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Neural responses to fearful eyes in children with conduct problems and varying levels of callous-unemotional traits

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	Conclusions: These data extend the evidence base suggesting that CU traits index meaningful heterogeneity in conduct problems. Focusing on regulating reactive emotional responses may be a fruitful strategy for children with CP/LCU.

Neural responses to fearful eyes in children with conduct problems and varying levels of callous-unemotional traits

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Abstract

Background: Children with conduct problems are a heterogeneous group. Those with high levels of callous-unemotional traits (CP/HCU) appear emotionally under-reactive at behavioural and neural levels, while those with low levels of CU traits (CP/LCU) appear emotionally over-reactive, compared with typically developing (TD) Controls. Investigating the degree to which these patterns of emotional reactivity are malleable may have important translational implications. Instructing participants with CP/HCU to focus on the eyes of fearful faces (i.e. the most salient feature) can ameliorate their fear recognition deficits, but it is unknown whether this is mediated by amygdala response. It is also unknown whether focusing on fearful eyes is associated with increased amygdala reactivity in CP/LCU.

Methods: fMRI was used to measure neural responses to fearful and calm faces in children with CP/HCU, CP/LCU and TD Controls (n=17 per group). On half of trials participants looked for a blue dot anywhere within target faces; on the other half, participants were directed to focus on the eye region.

Results: Reaction time (RT) data showed that CP/LCU were selectively slowed in the fear/eyes condition. For the same condition, CP/LCU also showed increased amygdala and subgenual ACC/OFC responses compared with TD Controls. RT and amygdala response to fear/eyes were correlated in CP/LCU only. No effects of focusing on the eye region were observed in CP/HCU.

Conclusions: These data extend the evidence base suggesting that CU traits index meaningful heterogeneity in conduct problems. Focusing on regulating reactive emotional responses may be a fruitful strategy for children with CP/LCU.

Introduction

Conduct disorder (CD) and conduct problems (CP) refer to a persistent pattern of antisocial behaviour in young people, and represent a significant public health cost (Romeo et al., 2006). Children with conduct problems are a heterogeneous group. Levels of callous-unemotional (CU) traits, i.e. a lack of guilt and empathy, have been shown to differentiate individuals with conduct problems in terms of aetiology, behaviour, and neurocognitive processing (Frick & Viding, 2009).

Research suggests that affective processing styles differ between children with conduct problems and low levels of CU traits (CP/LCU) and those with high levels of CU traits (CP/HCU). Behavioural data indicate that children with CP/HCU show a *hypo*-reactive response profile to affective cues (Loney et al., 2003; Sharp et al., 2006) coupled with difficulties in processing and recognising others' fearful and sad facial and vocal expressions (Blair et al., 2001; Blair et al., 2005). In contrast, CP/LCU children may show an exaggerated or *hyper*-reactive response profile to emotional stimuli, and a hostile attribution bias where neutral stimuli are construed as threatening (Frick et al., 2003a; Dadds et al., 2006). This emotional reactivity is often coupled with poor emotion regulation skills (Frick & Morris, 2004), resulting in aggression that is usually reactive in nature (Frick et al., 2003b). In contrast, aggressive behaviour in CP/HCU children may either be reactive or proactive, i.e. used in pursuit of a goal (Frick & Viding, 2009).

Neuroimaging data have also shown neurocognitive differences in affective processing between CP/CU subtypes. Studies contrasting CP/HCU against typically developing (TD) controls have found evidence for reduced amygdala response to others' fearful facial expressions (Marsh et al., 2008; Jones et al., 2009), mirroring behavioural evidence of emotional hypo-reactivity in this group. Similarly, one recent study from our group directly contrasting CP/HCU and CP/LCU found a significantly greater amygdala response to fearful faces presented below the level of conscious awareness in children with CP/LCU compared with CP/HCU (Viding et al., 2012).

However, findings from studies investigating conduct problems independent of CU present a mixed picture, with some reporting reduced amygdala responses to negative facial expressions (Passamonti et al., 2010) and negatively valenced pictures (Sterzer et al., 2005) relative to TD controls, while others report increased amygdala response using similar stimuli (Herpertz et al. 2008). One potential explanation was suggested by a recent study (Sebastian et al., 2012), which found that amygdala response to negatively valenced cartoon stimuli in CP children was positively associated with CP symptoms after controlling for CU traits, and negatively associated with CU traits after controlling for CP symptoms. Patterns of opposing influences on amygdala reactivity may thus exist within the same CP sample.

Behavioural and neuroimaging data have converged on fear processing as an important source of difference between CP/LCU, CP/HCU and TD controls (Marsh et al., 2011). However, the cognitive mechanisms underpinning these differences remain a subject of debate. Facial fear is unique in that it is identified chiefly by eye region information (Adolphs et al., 2005). One study found that a deficit in recognising fearful expressions in adolescent males with high levels of CU traits could be temporarily ameliorated by instructing participants to attend to the eye region of the face (Dadds et al., 2006). A follow-up study using eye-tracking (Dadds et al., 2008) found that (non-CP) adolescents with high CU scores made fewer and shorter fixations to the eye region of fearful faces under free viewing conditions than those with low CU scores. It is therefore possible that reduced amygdala response to fear in CP/HCU children (Marsh et al., 2008; Jones et al., 2009) is secondary to reduced attention to the eyes (Moul et al., 2012). One aim of the current study was to investigate whether directing attention to the eye region of a fearful face would normalise amygdala response in CP/HCU **relative to TD controls**.

A second important aim was to investigate the effects of directing attention to the eye region in children with CP/LCU. While there is evidence to suggest increased emotional reactivity to emotional

stimuli in this group (e.g. Frick et al., 2003a, 2003b), few neuroimaging studies have explored the mechanisms underlying this reactivity, or how this reactivity may be modulated. For example, directing attention to the eyes might have no effect on amygdala response. Equally, however, attending to eyes may serve to augment amygdala response relative to the degree of activation observed when attending to the whole face. In the current study we investigated whether instruction to focus on the eye region during fear processing interfered with performance of a concurrent task, predicting that this effect would be greater in the CP/LCU group relative to TD controls.

We devised a task in which participants judged whether a blue dot was present or absent from target faces which were either fearful or calm. In half of blocks of each valence (fear vs. calm), the dot was presented anywhere within the face (i.e. the whole face, including the eyes, needed to be scanned); while in the other half the dot was presented in the eye region only. Participants were directed to attend to either the whole face or the eye region accordingly. Our rationale for using the dot task was two-fold: first, accurate performance ensured that participants were focusing on the instructed region of the face; second, it introduced an implicit emotion regulation component, in which successful task performance depends on automatically regulating responses to distracting affective information (fearful faces) (Ochsner & Gross, 2005). This allowed us to test two hypotheses. First, we hypothesised that CP/HCU would activate the amygdala to a greater extent to fearful faces when instructed to focus on the eye region compared with the whole face. Second, given evidence of emotion regulation deficits in CP/LCU, we predicted that this group would show a greater amygdala response **(relative to TD controls)** to fearful faces when instructed to focus on the eye region compared with other conditions; and that this would be accompanied by a selective reduction in task performance, representing a reduced ability to implicitly regulate emotion in pursuit of a goal.

