Adjusted R^2	0.074			0.031			**0.082**			**0.086**		
Model F	1.847			1.343			**1.945**			**1.995**		
Model *p*	0.062			0.219			**0.048**			**0.042**		
R^2 change	**0.068**			**0.051**			**0.039**			**0.076**		

Follow-up regressions testing genetic profile scores (GPS) × puberty × sex interactions predicting fearful–neutral face activity: unstandardized (b) and standardized (*β*) regression coefficients and their associated *p*-value are presented for the final step of each model. Model statistics are listed below each set of predictor statistics. R^2 change indicates the change in model R^2 adding the three-way genetic profile scores × puberty × sex interaction to the model after all other predictors listed. Effects significant at $p < 0.05$ are in bold and effects reaching significance after false discovery rate (FDR) correction ($q < 0.05$) are shaded gray (correcting for the three-way interaction tested in each of four regions). FDR corrected *p*-values for each regions were: left amygdala $p = 0.012$, right amygdala $p = 0.028$, left hippocampus $p = 0.036$, and right hippocampus $p = 0.012$.

study their polygenic effects on brain structure and function, where single polymorphisms alone may not be significantly predictive (Nikolova et al, 2011). We created an additive genetic profile score from 10 SNPs within 4 integral HPA axis genes; higher scores indicate more alleles previously associated with increased cortisol, depression prevalence/severity, and/or related phenotypes (e.g. antidepressant treatment response, suicidality, etc.). These 10 SNPs were narrowed down from a larger set of 15 to reduce linkage disequilibrium (all pairwise $r^2 < 0.49$). Unweighted sum scores were created from the 10 SNPs of interest. Indicative of their construct validity, higher GPS predict elevated cortisol reactivity to a stressor in PDS participants (Pagliaccio et al., 2013a). The variants of interest included SNPs from *CRHR1* (rs4792887, rs110402, rs242941, rs242939, rs1876828), *NR3C2* (rs5522), *NR3C1* (rs41423247, rs10482605, rs10052957), and *FKBP5* (rs1360780). For more background on each SNP and linkage disequilibrium plots, see Pagliaccio et al. (2013a).

Facial emotion processing task

Participants completed a neuroimaging battery including high-resolution structural, resting state, and functional task scans. Only data from the Facial Emotion Processing Task was used for the current analysis. Directly following a sad mood induction and elaboration as described below (Furman et al., 2011), children completed a facial emotion processing task during which they were shown a series of 90 neutral and emotional faces (45 stimuli during each of 2 task runs) and were asked to judge the gender of the face, responding via a fiber optic button box to indicate whether the face was male or female. This task was chosen as previous research has indicated that those with or at-risk for depression show more robust amygdala activity than healthy controls in response to viewing emotional faces when attention was not constrained to the emotional content of the images (Fales et al., 2008; Monk et al., 2008). This task was also preferable to a passive viewing task as the active gender judgment helps to ensure engagement with the visual stimuli.

Face stimuli were drawn from the MacArthur Network Face Stimuli Set, a validated stimulus set containing images of 43 different actors from different ethnic backgrounds (Tottenham et al., 2009). Children saw faces with neutral, sad, angry, happy, and fearful expressions, equally distributed across task runs, from 10 of the individuals in this stimulus set. Each stimulus was presented for 2250 ms, followed by an inter-trial interval of 250 ms, 2750 ms, or 5250 ms (each occurring at equal frequency); each task run lasted 247.5 s.

One original goal of the PDS was to probe potential emotional biases relating to preschool-onset depression apparent with varying intensity of emotional facial expressions. To this end, children viewed both full- and half-intensity emotional faces. However, as we did not have specific hypotheses about emotional face intensity in the current analysis, we collapsed across the full- and half-intensity faces for each emotion type to increase our power.

Prior to the Facial Emotion Processing task, children underwent a mood induction and elaboration paradigm. The methods and results of this prior task have been discussed previously (Pagliaccio et al., 2011). Briefly, children watched a short clip from the film, *My Girl*, intended to induce sad mood followed by a series of verbal prompts to have the children mentally elaborate on the induced mood. fMRI scanning was performed during the elaboration period. After the elaboration, children began the Facial Emotion Processing task. Given that the original goals of the PDS included exploring the effects of a history of preschool-onset major depression on the brain, the mood induction was of interest because previous work has shown that negative mood induction can reactivate affective processing biases (Scher et al., 2005) and amygdala responses to emotional stimuli (Ramel et al., 2007) specifically in patients with a history of depression. Of note however, there were no correlations between induction-related activity during the elaboration period and fearful–neutral face activity in the Facial Emotion Processing task in our regions of interest (all ps > 0.18). Induction success did not differ as a function of sex, ethnicity, GPS, or life events (all ps > 0.26). Furthermore, the main results described below held when controlling for diagnostic status, mood ratings following mood induction, and induction-related activity during elaboration (data not shown). Further, GPS did not significantly differ based on the presence of depressive, anxious, or externalizing disorders nor did they predict mood ratings (ps > 0.10).

