Psychosocial stress and brain function in adolescent psychopathology

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Abstract

Objective: To explore how conduct, hyperactivity/inattention, and emotional symptoms are associated with neural reactivity to social-emotional stimuli, and the extent to which psychosocial stress modulates these relationships.

Method: Participants were community adolescents recruited as part of the European IMAGEN study. Bilateral amygdala regions of interest were used to assess the relationship between the three symptom domains with fMRI neural reactivity during passive viewing of dynamic angry and neutral facial expressions. Exploratory functional connectivity and whole-brain multiple regression approaches were used to analyze how the symptoms and psychosocial stress relate to other brain regions.

Results: In response to the social-emotional stimuli, adolescents with high levels of conduct or hyperactivity/inattention symptoms showed hyperactivity of the amygdala, and several regions across the brain, when they experienced a greater number of stressful life events. This effect was not observed with emotional symptoms. A cluster in the mid-cingulate was found to be common to both conduct problems and hyperactivity symptoms. Exploratory functional connectivity analyses suggested amygdala-precuneus connectivity is associated with hyperactivity/inattention symptoms.

Conclusions: The results link hyperactive amygdala responses, and regions critical for top-down emotional processing, with high levels of psychosocial stress in individuals with greater conduct and hyperactivity/inattention symptoms. This work highlights the importance of studying how psychosocial stress impacts functional brain responses to social-emotional stimuli, particularly in adolescents with externalizing symptoms.

Introduction

Common mental health problems that emerge during adolescence, such as symptoms of depression, anxiety, attention deficit hyperactivity disorder (ADHD) and conduct problems are frequent and debilitating (1). These symptom domains are associated with negative adult outcomes including substance dependence (2-4), familial discord (2, 4), poor educational attainment (2, 5) and poor vocational attainment (2, 4, 5). Psychosocial stress is an important contributor to the emergence of child and adolescent psychopathology. Family, personal and interpersonal stressors as well as trauma have been associated with externalizing symptoms, such as conduct problems and ADHD symptoms, as well as internalizing symptoms of depression and anxiety (6, 7). Therefore, identifying the interplay between symptoms of psychopathology, the environment, and biology is important to help prevent or ameliorate mental illness.

Adolescents with externalizing symptoms have difficulties with emotion recognition, particularly for anger and disgust (8, 9), while people with internalizing symptoms, are more accurate at recognizing sad and angry faces and tend to misinterpret neutral faces as angry or sad (10). Functional neuroimaging (fMRI) research suggests that the amygdala, an area crucial for emotional processing and emotional response, exhibits different activation patterns in individuals with externalizing and internalizing symptoms, versus controls (11-15). While these findings provide insight into neural differences associated with both internalizing and externalizing psychopathology they have not taken into account important moderators, such as psychosocial stress, which itself is known to increase face processing-related amygdala activation (16-18). Despite evidence that psychopathology and psychosocial stress may affect emotion perception at behavioural and neural levels, there are, to our knowledge, no studies exploring how psychopathology-related symptoms and psychosocial stress interact with one another to modulate amygdala activation related to emotional processing. We, therefore, sought to better understand the effects of psychosocial stress and psychopathology on brain

responses to angry and ambiguous faces, as well as the interaction of stress and psychopathology on brain function. We conducted amygdala-based region of interest analyses and PPI functional connectivity analyses, as well as exploratory whole-brain fMRI analyses, in 1288 adolescents from the IMAGEN Study, a large community-recruited European cohort. We hypothesised that the relationship between adolescent externalizing (conduct symptoms, hyperactivity/inattention symptoms) and internalizing (emotional) symptoms and neural reactivity to emotional stimuli is influenced by the experience of psychosocial stress.

Methods

Participants

Data that passed quality control checks for neuroimaging and behavioural tests were included; 1288 community-recruited adolescents were eligible (583 males) and their mean age was 14.4 years old (SD 0.40; range 13.18-15.43). Participants were assessed at eight study sites in France, Germany, Ireland and the United Kingdom. Each site sought approval from the local research ethics committee. Written consent was obtained from both participant and their parent/guardian; a detailed description of recruitment and assessment methods is found elsewhere (19).