Methods

Participants: Participants largely overlapped with a sample reported previously (Sebastian et al., 2012; Viding et al., 2012). Full details of sample recruitment are reported in these studies and in the supplementary material. Participant characteristics are displayed in Table 1. The study was approved by the University College London Research Ethics Committee (Project ID: 0622/001).

Fifty-five males aged 10-16 were scanned: 38 with a research **classification** of current conduct problems (CP) based on combined parent- and teacher- report on the Child and Adolescent Symptom Inventory (CASI-4R; Gadow & Sprafkin, 2009) Conduct Disorder subscale (CASI-CD); and 17 age-, IQ- handedness- and SES-matched typically developing (TD) controls. Data from CP children were excluded due to: excessive motion and poor task accuracy (1 CP); motion plus suspected autism spectrum and tic disorder (1 CP); scanner refusal (1 CP) and technical problems (1 CP). The 34 remaining participants with CP were assigned to low vs. high callous-unemotional trait groups (CP/LCU vs. CP/HCU, n=17 per group) on the basis of a median split on combined parent- and teacher- reported scores on the Inventory of Callous-Unemotional Traits (ICU; Essau et al., 2006). Median ICU score within the CP group was 44.5: all TD Controls scored below this CP group median.

For all groups, exclusion criteria included a previous diagnosis of any neurological or psychotic disorder, or a current prescription for psychiatric medication. (We later found that three participants (2 CP/LCU, 1 CP/HCU) had been medicated for ADHD symptoms during scanning. However, analyses conducted with and without these participants were very similar, and so their data were retained in reported analyses). To recruit a representative sample of children with conduct problems, common co-morbidities (ADHD, generalised anxiety disorder (GAD), major depressive disorder (MDE) and substance/alcohol abuse) were not used as exclusion criteria, but current parent-reported symptom counts were obtained using the CASI-4R.

**********Table 1**********

Experimental Task: Stimuli comprised fearful and calm faces of four individuals taken from the NimStim (two male, two female; mouths closed). **Face stimuli were presented as ovals measuring 7.5cm by 5cm**, in greyscale and with hair cropped. Stimuli were presented on a white background. Four block types were presented using a 2x2 factorial design with factors Emotion (fear, calm) and Region (eye region, whole face). Sixteen blocks were presented in four sets of four blocks containing one of each condition: fear/eyes, calm/eyes, fear/face, calm/face. Block order was randomised within each set of four blocks.

Participants indicated with a keypress response on every trial whether there was a blue dot present on the face or not. In the 'eyes' blocks, half the stimuli had a dot present within the eye region of the face (but not covering the eye), while for the other half there was no dot present. In 'face' blocks, the blue dot was located in the wider face area. The location of the dot varied and was counterbalanced across Emotion conditions. Each block lasted 30s, comprising 2.5s instructions, 20s face stimuli, and 7.5s fixation cross between blocks. The instruction screen reminded participants of the correct keypress responses. It also told participants whether to look at the eyes or the face for the coming block. Participants knew to expect that during 'eyes' blocks the blue dot would only be presented near the eye region, while during 'face' blocks it would only be presented in the wider face. The stimuli comprised 8 trials of 2500ms each (1750ms face presentation and 750ms ISI fixation cross). The 8 trials consisted of the two male and two female faces presented both with and without a dot present. On 'dot present' trials, the dot appeared concurrently with the face. Trial order within each block was pseudorandomised to prevent all stimuli of one type (i.e. dot, no-dot, male or female) being presented together. Participants practised the task outside the scanner, with calm faces of differing identities to those shown in the full experiment.

Psychometric and questionnaire measures: Participants completed the Wechsler Abbreviated Scale of Intelligence (Weschler, 1999) two-subtest version for group matching purposes, as well as Alcohol/Drug Use Disorder Identification Tests (AUDIT and DUDIT; Babor et al., 2001; Berman et al., 2005). A parent or guardian also completed the CASI-4R scales for ADHD, GAD and MDE in order to ascertain symptom counts for common co-morbidities with conduct problems (Table 1).

fMRI data acquisition: A Siemens Avanto 1.5T MRI scanner with a 32 channel head coil was used to acquire a 5.5 min 3D T1-weighted structural scan, and 209 multislice T2*-weighted echo planar volumes with BOLD contrast (1 run of 10 mins). The EPI sequence was designed to optimise signal detection and reduce dropout in OFC and amygdala (Weiskopf et al., 2006), and used the following acquisition parameters: 35 2mm slices acquired in an ascending trajectory with a 1mm gap, TE=50ms; TR=2975ms; slice tilt=-30° (T>C); flip angle=90°; field of view=192mm; matrix size=64x64. Fieldmaps were also acquired for use in the unwarping stage of data pre-processing.

fMRI data analysis: Imaging data were analysed using SPM8 (www.fil.ion.ucl.ac.uk/spm). Pre-processing followed a standard sequence: the first five volumes were discarded; data were realigned; unwarped using a fieldmap; normalised via segmentation of the T1 scan with a voxel size of 2x2x2mm; and smoothed with an 8mm Gaussian filter. A block analysis compared neural activity in a 2x2 factorial design with regressors representing fear/eyes, calm/eyes, fear/face and calm/face conditions, with each block of 20s duration. Two additional regressors of no interest were included, modelling fixation (duration 7.5s) and instructions (duration 2.5s). These six regressors were modelled as boxcar functions convolved with a canonical haemodynamic response function. The six realignment parameters were modelled as effects of no interest. For 13 participants (3 TD Controls, 6 CP/LCU, 4 CP/HCU), extra regressors were included to model a small number of corrupted images resulting from excessive motion. These images (no more than 10% of each participant's data) were

removed and the adjacent images interpolated to prevent distortion of the between-subjects mask. Data were high-pass filtered at 128s to remove low-frequency drifts.

At the first level, main effects of each factor (Emotion and Region) were calculated, as well as the interaction term (Emotion*Region). Contrast images were entered into separate second-level analyses, where Group (TD Control, CP/LCU, CP/HCU) served as a between-subjects variable in one-way ANOVAs. For whole brain analyses, an initial threshold of $p<.005$, $k\geq10$ (uncorrected) was used (Lieberman & Cunningham, 2009), with results reported as significant if they reached $p<.05$, FWE-corrected at the cluster level. As the amygdala was the a priori region of interest, we also conducted region of interest analyses in this region bilaterally using two 3mm radius spheres centred on anatomically defined central amygdala co-ordinates used in a previous study contrasting fearful and calm faces (Phillips et al., 2001) (+/- 20 -8 -16, after conversion from co-ordinates reported in Talairach space (+/- 20 -8 -13)). Results are reported if they survive small volume correction across the bilateral mask at $p<.05$, FWE-corrected.

Results

Behavioural Data

Mean reaction times (RTs) and percentage errors were calculated for each participant for each of the four conditions: fear/eyes, calm/eyes, fear/face, calm/face. Missed trial rates were low (mean across all groups and conditions=0.98%, SD=1.94), and were excluded from subsequent analyses.