MRI acquisition

Structural and functional imaging data were collected using a 3.0 Tesla TIM TRIO Siemens whole body scanner at Washington University in St. Louis. T1-weighted structural images were acquired in the sagittal plane using an MPRAGE 3D sequence (TR = 2400 ms, TE = 3.16 ms, flip angle = 8°, slab = 176 mm, 176 slices, matrix size = 256 × 256, field of

view (FOV) = 256 mm, voxel size = 1 × 1 × 1 mm; interslice skip = 0). Functional images were collected during the face processing task with a 12-channel head coil using a T2*-weighted gradient-echo echo-planar sequence in the axial plane (TR = 2500 ms, TE = 27 ms, flip angle = 90°, FOV = 256 mm, voxel size = 4 × 4 × 4 mm, interleaved slice acquisition, transverse axial alignment). T2-weighted images were collected for registration purposes using a 3D SPACE acquisition (TR = 3200 ms, TE = 497 ms, 160 slices, FOV = 256, voxel size = 1 × 1 × 1 mm).

fMRI pre-processing

Imaging data were preprocessed using the following steps: (1) correction for slice-dependent time shifts; (2) removal of first 4 images of each run to allow BOLD signal to reach steady state; (3) elimination of odd/even slice intensity differences due to interpolated acquisition; (4) realignment of data acquired from each participant within and across runs to compensate for rigid body motion (Ojemann et al., 1997); (5) image intensity normalization to a whole-brain mode value of 1000; (6) registration of the 3D structural volume (T1) to an atlas template (WU "711-2B") in the Talairach coordinate system (Talairach and Tournoux, 1988) using a 12-parameter affine transform and resampling to 1 mm cubic representation (Buckner et al., 2004; Ojemann et al., 1997); (7) co-registration of the 3D fMRI volume to the T2, and the T2 to the participant's structural image; (8) transformation of the fMRI data to 3 × 3 × 3 mm voxel atlas space using a single affine 12-parameter transform; and (9) spatial smoothing using a 6 mm full-width half-maximum Gaussian filter.

Stringent data quality criteria were used for data inclusion in the current analyses. The signal-to-noise ratio (SNR: mean signal/standard deviation across each BOLD run, computed for each slice and then averaged across all slices) for each of the two task runs was calculated using in-house software following preprocessing. Only task runs with an SNR above 200 were included in the current analyses (mean SNR for included first runs: 536.778 ± 192.336, minimum = 202; mean SNR for included second runs: 493.634 ± 188.070, minimum = 216).

Additionally, we applied previously validated corrections for head motion, termed "motion scrubbing" (Siegel et al., 2013). The motion scrubbing procedure assesses frame-wise displacement based on the movement parameters used in pre-processing step 4. For any given frame (i.e. timepoint), this represents the differential head motion from the previous frame summing across linear (x,y,z) and rotational displacements (yaw, pitch, roll, where degrees of rotation are converted to millimeters of movement by calculating displacement on the surface of a sphere with a radius of 50 mm). A temporal mask removed any frame with a sum displacement greater than 0.9 mm from analysis. If >40% of a participant's total number of frames or fearful or neutral face trial frames were censored due to motion, that participant was excluded from analysis ($n = 12$). Therefore, frames with high motion were censored allowing us to retain participants who otherwise would contribute poor quality data, while participants with excessive data loss due to motion/numbers of frames censored were excluded. Details on the validity and efficacy of this procedure for the Facial Emotion Processing Task data in a subsample of psychiatrically healthy children from the PDS have been published previously (Pagliaccio et al., 2013b).

fMRI analysis

Analysis of fMRI data was performed using in-house software (FIDL analysis package, http://www.nil.wustl.edu/labs/fidl/index.html; Ollinger et al., 2001). A voxel-wise general linear model (GLM) approach was used, which incorporated regressors for linear trend and baseline shifts. Only those trials on which the participant made a correct gender judgment were included in the analysis, though there were very few incorrect trials (mean error rate ~4%). We assumed a canonical SPM hemodynamic response for this analysis, which results in beta estimates of brain responses to each of the five face types (neutral, sad, happy, fear, angry). The primary contrast of interest in these analyses was response magnitude to fearful–neutral faces to specifically assess amygdala and hippocampus responses to fear/threat-related stimuli (i.e. fearful faces) by subtracting neutral faces, which are expected to not carry threat-related social signals (though they may in some contexts or for some people) but rather control for general activity to face stimuli. For follow-up analyses, we examined responses to sad–neutral faces to assess whether our predictors of interest related more generally to negative emotional stimuli.

We used FreeSurfer v5.1 (Fischl et al., 2002, 2004) to create anatomical region of interest (ROI) masks by segmenting each participant's T1 anatomical image and extracting bilateral amygdala and hippocampal segmentations. Each participant's ROIs were down-sampled to match the functional resolution of the atlas space (3 × 3 × 3 mm) and registered to the common atlas space. We extracted beta estimates of responses to each face type from each participant's four individually defined anatomical ROIs (left and right amygdala and hippocampus) for subsequent data analysis. Supplementary Fig. 1 shows a heat map of the overlap of individual subject ROIs in atlas space for illustration purposes.