Clinical Symptoms

As adolescents were community-recruited, we used continuous severity scores of internalizing and externalizing symptoms using the Strengths and Difficulties Questionnaire (SDQ; (20)). For externalizing symptoms, we used the conduct symptom and hyperactivity/inattention symptom subscales. For internalizing symptoms, we used the emotional symptom subscale. Combined informant-parent symptom scores were calculated for these three symptom domains during the six months prior to the assessment (see Supplement). The majority of participants' scores were within the average range (see Table S1).

Psychosocial Stress Score

A self-report measure (Life Events Questionnaire) was used to record the occurrence of stressful events during the adolescent's life span and in the previous 12 months (21). A score was calculated for number of stressful life events that had occurred during the last 12 months only to avoid inaccurate recall (see Supplement).

Additional covariates

Covariates of no interest included pubertal status (22), socioeconomic status indexed using the family stresses sub-section of the Development and Wellbeing Assessment (DAWBA; (23)), and verbal IQ (WISC-IV; (24)). Substance use was measured using the European School Survey Project on Alcohol and Drugs (25) and was defined in a binary fashion (i.e., had they ever/never smoked cigarettes, drank alcohol, or used drugs). Drugs included cannabis, glue, tranquilizers, amphetamines, LSD, mushrooms, crack, cocaine, heroin, narcotics, MDMA, ketamine, GHB, and anabolic steroids.

Emotional Reactivity fMRI Task

This task was adapted from Grosbras and Paus (26). Participants watched 18-second blocks of either a face movie (depicting anger or neutrality) or a control stimulus. Each face movie showed black and white video clips (200-500ms) of male or female faces. Five blocks each of angry and neutral expressions were interleaved with 9 blocks of the control stimulus. Each block contained 8 trials of 6 face identities (3 female). The same identities were used for the angry and neutral blocks. The control stimuli were black and white concentric circles expanding and contracting at various speeds that closely matched the contrast and motion characteristics of the face clips (see Figure S1).

Although some groups report significant activation in neural structures involved in threat detection such as the amygdala (11, 27), findings are mixed regarding neural reactivity to neutral stimuli in people with and without mental health problems. Therefore, we explored neural reactivity associated with the neutral stimuli in our task and found that while our target region of interest (amygdala) was significantly activated in two of the contrasts (angry faces vs. control and neutral faces vs. control), there was no significant activation of the amygdala in the angry faces vs. neutral faces contrast (see Table S2a). As a result, we proceeded with the analysis in the angry faces vs. control and neutral faces vs. control

contrasts, acknowledging that the analysis would be of the neural response to an angry or neutral face as a whole, rather than specifically isolating the emotion.

fMRI Measurement and Processing

Structural and functional MRI data were acquired with 3T MRI scanners (Siemens, Philips, General Electric & Bruker). Four sites (GE and Philips scanners) used an 8-channel coil while four sites (Siemens scanners) used a 12-channel coil. All sites used the same scanning protocol. High-resolution T1-weighted 3-dimensional structural images were acquired for anatomical localisation and registration with the functional time series. Data were pre-processed centrally (Neurospin, CEA) using Statistical Parametric Mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm/); see Supplement for further information. Individuals with anatomical abnormalities or poor realignment (e.g., greater than 3 mm head motion in at least one of the translations; n = 20) did not pass QC and were not included in these analyses.

Statistical Analysis

Behavioural Analysis: We used separate multiple regression models to establish the relationship between each of the symptoms and the number of stressful life events. Sex, study site, verbal IQ, socioeconomic status and pubertal status were included as covariates of no interest in all analyses.

fMRI Analysis: Functional MRI data were analysed using SPM8 version 6313. We used a separate group of IMAGEN participants (n=326; see Table S2b for demographics), for whom full phenotypic data were not available, to define a functional amygdala region of interest. There was robust bilateral activation (see Supplement). Using the peak MNI coordinates we created an amygdala region of interest with an 8mm sphere using MarsBaR ((28); http://marsbar.sourceforge.net/) and extracted summarised beta values in the full sample for both contrasts for analysis in SPSS.