Reaction Times: For RT data, a mixed-model ANOVA was conducted with within-subjects factors of Emotion (fear, calm) and Region (eyes, face); and with a between-subjects factor of Group (TD Control, CP/LCU, CP/HCU). There were no main effects of Region ($p=.46$) or Group ($p=.82$), but there was a marginal main effect of Emotion ($F(1,48)=3.25$, $p=.078$), with marginally slower RTs across fear stimuli as a whole. There was also a significant Emotion*Region interaction ($F(1,48)=5.41$, $p=.024$),

and a trend level three-way interaction between Emotion, Region and Group ($F(2,48)=2.30$, $p=.11$). Since we had a priori hypothesis of group differences, we deconstructed the three-way interaction into separate Emotion*Region analyses in each group. These data showed that the Emotion*Region interaction in the full sample was driven by the CP/LCU group only ($F(1,16)=8.80$, $p=.009$). In the TD Controls, $F(1,16)=1.59$, $p=.23$, and in CP/HCU, $F(1,16)=.001$, $p=.97$. Post hoc t-tests in CP/LCU showed that the interaction in this group was driven by significantly slower responses to fear/eyes than calm/eyes (mean RT for fear/eyes=728ms and for calm/eyes=697ms, $t(16)=3.22$, $p=.005$). Mean RTs to fear/face vs. calm/face stimuli did not differ in the CP/LCU group ($p=.25$) (Figure 1).

**********Figure 1**********

Errors: Total percentage error rate across groups and conditions was 3.17% (SD=3.65). A mixed-model ANOVA was conducted as for the RT data above. No main effects were significant (for Emotion $p=.80$; for Region $p=.78$; for Group, $p=.12$). No interactions reached significance, although there was a marginal Region*Group interaction ($p=.11$) and a marginal three-way interaction between Emotion, Region and Group ($p=.095$). On the basis of an a priori hypothesis for a three-way interaction, we explored further. While the CP/LCU group made significantly more errors than TD Controls for the calm/eyes condition ($p=.031$), no other effects were significant, and error data are not discussed further.

fMRI Data

Main effects: For completeness, main effects across all groups are reported in the Supplementary Information using whole brain analyses with a threshold of $p<.005$ uncorrected, $k\geq10$. The primary contrast of interest was the Emotion*Region contrast (fear/eyes>calm/eyes)>(fear/face> calm/face). This mirrors the behavioural data interaction analysis above, and indicates increased response to

fearful eyes relative to the other conditions. We also report overall responses to fear>calm (and the reverse).

Region of interest analyses: **We explored fMRI data in relation to the two specific hypotheses regarding amygdala response,** using region of interest analyses with bilateral amygdala spheres as described in the Method**.**

The first hypothesis was that directing attention to the eyes would lead to increased amygdala response to fear in CP/HCU. **Looking within CP/HCU only, there was no** Emotion*Region interaction effect for (fear/eyes>calm/eyes)>(fear/face>calm/face) **in the amygdala, and also no significant difference when looking at** responses to the simple effect fear/eyes>fear/face in CP/HCU (at either $p<.05$ FWE-SVC, or at $p<.005$, uncorrected, $k\geq10$). **We then looked at comparisons between CP/HCU and TD Controls on these two contrasts. Neither contrast showed an effect in the amygdala when data were collapsed across groups. There were also no group differences for the Emotion*Region interaction contrast (fear/eyes>calm/eyes)>(fear/face> calm/face). However, for the fear/eyes>fear/face contrast, CP/HCU showed a significantly greater response to fear/eyes relative to fear/face than did TD Controls in the right amygdala (peak=[20 -10 -14], *k*=5 *t*=3.04, *z*=2.89, FWE-SVC *p*=.018). This was driven by the TD Controls, who showed a significantly greater response to fear/face than to fear/eyes (*t*(16)=-3.56, *p*=.003, based on mean contrast estimates across the cluster). Responses in CP/HCU did not differentiate between conditions (*t*(16)=.83, *p*=.42).**

The second hypothesis was that CP/LCU would show a greater amygdala response to fear/eyes relative to other conditions than would TD Controls. For the Emotion*Region interaction contrast (fear/eyes>calm/eyes)>(fear/face>calm/face), there was a significant effect in the left amygdala in the direction CP/LCU>TD Controls, suggesting an increased response to fearful eyes in CP/LCU

(peak=[-18 -8 -18], *k*=6, *t*=3.16, *z*=2.99, FWE-SVC *p*=.013). Mean contrast estimates across the cluster (Figure 2) show that the interaction was driven by a significantly greater amygdala response to fear/eyes>calm/eyes than to fear/face>calm/face in CP/LCU (*t*(16)=2.19, *p*=.043), and a significant difference in the opposite direction in TD Controls (*t*(16)=-2.22, *p*=.041). Comparing TD Control and CP/LCU groups directly, there was a significantly greater response to fear/eyes>calm/eyes in CP/LCU than in TD Controls (*t*(32)=2.21, *p*=.034), and to fear/face>calm/face in TD Controls compared with CP/LCU (*t*(32)=2.09, *p*=.045). The only significant simple effect was a greater response to fear/eyes relative to calm/eyes in CP/LCU (*t*(16)=2.51, *p*=.023).

Although we had no specific hypotheses regarding amygdala response comparing CP/HCU and CP/LCU, for completeness we report that there were no significant differences between these groups for the Emotion*Region contrast within our region of interest, even at uncorrected levels.

**********Figure 2**********

Whole brain analyses: We report results from **exploratory** whole brain analyses **for the contrast (fear/eyes>calm/eyes)>(fear/face>calm/face),** which survived cluster-level FWE-correction at the whole brain level after initial thresholding at *p*<.005, *k*>10. **Note that results are not further corrected for multiple comparisons across groups. There were no differences between CP/HCU and TD Controls.** For the contrast CP/LCU>TD Controls, a response was seen in subgenual anterior cingulate cortex extending into orbitofrontal cortex (sgACC/OFC) indicating a greater response to (fear/eyes>calm/eyes)>(fear/face>calm/face) in CP/LCU (peak=[4 30 -14], *t*=4.18, *z*=3.84, FWE-corrected *p*<.001, *k*=1542; Figure 3a). **No significant differences were seen for the reverse contrast TD Controls>CP/LCU.** The contrast CP/LCU>CP/HCU yielded one cluster surviving cluster-level FWE-correction in left middle temporal gyrus (peak=[-48 -14 -22], *t*=4.69, *z*=4.23, *p*=.019, *k*=570; Figure 3b). **No significant differences were seen for the reverse contrast CP/HCU>CP/LCU.** Post-hoc

analyses **on significant effects** showed that all interactions were driven by crossover effects (see Figure 3 and the Supplementary Information).

