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Dual Cognitive and Biological Correlates of Anxiety in Autism Spectrum Disorders

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The authors declare that they have no conflict of interest.

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Short Title: Dual Correlates of Anxiety in ASD

Abstract

Young people with autism spectrum disorder (ASD) have a high prevalence (~40%) of anxiety disorders compared to their non-ASD peers. It is unclear whether cognitive and biological processes associated with anxiety in ASD are analogous to anxiety in typically developing (TD) populations. In this study 55 boys with ASD (34 with a co-occurring anxiety disorder, 21 without) and 28 male controls, aged 10 – 16 years and with a full-scale IQ ≥70, completed a series of clinical, cognitive (attention bias/interpretation bias) and biological measures (salivary cortisol/HR response to social stress) associated with anxiety in TD populations. Structural equation modelling (SEM) was used to reveal that that both attentional biases and physiological responsiveness were significant, but unrelated, predictors of anxiety in ASD.

Keywords: Attention, Comorbidity, Cortisol, Emotion, Stress,

Abstract

Young people with autism spectrum disorder (ASD) have a high prevalence (~40%) of anxiety disorders compared to their non-ASD peers. It is unclear whether cognitive and biological processes associated with anxiety in ASD are analogous to anxiety in typically developing (TD) populations. In this study 55 boys with ASD (34 with a co-occurring anxiety disorder, 21 without) and 28 male controls, aged 10 – 16 years and with a full-scale IQ ≥70, completed a series of clinical, cognitive (attention bias/interpretation bias) and biological measures (salivary cortisol/HR response to social stress) associated with anxiety in TD populations. Structural equation modelling (SEM) was used to reveal that that both attentional biases and physiological responsiveness were significant, but unrelated, predictors of anxiety in ASD.

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Introduction

Children and adolescents with autism spectrum disorder (ASD) are known to suffer from a high prevalence of co-occurring anxiety disorders (estimated prevalence ~ 40%) (van Steensel, Bögels, & Perrin, 2011). However, prevalence of anxiety in ASD varies across different anxiety diagnoses, with specific phobias, social anxiety disorder, and obsessive compulsive disorder (OCD) being the most common (Simonoff et al., 2008; van Steensel et al., 2011). Overall, the prevalence of any anxiety diagnosis is elevated relative to the general population (Merikangas et al., 2010). There are a number of challenges in the assessment of anxiety in people with ASD, including the young person's difficulty in effectively identifying or labelling emotional symptoms (Losh & Capps, 2006) and considerable behavioural overlap between symptoms of ASD and various anxiety disorders (Wood & Gadow, 2010). While the diagnosis of anxiety disorders is reliant on symptom self-report, models of anxiety in the non-ASD (typically developing – TD) population suggest that these symptoms reflect underlying differences in physiological reactivity and maladaptive cognitions (Lang, 1985). Despite some emerging evidence that anxiety in autism may present atypically (Kerns et al., 2014; White et al., 2014) there have been few empirical studies investigating whether the cognitive and biological correlates of anxiety disorder in TD children are similar or different in those with ASD.

Cognitive processing biases in childhood anxiety disorders and ASD

Several studies in TD children and adolescents with anxiety disorders have found significant attentional biases using the popular dot-probe task with threatening faces (Salum et al., 2013; Waters, Henry, Mogg, Bradley, & Pine, 2010) or words (Dalgleish, Moradi, Taghavi, Neshat-Doost, & Yule, 2001; Dalgleish et al., 2003). The

dot-probe task consists of presenting an emotional stimulus, paired with a neutral stimulus, followed by a probe in a position previously occupied by either the emotional or neutral stimuli. Reaction time is then used as a measure of the attentional resources captured by the emotional versus neutral stimuli. A significant attentional bias to threat occurs when reaction time is greater to a threat stimulus than to a neutral stimulus. This paradigm has been shown to be relatively consistent in identifying attentional biases in young people with anxiety problems (Lau et al., 2012).

Furthermore, studies comparing the interpretations of ambiguous scenarios have also found that children with anxiety disorders make more threatening interpretations compared to controls (Bögels, Snieder, & Kindt, 2003; Dodd, Hudson, Morris, & Wise, 2012; Waters, Craske, Bergman, & Treanor, 2008). However, there are some exceptions to this finding which suggest that children with anxiety disorder do not interpret ambiguous pictures as significantly more negative than controls, but instead rate their own feelings when viewing the stimuli as more negative and feel more arousal than controls (In-Albon, Dubi, Rapee, & Schneider, 2009).

Despite strong evidence for the role of cognitive biases in children with an anxiety disorder there is a lack of empirical investigation into the cognitive factors that may underlie the high prevalence of anxiety in ASD. In one previous study there was no significant difference in biases towards either threat faces or words in a sample of ASD children and adolescents compared to controls; furthermore, within the ASD group, there was no relationship between attention bias and parent- or self-reported anxiety symptoms (Hollocks, Ozsivadjian, Matthews, Howlin, & Simonoff, 2013). However, in that study, no information was collected about the presence of clinically defined anxiety disorders and participants were tested in their familiar school environments, possibly minimising levels of state anxiety which are shown to be

related to performance on this task (Bar-Haim et al., 2010). Consistent with this, a more recent study by May and colleagues (May, Cornish, & Rinehart, 2015) found no significant relationship between anxiety symptoms and attentional bias to threat faces in children with ASD. However, again anxiety in this study was assessed using a questionnaire measure only, and did not examine the presence of anxiety diagnoses.

