Baseline and Affective Startle Modulation by Angry and Neutral Faces
in 4-8 Year Old Anxious and Non-Anxious Children

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Abstract

The present study examined the magnitudes of startle blink reflexes and electrodermal responses in 4 to 8 year old high anxious children ($N = 14$) and non-anxious controls ($N = 11$). Responses were elicited by 16 auditory startle trials during a baseline phase prior to an affective modulation phase involving 12 startle trials presented during angry and neutral faces. Results showed significant response habituation across baseline trials and equivalent response magnitudes between groups during the baseline phase. The modulation of response magnitudes during angry and neutral faces did not differ significantly in either group. However, high anxious children showed larger responses overall compared with non-anxious control children during the affective modulation phase. Moreover, greater anxiety severity and larger startle reflexes were associated with poorer accuracy in rating neutral faces as neutral in high anxious children. Results may reflect elevated reactivity to threat contexts in 4 to 8 year old high anxious versus non-anxious children.

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Anxiety disorders are the most commonly diagnosed psychiatric disorders and one of the most significant health problems in terms of global burden of disease, exceeding the vast majority of physical health diseases (Murray & Lopez, 1996). Childhood-onset anxiety is a debilitating condition affecting up to 15-20% of youths and is a significant risk factor for other emotional and behavioural disorders including adolescent and adult anxiety (McGee, Feehan, Williams, & Anderson, 1992), depression (Hayward, Killen, Kraemer, & Taylor, 2000; Pine, Cohen, Gurley, Brook, & Ma 1998), eating disorders (Patton, 1998) and substance disorders (Merikangas, Avenevoli, Dierker, & Grillon, 1999). Childhood anxiety disorders are also associated with debilitating academic and vocational functioning (Kessler, Foster, Saunders, & Stang, 1995), impaired social competence (Spence, Donovan, & Brechmann-Touissant, 1999), and if left to persist into adulthood, a range of socio-economic costs including unemployment, days lost from work, hospitalisation and medication (Waghorn, Chant, White, & Whiteford, 2004).

Although research on childhood anxiety disorders lags behind research on adult anxiety in general, a combination of both genetic and environmental influences are thought to play contributory roles to the development and maintenance of childhood anxiety disorders (see Craske & Waters, 2005, for a review). The understanding of neurophysiological processes that underlie childhood anxiety disorders is particularly limited and based primarily on studies of children in mid- to late-childhood and adolescence (see Ornitz, 1999, for review). The current study extends this literature by examining baseline and affective modulation of the startle reflex in high anxious and low anxious control children between 4 and 8 years of age.

The magnitude of the human startle reflex indexes defensive responding to aversive stimuli and associated contexts (see Davis, 1998, and Grillon & Baas, 2003, for reviews). Rodent studies have shown that modulation of the startle reflex by *aversive contexts* (such as long duration bright lights) is mediated by the bed nucleus of the stria terminalis, whereas fear-induced modulation of the startle reflex by *explicit threat cues* (such as a cue previously paired with an electric shock) is mediated by the central nucleus of the amygdala (Davis, 1998; Walker, Toufexis, & Davis, 2003). As tendencies to react with fear and avoidance in a range of situations associated with anxiety-provoking stimuli are hallmarks of emotional and behavioural characteristics of anxious children (American Psychiatric Association, 1994), the study of startle reflex modulation by explicitly aversive stimuli and associated contexts may elucidate the neurophysiological processes that underlie anxiety disorders in children. Moreover, as an involuntary response present from birth, the startle reflex is a neurophysiological measure especially suited to the study of defensive responding in young children (see Ornitz, 1999, for a review).

Numerous studies have demonstrated increased startle reflex magnitude in adults with anxiety disorders in contexts associated with threat but not in response to explicit threat cues. For example, Grillon and colleagues demonstrated that adults with panic disorder and post-traumatic stress disorder (PTSD) showed sustained elevations in “baseline” startle reflexes elicited at the commencement of the experimental session that later involved the delivery of unpleasant electric shocks signalled by a cue. In contrast, participants with anxiety disorders did not differ from controls in startle reflex magnitude when elicited during explicit cues of threat (e.g., Grillon, Ameli, Goddard, Woods, & Davis, 1994; Grillon, Morgan, Davis & Southwick, 1998; Grillon & Ameli 1998; Grillon & Morgan, 1999). Baseline startle reflexes were not elevated when participants with PTSD were explicitly informed that no aversive stimuli would be presented during the entire experimental procedure (e.g., Grillon et al., 1998). Another study of police officers (Pole, Neylan, Best, Orr, & Marmar, 2003) demonstrated that severity of

PTSD symptoms correlated with startle reflex magnitude during low and medium threat conditions, when informed that no shock would be delivered, but did not correlate with startle reflex magnitude during high threat conditions that explicitly signalled potential shock. Thus, in all of these studies, adults with anxiety disorders did not show larger startle reflexes compared with low anxious adults in response to cues signalling explicit threat of electric shock. Instead, they showed elevated responding in contexts associated with threat (see Grillon, 2002).