**********Figure 3**********

Relationships Between Behavioural and fMRI Data: **In support of hypothesis 2,** both RT and fMRI data showed a disproportionate response to fear/eyes in CP/LCU relative to other experimental conditions **and TD Controls**. We explored potential relationships between RTs and the amygdala effect hypothesised a priori by creating a single metric for each variable reflecting difference values for (fear/eyes>calm/eyes)> (fear/face>calm/face). A positive value on this metric indicates slower RTs/greater amygdala response to fear/eyes relative to other conditions. **Amygdala response was defined as for Figure 2, i.e. mean contrast estimates across the cluster surviving small volume correction for (fear/eyes>calm/eyes)>(fear/face>calm/face) in the direction CP/LCU>TD Controls.**

Bivariate correlations between RT and amygdala response showed a significant positive correlation in CP/LCU (r=.50, p=.043), but no significant relationship in TD Controls (r=-.42, p=.093). The correlation in CP/LCU could not be explained by co-morbid anxiety, depression, or ADHD symptoms: including these as covariates r=.58, p=.030. **To test for a significant difference between the slopes, a custom univariate ANOVA was conducted with amygdala response as the dependent variable, mean-centred RT as a covariate and Group as a fixed factor (including CP/LCU and TD Controls). After accounting for main effects, there was a significant interaction between RT and Group (F(1,30)=7.59, p=.01), showing a significant group difference in slopes indexing the relationship between RT and amygdala response (Figure 4).**

**********Figure 4**********

Discussion

The current study investigated behavioural and neural consequences of directing attention to the eye region of fearful vs. calm faces in children with conduct problems and differing levels of callous-unemotional traits. Contrary to our first hypothesis, amygdala response to fearful faces in children with conduct problems and high CU traits (CP/HCU) did not increase when participants looked for a dot near the eye region of fearful faces compared with searching across the whole face. However, in line with our second hypothesis, children with conduct problems and low CU traits (CP/LCU) showed increased left amygdala response to the fear/eyes condition relative to both other conditions and TD Controls. This was accompanied by increased RTs, with the RT increase specific to fearful eyes correlating with amygdala response in CP/LCU but not in TD Controls. CP/LCU also showed increased neural responses to fearful eyes in the subgenual anterior cingulate/orbitofrontal cortex (sgACC/OFC) (relative to TD Controls), and left middle temporal gyrus (relative to CP/HCU).

It is important to consider why directing attention to the eye region during fear processing did not result in increased amygdala response in CP/HCU. One interpretation is that amygdala response in this group is largely immutable to the effects of manipulating attentional focus. Under this interpretation, improved fear recognition when focusing on the eye region (Dadds et al., 2006) would not be mediated by increased amygdala response. An alternative explanation relates to the nature of task demands. It has been suggested that fear processing deficits in CP/HCU are associated with a reduced ability to reflexively shift attention to the salient eye region, a process potentially mediated by the basolateral amygdala (Gamer & Buchel, 2009; Moul et al., 2012). In the fear/eyes condition, attention was already focused on the eye region, meaning no amygdala-mediated reflexive shift was needed; **in contrast, the fear/face condition may paradoxically elicit greater amygdala response owing to the need for a reflexive gaze shift**. **This idea is supported by the pattern of results seen in TD Controls in analyses for hypothesis 1. This group showed increased right amygdala response to fear/face relative to fear/eyes, while CP/HCU showed no difference**

between conditions. Although speculative, the pattern of results in TD Controls may reflect a typical orienting response that involves the amygdala, while the lack of difference between conditions in CP/HCU could reflect atypical processing.

It is also important to consider that the instruction to look for a dot may have introduced unforeseen processing biases **that limited modulation of amygdala responses to fear. One recent study investigating CP/HCU responses to fearful eye gaze during a spatial attention task (White et al., 2012) found reduced activation in a dorso-parietal orienting network compared with controls, but no effect in the amygdala. It was suggested that CP/HCU amygdala hypoactivity may be specifically elicited when task demands are low. Similarly,** it may be that the **present** task was not optimised for detecting conditions under which a fear processing deficit **in the amygdala** might be elicited or ameliorated in CP/HCU.

Previous studies have shown a hyper-reactive affective profile in CP/LCU (Frick et al., 2003a; Dadds et al., 2006). The current data suggest that emotional reactivity may be augmented when attention is directed to the eye region, which is high in affective salience (Adolphs et al., 2005). RT data further show a specific slowing during the fear/eyes condition. The positive relationship between RTs and amygdala reactivity in CP/LCU suggests that increased reactivity as indexed by amygdala response is associated with a reduction in task performance. It is unlikely that these results are driven by anxiety, since TD and CP/LCU groups did not differ on this measure. It is also unlikely that results can be explained by other symptoms on which the groups differed (i.e. ADHD and MDE), since the CP/HCU group also showed elevated symptoms but did not show the same pattern of results. Instead, increased amygdala reactivity and slower RTs suggest that children with CP/LCU have difficultly implicitly or automatically regulating emotional responses in pursuit of a goal (Ochsner & Gross, 2005). This complements well-documented reports of difficulties with explicit emotion regulation in everyday life (Frick & Morris, 2004). Indeed, difficulties with automatic emotion

regulation may contribute to the development of expressed behaviours such as reactive aggression (Eisenberg et al., 2010).

These data are in line with a recent study exploring interactions between attention and affective processing in adults with externalizing behaviours (Baskin-Sommers et al., 2012). Using an instructed fear paradigm, this study found that externalizing behaviours were not associated with a global hyper-reactivity effect. Rather, increased emotional reactivity and amygdala response, relative to low-externalising participants, was seen specifically when attention was focused on threat-related information. Together, these data suggest the importance of understanding the specific conditions under which emotional hyper-reactivity is seen in externalizing conditions such as CP/LCU. This is necessary to elucidate neurocognitive mechanisms underpinning such behaviours, and may provide insights that will improve current approaches to intervention.

While not predicted a priori, increased neural responses to fear/eyes were seen in CP/LCU in sgACC/OFC (relative to TD Controls) and in the left middle temporal gyrus (relative to CP/HCU). The sgACC and medial OFC form part of an extended network involved in the experience and regulation of emotional states (Drevets et al., 2008). More specifically, OFC and amygdala are involved in directing attention to affective stimuli (Zikopoulos & Barbas, 2012) and in the integration of emotion and cognitive control (Pessoa, 2008). Our data suggest aberrant cognitive control of emotion in CP/LCU, which may include sgACC/OFC dysfunction. Future studies should investigate further the role of these regions in reactive aggressive conduct problems. The difference between CP/LCU and CP/HCU in the middle temporal gyrus is difficult to interpret since this region is not typically activated during facial emotion processing, and indeed was not activated under any condition in the TD Controls.

Limitations of the present study include the use of a community sample of males: extension to clinically diagnosed and female participants would be of interest. Additionally, CU trait groupings relied on a median split, meaning assignment depended on sample characteristics rather than independently agreed cut-offs. Finally, relative to CP/LCU, the CP/HCU group showed elevated conduct disorder symptoms as well as CU traits. This is unsurprising, since CU traits index a particularly severe subgroup of children with conduct problems (Frick & Viding, 2009). Moreover, severity of conduct problem symptoms is unlikely to explain the observed pattern of results, with the greatest RT and neural responses to fearful eyes seen in CP/LCU, not CP/HCU.

In summary, this study compared CP/LCU, CP/HCU and TD Control children on a facial emotion processing task including an implicit emotion regulation component. Children with CP/LCU, associated with reactive aggression, showed increased amygdala reactivity compared with TD Controls, specifically in response to fearful eyes. This was correlated with longer RTs in the fear/eyes condition relative to control conditions. These data are in line with cognitive and behavioural profiles showing increased emotional reactivity in CP/LCU, and extend our knowledge to suggest specific conditions under which hyper-reactivity may be elicited in neural circuitry engaged in emotion-cognition interactions.