Physiological correlates of anxiety in childhood anxiety disorders and ASD

The most common task used to measure anxiety-related physiological differences is a psychosocial stress test (PST), e.g., Kirschbaum, Pirke, & Hellhammer, 1993. This typically consists of a baseline period, followed by preparing and giving a speech, and then completing an arithmetic task under observation. During the stressful task a number of physiological parameters are measured, including changes in salivary cortisol and heart rate.

Studies of non-ASD children and adolescents with anxiety disorders that have measured salivary cortisol in response to psychosocial stress are inconsistent and include reports of: cortisol hyper-responsiveness in pre-pubertal anxious children compared to controls (van West, Claes, Sulon, & Deboutte, 2008), a non-significant trend for a blunted cortisol response in similarly aged anxious children (Krämer et al., 2012), and no significant group difference in cortisol response in adolescent girls with social phobia (Martel et al., 1999). Studies measuring heart rate are also inconsistent, with findings in social phobia varying from increased HR (Schmitz, Krämer, Tuschen-Caffier, Heinrichs, & Blechert, 2011), decreased HR (Krämer et al., 2012) and no difference compared to healthy controls.

Studies including young people with ASD, but without co-occurring anxiety, are rather more consistent in showing a significantly reduced cortisol response to social

stress (Corbett, Schupp, & Lanni, 2012; Jansen et al., 2006; Lanni, Schupp, Simon, & Corbett, 2012; Levine et al., 2012). Likewise, two of the three studies in ASD that have measured heart rate during a social stressor suggest an attenuated HR response (Jansen et al., 2006; Jansen, Gispen-de Wied, van der Gaag, & van Engeland, 2003), while the third failed to find any significant group differences (Levine et al., 2012). However, the general trend for studies using a non-social stressor or an at-rest measure is for a reduced parasympathetic input and therefore a higher mean HR (Bal et al., 2010; Ming, Julu, Brimacombe, Connor, & Daniels, 2005; Van Hecke et al., 2009).

Four studies (Bitsika, Sharpley, Andronicos, & Agnew, 2015; Bitsika, Sharpley, Sweeney, & McFarlane, 2014; Hollocks, Howlin, Papadopoulos, Khondoker, & Simonoff, 2014; Simon & Corbett, 2013) have specifically focused on the relationship between physiological parameters and anxiety in people with ASD. Studies using standardized questionnaire measures of anxiety have shown null results when looking at elicited stress (Simon & Corbett, 2013), but significant relationships between greater anxiety and cortisol concentration 30 minutes after waking (Bitsika et al., 2014) and between generalized anxiety and cortisol concentration measured across the day (Bitsika et al., 2015). In a previously published study using the current sample we reported that children and adolescents with ASD and co-occurring anxiety disorders had a significantly reduced heart rate and cortisol response to PST compared to adolescents with ASD only and healthy controls (Hollocks et al., 2014).

Despite strong evidence for the importance of both cognitive and physiological processes in anxiety disorders, the overlap between the two is rarely studied. This is surprising given that cognitive models of anxiety, particularly social anxiety, which is a commonly reported anxiety diagnoses in ASD (Salazar et al., 2015; Simonoff et al.,

2008), often incorporate physiological symptoms as an important maintaining factor of maladaptive cognitions (Rapee & Heimberg, 1997). Whether or not physiological differences may mediate cognitive biases is also an important issue when considering the development of effective interventions for anxiety in ASD, which could be targeted at cognitions (Sukhodolsky, Bloch, Panza, & Reichow, 2013; Wood et al., 2009), physiological responsiveness (Kodish, Rockhill, Ryan, & Varley, 2011) or both (Walkup et al., 2008). It is also relevant to consider this overlap in light of the Research Domain Criteria (RDoC) initiative, which provides a framework for studying mental disorders with an emphasis on different levels of analysis (Insel, 2014). In the case of the current study we are focusing on what are described as the cognitive and arousal systems.

As previously mentioned some people with ASD have substantial difficulties in labelling emotions (Losh & Capps, 2006). This makes the self-report of anxiety symptoms for those with a limited verbal ability especially challenging. This is problematic given the "gold-standard" assessment of childhood anxiety requires a multi-informant approach and emphasises the importance of self-report (Lyneham & Rapee, 2005). Therefore, it is also of interest to investigate the possibility of using cognitive tasks or psychophysiological measurements as surrogate markers for anxiety in ASD, particularly for those with a limited verbal ability. However, before such measures can be employed in this way their use requires validation in groups of higher functioning people with ASD, where verbal reports may be more reliable (Blakeley-Smith, Reaven, Ridge, & Hepburn, 2012; Ozsivadjian, Hibberd, & Hollocks, 2014).

The aim of this study is to combine our previously identified physiological correlates of anxiety in ASD with novel cognitive data, all of which were collected in a single experimental session, to investigate three hypotheses. Firstly, we will

investigate whether cognitive processing biases in attention and interpretation are associated with anxiety in ASD. Secondly, we will examine whether anxiety-related physiological responses to social stress mediate the relationship between cognitive biases and anxiety, or whether they are both independently related to anxiety symptoms. Finally, we will investigate the utility of these cognitive and physiological parameters as classifiers of anxiety disorders in ASD.