Statistical analysis

Outliers (more than three times the interquartile range away from the 25th or 75th percentile) were Winsorized before subsequent data analysis (1–3 outliers identified for life events and brain activity variables; main analyses remain when excluding these outliers instead). We used hierarchical linear regressions in IBM SPSS Statistics v20 (Armonk, NY: IBM Corp.) to explore the effects of interest predicting left and right amygdala and hippocampus activity (magnitude estimates). The first step in each regression included ethnicity (White vs. African American) and sex as predictors. Next, genetic profile scores and stressful/traumatic life events were added as predictors. Consistent with recent recommendations (Keller, 2013), interactions between the predictors of interest (i.e., GPS and stressful life events) and the covariates (i.e. ethnicity and sex) were added in the next step to better control for potential confounds. Finally, an interaction between genetic profile scores and life events was added. False discovery rate (FDR; Benjamini and Hochberg, 1995) correction was used to control for multiple comparisons for the 12 hypothesized tests (i.e., main effects of GPS and life events as well as their interaction across four brain regions) setting a maximum acceptable FDR of 0.05.

As noted in the results, we also pursued a follow-up analysis to explore a GPS × sex interaction that emerged in the above analyses. Specifically, we tested the hypothesis that pubertal status might further moderate this effect. To do this, we ran four regression models as above. The first step included ethnicity and sex. GPS were added in the second step, followed by interactions between GPS and the demographic factors. Life events were not of interest for these analyses given that they did not interact with sex or GPS in the initial models. Pubertal status was added in the fourth step, followed by interactions between GPS and pubertal status and between pubertal status and demographic factors. Next, the three-way interaction of interest, GPS × pubertal status × sex, was added. Finally, we controlled for all other three-way interactions (GPS × pubertal status × ethnicity, GPS × sex × ethnicity, pubertal status × sex × ethnicity). FDR correction was used to control for multiple comparisons for the GPS × sex × pubertal status effect tested in all four regions. We used the moderated moderation model from the PROCESS tool for SPSS (Hayes, 2013) to parse significant 3-way interaction effects by isolating simple slopes.

Power calculations were performed using G*Power 3 (Faul et al., 2009, 2007).

Results

Control analyses/demographic and clinical factors

Table 1 shows the means and standard deviations or counts of demographic and brain activity variables. We tested for any potentially confounding effects of demographic factors on our variables of interest. As noted in Supplemental Table 3, there were no significant differences in the variables of interest by sex or pubertal status (all $ps > 0.05$). There were significant ethnic differences where African American children had significantly higher genetic profile scores and stressful/traumatic life events experience. Additionally, it is important to note that there was no significant correlation between genetic profile scores and life events ($r(105) = 0.006$, $p = 0.952$). Finally, the percent of frames cut/retained from motion scrubbing did not correlate with activity in any of the four regions of interest (all ps > 0.38).

Regression results predicting fearful–neutral face activity

Supplementary Tables 4–7 present all steps of the regression models predicting fearful–neutral face activity in the left and right amygdala and hippocampus. These results indicated that the number of stressful/traumatic life events experienced by the time of scan positively predicted fearful–neutral face activity in the left amygdala ($b = 0.008$, $\beta = 0.368$, $t = 3.800$, $p < 0.001$; FDR corrected $p = 0.003$; see Fig. 1), but did not significantly predict activity in the right amygdala or left or right hippocampus (all ps and FDR corrected ps >0.10). This effect of life events remained significant in a follow-up regression step controlling for age at scan (months) and age × sex and age × ethnicity interactions (life events: $b = 0.009$, $\beta = 0.406$, $t = 4.035$, $p < 0.001$). Further follow-up analysis examining the influence of stressful life events and traumatic life events as separate predictors is presented in Supplementary Table 8. Additional follow-up analyses examine the potential role of family income as a proxy of socio-economic status (Supplementary Table 9). Briefly, higher family income correlates with lower experience of stressful life events, but income does not predict left amygdala activity whereas life events continue to predict activity even controlling for income.

In the main regression models, genetic profile scores did not significantly predict activity in any of the four regions (all ps and FDR corrected ps > 0.08). Across the four regions, the GPS × life events interaction only predicted left hippocampal activity ($b = -0.002$, $\beta = -0.206$, $t = -2.116$, $p = 0.037$, FDR corrected $p = 0.210$), but this effect did not pass FDR correction for multiple comparisons. Finally, though not hypothesized, a GPS × sex interaction predicted left ($b = 0.051$, $\beta = 0.426$, $t = 2.150$, $p = 0.034$) and right ($b = 0.051$, $\beta = 0.425$, $t = 2.145$, $p = 0.034$) hippocampal activity in step 3 of each regression (Supplementary Tables 6–7). This interaction was also a trend-level predictor of left ($b = 0.059$, $\beta = 0.342$, $t = 1.824$, $p = 0.071$) and right ($b = 0.071$, $\beta = 0.358$, $t = 1.777$, $p = 0.079$) amygdala activity in regression step 3 (Supplementary Tables 4–5). Simple slope analyses for the left and right hippocampus indicated a positive relationship between genetic profile scores and activity among females (left: $b = 0.067$, $\beta = 0.219$, $t = 2.368$, $p = 0.019$; right: $b = 0.062$, $\beta = 0.194$, $t = 2.176$, $p = 0.032$) but a negative relationship among males (left: $b = -0.026$, $\beta = -0.137$, $t = -0.911$, $p = 0.364$; right: $b = -0.020$, $\beta = -0.194$, $t = -0.688$, $p = 0.493$).