Using separate regression models we explored the extent to which the psychosocial stress score and symptom count scores for each of the three symptoms (conduct, hyperactivity/inattention and emotional) were associated with fMRI activation in the amygdala as main effects. We also explored the extent to which psychosocial stress moderated the relationship between each symptom and amygdala activation as an interaction. Independent variables (conduct, hyperactivity/inattention and emotional symptoms and psychosocial stress score) were mean centred. Interaction terms were calculated in SPSS for input into the second-level regression models. To adjust for multiple testing we applied a Bonferroni correction threshold of $p<0.05/12$ (two amygdala ROIs, two fMRI contrasts, three symptom domains).

To account for comorbidity among the three symptoms we carried out *post-hoc* regression models for each symptom controlling for the other two symptoms. We also carried out *post-hoc* regression models to examine the effects of site, gender, and substance use on our results.

We conducted exploratory psychophysiological interaction (PPI) functional connectivity analyses to investigate potentially distinct amygdala networks involved in the symptom by stress results. PPI analyses compute the interaction between the seed BOLD time series and a chosen condition-specific interaction factor when predicting each voxel BOLD time series. Generalized PPI (gPPI) regression analyses were carried out via the SPM-based CONN toolbox ((29); http://www.nitrc.org/projects/conn/) using the same amygdala ROI as our *a priori* seed region (see Supplement) and same covariates of no interest. To adjust for multiple testing we applied the same Bonferroni correction ($p<0.05/12$).

We conducted using exploratory whole-brain multiple regression analyses of the same main and interaction effects. The same covariates of no interest were used as in the ROI and PPI analyses. To adjust for multiple testing we applied the same Bonferroni correction ($p<0.05/12$).

Results

Behavioural Analysis

We used regression models to establish the relationship between conduct, hyperactivity/inattention and emotions symptoms, and the psychosocial stress score. Among these three symptom domains psychosocial stress had the strongest association with conduct symptoms (t=10.55, model r^2=0.12, p=5.51x10^{-25}), followed by hyperactivity/inattention (t=8.36, model r^2=0.11, p=1.59x10^{-16}) and emotional symptoms (t=4.7, model r^2=0.08, p=3.0x10^{-6}). Other significant predictors for psychopathology symptoms included verbal IQ, male sex for conduct and hyperactivity/inattention symptoms, and female sex and socioeconomic status for emotional symptoms, see Table 1. Descriptive statistics and differences by site are provided in the Supplement (text and Tables S3a, S3b).

fMRI Region of Interest Analysis

To understand the relationship between the three symptom domains, psychosocial stress, and amygdala reactivity to our face stimuli we conducted region of interest fMRI regression analyses in bilateral amygdalae. We first examined these relationships in all participants and then also examined gender differences *post-hoc*. As gender was a covariate in the regression models, we also checked if there were any main effects of gender on amygdala activation but found no such effects. We examined gender differences in amygdala activation during emotional face processing (30) and found that males had greater right amygdala activation during the angry vs. control contrast (t=2.82, $p_{\text{family-wise error-corrected}}$=0.005, $p_{\text{Bonferroni-corrected}}$=0.04). Including substance use in the regression models did not change results (see Methods and online Supplement). As no clusters survived whole-brain correction, analyses were thresholded at p<0.001 (voxel-level uncorrected) and statistically significant clusters reported at p<0.05 (family-wise error corrected).

All main effect and interaction test statistics are shown in Table 2. There were neither significant main effects of conduct symptoms, hyperactivity/inattention symptoms and emotional symptoms, nor psychosocial stress, on amygdala activation in either the angry vs. control or neutral vs. control fMRI contrasts. We did, however, find the following interactions:

Conduct Symptoms: We found a significant interaction between conduct symptoms and psychosocial stress score in the right amygdala (Figure 1A: t=3.11, $p_{\text{family-wise error-corrected}}$=0.002; $p_{\text{Bonferroni-corrected}}$=0.024) in the angry vs. control contrast. The greater the number of stressful life events experienced by individuals with severe conduct symptoms (Table S1), the greater the amygdala activation (Figure 1B). The interaction in the left amygdala was not significant ($p_{\text{family-wise error-corrected}}$=0.053). We also found an interaction effect in the neutral vs. control contrast in the left amygdala (t=3.24, $p_{\text{family-wise error-corrected}}$=0.0012; $p_{\text{Bonferroni-corrected}}$=0.014), but not in the right amygdala ($p_{\text{family-wise error-corrected}}$=0.06). Controlling for hyperactivity and emotional symptoms did not affect the result in either the angry vs. control (t=3.12, $p_{\text{family-wise error-corrected}}$=0.002, $p_{\text{Bonferroni-corrected}}$=0.024) or neutral vs. control (t=3.21, $p_{\text{family-wise error-corrected}}$=0.0014, $p_{\text{Bonferroni-corrected}}$=0.017) contrast.

To explore gender differences in the aforementioned results, we split the sample and compared the interaction of conduct symptoms and psychosocial stress on amygdala activation in males and females. Males had a stronger symptom by stress interaction on amygdala activation (right amygdala, angry vs. control contrast: r=0.133, $p_{\text{family-wise error-corrected}}$=0.001, $p_{\text{Bonferroni-corrected}}$=0.024; left amygdala, neutral vs control contrast: r=0.146, $p_{\text{family-wise error-corrected}}$=0.0005, $p_{\text{Bonferroni-corrected}}$=0.012) than females (right amygdala, angry vs. control contrast: r=0.022, $p_{\text{family-wise error-corrected}}$=0.558; left amygdala, neutral vs. control contrast: r=0.030, $p_{\text{family-wise error-corrected}}$=0.424). These differences between the genders were significant: right amygdala, angry vs. control contrast: Z=1.99, p=0.047; left amygdala, neutral vs. control contrast: Z=2.09, p=0.037; two-tailed Fisher's Z test).

Hyperactivity/Inattention Symptoms: We identified the same interaction effect between hyperactivity symptoms and psychosocial stress in the left amygdala (t=3.28, $p_{family\text{-}wise\ error\text{-}corrected}$=0.0011, $p_{Bonferroni\text{-}corrected}$=0.013) in the angry vs. control contrast (Figure 1C). The interaction in the right amygdala was not significant ($p_{family\text{-}wise\ error\text{-}corrected}$=0.312). There was no significant interaction in the neutral vs. control contrast after correction for multiple testing (Table 2). Controlling for conduct and emotional symptoms did not affect the result (t=3.18, $p_{family\text{-}wise\ error\text{-}corrected}$=0.002, $p_{Bonferroni\text{-}corrected}$=0.024). There were no gender differences.

Emotional: We found no significant symptom by stress interactions in amygdala activation in either fMRI contrast (Table 2). The main effects and interactions remained non-significant when controlling for conduct and hyperactivity symptoms. There were no gender differences.

PPI Functional Connectivity Analysis

We carried out exploratory PPI functional connectivity analysis to explore potential amygdala networks related to conduct, hyperactivity/inattention, and emotional symptoms, psychosocial stress, and angry/neutral face stimuli. We found a positive relationship between hyperactivity/inattention symptoms and connectivity between the right amygdala and left ventral precuneus in the angry condition compared to the control condition ($p_{family\text{-}wise\ error\text{-}corrected}$=0.0027, $p_{Bonferroni\text{-}corrected}$=0.032, $p_{5000nonparametric\text{-}permuted}$=0.026; xyz -8 -56 18, k_e=150). There were neither main effects nor any significant symptom by stress interactions in the hyperactivity/inattention or emotional symptoms, or the neutral vs. control contrast (Table 3).

fMRI Whole-Brain Analysis

We conducted exploratory whole-brain regression analyses of the same main and interaction effects as in the region of interest and PPI analyses. As no clusters survived whole-brain correction, analyses were thresholded at $p<0.001$ (voxel-level uncorrected) and statistically significant clusters reported at $p<0.05$ (family-wise error corrected).