Methods

This research was reviewed and approved by the South East London Research Ethics Committee (REC 4: 10/H0870/67). The study includes 83 male participants (55 ASD and 28 control) aged 10 – 16 years with a full-scale IQ (FSIQ) and reading level ≥ 70. Exclusion criteria included current use of any mood-stabilizing, anti-depressant or anxiolytic medication. ASD diagnoses were made by local clinicians, and in 30/55 cases were confirmed using either the Autism Diagnostic Interview – Revised [ADI-R (Lord, Rutter, & Le Couteur, 1994)] and/or Autism Diagnosis Observation Schedule-Generic [ADOS-G (Lord et al., 2000)] algorithm scores. In the absence of ADOS/ADI-R confirmed diagnosis, a Social Communication Questionnaire [SCQ (Rutter, Bailey, & Lord, 2003)] score of ≥15 in combination with a clinical diagnosis from a psychiatrist/psychologist was required.

ASD participants were primarily recruited from National Health Service (NHS) clinics in London and the south-east of the United Kingdom. Control participants were recruited from local schools and public advertisement and had no history of psychiatric or neurological problems based on parental report. FSIQ was measured using the two-subtest version of the Wechsler Abbreviated Scale of Intelligence [WASI (Wechsler,

1999)] and reading ability was assessed using the word reading test from the Wechsler Individual Achievement Test [WIAT (Wechsler, 2005)].

Measures

Anxiety measures

The Child and Adolescent Psychiatric Assessment (CAPA) (Angold & Costello, 2000) is a semi-structured psychiatric interview focused on symptoms present within the *3 months* prior to the interview date, referred to as *current* diagnoses. In this study the CAPA parent-version rather than child-version was used because of the frequent difficulty experienced by young people with ASD in giving accurate and detailed descriptions of emotional symptoms. The CAPA was administered by trained researchers and was chosen as it requires detailed behavioural description in order to endorse individual symptoms. This makes it possible to take into account any behavioural overlaps between symptoms of anxiety and ASD when coding the interviews. For example, in the case of social phobia the participant would have had to display a clear avoidance of social events due to fear of evaluation/embarrassment and not due only to disinterest in social interaction. The CAPA was used to generate diagnoses of panic / agoraphobia, generalized anxiety disorder (GAD), separation anxiety, simple phobia, social phobia, obsessive compulsive disorder (OCD) and post-traumatic stress disorder (PTSD). The CAPA was also administered to parents of control children to rule out any psychiatric diagnoses.

The *Spence Children's Anxiety Scale*, (parent and child versions, SCAS-P and SCAS-C;(Spence, 1998)) is a 44-item questionnaire widely used in research to screen for anxiety disorders and includes questions addressing a range of anxiety symptoms.

In this study we used the SCAS-P/SCAS-C as a continuous measure of anxiety symptoms in both ASD and control groups.

Cognitive measures

The dot-probe tasks were included as measures of attentional bias towards emotion. This is measured by the relative response latency (ms) to emotion stimuli (e.g., an angry or happy face) versus neutral stimuli (e.g., a neutral face), with a positive value indicating a bias towards emotion. It is suggested that non-ASD children with anxiety disorders demonstrate a greater attentional bias towards angry faces compared to controls (Taghavi, Neshat-Doost, Moradi, Yule, & Dalgleish, 1999; Waters et al., 2010).

Dot-probe emotional faces. For a detailed description of this procedure see Hollocks et al., 2013. In total there were 32 angry-neutral face-pairs, 32 happy-neutral face pairs and 16 neutral-neutral face pairs taken from the NimStim face set (Tottenham et al., 2009). Each trial began with a 500 milliseconds (ms) fixation cross followed by the presentation of paired face stimuli. Each stimulus was presented for 500ms and then replaced by a probe on either the left or right hand side of the screen in the spatial location of one of the previous stimuli and the response latency was measured in ms.

Dot-probe emotional words. This task was of similar design to that described above and used 56 emotional words and 48 filler trials. The emotional words comprised 16 social threat-neutral pairs, 16 physical threat-neutral pairs and 16 positive-neutral pairs. The words were matched on length and selected from a list of words rated as neutral, happy, or related to either social or physical threat by a panel of researchers. Stimuli were presented one above the other for 1500 ms separated by

a vertical axis of 3 cm, consistent with their use in previous research (Dalgleish et al., 2003).

The analysis of dot-probe data was based on reaction times (RT), i.e. response latency to the visual probe. RTs from trials with an incorrect response were excluded as were trials in which responses were considered unreliable (<200ms or >3 SD above the individual participant's mean RT). Bias scores were calculated by deducting the mean RT for congruent trials from the mean RT for incongruent trials. Positive bias values indicate an attentional bias towards the emotional stimuli; negative values indicate a bias away from the stimuli.

Ambiguous Situations Task. Interpretation biases were assessed using the forced choice component of the Ambiguous Situations Interview used previously in anxious children (Barrett, Rapee, Dadds, & Ryan, 1996). Twelve ambiguous scenarios (6 social threats, 6 physical threats) were presented to each participant on a laptop computer. For each scenario four response options, two neutral and two threatening, were then presented and the participants were asked to select the one they thought was most likely to be true. A threat interpretation was scored as 1 and neutral as 0, generating three scales: social threat (0-6), physical threat (0-6) and total (social and physical) threat bias score (0-12).