Elevated baseline startle reflexes in individuals with anxiety disorders is thought to represent increased anxiety about the laboratory context associated with threat of an aversive stimulus (Grillon et al., 1998b). Indeed, Grillon, Baas, Lissek, Smith, and Milstein (2004) concluded that “sustained contextual anxiety, but not phasic explicit cue fear, differentiates anxiety-disordered from non-anxiety-disordered individuals” (p. 916). This conclusion reflects what has been called the “strong situation” effect (Lissek, Pine, & Grillon, 2006), in which anxious and non-anxious individuals respond equally to intensely aversive stimuli, whereas only anxious individuals also respond strongly to low intensity aversive stimuli relative to non-anxious individuals.

A small literature exists on fear-potentiated startle modulation in children with most studies based on children at risk for anxiety disorders by virtue of parental anxiety. For example, child and adolescent offspring of parents with anxiety disorders (age range 7 to 18 years) have exhibited elevated startle reflexes during the preceding baseline phase as well as during darkness-induced fear-potential protocols and during threat of an air blast to the larynx compared with low-risk offspring (Grillon, Dierker, & Merikangas, 1997; 1998; Merikangas et al., 1999). Similarly, whereas there were no group differences in startle reflex magnitudes during pictures of fear-relevant visual (i.e., snake picture) and auditory (i.e., 1000 Hz, 100 dB tone) stimuli, offspring of anxious parents (age range 7 to 12 years) displayed significantly higher electrodermal activity during the resting baseline and during the inter-trial intervals (Turner, Beidel, & Roberson-Nay, 2005).

A significant limitation in applying fear-potential protocols with young children is that threat of electric shock, air blasts to the larynx, and periods of time in complete darkness may be too aversive for young children to tolerate. One avenue for overcoming this limitation has been to employ a picture viewing paradigm that assesses affective modulation of the startle reflex (e.g., Bradley, Cuthbert, & Lang, 1993; Cuthbert, Bradley, & Lang, 1996). An extensive literature with adults has shown repeatedly that blink reflexes are larger while viewing unpleasant pictures in comparison to blinks elicited during neutral and pleasant pictures (Cuthbert et al., 1996). Affective modulation of the blink reflex is thought to reflect the activation of defensive motivational processes by the match in affective valence between unpleasant picture stimuli and the aversive auditory startle-eliciting stimulus (Bradley, Cuthbert, & Lang, 1999). Moreover, adult studies have demonstrated a robust relationship between enhanced affective startle modulation and high fearfulness (e.g., Cook, Hawk, Davis, & Stevenson, 1991; Hawk, Stevenson, & Cook, 1992).

Studies of affective modulation in normally developing children have produced results divergent from those with adults. McManis and colleagues were unable to demonstrate startle facilitation during unpleasant compared with neutral pictures in 7 to 10 year old children (e.g., McManis, Bradley, Cuthbert, and Lang, 1995) and found that girls but not boys showed the expected increase in startle reflex magnitude during unpleasant than pleasant pictures (McManis, Bradley, Berg, Cuthbert, and Lang (2001). Cook, Hawk, Hawk, and Hummer (1995) also did not find the adult pattern using affectively valent script-induced imagery in school-age children; almost identical startle magnitude was found during imagery of pleasure, joy, sadness, fear, and anger. Finally, Waters, Lipp, and Spence (2005) found that startle reflex magnitude did not differ significantly during unpleasant compared with neutral or pleasant pictures in 8 to 12 year old children.

Studies examining anxiety-related differences in affective modulation of startle in children have also produced conflicting results. Cook et al. (1995) found that startle responses of

children who scored higher on a fear survey schedule were smaller during unpleasant than pleasant imagery and were smaller in high fear compared with low fear children. Waters et al. (2005) found that anxious children showed larger blink reflexes overall during unpleasant, neutral and pleasant pictures compared with low anxious children. As affective startle modulation is strongest for startle reflexes elicited during highly arousing affective stimuli (Cuthbert et al., 1996), inconsistent results between children and adults have been attributed to the less arousing picture stimuli used with children compared with adults and the unreliability of imagery procedures with children (see McManis et al., 2001; Waters et al., 2005).

Emotional face stimuli in a picture viewing paradigm may be an approach that overcomes these limitations with very young children. Other advantages include the greater ecological validity of face stimuli than other emotion-evoking stimuli (Mogg & Bradley, 1998) and that emotional faces, such as angry ones, receive preferential processing over other salient stimuli due to their evolutionary association with threat to the safety of humans across the lifespan (see Öhman & Mineka, 2001, for a review). Recent functional neuroimaging and cognitive science studies with children and adolescents between 8 and 18 years of age show perturbed amygdala activation and attention allocation to angry and fearful faces compared with neutral ones in anxious versus non-anxious youths (e.g., Hadwin et al., 2003; Stirling, Eley, & Clark, 2006; Monk et al., 2006; Thomas et al., 2001; Waters & Lipp, in press; Waters, Mogg, Bradley, & Pine, in press). Moreover, recent studies of startle modulation by emotional faces with non-selected adults demonstrated larger startle reflex magnitudes when participants viewed angry faces versus other types of expressions (i.e., fearful, neutral, and happy) (Springer, Rosa, McGetrick, & Bowers, 2007). These findings suggest that emotional faces may possess greater sensitivity than broad-based affective picture stimuli for assessing neurophysiological processes that underlie anxiety in children, such as enhanced defensive responding to threat and associated contexts.