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Tables and Figure Legends

Tables

Table 1: Demographic data, presented by Group.

Figures

Figure 1: Mean RT differences plotted by Group and Condition. The significant Emotion*Region interaction is driven by the CP/LCU group, who showed significantly slower RTs to fear/eyes than calm/eyes (the dark bar for CP/LCU). This group also showed significantly slower RTs for the interaction term (fear/eyes>calm/eyes)>(fear/face>calm/face) (the difference between the dark and pale bars) i.e. the fear/eyes condition had a disproportionate slowing effect on RTs in CP/LCU, but not in the other two groups.

Figure 2: Emotion*Region*Group interaction in the left amygdala (peak=[-18 -8 -18]), driven by a significantly greater response for (fear/eyes>calm/eyes)>(fear/face>calm/face) in CP/LCU relative to TD Controls. Top: Bars indicate mean contrast estimates across the cluster (*k*=6) surviving FWE correction within a 3mm-radius bilateral sphere centred on central amygdala co-ordinates [+/- 20 -8 -16]. Bottom: Overlay shows the significant cluster overlaid on a mean T1 scan from all participants.

Figure 3: Regions showing an Emotion*Region*Group interaction at a whole brain cluster-corrected threshold of *p*<.05, FWE. Overlays are displayed at the initial threshold of p<.005, k≥10. a) Significantly greater response in CP/LCU than in TD Controls in the subgenual ACC, extending into the OFC. b) Significantly greater response in CP/LCU than in CP/HCU in the middle temporal gyrus.

Figure 4: Relationship between RT and amygdala response for the contrast (fear/eyes>calm/eyes)> (fear/face>calm/face), in **CP/LCU and TD Controls**. Slopes differed significantly between groups, with a significantly positive relationship between RT and amygdala response in CP/LCU and no relationship in the TD controls.

Figure 1
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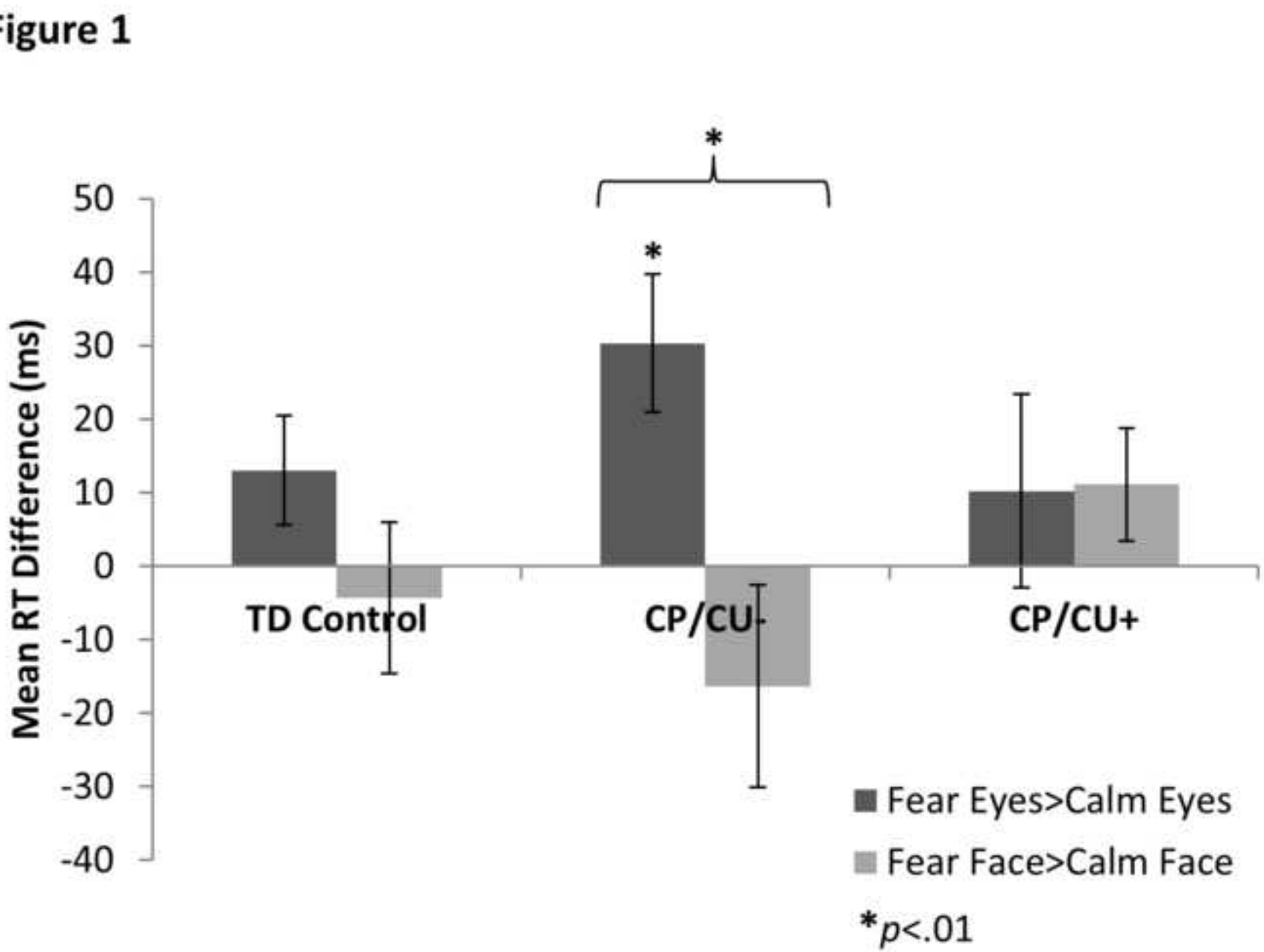