Physiological measures. A detailed description of the Psychosocial Stress Paradigm used in this study has been published previously (Hollocks et al., 2014). Briefly, participants completed a stress paradigm preceded and followed by 40 minutes of relaxation/recovery. The stress paradigm included: 1) copying a complex figure drawing; 2) 10 minutes to prepare a speech about themselves; 3) a 5-minute presentation; and 4) remembering the complex figure drawing. This is an adapted form

of the traditional Trier Social Stress Test (Kirschbaum et al., 1993) with the primary adaptation being the use of a drawing task rather than an arithmetic task. This decision was made based on our clinical experience administering cognitive assessments that some of our ASD sample may have found the arithmetic task less stressful than controls. This task was carried out in the afternoon beginning between 13:00 h and 14:00 h, to reduce the impact of diurnal cortisol variation. Participants were asked not to consume any food or drink within 30 min of the task initiation. During the stress test, up until the end of the preparation phase, two researchers were present in the room; once the speech preparation was completed, a third person (unknown to the participant) who was presented as the evaluator entered the room and asked the participant to begin their presentation. Six salivary cortisol samples were taken throughout the test: two during rest (-40 min pre-stressor, -20 min pre-stressor), pre-stressor (0 min), post-stressor (+20 min) and two during recovery (+40 min, +60 min). Saliva samples were collected in plain Sarstedt salivettes which were stored at -40 °C until analysis. Saliva cortisol concentrations were determined using the ‘‘Immulite’’, Siemen’s Immunoassay system (www.dignostics.siemens.com) as using a previously described methodology (Mondelli et al., 2010). Heart rate was recorded continuously throughout the psychosocial stress test using the Zephyr BioHarnessTM wireless telemetry system. The ECG signal was recorded and analyzed via ADInstruments Labchart, version 7 (ADInstruments Pty. Ltd., Bella Vista, Australia).

Statistical analysis

The inferential statistical analysis for this study was divided into three components. Firstly, we conducted regression analyses to examine group differences [control vs. ASD (ASD) vs. ASD with an anxiety disorder (ASDanx)] in our key predictor

variables; attention bias, interpretation bias and the PST response variables (HR response/cortisol response). To analyse the time-series physiological data a three-piece linear mixed model was fitted to both the HR and cortisol data, using two knot points, one at just before the initiation of the psychosocial stress and the other corresponding to the stress response profile. To generate a responsiveness variable from the model to use in the current analysis we extracted the fixed- and random-effects of the response slope from the piecewise model (see Hollocks et al, 2014, for further details).

Secondly, we conducted a within-ASD group analysis to examine the independent relationship between information processing biases, physiological responsiveness, and levels of anxiety. To maximize power, for this analysis we collapsed the ASD-only and ASDanx groups into a single sample and used the SCAS-P/SCAS-C questionnaires as continuous measures of anxiety symptoms. We included only variables that showed significant group differences as independent variables in a structural equation model (SEM) predicting a dependent continuous variable of anxiety symptoms (SCAS scores). The two physiological response variables were collapsed into a single latent construct. The justification for this is based on our previous findings that they were highly correlated in our previous analysis and also had a similar relationship with anxiety in ASD (Hollocks et al., 2014). In addition, the HPA axis and ANS, while being distinct systems, operate in an interactive way during a stress response (Ulrich-Lai & Herman, 2009). In contrast, for this analysis the cognitive variables remained as observed variables. Our initial full model (Figure 1) predicted independent associations between anxiety symptoms and both information processing (attention and interpretation) biases and physiological responsiveness. The physiological responsiveness latent variable was included as predictor of anxiety and

also as a possible mediator of the relationship between cognitive biases and anxiety. The model allowed correlations between the cognitive variables, and FSIQ was regressed onto all predictors. Models were fit to raw data using full information maximum likelihood to account for data missing at random and alternative models were compared using the chi-square likelihood ratio test of comparative model fit, comparative fit index (CFI), and root mean square error of approximation (RMSEA). SEM analysis was conducted in Mplus 5th Edition (Muthén & Muthén, 2012).

Finally, we explored whether the cognitive and physiological parameters could identify the presence of anxiety disorders within the ASD group by conducting logistic regressions in which we regressed each predictor onto the dichotomous variable of anxiety disorder, present or absent. We plotted Receiver Operating Characteristics (ROC) curves for each measure using the *roctab* command in STATA 13 (StataCorp.13, 2013) and compared the area under the curve (AUC) using the *roccomp* command.

Results

Descriptive statistics

The final sample of adolescent boys comprised 28 non-ASD controls (mean age = 13.9, SD = 1.8), 21 ASD participants without an anxiety disorder (ASD-only: mean age = 13.0, SD = 1.9) and 34 participants with ASD and one or more anxiety disorders based on the CAPA (ASDanx; mean age = 12.7, SD = 1.9). Diagnoses in the ASDanx group included panic / agoraphobia (n = 21), GAD (n = 22), separation anxiety (n = 14), simple phobia (n = 2), social phobia (n = 4) and OCD (n = 11). No participants met criteria for PTSD. Specific phobia always occurred in combination with another anxiety disorder and one participant was diagnosed solely with OCD;

exclusion of this individual had no impact on the results presented below. As previously reported in ASD and other samples, anxiety disorders typically aggregate (Simonoff et al., 2008). Twenty-six out of 34 (76%) of the ASDanx participants had more than one co-occurring anxiety disorder and 10 (29%) met criteria for three or more anxiety disorders. The ASD groups differed significantly from the control group on parent- and child-reported anxiety (SCAS), SCQ scores, FSIQ and age (Table 1*)*. In the ASD groups, scores on the SCAS-P were not significantly correlated with FSIQ (r = .02, *p* =.88) or the SCQ score (r = .19, *p* = .18); in the control group there was a borderline significant association between parent-reported anxiety and the SCQ total score (r = .39, *p* = .05). Consistently, SCAS-C scores were not significantly correlated with FSIQ (r = -.08, *p* =.52) or the SCQ score (r = .04, *p* = .73) in either the ASD participants, nor in the control group (FSIQ, r = .03, *p* = .87; SCQ, r = .06, *p* = .78).