Exploratory analysis with pubertal status

Given that puberty is a key transitional period when sex differences in depression prevalence tend to develop (e.g. Angold et al., 1998; Angold and Worthman, 1993), we conducted follow-up analyses to examine whether puberty further moderated the GPS × sex interaction

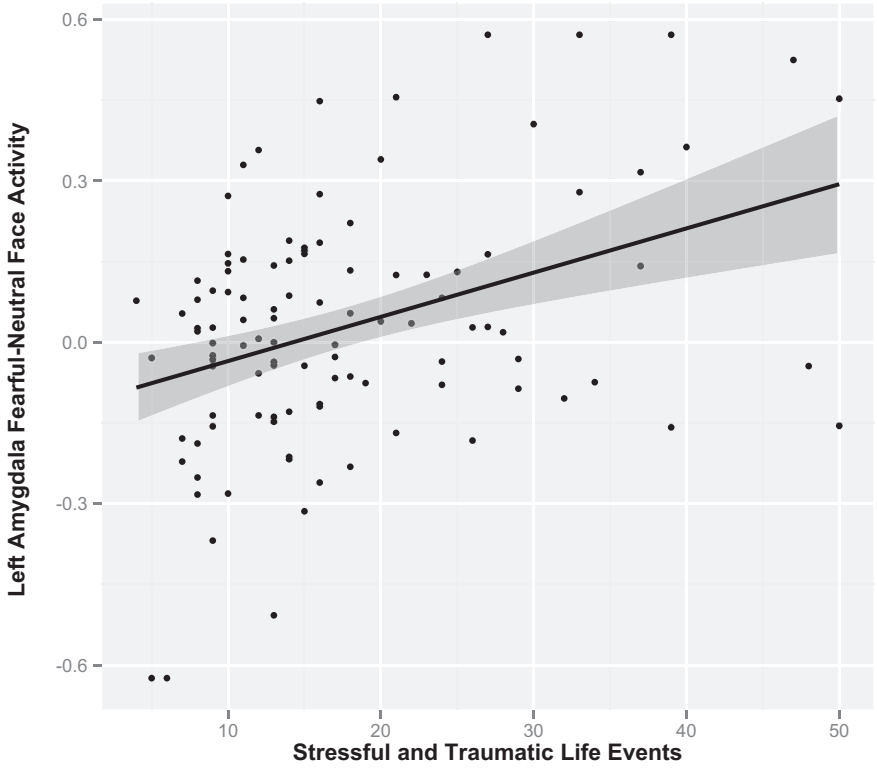

Fig. 1. Effect of life events experience on left amygdala activity. This figure displays the relationship between sum counts of stressful and traumatic life events experienced by the time of scan and fearful–neutral face activity in the left amygdala (difference in magnitude estimates for fearful face vs. neutral face contrasts). The shaded region represents the 95% confidence interval around the fit line.

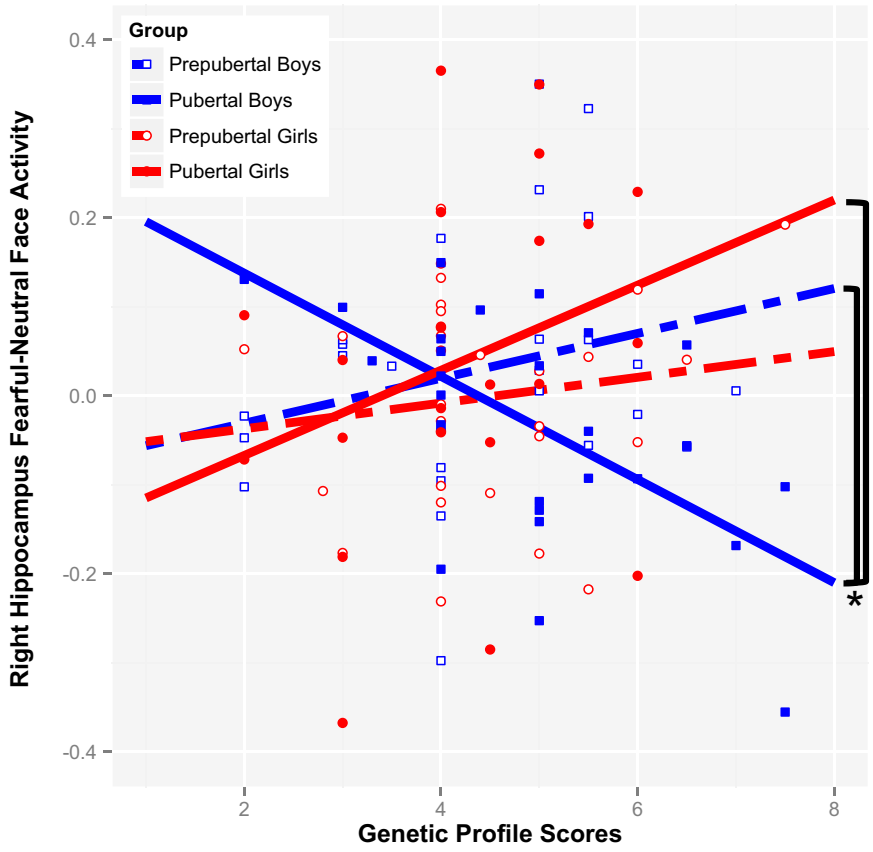

Fig. 2. Effects of genetic profile scores on right hippocampus activity. This figure shows the relationship between genetic profile scores and fearful–neutral face activity in the right hippocampus split by sex and pubertal status. Females are plotted with a circle and males with a square; pubertal children are denoted by filled symbols and prepubertal children by empty symbols. Brackets indicate relationships that are significantly different (i.e. there is a genetic profile score × sex interaction among pubertal children and a genetic profile score × pubertal status interaction among males). Pubertal males also show a significant simple slope effect * $p < 0.05$.