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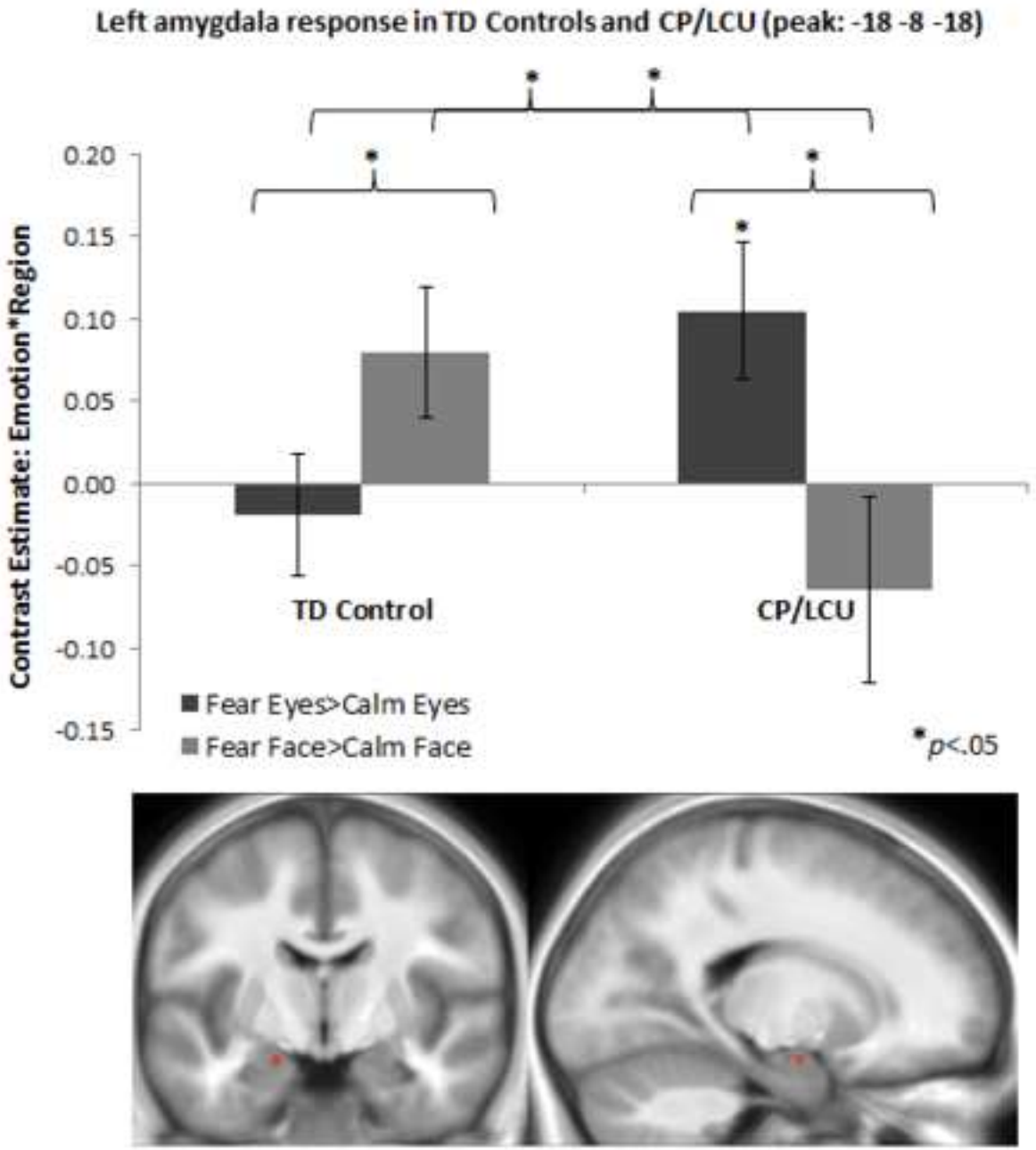
Figure 2



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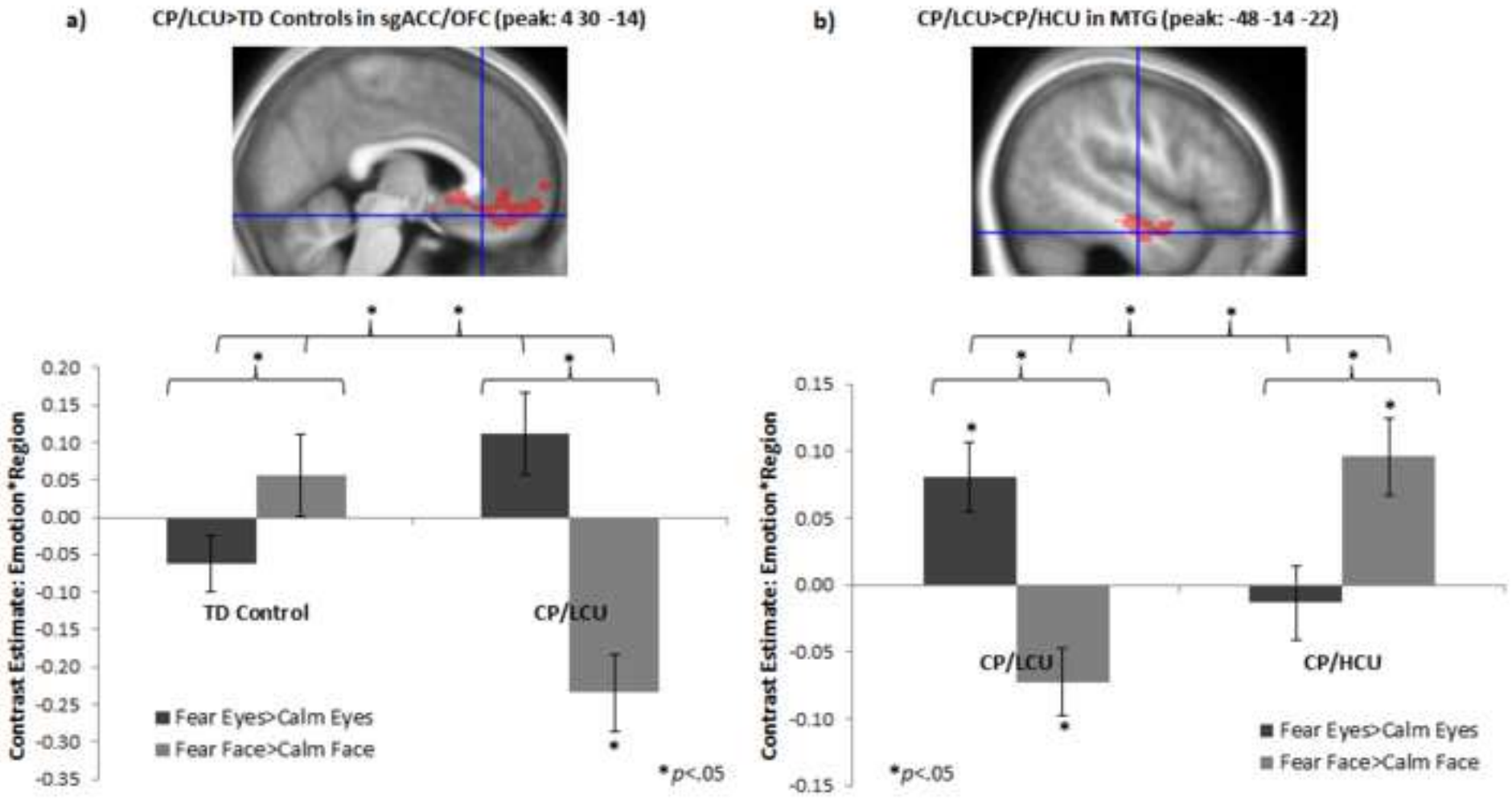