Group differences in cognitive and physiological variables

Emotional faces bias. There were significant group differences in attentional bias towards threatening (F (2, 78) = 3.37, *p* = .04), but not happy faces (F (2, 77) = .39, *p* = .67). Specifically, *post hoc* analyses showed that the ASDanx group had a significantly greater threat bias towards angry faces compared to both the ASD-only (*p* =.04) and the control group (*p* =.01), while the ASD-only and control groups did not differ (*p* =.17) (*see Table 2)*.

Emotional words bias. There were no significant group differences in bias toward either the physical threat words (F (2, 77) = 1.40, *p* = .25), social threat words (F (2, 79) = 1.12, *p* = .33) or the happy words (F (2, 79) = 0.80, *p* = .45).

Interpretational bias. There was a significant group difference in total negative bias score (F (2, 76) = 3.98, *p* = .02; *see Table 2*). Specifically, *post hoc* analyses showed

that the ASDanx group made significantly more negative interpretations compared to the control group (p =.02), but not the ASD-only group. Furthermore, there was no significant difference between the ASD-only group and the other two groups. The same pattern of results was observed when using the social situations only (F (2, 76) = 4.75, p = .01); again *post-hoc* analyses revealed that the ASDanx group made more negative social interpretations than the control group ($p \leq .01$), but not the ASD only-group. There were no significant group differences in the interpretation of physical threat scenarios (F (2, 76) = .36, p = .36).

Physiological responsiveness. There were significant group differences when using both the HR (F (2, 69) = 457.0, $p \leq .01$) and cortisol responsiveness variables (F (2, 71) = 73.4, $p \leq .01$). Both variables showed a consistent pattern whereby those in the ASDanx group had the lowest level of both HR and cortisol response versus both the ASD-only ($p \leq .01$) and control groups ($p \leq .01$). The ASD-only group demonstrated an intermediate response profile that was significantly lower than the control group ($p \leq .01$) but not that of the ASDanx group.

SEM of independent cognitive and physiological pathways to anxiety in ASD and the possible mediating effect of physiology

The aim of the SEM was to investigate the independent contributions of our cognitive variables to anxiety within the ASD group, while accounting for the role of physiological responsiveness as a possible mediating factor. Inter-correlations for all variables included in the model, limited to the ASD sample, are presented in *Table 3.* Our initial full model (see Figure 1) which predicted independent associations between anxiety symptoms and both cognitive variables and physiological responsiveness had a good fit (x^2 (7) = 6.4; CFI = 1.0; RMSEA = .00, 90% CI = 0.0, 0.16) and showed that

both reduced physiological responsiveness (standardized coefficient = -.70, $p < .01$) and greater attentional bias to threat faces (standardized coefficient = .37, $p < .01$) were related to higher levels of anxiety symptoms (see Figure 1). There was a non-significant association between the interpretational bias score and anxiety symptoms (standardized coefficient = -.06, $p = .69$). The model revealed that the cognitive variables were not significantly inter-correlated. In addition, there was a significant association between reduced physiological responsiveness and more negative interpretation biases (standardized coefficient = -.32, $p = .04$), but there was no significant mediating effect of the physiology latent variable on attentional bias. The non-significant paths were excluded, and models were compared using the likelihood ratio x^2 test to determine more parsimonious models. However, the initial full model provided the best fit to the data, indicating the role of both physiological and information processing factors in anxiety symptoms amongst young people with ASD (Figure 1).

Using cognitive and physiological measures to predict anxiety diagnoses in ASD.

Based on the results of our SEM we conducted a ROC analysis using logistic regression to examine how well attentional threat bias, HR responsiveness and/or cortisol responsiveness predicted the presence of anxiety disorder (as opposed to symptoms) within our ASD sample. In bivariate analyses, both cortisol ($\beta = 19.3$, $p \leq .01$; AUC = 0.89) and HR ($\beta = 6.3$, $p \leq .01$; AUC = 0.94) responsiveness were significant predictors of anxiety disorder. However, attentional bias was a poor predictor of anxiety diagnosis with an AUC just above chance level ($\beta = 0.01$, $p = .42$; AUC = 0.56). Comparison of the AUCs using x^2 statistics to identify the single best predictor revealed no significant difference between the cortisol and HR variables ($x^2 = 0.59$, $p = .44$), but both were superior to the attentional bias variable at predicting

anxiety diagnosis [cortisol (x^2 = 18.6, $p \leq .01$), HR (x^2 = 15.9, $p \leq .01$)]. In order to investigate the possible utility of combining the cortisol and HR variables into a single predictor, we generated a combined score by creating standard scores for both variables and then taking the average. This resulted in a marginally higher AUC of 0.95, which was not significantly better than using HR alone (x^2 = 0.16, p = .69).

Discussion

The aim of this study was to investigate whether cognitive processing biases were significantly related to anxiety in children and adolescents with ASD, and whether such biases are mediated by physiological responsiveness to psychosocial stress. Our results indicated that while both increased attentional biases to threat stimuli and a blunted HR and cortisol response to social stress are strongly related to anxiety symptoms, they represent independent pathways in our sample. Indeed, while both cognitive and physiological factors are related to the degree of anxiety symptoms reported in this sample, only the physiological parameters were significant predictors of current anxiety disorders.