The present study

This study examined baseline and affective startle modulation in young anxious and non-anxious children between 4 and 8 years of age. This age range was selected to expand on previous studies that have not assessed children below 7-8 years of age. The study assessed whether high anxious children would show larger startle reflexes compared with non-anxious control children during a baseline phase prior to an affective modulation phase in which angry faces were presented. The study also examined whether high anxious children would show enhanced affective startle modulation relative to non-anxious control children, as indexed by larger startle reflexes during angry compared with neutral faces. Angry faces were selected because they are stronger signals of the presence of threat than are fearful faces (Whalen et al., 2001) and have been shown to elicit larger startle reflex magnitudes than fearful, happy or neutral expressions in adults (Springer et al., 2007). Moreover, in the absence of affective modulation studies in children using face stimuli, we compared responses during angry faces with those during neutral faces based on the cognitive science literature which has shown reliable anxiety-related attentional bias effects for angry faces compared with neutral ones in children (e.g., Hadwin et al., 2003; Stirling et al., 2006; Monk et al., 2006; Waters et al., in press). Skin conductance responses were also recorded as an adjunct or complementary measure to the startle blink reflex because it will reflect a defensive response to the startle eliciting stimulus. Similar results across the two measures were expected.

Method

Participants

Participants were 25 children aged 4 years, 4 months to 8 years, 6 months (12 girls; 13 boys). Of these children, 14 had a clinically-significant diagnosis of an anxiety disorder (M age = 6.08, SD = 1.44; 7 girls; 7 boys) and 11 were non-anxious controls (M age = 6.00; SD = 1.41; 5 girls; 6 boys). Written informed consent was obtained from parents prior to children's participation in an experimental protocol that was approved by the Institutional Human Research Ethics Committee. High anxious children were referred by paediatricians, local

community mental health agencies, school guidance counsellors and parents to the Griffith University Child and Adolescent Anxiety Disorders Research Program for assessment and treatment. Non-anxious control children were recruited from first year undergraduate psychology students who had children 4 to 8 years old. These students received course credit in exchange for their child's participation.

All children were born in Australia and spoke English as their first language. Twenty-one children (85%) lived with parents who were married and four children (16%) had parents who were separated/divorced. Children tended to come from average income Australian families according to the Daniel Prestige Scale (1983), a measure of Australian occupational prestige. There were no significant differences between groups on demographic variables.

Diagnostic status of high anxious children was determined using the parent interview schedule of the Anxiety Disorders Interview Schedule for DSM-IV (ADIS-C; Silverman & Albano, 1996) in which a clinician severity rating (CSR) of 4 or greater (scale 0 to 8) for at least their principal diagnosis was used to determine clinical significance (Silverman & Albano, 1996). Thus, inclusion criteria for anxious children was a principal diagnosis of an anxiety disorder with an ADIS-C CSR of 4 or greater, in the absence of externalising disorders, developmental disorders, psychosis, organic brain damage or vision impairments. Major depressive disorder (MDD) was not a reason for exclusion as long as it was not the principal diagnosis. No child met criteria for MDD. Thus, of the 14 high anxious children, six had a principal diagnosis of specific phobia, three had generalised anxiety disorder, three had separation anxiety disorder, and two had social phobia. The mean ADIS-C CSR of children's principal anxiety diagnosis was 6.43 ($SD = 1.91$) and comorbidity between anxiety disorders was high with children having an average of 3.64 anxiety diagnoses ($SD = 2.13$), predominantly specific phobias. The six children with a principal diagnosis of specific phobia all had comorbid diagnoses of either generalised anxiety disorder, social phobia or separation anxiety disorder, suggesting that emotional faces would be as pertinent to these children as those with principal

anxiety diagnoses that are interpersonally-focused. Parents of high anxious children also completed the parent version of the Spence Children's Anxiety Scale (SCAS-P; Nauta et al., 2004; Spence, 1998) which is a questionnaire containing a total score and subscale scores corresponding to each of the DSM-IV anxiety disorder diagnoses.

The non-anxious control group was determined by total scores within the non-clinical range on the SCAS-P (Nauta et al., 2004). Consistent with this, the mean SCAS-P total score of 31.53 ($SD = 13.52$) for high anxious children and 16.22 ($SD = 9.71$) for non-anxious control children are comparable to the normative means for anxiety-disordered children ($M = 31.8$, $SD = 14.1$) and non-clinical controls respectively ($M = 14.2$; $SD = 9.7$; Nauta et al., 2004).¹