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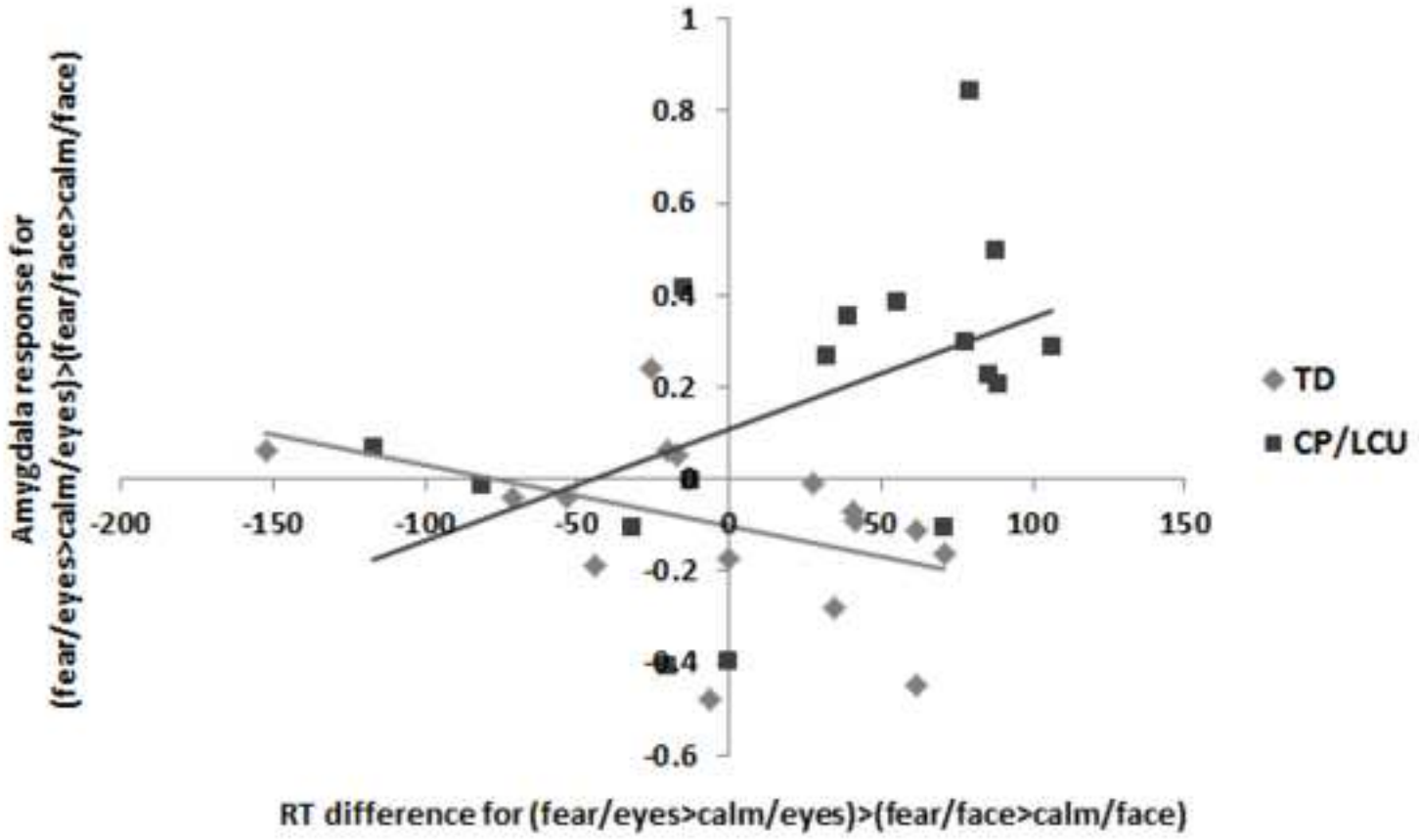

Table 1 - revised

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Table 1 - Demographic data, presented by Group

	Group					
	TD Controls[1]			CP/LCU[2]		
Characteristics and questionnaires	(n=17)			(n=17)		
	M	SD	Range	M	SD	Range
Age[b]	13.51	1.60	10-16	14.54	1.58	12-16
Socio-Economic Status[b]	2.73	.83	2-5	2.76	1.24	1-5
Full IQ score from 2-subtest WASI[c]	106.71	12.27	79-129	102.88	11.51	86-124
Ethnicity[b,e]	15:1:1	-	-	10:4:3	-	-
Handedness[b,f]	12:4:1	-	-	13:4:0	-	-
Inventory of Callous-Unemotional Traits[d]	24.00	5.81	15-36	35.35	7.87	15-44
Child and Adolescent Symptom Inventory						
Conduct Disorder[d]	0.53	0.8	0-2	8.14	3.64	4-16
Attention Deficit Hyperactivity Disorder[g]	9.71	6.04	1-21	21.84	11.44	7-41
Generalised Anxiety Disorder[g]	3.59	3.16	1-11	6.90	4.42	1-20
Major Depressive Episode[g,h]	2.71	1.93	2-10	5.73	3.41	2-13
Alcohol Use and Disorders[c]	1.18	1.7	0-6	4	5.61	0-21
Drug Use and Disorders[c]	0	0	0-0	2.47	5.27	0-21

* p <.05, Bonferroni corrected

[a]All p -values obtained using t -tests except for Ethnicity and Handedness (Fisher's exact tests used)

[b]Measures taken at screening phase, parent report

[c]Child at scanning session

[d]Measures taken at screening phase, parent and teacher report

[e]White:Black:Mixed

[f]Right:Left:Ambidextrous

[g]Measures taken at scanning session - parent report

[h]Missing data from 1 participant with conduct problems

CP/HCU[3] (n=17)			P value	Post hoc*
M	SD	Range		
13.99	1.94	10-16	.227	
3.12	1.08	2-5	.496	
98.35	11.64	79-120	.130	
13:1:3	-	-	.357	
15:2:0	-	-	.675	
53.35	5.60	45-62	<.001	1<2<3
13.36	6.77	6-26	<.001	1<2<3
30.29	9.64	12-45	<.001	1<2<3
8.24	5.02	1-17	.008	1<3
5.88	3.61	2-12	.006	1<2/3
4.47	7.13	0-25	.161	
1.00	2.55	0-10	.111	

Supplementary Materials for 'Neural responses to fearful eyes in children with conduct problems and varying levels of callous-unemotional traits'

Supplementary Methods

Males aged 10-16 were recruited from the community via newspaper advertisements and local schools. Screening questionnaires were administered to parents and teachers of 176 boys whose families expressed an interest in taking part and provided informed consent; and were scored by a trained research assistant according to standard published guidelines. These yielded: a research diagnosis of current conduct problems; dimensional assessment of CU traits; an overall psychopathology screen; demographic data for group matching purposes (socio-economic status, parent-defined ethnicity, and handedness); and information regarding previous neurological or psychiatric diagnoses. Current conduct problems were assessed using the Child and Adolescent Symptom Inventory (CASI-4R; Gadow & Sprafkin, 2009) Conduct Disorder subscale (CASI-CD), and CU traits were assessed using the Inventory of Callous-Unemotional Traits (ICU; Essau et al., 2006). Both were scored by taking the highest ratings from either the parent or the teacher questionnaire for any given item (Piacentini et al., 1992). The Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997) was used as a brief screening measure for psychopathology in the typically developing (TD) control group.

On the basis of the screening information participants were invited for an fMRI scan. CASI-CD symptom severity scores were used to make the research diagnosis of current conduct problems. Symptom severity cut-off scores for inclusion in the conduct problems group were 3+ (ages 10-14) and 6+ (ages 15-16). Scores of this magnitude and above are associated with a clinical diagnosis of conduct disorder **(Gadow & Sprafkin, 1998),** with an agreement between the screening cut-off scores for CASI-CD (completed by both parent and teacher) and clinical diagnoses of .95 (sensitivity) and .56 (specificity). There were no restrictions on ICU score for the conduct problems group. TD control participants were matched to conduct problems participants on verbal/performance IQ, age,

handedness, ethnicity and socio-economic status, but scored in the normal range for the CASI-CD and on each SDQ subscale. All control participants also scored below the conduct problems group median (=44.5) on the ICU.