Information processing biases in ASD and anxiety

Attentional and interpretational biases are commonly reported in relation to pediatric anxiety disorders (Bögels et al., 2003; Waters et al., 2008, 2010). In this study we found that young people with ASD and a co-occurring anxiety disorder had significant biases in both attention and interpretation compared with healthy controls. In the case of attentional biases, this was only when threat faces, and not threat words, were used. It is possible that the words used in this study lacked adequate emotional saliency to elicit a bias in the ASD sample. Research suggests that word stimuli should

be relevant to an individual's anxieties (Beck & Clark, 1997), and given that young people with ASD show some idiosyncrasies in their fears compared to children without autism (Ozsivadjian, Knott, & Magiati, 2012), future research may benefit from tailoring stimuli to the individual anxieties of a child with ASD.

Our results also indicate that children and adolescents with ASD and anxiety have more negative interpretational biases than healthy controls. It was also evident that this negative bias primarily relates to higher levels of social, but not physical threat in the ASD and anxiety group. This is particularly interesting given that social anxiety is sometimes reported to be one of the more prevalent anxiety disorders in ASD (Simonoff et al., 2008). We should note that the ASD participants with and without anxiety did not significantly differ on their degree of interpretation bias, and in fact, when re-analysed as a single ASD group (ASD vs. control) the difference with controls was statistically greater (data not presented). This suggests that this propensity to interpret ambiguous social situations as threatening may be more related to autism generally, rather than being anxiety-specific. However, this should be considered with the major caveat that social anxiety was not highly prevalent within this sample.

In fact, when comparing the scores across groups for the dot-probe faces task, the ASD group without anxiety were intermediate between the controls (low-bias score) and the ASD and anxiety group (high-bias score). Although differences between the ASD group without anxiety and controls were non-significant, the direction of these findings, if replicated in a larger sample, may suggest a greater propensity for a negative information processing style in ASD individuals generally. This may also explain why the attentional bias scores were not good predictors of anxiety diagnosis. It is also important to note that the SCAS scores in the ASD group were also intermediate between the control and ASDanx groups.

Reduced physiological responsiveness in ASD and anxiety

There have been a number of studies demonstrating that young people with ASD display a reduced HR and cortisol response to psychosocial stress compared with healthy controls. As we have previously reported, this pattern of physiological hypo-responsiveness relates to both elevated levels of anxiety symptoms and the presence of co-occurring anxiety diagnoses in young people with ASD (Hollocks et al., 2014), as well as elevated levels of irritability compared to TD controls (Mikita et al., 2015). These results, along with evidence from the same study that participants with ASD and controls experienced equivalent levels of subjective anxiety during the PST, may indicate a possible biological basis underlying the high prevalence of anxiety disorders in people with ASD.

While the mechanism by which a blunting of physiological responsiveness is related to anxiety in ASD is unclear, similar patterns of physiological response to psychosocial stress are associated with disorders of chronic stress including early childhood maltreatment (Danese & McEwen, 2012). Hence, the anxiety-related physiological response profile observed in ASD may relate to exposure to chronic stress over the course of childhood and therefore a blunted physiological response may occur as a consequence of high levels of long-standing anxiety in those with ASD. It is currently unclear whether or not this mechanism would be related more strongly to specific anxiety disorders or whether it is related more generally to anxiety severity or the number of co-occurring disorders.

One alternative explanation is that this pattern of reduced physiological responding, within a social context, may lead to the experience of anxiety. The normal

increases in the autonomic nervous system and hypothalamic-pituitary-adrenal axis under stress are biologically adaptive, and a physiological response that is less appropriate in a given situation may be maladaptive. While an interesting hypothesis, this suggestion is currently not supported by the literature. In order to investigate this hypothesis and to disentangle the problem of directionality further research is required using longitudinal designs and clinical trials. Regardless of whether these differences in physiological function are cause or effect, our results indicate that they are strong correlates of anxiety disorders in people with ASD.

Dual correlates of anxiety in ASD

The difference in the association of cognitive and physiological measures to anxiety disorders in ASD is consistent with the results of our SEM*,* which shows that attention bias and a blunted physiological response are significantly but independently related to anxiety symptoms. As alluded to above, this may reflect the possibility that each measure represents a different stage in the development of anxiety disorders. Some evidence suggests that cognitive biases precede the onset of an anxiety disorder, and that manipulating these biases can induce a positive or negative mood (Lothmann, Holmes, Chan, & Lau, 2011; Wilson, MacLeod, Mathews, & Rutherford, 2006). This has two implications for those with ASD; first, differences in cognitive processing style may be a risk factor for developing anxiety disorder, and second, these underlying cognitive differences may be good targets for early intervention. There is emerging evidence from the anxiety disorder literature that attentional bias modification training (ABMT) may reduce anxiety symptoms (Bar-Haim, Morag, & Glickman, 2011; Bar-Haim, 2010; See, MacLeod, & Bridle, 2009). The identification of dual cognitive and physiological pathways highlights that a combined cognitive and pharmacological approach to treatment of anxiety in ASD may be most appropriate.