Stimuli and Apparatus

Electrophysiological materials, equipment and data acquisition. Auditory startle-eliciting stimuli (105 dB, 0 ms rise time, 50 ms white noise bursts) were presented binaurally through stereophonic headphones (Sony, Model MDRV700). A Dell 19" CRT colour monitor was used to present the silent animal documentary used during the baseline phase and angry and neutral faces used during the modulation phase. To record electromyogram (EMG) activity of the orbicularis oculi associated with the startle reflex blink, two miniature Ag/AgCl electrodes (Med Assoc., TDE-023-48) were placed beneath the right eye approximately 10 mm apart edge to edge, and 9 to 11 mm below the lower lid margin (e.g., Ornitz, Guthrie, Kaplan, Lane, & Norman, 1986). The lateral electrode was placed 5 mm medial to the outer canthus. Vertical (lid movement) and horizontal (left and right eye gaze direction) electro-oculogram (EOG) activity associated with blink activity and eye movement was recorded for data quality control purposes (see Response Definitions, Data Screening and Statistical Analysis section), using two Ag/AgCl electrodes 1 cm in diameter. The vertical EOG electrodes were placed above the left eyebrow and on the cheek, equidistant from the pupil. The horizontal EOG electrodes were placed at the outer canthi. Skin conductance responses (SCRs) were recorded from two Ag/AgCl electrodes 1 cm in diameter (Grass, F-E9M-60-5), placed on the distal phalanx of the index and middle

fingers of the non-dominant hand. A ground electrode was placed on the centre of the forehead. The impedance level of electrodes was 15 KOhm or less.

Data from EMG, EOG, and SCR recordings were acquired using a Grass Instruments Amplifier System (Model 15RXI) and were digitized and sampled on-line using National Instruments LabVIEW Programming Software (v7) installed on a Dell Precision Workstation computer. EMG was full-wave rectified. EMG and EOG measures were AC amplified at a gain of 10,000 for EMG and horizontal EOG and 5000 for vertical EOG, using a Grass Instruments Model 15A54 Quad AC amplifier installed in the Grass Instruments Model 15 Amplifier System. The low and high frequency cut-off values were 30 Hz and 1000 Hz for EMG, .01 Hz and 30 Hz for vertical EOG, and 10 Hz and 30 Hz for horizontal EOG. SCR was DC amplified at a gain of 2000.

Face Stimuli. Still pictures of 3 male and 3 female young adults of Caucasian appearance taken from the NimStim Face Stimulus Set (www.macbrain.org) were used for each category of angry and neutral facial expressions (12 faces in total). Validity ratings of these faces indicate high agreement by both children and adults about the expressed facial emotions (Tottenham, Borscheid, Ellertson, Marcus, & Nelson, 2002). All facial images were digitized files sized to 506 pixels wide x 650 pixels high presented in full colour surrounded by a dark grey background in the centre of the 19" Dell computer monitor.

Subjective Ratings of Face Stimuli. Children provided ratings of the emotion shown on each of the 12 faces. Children were shown a colour picture of each face in random order and were asked to say what emotion they thought each face was showing, if any. The research assistant recorded if children correctly identified the relevant faces as angry or neutral and recorded verbatim any other emotions children reported. Children were told that they could say they were unsure if they did not know what emotion the face showed. All responses were subsequently coded into categories of neutral, angry, scared, sad, happy, surprised, and unsure.

Procedure

Upon initial contact with parents of high anxious children, a brief telephone screening was conducted based on the ADIS-C to ensure that anxiety was the child's principal problem and eligible families were invited to attend an assessment session at the Griffith University Psychology Clinic. High anxious children completed the experiment in a research laboratory opposite the psychology clinic in which parents completed questionnaires and the ADIS-C interview. The parent interview schedule of the ADIS-C was administered with the attending parent/s by a doctorate-level student in clinical psychology trained to reliability in the ADIS-C administration by matching diagnoses and clinical severity ratings for each diagnosis from interviews conducted by a trained diagnostician. All ADIS-C interviews were reviewed during clinical supervision for reliability and validity.

When parents of non-anxious control children contacted the research team in response to study advertisements, the same brief telephone screening was conducted to ensure that anxiety was not a principal concern for their child, and an appointment time was made with eligible families. Non-anxious control children completed the experiment in the same research laboratory as anxious children while their parents completed the SCAS-P.

After orientation to the laboratory setting, children and parent/s were informed that children's physical responses would be recorded while they heard loud, crackly sounds through headphones and watched a silent animal movie followed by seeing some faces of people showing different feelings, including calm and angry ones, presented on the computer monitor. Children were seated in an air-conditioned, experimental room adjacent to the control room and were in constant contact with a research assistant via a closed circuit camera and intercom system. After electrode connection, children completed a 5 min rest period during which time they sat quietly watching a silent DVD animal documentary on the computer monitor. The computer monitor was set approximately 1 m in front of the child at eye level. Children were then fitted with headphones and were informed they would hear a number of loud crackly sounds through the headphones while they continued to watch the silent animal documentary.

Children received a single startle stimulus to reduce initial reactivity prior to completing the experiment; it was excluded from analyses.