predicting fearful–neutral activity reported above. The GPS × pubertal status × sex interaction predicted fearful–neutral face activity in the left and right amygdala and left and right hippocampus (Fig. 2). This interaction effect passed FDR correction in all four regions (Table 2; all ps and FDR corrected ps < 0.04) and remained significant in the left amygdala ($b = 0.177$ $\beta = 1.022$, $t = 2.195$, $p = 0.031$), left hippocampus ($b = 0.116$, $\beta = 0.974$, $t = 2.092$, $p = 0.039$), and right hippocampus ($b = 0.128$, $\beta = 1.066$, $t = 2.291$, $p = 0.024$), when further controlling for the other three-way interactions (GPS × pubertal status × ethnicity, GPS × sex × ethnicity, pubertal status × sex × ethnicity, none of which were significant predictors themselves [ps > 0.06]). The spatial extent of this interaction is displayed in Supplementary Fig. 2.

Given the strong relationship between pubertal status and age, we added age at scan and interactions with age (age × ethnicity, age × sex, GPS × age, and GPS × age × sex) as covariates to the model as a further statistical control (Supplementary Table 10). The

Table 3
Interactions with pubertal status or sex and simple slope effects of genetic profile scores (GPS) predicting fearful–neutral face activity.

	Left amygdala			Right amygdala			Left hippocampus			Right hippocampus		
	b	*β*	*p*	*b*	*β*	*p*	*b*	*β*	*p*	*b*	*β*	*p*
GPS × puberty, split by sex												
Boys	−0.042	−0.212	0.354	−0.056	−0.258	0.300	−0.052	−0.422	0.102	**−0.087**	**−0.703**	**0.007**
Girls	**0.152**	**0.895**	**0.011**	**0.137**	**0.702**	**0.050**	0.050	0.428	0.216	0.056	0.476	0.172
GPS × sex split, by puberty												
Prepubertal	−0.039	−0.383	0.426	−0.026	−0.240	0.644	0.001	−0.041	0.973	−0.022	−0.278	0.517
Pubertal	**0.155**	**0.724**	**0.002**	**0.166**	**0.721**	**0.005**	**0.103**	**0.808**	**0.003**	**0.121**	**0.901**	**0.001**
Simple slope effects of GPS												
Prepubertal boys	−0.019	−0.053	0.576	0.015	0.114	0.704	0.007	0.077	0.762	0.021	0.208	0.373
Pubertal boys	−0.061	−0.265	0.058	−0.041	−0.144	0.282	**−0.045**	**−0.344**	**0.045**	**−0.066**	**−0.495**	**0.004**
Prepubertal girls	−0.058	−0.436	0.139	−0.011	−0.126	0.803	0.008	0.036	0.760	−0.001	−0.070	0.971
Pubertal girls	**0.094**	**0.459**	**0.040**	**0.126**	**0.577**	**0.020**	0.059	0.464	0.062	0.055	0.406	0.081

Interactions with pubertal status or sex and simple slope effects of genetic profile scores (GPS) predicting fearful–neutral face activity: for each of the four regions of interest, GPS × pubertal status interactions for each sex and GPS × sex interactions within each pubertal status group are presented. Additionally, the simple slope effects of GPS predicting fearful–neutral face activity for each pubertal status × sex subgroup are presented. These effects were extracted using the PROCESS tool from the full models presented in Table 2, i.e. accounting for all covariates. Unstandardized (*b*) and standardized (*β*) regression coefficients and their associated *p*-values are presented for each effect in each model. Effects significant at $p < 0.05$ are in bold.

three-way GPS × pubertal status × sex interaction remained significant in the left amygdala and left and right hippocampus ($p < 0.05$) and trend-level significant in the right amygdala ($p = 0.06$). Additionally, no significant main effects or interactions with age were found, suggesting that the above interaction is specific to pubertal effects rather than age in this sample. In a final statistical control, we found that the GPS × pubertal status × sex interaction held significant in all four regions (all $ps < 0.03$) when controlling for histories of major depressive disorder, anxiety disorders, and/or externalizing disorders (see Supplementary Table 11). Furthermore, we examined whether these effects were significant in subsets of children with histories of each of these types of disorders (Supplementary Table 12). The results also held significant when controlling for amygdala or hippocampal volume (data not shown).