Limitations, strengths and conclusions

The study has several limitations that should be considered when interpreting these results. These include the small sample size and the relatively large number of statistical comparisons conducted. The study design would have been strengthened by the inclusion of a non-ASD anxiety disorder group. This would have allowed for a direct comparison of task performance and provide more substantial evidence of either similar or different cognitive and physiological mechanisms in anxiety with and without ASD. Furthermore, this study included a heterogeneous sample of children and adolescents with ASD who suffered from a wide range of anxiety disorders, and in a few cases co-occurring symptoms of depression. While this may certainly add noise to our dataset, it does represent the clinical realities of working with this population. It is important to highlight that in our study the rate of social phobia was considerably lower than what has been suggested in the previous literature (Simonoff et al., 2008; van Steensel et al., 2011). However, other anxiety diagnoses which are less commonly reported in ASD, such as panic / agoraphobia, were particularly common in this sample. It is possible that young people with the most severe co-occurring social phobia may have refused to be enrolled in this study due to its challenging experimental paradigm. Furthermore, it is important to note that this study made use of a clinically selected sample and any inferences regarding the prevalence of various co-occurring anxiety problems should be made with caution.

Finally, there is some evidence that in adolescence sensitivity to tasks such as the psychosocial stress test increases (Sumter, Bokhorst, Miers, Van Pelt, & Westenberg, 2010). As this study includes both pre- and post-pubescent boys this may have had some influence on our results. However, the age range across each of the participant groups was similar and so the impact of this should be minimal. In a

related point, the current study included only male participants which may reduce our ability to generalize our findings to the whole ASD population. The decision to reduce potential variance by recruiting only males was made based on evidence of gender differences in cortisol response (Kirschbaum, Kudielka, Gaab, Schommer, & Hellhammer, 1999), which may be particularly pronounced in the adolescent period (Bouma, Riese, Ormel, Verhulst, & Oldehinkel, 2009). Given these limitations our results should both be interpreted with caution and independently replicated by other groups.

Nevertheless, this is the first study to demonstrate significant relationships between cognitive measures associated with anxiety in the typically developing population in a sample with ASD and co-occurring anxiety disorders assessed by semi-structured psychiatric interview. Previous studies have focussed only on anxiety symptoms measured by questionnaires. It is also the first study to combine both cognitive and physiological risk factors within a single model. In conclusion, we provide the first evidence for the role of both cognitive processing biases and differences in physiological responsiveness as pathways that may partially explain the high prevalence of anxiety in children and adolescents with ASD. These results also give some insight into possible treatment strategies that may be appropriate to manage anxiety in ASD. The presence of cognitive factors that are similar to those found in relation to anxiety in the non-ASD population supports studies that have suggested that CBT-based interventions for anxiety are of use in ASD (Sukhodolsky et al., 2013). However, consistent with findings in non-ASD youth that combining CBT with a pharmacological intervention is superior to intervention on its own (Walkup et al., 2008), an independent physiological pathway suggests that combining psychological therapies with such a pharmacological intervention may provide addition benefits as

well as offering an alternative approach for those who do not respond well to CBT or are of lower verbal ability.

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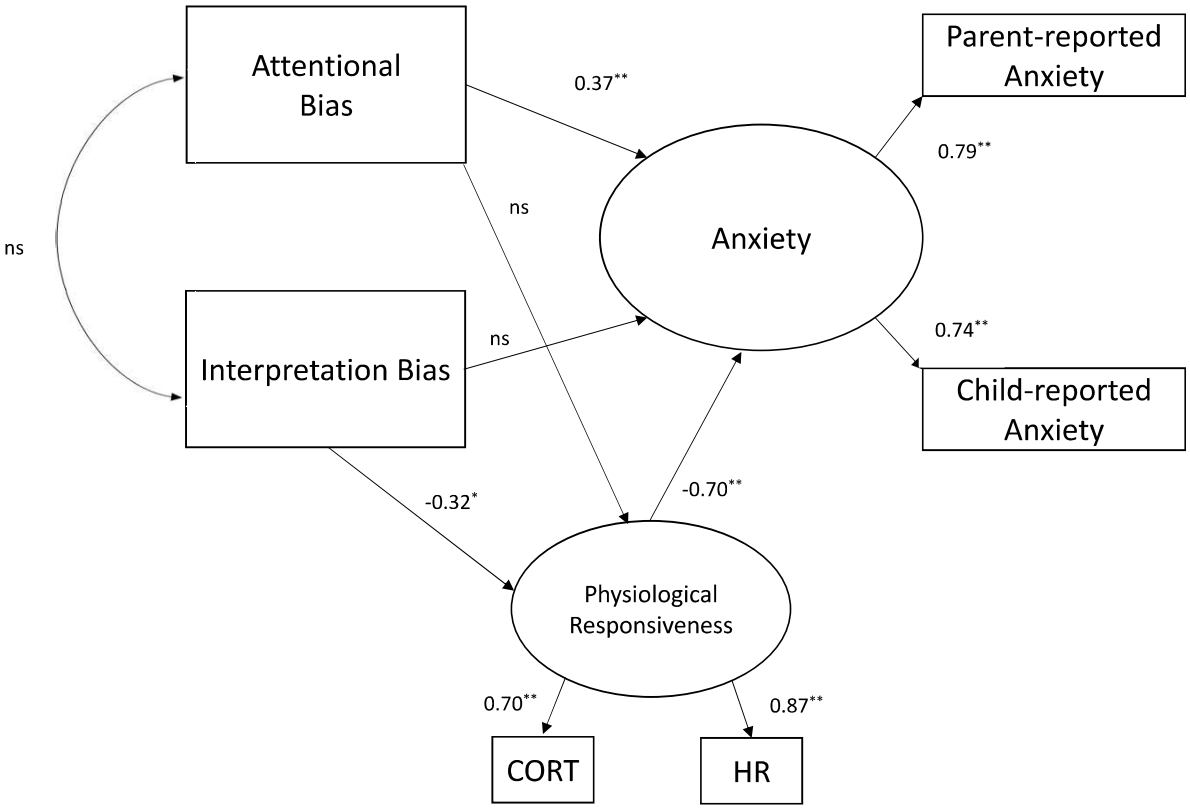
Figure 1; Final SEM model for ASD sample demonstrating independent significant pathways between both attention bias and physiological responsiveness to greater anxiety symptoms