Conduct Symptoms: Adolescents with more conduct symptoms showed significantly larger BOLD responses when they had also experienced a greater number of stressful life events. We found a significant interaction between conduct symptoms and stress frequency in the angry vs. control contrast in the superior temporal gyrus, thalamus, anterior cingulate cortex, superior frontal gyrus, and inferior frontal gyrus (Figure 2A and Table S3c). We also found a significant main effect for conduct symptoms in the precuneus and postcentral gyrus but no significant main effect for psychosocial stress score.

Hyperactivity/Inattention Symptoms: We found a significant interaction between hyperactivity/inattention symptoms and psychosocial stress score in the mid and anterior cingulate cortex. Youths with more symptoms showed larger BOLD responses when they had also experienced more stress (Figure 2B and Table S3c).

Emotional Symptoms: There were no significant main or interaction effects for emotional symptoms in either fMRI contrast.

We compared the size and location of the significant clusters from the whole-brain analyses of conduct and hyperactivity/inattention symptoms and found an overlapping portion of the mid-cingulate common to both conduct and hyperactivity symptoms (Figure 2C).

Discussion

In order to comprehensively characterise the relationship between adolescent mental health and psychosocial stress, we examined three behavioural symptom domains in a large sample of community-recruited adolescents. Consistent with previous reports, we found evidence to suggest that psychosocial stress is associated with greater conduct, hyperactivity/inattention, and emotional symptoms. Our primary research objective was to explore how the relationship between these symptoms and neural reactivity to emotional stimuli is influenced by the experience of psychosocial stress. Using an fMRI paradigm designed to target stress-related neural systems, we found that the degree to which adolescent brains respond to social-emotional stimuli depends on the type and severity of conduct and hyperactivity/inattention symptoms and also the amount of stress they experienced.

Our region of interest analyses showed heightened amygdala response to anger was related to severe conduct and hyperactivity/inattention symptoms, only when adolescents also had a higher psychosocial stress score. We observed this finding in the angry vs. control and to a lesser extent in the neutral vs. control contrasts, suggesting that the interaction of conduct symptoms or hyperactivity/inattention and stress may not only be related to affective stimuli, but may also involve processing of social stimuli. This finding may also suggest that previously observed altered response to social emotional stimuli in adolescents with conduct disorder (11, 31) and ADHD (32) might be related to increased psychosocial stress, and perhaps not to the externalising symptoms per se. For example, increased amygdala activation is found in stressed youths when viewing angry and neutral faces (33).

Our finding of a stress-dependent interaction of attention deficit/hyperactivity and conduct symptoms on social-emotional processing is not limited to heightened amygdala activation alone, but - as shown in the results of our whole brain analysis - extends to other brain regions related to the behavioural deficits observed in externalising behaviour. We found increased brain activity related to psychosocial stress

and conduct disorder in a network of regions that influence cognitive and emotional processes including perception (thalamus, superior temporal gyrus, middle temporal gyrus), interpretation (anterior cingulate cortex, insula, superior frontal gyrus), and inhibitory control (inferior frontal gyrus). Furthermore, the mid-cingulate cortex, an area identified in promoting aggressive behaviour in response to angry emotional expressions (34), was commonly activated for stressed youths with conduct symptoms and stressed youths with hyperactivity/inattention symptoms. These findings suggest that the impaired social functioning and emotional regulation observed in youths with externalising symptoms might be regulated by networks of brain activity related to hyperactivity/inattention symptoms and conduct symptoms, that are both common and distinct for these symptom domains. These networks involve both cortical and subcortical structures, which is consistent with the behavioural complexity of externalising symptoms.

In the exploratory PPI analyses, we observed greater functional connectivity between the amygdala and ventral precuneus in the angry vs. control condition with increasing hyperactivity/inattention (but not with conduct symptoms) that was independent of psychosocial stress. The precuneus has negative connectivity with the amygdala (35) and increased functional coupling between these regions is important for emotion regulation, particularly distraction (i.e., shifting attention away from emotional stimuli (36). Our finding is counterintuitive considering individuals with hyperactivity/inattention symptoms are described as having emotion regulation deficits (32). However, in the context of hyperactivity/inattention symptoms, adolescents may encounter angry faces more frequently, as stress levels are high in parents of ADHD children (37, 38). Therefore, it may be that individuals with hyperactivity/inattention symptoms are more adept at distraction as a means to regulate emotion.