The startle baseline phase involved the recording of physiological responses to 16 startle stimuli while children continued to view the silent animal documentary to maintain their focus (e.g., Ornitz, Russell, Yuan, & Liu, 1996). The mean interstimulus interval (ISI) between startle trials was 22 sec (range 20-24 sec). Children were then instructed they would continue to hear the crackly sounds through the headphones while faces appeared on the computer screen showing different feelings. Elapsed time between the two phases was no longer than 2 min on average. During the affective modulation phase, physiological responses were recorded to a further 12 startle stimuli also with a mean ISI of 22 sec (range 20-24 sec) delivered during 6 angry faces (3 male; 3 female) and 6 neutral faces (3 male; 3 female) shown individually on the computer monitor for 6 s each. Angry and neutral faces were presented across 3 blocks with one male and one female face per block. Angry and neutral face blocks were presented in alternating order and were counterbalanced across participants. A startle stimulus was delivered at random between 3 and 5 sec after the onset of each face. A white fixation cross set against a dark grey background was displayed in-between face presentations and no startle stimuli were delivered during these periods. After the affect modulation phase, the headphones and electrodes were removed. The children were presented with each of the 12 faces again in the same order as before and asked to say out loud what feeling they thought was shown on each face. Their responses were recorded verbatim. Children were then debriefed and returned to their parents. All anxious children commenced group-based, cognitive-behavioural treatment for anxiety disorders within two weeks after participating in this experiment.

Response definitions, data screening and statistical analysis

The startle reflex was measured by electromyogram (EMG) activity of the orbicularis oculi and by skin conductance responses (SCR). Eye blink measures the muscle response whereas SCR measures the sympathetically mediated arousal response to a startle stimulus (e.g.,

Samuels, Hou, Langley, Szabadi, & Bradshaw, 2007). Electro-oculogram (EOG) activity associated with vertical lid movement and left and right eye gaze direction was measured for quality control purposes when quantifying EMG (Gehricke, Ornitz, & Siddarth, 2002).

Startle blink reflex responses. After application of a 2 ms moving average, EMG onsets were computed as departures that were two SDs above the mean pre-startle EMG (computed for 200 ms before the startle stimulus) and did not drop below that level for more than 10 ms within a 20-80 ms response onset window following startle stimulus onset (e.g., Ornitz et al., 1986). Amplitudes of EMG were defined as the difference between the mean pre-startle EMG and the peak of the response, expressed in microvolts (μV). Data were log transformed to normalise the distribution (Ornitz et al., 1986).

Trials were rejected if (1) the tonic EMG exceeded 5 μV or the vertical EOG revealed spontaneous blinks or saccades during the 200 ms pre-startle period, (2) if the onset of the startle blink response seen on either the EMG or vertical EOG channels was earlier than 20 ms following startle stimulus onset, or (3) if the behavioural observations recorded during the experimental session via closed-circuit video camera indicated gross body movement or drowsiness prior to the startle stimulus. Behavioural observations leading to trial rejection were augmented by additional channels of recording (electrocardiogram and horizontal EOG) that were useful in revealing excessive muscle tension, trunk or head movement, and gaze deviations before or during the startle trials. Drowsiness was also documented by slow rolling eye movement in the EOG channels. Six percent of trials were rejected using these criteria. Trials were scored as zero (9% of all trials) if no observable blink activity was evident in the EMG channel during the 20-80 ms response onset window and there was no reason to reject the trial. Participants with more than one-third of trials rejected or scored as zero ($N = 2$ high anxious children; $N = 1$ non-anxious control children) were excluded from analyses. Thus, analyses of startle reflex magnitude data were based on 12 high anxious children and 10 non-anxious control children.

SCR. The magnitude of SCRs elicited by the startle stimulus was defined as the difference between the trough and apex of the curve, expressed in microsiemens (μS), and commencing within 1-4 s following startle stimulus onset (Christie & Venables, 1980; Neumann & Waters, 2006). Trials were rejected if behavioural observations recorded during the experiment and activity in other channels indicated excessive drowsiness, movement, or behaviour such as deep sighing, coughing and sneezing. Trials were scored as zero if there was no observable SCR activity within the 1-4 s onset window. For consistency across measures, analyses of SCR data were based on data from the same 12 anxious children and 10 control children who had available startle magnitude data. SCR magnitude data were square root transformed to normalise the distribution (Christie & Venables, 1980; Neumann & Waters, 2006).

Labelling of face stimuli. The sum of correct labelling of angry and neutral faces was computed for each participant and expressed as a percentage of the total number of labels for each type of face. Thus, higher percentage values reflected greater accuracy in labelling the faces as either angry or neutral. For consistency across measures, analyses of the labelling data were based on responses from the same 12 anxious children and 10 control children who had available startle reflex and SCR data.