To parse and understand this three-way GPS × pubertal status × sex interaction, we assessed two-way interactions with genetic profile scores in the sex and pubertal subgroups and isolated the simple slopes for GPS predicting fearful–neutral face activity for prepubertal and pubertal boys and girls (see Fig. 2 and Table 3). A significant GPS × sex interaction was present among pubertal children in all 4 regions but was absent among prepubertal children. Further, there were significant GPS × pubertal status interactions among girls in the left and right amygdala and among boys in the right hippocampus ($p = 0.007$). Pubertal boys ($N = 28$) showed a negative relationship between genetic profile scores and activity in all four regions (e.g. right hippocampus: $b = -0.066$, $\beta = -0.495$, $t = -2.993$, $p = 0.004$) whereas pubertal girls ($N = 24$) show the hypothesized positive relationship in all four regions (e.g. right amygdala: $b = 0.126$, $\beta = 0.577$, $t = 2.375$, $p = 0.020$).

To examine whether the results represented differential effects on fearful and/or neutral faces, we examined whether the GPS × sex interaction predicted fearful-baseline and neutral-baseline activity among the pubertal children (given that all four regions showed a GPS × sex interaction only among pubertal children). We tested this with a GLM with emotion type as a 2-level within-subject factor (fearful face vs. baseline activity and neutral face vs. baseline activity), sex and ethnicity as binary between-subject factors, and GPS as a continuous predictor. The results indicated an emotion type × GPS × sex interaction in all four regions (see Supplementary Table 13). Fig. 3 shows the relationships between GPS and fearful face and neutral face activity separately for pubertal boys and girls for the right hippocampus. For this, and the other regions, pubertal girls tended to show a *positive* relationship between GPS and *fearful* face activity while pubertal boys tend to show a *positive* relationship between GPS and *neutral* face activity. In other words, the positive relationship between GPS and fearful–neutral face activity among pubertal females was driven mainly by a positive effect on fearful face activity. Conversely, the *negative* relationship between GPS and fearful–neutral face activity observed among pubertal males was driven mainly by a positive effect on neutral face activity. Thus, more risk-conferring alleles among stress- and MDD-related genetic variants predicted greater responses to emotional faces among females, but greater responses to neutral faces among males.

Specificity of results to fearful faces

As a follow-up analysis, we tested whether these associations were specific to fearful faces or whether these stress-related factors predicted increased reactivity to negative emotional faces more generally. To do so, we performed the same sets of regressions as above but predicting *sad*–neutral face activity. Supplementary Table 14 shows a summary of the final step of each of the regression models including demographics, life events, GPS, and interactions predicting sad–neutral faces. Here, we found no significant main effects of or interactions with stressful life events. There was a significant GPS × sex interaction predicting left amygdala ($b = 0.091$, $\beta = 0.534$, $t = 2.669$, $p = 0.009$) and right amygdala activity ($b = 0.081$, $\beta = 0.432$, $t = 2.106$, $p = 0.038$); this was also trend level significant when predicting the left and right hippocampal activity ($ps < 0.1$). The pattern of simple slopes was similar to that predicting fearful–neutral faces, i.e. there was a positive relationship between GPS and activity among females but a negative relationship among males.

Next, we performed the same exploratory regression models predicting sad–neutral face activity to test the specificity of the three-way interactions to fearful–neutral faces (Supplementary Table 15). While each region showed a significant genetic profile score × sex effect (all $ps < 0.05$), we found that the GPS × pubertal status × sex interaction