Although psychosocial stress frequency was associated with emotional symptoms, the magnitude of this association was less than for conduct and hyperactivity/inattention symptoms. We found neither

main effects of emotional symptoms nor emotional symptoms by stress interaction on amygdala activation or connectivity. Considering the task used in this study has an emotional component and targets stress-related neural systems, the absence of significant findings was unexpected. One reason for the disparity between conduct and hyperactivity/inattention symptoms, stress, and neural reactivity compared to emotional symptoms may be due to the nature of the emotional items from the SDQ and the specific fMRI contrasts used. The SDQ emotional subscale items are more reflective of anxiety and irritability than depression (e.g., 'I worry a lot'; 'I am nervous in new situations'). Of interest, youths with high anxiety tend to orient away from emotional faces (39, 40), which may help explain our null findings; participants with emotional symptoms may not have been engaged with the fMRI task.

The study was limited by the fMRI paradigm in terms of the range of emotional stimuli available for analysis. Future studies may expand these findings to explore reactivity in response to different emotional expressions. Although potentially informative, we did not examine/account for potential within-session amygdala habituation effects (41). Although behavioural data during the viewing of angry and neutral faces were not collected, participants were asked after the scanning session to identify whether or not they had seen a set of faces; 99% were found to have good reliability. Site differences were observed in key demographic covariates such as verbal IQ and socioeconomic status. These can reflect differences in recruitment strategies or specific cultural attitudes and environment of the area. While we controlled for the effect of site on our results, and did not detect any systematic bias, we acknowledge that multisite studies add heterogeneity to the data. Despite these limitations, this study was strengthened by the use of quantitative analysis of clinical symptoms and psychosocial stress frequency, allowing us to explore how all adolescents responded, including those with few or no pathological symptoms, rather than simply those at the high end of the distribution. The exploratory analyses, while not hypothesis-driven, are hypothesis generating and in need of further exploration and eventual replication.

The current results highlight the importance of studying how environmental stress impacts functional brain responses to social-emotional stimuli, particularly in adolescents with externalizing symptoms. The observed heightened amygdala activation, and associated networks implicated in top-down control of emotion regulation, may impact an individual's ability to effectively assess risk and may contribute to aggressive or fearful behavioural responses to incoming stimuli. Improved understanding of how stress and externalizing symptoms influence social-affective neurobiological processes may inform the development of future therapies that enhance emotional awareness and reduce disproportionate neural reactions in challenging social situations.

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Table 1 Demographic and clinical symptom characteristics. Female N=705, Male N=583; Age = Mean (SD): 14.40 (0.40); Range = 13.18-15.43.

			Test statistics – Symptom domains								
	Descriptive Statistics		**Conduct symptoms**			**Hyperactivity/Inattention symptoms**			**Emotional symptoms**		
	Mean	**SD**	**β**	**t**	**P**	**β**	**t**	**P**	**β**	**t**	**P**
Sex			-0.12	-3.70	2.25×10^{-4}	-0.06	-1.99	.047	0.17	5.09	4.03×10^{-7}
Stressful Life Event Frequency	3.35	2.08	0.29	10.5	5.51×10^{-25}	0.23	8.36	1.59×10^{-16}	0.13	4.70	3.0×10^{-6}
Verbal IQ	111.98	14.82	-0.10	-3.50	4.83×10^{-4}	-0.17	-5.90	4.53×10^{-9}	-0.10	-3.52	4.53×10^{-4}
Socioeconomic Status	0.66	1.06	0.04	1.50	0.13	0.01	0.36	.72	0.11	3.82	1.4×10^{-4}
Puberty Development Stage	3.64	0.70	0.01	0.25	0.81	-0.03	-0.79	0.43	-0.05	-1.55	0.12
Conduct Problems	2.49	1.58									
Hyperactivity/ Inattention Problems	4.34	2.17									
Emotional Problems	2.24	2.30									