Analyses. Startle reflex and SCR magnitude data acquired during the startle baseline phase were divided into four blocks averaged over four trials per block and were analysed with separate 4 [Block; first, second, third, fourth] x 2 [Group: high anxious; low anxious control] repeated-measures analyses of variance (ANOVA). Independent samples *t*-tests were performed on startle reflex and SCR magnitude data during the first block of baseline trials to test for group differences in initial reactivity. Startle reflex and SCR magnitude data acquired during the affect modulation phase were averaged separately across the six trials for angry and neutral faces as preliminary analyses revealed that two consecutive trials of the same emotional face over three blocks did not produce a block effect. Similarly, as preliminary analyses indicated that whether

the face was male or female had no effect on results, face gender was not included as a factor in the final analyses. Thus, data were analysed with separate 2 [Face Emotion: angry; neutral] x 2 [Group: high anxious; low anxious control] repeated measures ANOVAs. Percent correct labelling of angry and neutral faces were analysed using a 2 [Face Emotion: angry; neutral] x 2 [Group: high anxious; low anxious control] repeated measures ANOVA. Correlation analyses, corrected for multiple comparisons with Bonferroni adjustments, were also performed between startle reflex and SCR data and children's percent correct labelling of the face stimuli and SCAS-P total scores.

Results

Startle baseline phase

Startle reflexes. As reported in previous studies of children (e.g., Ornitz et al., 1996), there was wide variation in startle reflex amplitude, with mean responses varying from 5.3 to 359.1 μV . Log transformations reduced this variability substantially. Figure 1, left panel, displays the mean startle reflex magnitudes expressed in log transformed units across four blocks of four trials. The repeated measures ANOVA revealed a significant Block main effect, $F(3, 18) = 3.67, p = .03, \eta_p^2 = .38$, reflecting that startle reflex magnitude decreased in all children across consecutive blocks of trials. However, there were no main effects or interactions involving the Group factor, all F 's < 1.92 , ns. The independent samples t -test comparing groups over the first block of trials was not significant, $t = .30$, ns.

Insert Figure 1 about here

SCR. Figure 1, right panel, displays the mean square root transformed SCR magnitude data across four blocks of four trials. The repeated measures ANOVA revealed a significant Block main effect, $F(3, 18) = 11.90, p < .001, \eta_p^2 = .67$. As with EMG magnitude, there were no

significant main effects or interactions with Group, all F 's < 1.09, ns. The independent samples t -test comparing groups over the first block of trials was not significant, $t = .35$, ns.

Affective modulation phase

Startle reflexes. Figure 1, left panel displays the mean log transformed startle reflex magnitude values during angry and neutral faces for each group. As can be seen, high anxious children showed larger startle reflexes overall compared with non-anxious control children, significant Group main effect, $F(1, 20) = 4.39$, $p = .04$, $\eta_p^2 = .18$. There were no significant main effects or interactions involving the Face Emotion factor, all F 's < 2.20, ns.

SCR. Figure 1, right panel also shows the mean square root transformed SCR magnitude values during angry and neutral faces for both groups. Similar to EMG magnitude data, the analysis revealed a significant Group main effect, reflecting larger SCRs in high anxious compared with non-anxious control children, $F(1, 20) = 4.71$, $p = .04$, $\eta_p^2 = .19$, whereas no other effects were significant, all F 's < 1.81, ns.

Response habituation across the entire experimental procedure

Additional analyses were performed to examine group differences in startle reflex and SCR habituation across the entire experimental procedure. As is evident in Figure 1, analyses for each group separately revealed that significant Block main effects for startle reflexes and SCR were associated with significant linear components in non-anxious control children (EMG: $F(1, 9) = 10.84$, $p = .009$, $\eta_p^2 = .45$; SCR: $F(1, 9) = 25.80$, $p < .001$, $\eta_p^2 = .74$) but significant linear and quadratic components in high anxious children (EMG: $F(1, 11) = 2.90$, $p = .05$, $\eta_p^2 = .30$; $F(1, 11) = 3.21$, $p = .04$, $\eta_p^2 = .31$; SCR: $F(1, 11) = 18.64$, $p < .001$, $\eta_p^2 = .63$; $F(1, 11) = 11.46$, $p = .006$, $\eta_p^2 = .51$). These results suggest that whereas non-anxious control children habituated across the entire experiment, habituation was interrupted by the presentation of emotional faces in high anxious children.

Labelling of emotional faces

Table 1 displays the mean percent correct labelling of angry and neutral faces for both groups. The analysis revealed a significant main effect of Face Emotion, $F(1, 20) = 16.58, p < .001, \eta_p^2 = .45$, reflecting significantly more accurate labelling of angry faces (97%) compared with neutral faces (64.4%) by all children combined. No other effects were significant, all F 's < 1 , ns. Of the incorrect labelling of neutral faces (see Table 1), 13.6% were labelled as sad, 14.4% as happy, and 3.8% as angry. Independent samples t -tests revealed no significant group differences in the emotion children incorrectly reported for neutral faces (all $p > .29$), and there were no significant differences in the percentages of different emotions chosen within each group, all F 's < 1.92 , ns.

Correlation analyses

Correlations between startle reflex and SCR magnitude data and percent correct labelling of angry and neutral faces for each group separately revealed a significant negative correlation for high anxious children between startle reflex magnitude during neutral faces and neutral labelling ($r = -.60, p = .01$). There were no significant correlations for non-anxious control children (all r -values $< .29$, ns) or for other measures in anxious children (all r -values $< .41$, ns). The significant correlation suggested that high anxious children who showed larger startle reflexes to neutral faces were also less accurate in labelling the faces as neutral. Additional correlations assessed the relationship between startle reflex and SCR magnitude and percentages of incorrect labelling of neutral faces as happy, angry and sad. Whereas there were no significant correlations for non-anxious control children (all r -values $< .47$, ns), larger startle reflex magnitudes were significantly associated with increased labelling of neutral faces as sad in high anxious children ($r = .68, p = .02$). There were no other significant correlations for anxious children (all r -values $< .46$, ns).