We obtained written informed consent from parents and written assent from participants. We scanned a total of 55 children (38 with conduct problems, 17 typically developing controls), yielding a final sample of usable data from 34 boys with conduct problems and 17 controls (exclusions as described in the main text). Assignment to CU group took place after these exclusions had been made on the basis of a median split on ICU scores, and yielded two groups: conduct problems with low CU traits (CP/CU-, N=17) and conduct problems with high CU traits (CP/CU+, N=17).

Supplementary Results

*fMRI Data: Emotion*Region*Group interaction: deconstructing interactions in non-predicted regions*

There was an effect of CP/CU->TD Controls for the contrast (fear/eyes>calm/eyes)> (fear/face>calm/face) in a cluster encompassing subgenual anterior cingulate cortex and orbitofrontal cortex (sgACC/OFC) (peak=[4 30 -14], t=4.18, z=3.84, p<.001 FWE-corrected at the cluster level, k=1542). As can be seen in Figure 3a, this effect was driven by a significantly greater response across the cluster to (fear/eyes>calm/eyes) than (fear/face>calm/face) in CP/CU- (t(16)=4.28, p=.001), and the reverse pattern in TD Controls (t(16)=-2.59, p=.02). Comparing groups, there was also a greater response to (fear/eyes>calm/eyes) in CP/CU- than TD controls (t(32)=2.66, p=.012), and a greater response to (fear/face>calm/face) in TD controls than in CP/CU- (t(32)=-3.84, p=.001). Looking at simple effects showed that the result was driven largely by CP/CU-, who showed a significantly larger response to calm/face than fear/face (t(16)=-4.55, p<.001, and a marginally greater response to fear/eyes than calm/eyes (t(16)=2.07,p=.055. Simple effects within the TD Control group were not significant.

There was also an effect of CP/CU-> CP/CU+ for the contrast (fear/eyes>calm/eyes)> (fear/face>calm/face) in left middle temporal gyrus (peak=[-48 -14 -22], t=4.69, z=4.23, p=.019, k=570). As can be seen from Figure 3b), this effect was driven by a significantly greater response across the cluster to (fear/eyes>calm/eyes) than (fear/face>calm/face) in CP/CU- (t(16)=3.59, p=.002), and the reverse pattern in CP/CU+ (t(16)=-3.47, p=.003). Comparing groups, there was also a greater response to (fear/eyes>calm/eyes) in CP/CU- than CP/CU+ (t(32)=2.48, p=.019), and a greater response to (fear/face>calm/face) in CP/CU+ than in CP/CU- (t(32)=-4.39, p<.001). Simple effects showed a significantly greater response to fear/eyes than calm/eyes in CP/CU-, but the reverse pattern for faces (calm/face>fear/face) (*ps*<.05). In CP/CU+, there was a greater response to fear/face than calm/face (t(16)=3.30, p<.005), but no difference between fear/eyes and calm/eyes.

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Supplementary Tables

Supplementary Table 1: Regions showing a main effect across all groups for the contrasts Fear>Calm and Calm>Fear. Results are reported at a threshold of *p*<.005, *k*>10. BA=Approximate Brodmann area in which the peak voxel is located. L/R=left/right, peak voxel=MNI co-ordinates, k=cluster size, ext.=extends into adjacent region.

Brain Region	BA	L/R	Peak Voxel			k	t	z
Fear>Calm (across Eyes/Face conditions)								
Fusiform gyrus (ext. inferior occipital gyrus)	20, ext. 19	R	44	-36	-16	417	4.41	4.02
Superior temporal gyrus	22	R	50	-52	6	463	4.32	3.95
Temporal pole (ext. inferior frontal gyrus)	38, ext. 47	R	40	22	-28	167	4.23	3.88
Occipital gyrus	18	L	-30	-86	-20	288	3.95	3.66
Superior temporal gyrus	21	R	48	-26	-6	47	3.83	3.56
Fusiform gyrus (ext. parahippocampal gyrus)	37, ext. 19	L	-34	-38	-18	78	3.71	3.46
Superior frontal gyrus	10	R	8	64	28	29	3.16	3.00
Middle temporal gyrus	37	R	38	-60	0	29	3.06	2.91
Amygdala	-	L	-20	-8	-14	20	3.00	2.85
Middle frontal gyrus	46	R	58	30	22	24	2.99	2.85
Superior frontal gyrus	9	-	0	56	38	13	2.88	2.76
Calm>Fear								
Superior frontal gyrus	10		24	44	20	128	4.02	3.71
Cingulate gyrus	24		-14	14	36	64	3.94	3.65
Caudate head (ext. bilaterally)	-		10	14	4	270	3.90	3.61
Anterior cingulate	24		-8	30	20	103	3.72	3.47
Thalamus	-		-20	-20	4	43	3.72	3.47
Cerebellum	-		10	-62	-26	25	3.11	2.95
Superior temporal gyrus	41		-46	-38	16	24	3.07	2.92
Superior frontal gyrus	8		18	28	42	14	2.92	2.79

Supplementary Table 2: Regions showing an Emotion*Region interaction effect (fear/eyes>calm/eyes)>(fear/face>calm/face) across groups. Results are reported at a threshold of $p<.005$, $k>10$. BA=Approximate Brodmann area in which the peak voxel is located. L/R=left/right, peak voxel=MNI co-ordinates, k=cluster size, ext.=extends into adjacent region.

Brain Region	BA	L/R	Peak Voxel			k	t	z
(Fear/Eyes>Calm/Eyes)>(Fear/Face>Calm/Face)								
Anterior cingulate	24	R	10	16	24	99	5.17	4.59
Posterior cingulate	30, ext. 29	L	-10	-54	12	345	4.88	4.38
Middle frontal gyrus (inc. white matter)	9	L	-26	18	26	361	4.34	3.97
Anterior cingulate (ext. medial frontal gyrus)	32, ext. 10	L	-14	34	2	598	4.26	3.90
Mid-cingulate gyrus	24	L	-10	-4	40	146	3.77	3.51
Precentral gyrus	6	L	-44	-18	28	110	3.69	3.44
Middle temporal gyrus	21	R	62	2	-26	31	3.68	3.43
Postcentral gyrus	43	L	-58	-12	18	107	3.45	3.24
Medial frontal gyrus	10	L	-14	50	-6	35	3.37	3.18
Caudate	-	R	14	14	10	29	3.29	3.11
Postcentral gyrus	2	R	58	-24	38	20	3.23	3.06
Thalamus	-	L	-14	-22	8	25	3.19	3.02
Thalamus	-	-	0	-6	12	46	3.13	2.98
Middle frontal gyrus	9	R	30	22	28	13	3.06	2.91
Medial frontal gyrus	10	R	14	54	6	32	3.05	2.90
Precentral gyrus	6	R	36	-12	36	22	3.03	2.88
Middle temporal gyrus	21	L	-62	0	-18	11	2.98	2.84
Lingual gyrus	19	L	-14	-64	-10	24	2.97	2.83
Superior temporal gyrus	22	L	-56	-6	-8	12	2.95	2.81
Superior temporal gyrus	41	R	48	-28	8	18	2.95	2.81
Superior frontal gyrus	8	R	18	16	46	10	2.88	2.75