Table 2 Results of amygdala ROI regression analyses

		Angry vs. control, L amygdala			Angry vs. control, R amygdala			Neutral vs. control, L amygdala			Neutral vs. control, R amygdala		
		β	**t**	**p**	**β**	**t**	**p**	**β**	**t**	**p**	**β**	**t**	**p**
Conduct problems													
	Main effect	0.008	0.272	0.786	-0.048	-1.59	0.111	-0.015	-0.517	0.605	0.003	0.109	0.913
	Stress main effect	-0.001	-0.027	0.978	$4x10^{-4}$	-0.013	0.99	-0.037	-1.22	0.224	-0.032	-1.04	0.297
	Conduct x stress	0.081	2.86	0.0044	0.089	3.11	0.0019	0.093	3.27	0.001	0.081	2.84	0.005
Hyperactivity problems													
	Main effect	-0.004	-0.125	0.901	0.007	0.224	0.823	-0.047	-1.60	0.110	0.019	0.638	0.523
	Stress main effect	0.009	0.315	0.753	-0.005	-0.167	0.867	-0.020	-0.664	0.507	-0.026	-0.887	0.375
	Hyperactivity x stress	0.091	3.28	0.0011	0.062	2.23	0.026	0.070	2.49	0.013	0.070	2.52	0.012
Emotional problems													
	Main effect	0.016	0.542	0.588	-0.001	-0.045	0.964	0.035	1.20	0.230	0.055	1.88	0.060
	Stress main effect	0.008	0.262	0.793	-0.003	-0.092	0.927	-0.034	-1.16	0.246	-0.031	-1.05	0.296

	Emotion x stress	0.045	1.57	0.117	0.032	1.14	0.254	0.034	1.18	0.240	0.052	1.82	0.070

β = standardised beta coefficient, t = t-test statistic, p = significance value

Table 3 Results of PPI functional connectivity analyses

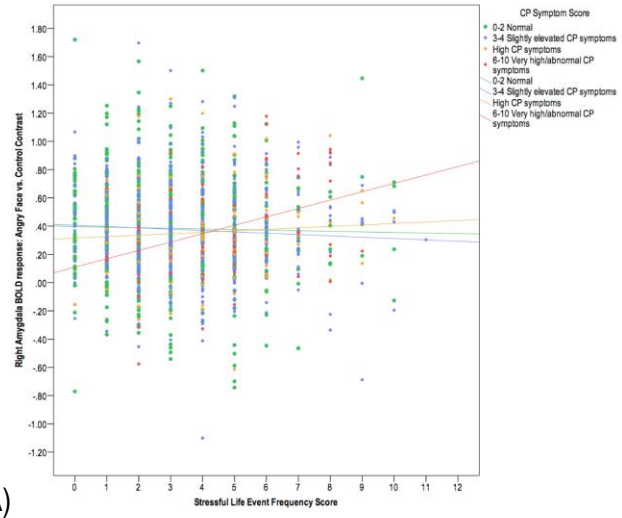
		Region	Left/Right	Coordinates of Peak Activation (MNI)	*t*	β	Cluster Size (*k*)	p
Angry vs control, L amygdala	**Positive or Negative PPI**							
		Main effect: Conduct Problems						
	Positive	L Frontal Pole	Left	-38 -10 -18	5.22	.043	117	.016
Angry vs. control, R amygdala	**Positive or Negative PPI**							
		Main effect: Hyperactivity Symptoms						
	Positive	Precuneus*	Left	-8 -56 18	4.10	.034	150	.0027*
Neutral vs. control, L amygdala								
	Positive	**Main effect: Hyperactivity Symptoms**						
	Positive	Caudate	Left	-6 10 0	4.51	.042	96	.044