Correlations between children's anxiety severity and startle reflexes, SCR and correct and incorrect labelling of angry and neutral faces were performed using the SCAS-P total score given that parents of children in both groups completed this measure and because the ADIS-C

CSR ratings for high anxious children only has five values in the clinical range (i.e., 4 – 8), with the majority of anxious children (78%) obtaining the median score (6) or above. Correlations between SCAS-P total scores and anxious children's labelling of neutral faces revealed a significant negative correlation ($r = -.62, p = .01$), indicating that more severely anxious children were less likely to label neutral faces as neutral. There were no other significant correlations (all r -values $< .40$).

Discussion

Results revealed that 4 to 8 year old high anxious and non-anxious control children showed significant habituation of startle blink reflexes and electrodermal responses over 16 trials during the baseline phase. During the affective modulation phase, responses were undifferentiated during angry faces compared with neutral ones in both groups of children. However, responses were larger overall in high anxious children compared with non-anxious controls during this phase; a group difference that was not observed during the baseline phase. Finally, the labelling data revealed that both groups were highly accurate (97%) in judging angry faces, whereas they were marginally better than chance at categorising neutral faces (65%). For 35% of neutral faces, children labelled them as emotional (happy, sad, or angry). Moreover, in high anxious but not non-anxious control children, those with greater anxiety severity and larger startle blink reflexes were less accurate in labelling neutral faces as neutral and were more likely to label them as sad.

Results of the present study indicate that after receiving explicit instruction about the impending presentation of emotional faces, startle blink reflexes and skin conductance responses were larger in high anxious than non-anxious control children. Moreover, this appeared to reflect that whereas non-anxious control children habituated across the entire experiment, i.e., they showed no effect of the introduction of the emotional faces, habituation was interrupted by the presentation of emotional faces in high anxious children (see Figure 1). These results suggest that in an experimental procedure in which an affective modulation phase follows a startle

baseline phase, elevated anxious responding to emotionally threatening and neutral faces can interfere with response habituation in high anxious children.

These findings present important similarities with previous studies of fear-potentiated startle modulation in older at-risk youths and anxious adults (e.g., Grillon et al., 1994; 1997, 1998; 2005; Grillon & Ameli, 1998; Merikangas et al., 1999). In all of those studies, anxious and at-risk participants have shown elevations of the startle reflex during *contexts* associated with threat. One could argue that larger response magnitudes during angry and neutral faces in high anxious compared with non-anxious control children in the present study reflects a similar context effect since elevated responding was present also during neutral faces. A cue-specific effect would suggest that startle reflexes would have been larger during angry than neutral faces. However, prior studies have found that startle reflex magnitudes are elevated during the baseline phase prior to, as well as throughout, fear-potentiation protocols involving threat of electric shock, blasts of air to the larynx, or periods in complete darkness (e.g., Grillon et al., 1994; 1997; Merikangas, 1999). In the present study, there were no group differences in response magnitudes during the baseline phase. One explanation for this difference is that instruction about forthcoming presentations of emotional faces is less intensely aversive compared with upcoming electric shocks, air blasts and darkness. All of these “traditional” aversive stimuli have direct associations with potential physical harm and may evoke stronger defensive responding earlier in the experimental session in anxious individuals. Notably, in a study of aversive learning in anxious and non-anxious children (age range 7-14 years) (Liberian, Lipp, Spence, & March, 2006), significant group differences in startle reflex or SCR magnitudes were not found during a 12 trial startle baseline phase prior to a Pavlovian conditioning task involving a loud tone unconditional stimulus; a stimulus that also is less aversive than electric shock, air blasts and darkness (see Neumann & Waters, 2006). Another obvious difference between the present and previous studies in which elevated baseline responding in anxious and at-risk children was found is children’s age. Instruction about an event that will occur later in the

experimental session may be too abstract for very young children to comprehend. Further studies with adults and older youths that compare startle modulation by emotional faces versus the more traditional stimuli (e.g., electric shock) would help clarify these possibilities.

Startle reflex magnitude and electrodermal responses were undifferentiated during angry and neutral faces in both high anxious and non-anxious control children, findings that differ from a recent study with adults showing larger startle reflexes during angry faces than other emotional expressions (e.g., Springer et al., 2007). Instead, the present results appear consistent with mixed results on affective startle modulation in children (e.g., Cook et al., 1995; McManus et al., 1995; Waters et al., 2005). The lack of differential modulation to angry and neutral faces in children, but not adults, may reflect that children perceive neutral faces as more negative than do adults. Supporting this interpretation was the analysis of the children's labelling data, which indicated that they interpreted neutral faces with only 65% accuracy, instead describing about one-third of faces (35%) as emotional (predominantly happy and sad). Inspection of children's incorrect labelling data confirmed this was not associated with particular neutral faces over others. That there were no significant group differences is consistent with recent evidence of unperturbed facial expression labelling in anxious children relative to controls (Guyer et al., 2007). Thus, whereas children were highly accurate in discriminating neutral and angry expressions, neutral faces were emotionally ambiguous to children.