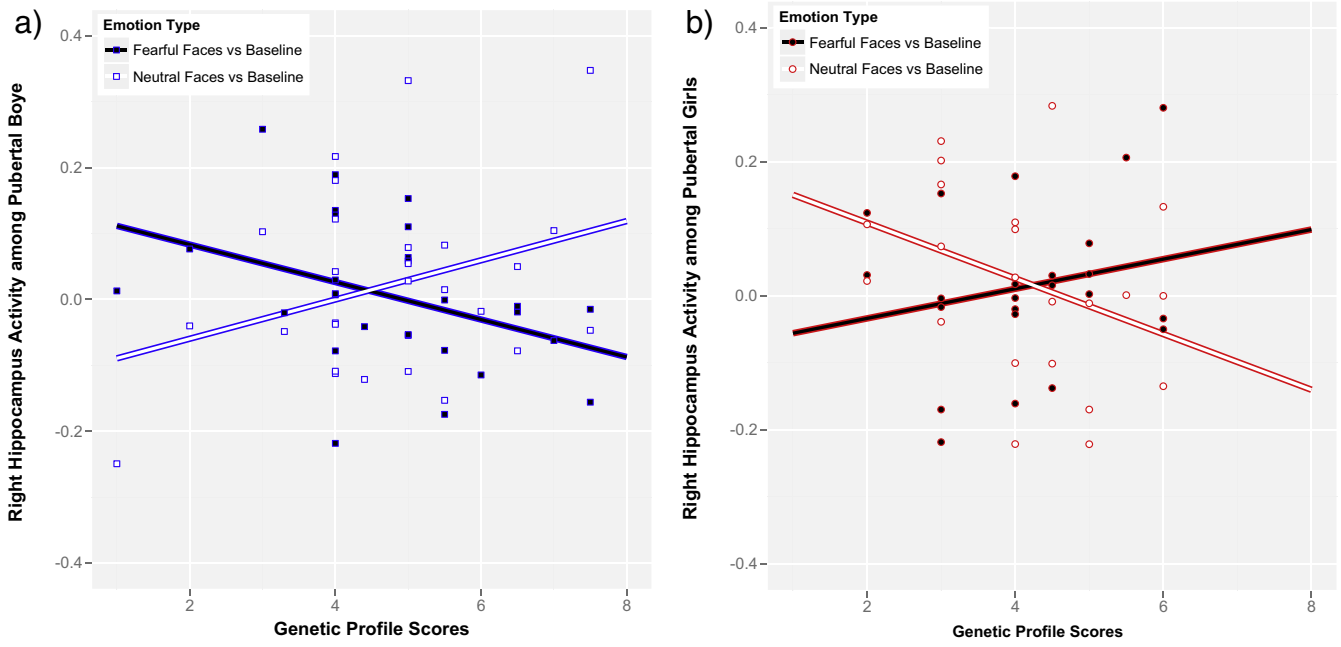

Fig. 3. Effects of genetic profile scores on fearful and neutral face activity among pubertal children. This figure shows the effects of genetic profiles scores on fearful face vs. baseline (filled black) and on neutral face vs. baseline activity (filled white) in the right hippocampus. These effects are shown only for pubertal children and are displayed separately for males (a) and females (b).

predicted sad–neutral face activity in the left hippocampus ($b = 0.098$, $\beta = 0.835$, $t = 2.109$, $p = 0.038$) and was a trend-level significant predictor of left amygdala and right hippocampus activity ($p < 0.1$). This suggests that the differential effects of stress-system genes by sex and pubertal status may generalize to negative emotional face stimuli, though pubertal effects were less strong.

Power calculations

Consistent with current reporting standards for gene × environment studies, we calculated estimates of post-hoc power to detect our initial GPS × life events hypothesis ($N = 107$, 9 total predictors, assuming all other predictors account for 15% of variance). We would have over 80% power to detect a 6% or greater increase in variance explained by the addition of the GPS × life events interaction, but about 20% power to detect a 1% increase in variance explained. We estimated our power at 70% to detect the GPS × sex interaction among the pubertal children for a 10% increase in variance explained ($N = 52$, 5 total predictors, assuming all other predictors account for 10% of variance). Yet, our power was limited (20%) to detect the simple slope effects, expecting a 5% increase in variance explained ($N = 26$, 3 total predictors, assuming all other predictors account for 10% of variance).

Discussion

Genetic profile scores, sex, and puberty

HPA axis SNPs may moderate effects of life stress/adversity on amygdala reactivity (Bogdan et al., 2012; White et al., 2012). While we did not observe a GPS × life events interaction here, it is possible that this type of effect is not detectable this early in childhood. Yet, we instead found a three-way interaction between genetic profile scores × pubertal status × sex. GPS predicted activity more strongly among pubertal than prepubertal children, even using a relatively coarse grouping based on Tanner Stages. Among pubertal children, higher GPS predicted higher fearful face activity among females but predicted higher neutral face activity among males. While this particular pattern of results was not anticipated, it may still be conceptually consistent, where both males and females with more genetic 'risk' variants show greater amygdala and hippocampus reactivity. Specifically, females showed an expected positive association between GPS and negative emotional stimuli, while males showed greater responses to stimuli that we expect to be emotionally neutral, but that may be perceived as more negative in the context of other negative faces.

While this type of sex-moderated effect of genetic risk on amygdala and hippocampus reactivity is novel, there is potentially relevant precedent for sex differences in neural response to emotional stimuli. For example, boys but not girls may exhibit amygdala habituation to fearful faces (Thomas et al., 2001a) where this type of sex difference may relate to the apparently differential effects on fearful vs. neutral face activity. Further, there is much evidence for sex differences in stress-system and amygdala and hippocampus function. In previous work, we showed that GPS were a significantly stronger positive predictor of cortisol reactivity among females than males (Pagliaccio et al., 2013a). Sex also moderates cortisol reactivity to acute stressors (e.g. Kirschbaum et al., 1995, 1992) and the effects of childhood trauma and *CRHR1* variation on cortisol reactivity and depression (Heim et al., 2009). Sex is also a major moderator of the effects of environmental stress and stress-system genes on the HPA axis and the amygdala and hippocampus in a variety of animal studies (e.g. Bourke et al., 2013; Shors et al., 2001; Zohar and Weinstock, 2011).

The relationship between puberty and neural/emotional development is also key in this age range, and likely interacts with factors like sex and stress. Structurally, the amygdala and hippocampus exhibit non-linear growth rates across pubertal development where females tend to show large increases in volume in early puberty which peak in mid puberty whereas males show increasing subcortical volumes throughout puberty (Goddings et al., 2014). It has been suggested that amygdala responses to emotional faces also show a U-shaped developmental curve where adolescents show greater responses to emotional faces than children or adults (Guyer et al., 2008; Hare et al., 2008). Further, pubertal development specifically has been positively correlated with amygdala responses to emotional and neutral faces in early adolescence but not in late childhood (Moore et al., 2012). In contrast, we observed interactions with pubertal status rather than main effects. Nonetheless, it will be important to test whether these main effects and/or interactions with puberty are observed longitudinally across development.