Neutral vs. control, R amygdala								
		Main effect: Stress in Conduct Symptoms model						
	Positive	Angular gyrus	Right	52 -50 28	4.99	.040	152	.004
		Main effect: Stress in Hyperactivity/Inattention Symptoms model						
	Positive	Angular gyrus	Right	52 -50 28	4.79	.037	132	.008
		Interaction effect: Hyperactivity/Inattention Symptoms x Psychosocial Stress Frequency						
	Positive	Frontal Pole	Right	0 64 6	3.73	.023	86	.066
		Main effect: Stress in Emotional Symptoms model						
	Positive	Angular gyrus	Right	52 -50 28	4.66	.036	118	.015

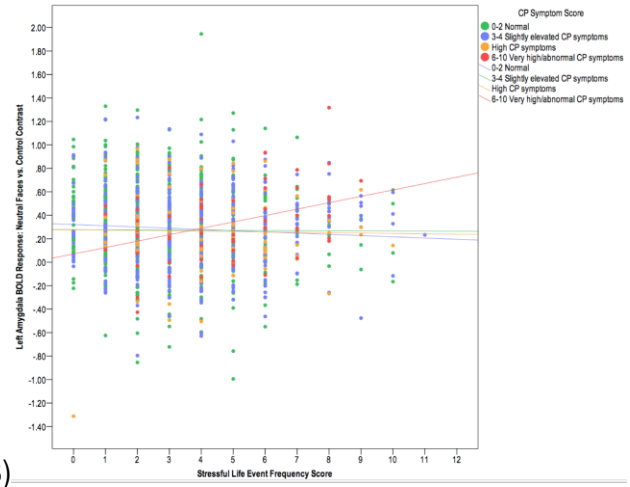
MNI, Montreal Neurological Institute; [a]Coordinates refer to the voxel with the maximum signal intensity. β = standardised beta coefficient, t = t-test statistic, p = significance value

* Indicates result survives correction for multiple comparisons (p<.0041). † indicates group result survived correction for multiple comparisons (p<.001).

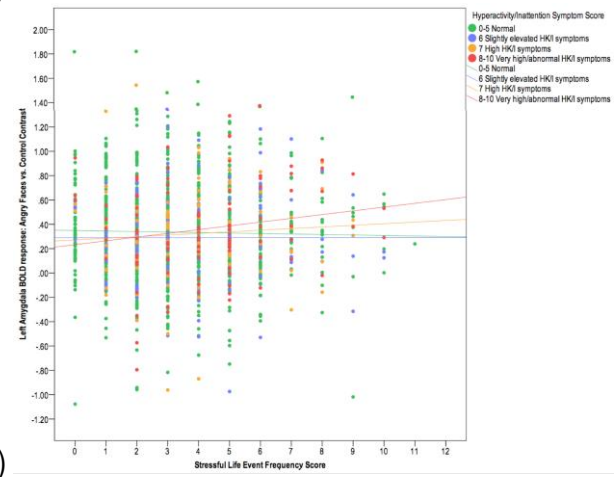
Figure 1 (A) Scatter plot depicting the interaction effect between conduct symptoms and stress on contrast estimates (angry face vs. control) in the right amygdala. (*B*) Scatter plot depicting the interaction effect between conduct and stress on contrast estimates (neutral face vs. control) in the left amygdala. (*C*) Scatter plot depicting the interaction effect between hyperactivity/inattention symptoms and stress on contrast estimates (angry face vs. control) in the left amygdala.



(A)



(B)



(C)

Figure 2 Statistical parametric map overlaid on a T1-weighted structural brain image. *(A)* Youths with conduct symptoms show increased fMRI BOLD responses with greater psychosocial stress (angry face vs. control). The image is centred at the middle cingulate cluster (xyz 3 5 37) and only clusters showing a spatial extent of at least 38 contiguous voxels are shown for visualisation purposes. (*B*) Youths with hyperactivity/inattention symptoms show increased fMRI BOLD responses with greater psychosocial stress (angry face vs. control). The image is centred at the middle cingulate cluster (xyz -3 2 37) and only clusters showing a spatial extent of at least 48 contiguous voxels are shown for visualisation purposes. (*C*) The overlap between the two significant mid-cingulate clusters common to both hyperactivity/inattention and conduct symptoms. The image is centred at xyz 0.8 3.5 37 and k_e is 272 voxels.