Note: full-scale IQ is regressed onto all independent variable in the model but not shown here

Table

Table 1, Descriptive variables and anxiety scores across groups, Mean (S.D.; range)

	Control	ASD	ASDanx	F-test, (df)
Age in Years	13.9 (1.8;10-17)**a**	13.0 (1.9;10-16)**a**	12.8 (1.9;10-16)**a**	2.69 (2,80)
Full-Scale IQ	116 (9.5; 96-136)**a**	103 (16.7;76-138)**b**	99.7 (10.9;83-128)**b**	14.9 (2,80)
SCQ	1.9 (1.99;0-7)**a**	19.4 (5.7;10-31)**b**	24.7 (5.8;13-36)**c**	167.5 (2,80)
SCAS-P Total	7.0 (4.9;0-24)**a**	20.1 (11.7;3-45)**b**	41.8 (19.1;12-88)**c**	48.9 (2,77)
SCAS-P GAD	1.4 (0.1;0-4)**a**	3.9 (2.1;1-9)**b**	7.5 (3.5;1-14)**c**	44.5 (2,77)
SCAS-P Panic	0.3 (0.7;0-3)**a**	1.9 (2.1;0-6)**b**	5.8 (4.6;0-18)**c**	24.6 (2,77)
SCAS-P Separation Anxiety	0.6 (0.9;0-3)**a**	3.3 (2.7;0-11)**b**	7.2 (4.3;0-16)**c**	34.9 (2,77)
SCAS-P Social Phobia	2.6 (1.8;0-6)**a**	5.9 (3.4;1-12)**b**	9.7 (4.4;1-17)**c**	31.6 (2,77)
SCAS-P OCD	0.3 (0.7;0-3)**a**	2.2 (2.01;0-8)**b**	6.2 (3.7;1-16)**c**	40.8 (2,77)
SCAS-P Physical Threat	1.6 (1.9;0-8)**a**	3.3 (2.3;0-9)**a**	5.6 (3.6;0-15)**b**	15.3 (2,76)
SCAS-C Total	12.2 (6.9;0-25)**a**	23.7 (11.2;3-38)**b**	35.9 (16.9;5-72)**c**	25.3 (2,76)

a,b,c = different letters indicate significant group differences at $p \leq .05$

Table

Table 2, Mean attention and interpretation bias scores for the control, ASD only and ASDanx groups, Mean (S.D., Range)

Attentional Bias (ms)	Control	ASD	ASDanx	F-test, (df)
Threat face	-1.6 (23.1,-56.9–34.7)**a**	9.5 (21.01, -41.7-56.5)**b**	16.0 (31.5,-43.2–91.4)**c**	3.1 (2,78)
Happy face	1.4 (18.1, -45.4–48.7)**a**	-.2.9 (22.1, -61.9–46.5)**a**	3.1 (27.5, -41.7 – 96.3)**a**	0.3 (2,77)
Social threat words	-8.9 (43.1,-130–82.7)**a**	-6.3 (47.9,-109 – 92.8)**a**	7.03 (62.1, -173 – 149)**a**	0.7 (2,79)
Physical threat words	1.0 (30.4,-58.3–82.2)**a**	-20.6 (57.8, -206 – 62)**a**	-.5.25 (57.4, -172-143)**a**	1.1 (2,77)
Happy words	2.4 (24.9, -34.2-55.4)**a**	7.4 (43.9, -107 – 77.7)**a**	-8.6 (38.04, -87.0-86.1)**a**	0.7 (2,79)
Interpretation Bias				
Total Threat (0-8)	2.2 (1.9, 0–7)**a**	3.2 (1.8, 0–7)**b**	3.6 (2.1, 0–7)**b**	3.6 (2,76)
Social Threat (0-4)	1.4 (1.3, 0–4)**a**	2.0 (1.4, 0–4)**b**	2.4 (1.4, 0–5)**b**	4.8 (2,76)
Physical Threat (0-4)	0.8 (0.9, 0–3)**a**	1.2 (1.2, 0–4)**a**	1.2 (1.1, 0 – 3)**a**	.90 (2,76)

a,b,c = different letters indicate significant group differences at $p \leq .05$

Table 3. Correlation matrix for ASD sample displaying inter-correlations between all variables included in the SEM model

	Parent-reported anxiety	Child-reported anxiety	Threat Attention Bias	Interpretational Bias	Cortisol Response	HR response
Child-reported anxiety	0.72*					
Threat Attention Bias	0.38*	0.35*				
Interpretation Bias	0.23	0.31*	0.11			
Cortisol Response	-0.63*	-0.59*	-0.32*	-0.39*		
HR response	-0.67*	-0.61*	-0.33*	-038*	0.75*	
FSIQ	-0.29*	-0.33*	-0.07	-0.29*	0.44*	0.58*

FSIQ = Full-scale IQ, * = p ≤ .01

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