It should also be noted that interactions with genetic profile scores also predicted sad–neutral face activity. Thus, these genetic 'risk' factors may play a role in amygdala and hippocampus responses to emotionally salient stimuli more generally, rather than specifically relating to threat-related stimuli. These differences in the effect of HPA axis genetic risk factors and amygdala and hippocampus function based on sex and pubertal development may be particularly salient in understanding the increasing rates of internalizing psychopathology during puberty/adolescence and the increasingly high prevalence rates among females relative to males during this period (e.g. Angold et al., 1998; Hankin et al., 1998).

Stressful and traumatic life events

We found that the experience of more stressful and traumatic life events across childhood predicted higher fearful–neutral face activity in the left amygdala. This is consistent with previous work showing that the experience of severe adversity/trauma predicted greater amygdala responses to negative faces among children, adolescents, and adults (Ganzel et al., 2013; Grant et al., 2011; Tottenham et al., 2011). Other work in this sample has explored the separate relationships between stressful vs. traumatic life events on responses to emotional faces in the amygdala, hippocampus, and other regions (Suzuki et al., 2014). These and our findings build upon prior results by suggesting that not only severe traumatic events relate to amygdala reactivity but particularly stressful life events predict amygdala reactivity even in school-age children. Additionally, we found that this effect was specific to fearful–neutral faces (i.e. life events did not predict sad–neutral face activity). While the amygdala generally responds to different facial emotion types (for meta-analysis, see Sergerie et al., 2008; for results in a healthy subsample of school-age children from the PDS, see Pagliaccio et al. (2013b); these results suggest that the effect of life stress may be particularly important for amygdala response to threat-related stimuli rather than for negative emotional expressions more generally. Additionally, it is important to note that lack of a GPS × life events interaction may indicate more independent effects of stress-system genetics and childhood stressors, but may also be due to low power to detect interactions of small effect sizes.

Limitations and future directions

As previously discussed in greater detail (Pagliaccio et al., 2013a), there are several limitations to using single summary variables to encapsulate genetic variation or stressful/traumatic life events. Though this approach can increase power by combining multiple sources of variance and reducing the number of tests to be performed, it assumes that the effects of stressors or of SNPs sum additively with equal weights. Refining this approach is an important future direction that requires optimizing the relative weighting of life events or SNPs for testing in independent samples. To this end, Supplementary Table 16 presents the effects of each SNP independently predicting left amygdala fearful–neutral face activity split by sex and pubertal status. These results should be interpreted with caution as the counts of each genotype by subgroup are relatively small, but these regression coefficients may

be useful in creating better-informed genetic profile scores in the future. Additionally, we have previously presented the relationship between these individual SNPs and cortisol reactivity and brain volumes (Pagliaccio et al., 2013a).

Additionally, the mood induction task used prior to the Emotional Face Processing Task and the presence of both half- and full-intensity emotional faces may introduce additional sources of variance into the effects of interest. Another limitation is that we examined pubertal status in a relatively coarse manner by collapsing across Tanner Stages 2–5. While we did not have sufficient sample sizes within each stage (as would be expected in this age range) to adequately test incremental changes across puberty, longitudinal data as prepubertal children transition into puberty would be key to truly confirm our results. Using measures of gonadal hormones may also be useful for exploring the underlying mechanisms of puberty's moderating effects on stress.

From a genetic perspective, both the likelihood of low power contributing to false negatives and also an increased false discovery rate contributing to false positives need to be considered. Our calculations indicate that we would have good power to detect medium to large effect sizes but that we lack power at smaller effect sizes. Thus, we may have false negatives in our results if we are unable to detect genetic influences of small effects. Second, while we were highly cautious in selecting polymorphisms for the GPS, one might argue that the priors associated with their inclusion may not be satisfactory (e.g. these SNPs have not emerged in genome-wide association studies of depression), which may increase the likelihood of false positives. All these issues highlight the importance of future replication regarding interactions with GPS. Furthermore, it is important to consider how effects may differ by ethnicity. While we did not find differences in brain activity by ethnicity, GPS and life events scores were higher among the African American children in the sample. As we did not have large enough sample sizes split by ethnicity, future studies will need to test the specificity and generalizability of these effects across ethnicity or genetic ancestry.

Conclusions

We found that having more 'risk' alleles in HPA axis genes predicted higher amygdala and hippocampus reactivity, especially among pubertal school-age children. This interacted with sex, such that higher genetic profile scores predicted higher fearful (and sad) face activity among girls but predicted higher neutral face activity among boys. The experience of more stressful/traumatic life events predicted higher left amygdala reactivity to fearful–neutral faces (but not sad–neutral faces). These findings help elucidate effects of normative genetic and environmental factors on individual differences in amygdala and hippocampus reactivity. Further, the results underscore that sex and puberty may be key factors to consider in studies of the neural measures of emotion reactivity in children. Overall, the current results suggest that how stress-related risk factors impact the neural underpinnings of emotion processing may be key to understanding the normative individual differences in neural responding to emotional stimuli with potential salience for the developmental psychopathology of internalizing disorders, especially in the peripubertal period.

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Conflict of interest

The authors report no biomedical financial interests or potential conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.neuroimage.2015.01.017.

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