Startle reflexes can be potentiated by emotional processes, such as the affective valence of foreground stimuli (e.g., Bradley et al., 1999) and by attentional properties, such as stimulus interest value (e.g., Lipp, Siddle, & Dall, 1998). One possibility is that startle reflexes were enhanced during neutral faces due to increased vigilance associated with attempts to interpret ambiguous expressions. Thomas et al. (2001) found greater amygdala activation during neutral than fearful faces and poor categorisation of these expressions in children compared with reversed effects in adults. They concluded that because neutral faces were highly ambiguous to children, they produced amygdala activation due to continued efforts to interpret them.

However, larger startle reflex magnitudes and increased anxiety severity in high anxious children were associated with less accurate judgments of neutral faces and increased interpretations of them as sad. Therefore, another possibility is that emotionally negative interpretations of neutral faces may have modulated startle reflexes, particularly in high anxious children, which would suggest an emotional modulation account (e.g., Bradley et al., 1999). As the present study cannot elucidate the underlying processes that mediate these results, further research with anxious and non-anxious children in this age range will be required before firm conclusions can be drawn.

Although the present study extends previous investigations of defensive responding in threat contexts in anxious adults and older at-risk youths to very young children, there are notable limitations. The present study does not address the aetiology of group differences between anxious and control children. Although studies of older at-risk offspring of anxious parents demonstrate similar findings (e.g., Grillon et al., 1997; 1998; 2005; Merikangas et al., 1999), studies of younger children at risk are required to examine whether sensitivity to threat contexts and emotionally ambiguous faces reflects on neurophysiological vulnerabilities to childhood anxiety disorders. Future investigations will also need to include other negative emotional faces (e.g., sad faces) to examine differential reactivity to varying negative human emotions (e.g., Springer et al., 2007). Given that neutral faces appear to be highly ambiguous to children, future study designs should compare responses elicited during negative emotional faces with those elicited during happy expressions (Balaban, 1995) or during intertrial intervals, which may be a better “neutral” condition than potentially ambiguous neutral faces. It is also possible that making judgments of faces as “neutral” may be difficult for children in part because of demand characteristics and because the majority of faces were emotional which tend to be more salient in general. Future studies should also include a dimensional rating of emotion elicited by the faces (e.g., from unpleasant to pleasant). Such a rating may be more sensitive to anxiety-related differences in young children than categorical labelling of the faces. Another

consideration for future studies is whether adult versus child angry faces differentially influence responding in anxious relative to control children. Adult angry faces may be more likely than child angry faces to provoke anxiety and contextual threat in anxious children. Studies with larger sample sizes and equivalent age and sex distributions will also be important for examining developmental effects (Balaban, 1995; Springer et al., 2007) and to extend on previous studies showing sex differences in face processing (e.g., Thomas et al., 2001; McClure, 2000; McClure, et al., 2004) and fear-potentiated startle (e.g., Grillon et al., 1998; 2005). Moreover, as adult studies have shown differences in the physiology of various anxiety disorders (e.g., Cuthbert et al., 2003), future studies should include a larger sample that would permit these comparisons in anxious children. Future research may also benefit from utilising sound stimuli instead of, or in conjunction with, face stimuli. For example, Neumann, Waters and Westbury (in press) have shown that an unpleasant sound of metal scraping on slate was both tolerable and highly effective in eliciting unconditional responses in adolescents. Lau et al. (in press) have shown that a scream sound coupled with a fearful face produced anxiety-related differences in a fear conditioning paradigm in adolescents. Future designs should also include more than three blocks of two trials for each emotional face and attempt to assess children's judgments of emotional faces "on line" rather than after the experimental procedure. Finally, examination of the time course of processing emotional faces is an important direction for future studies given previous evidence that anxious children and adults show elevated startle magnitudes during unpleasant compared with neutral pictures and words very early during stimulus processing (i.e., 60 ms lead intervals) (e.g., Aitken, Siddle, & Lipp, 1999; Waters, Lipp, & Cobham, 2000; Waters et al., 2005).

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Footnotes

¹ Although the psychometric properties of the SCAS-P have been derived from large samples of anxious and non-anxious children between 6 and 18 years of age (e.g., Nauta et al., 2004; Spence, 1998), the SCAS-P was completed by all parents in the present study to avoid having incomparable anxiety measures for children below and above 6 years of age. Parents of children younger than 6 years of age had no difficulty completing the SCAS-P in relation to their child and their scores were similar to those relating to older children in their respective group.

Table 1

Percent correct ratings of angry and neutral faces and percent of neutral faces judged incorrectly as happy, sad or angry as a function of group.

Group	Percent Correct Judgments of Angry Faces	Percent Correct Judgments of Neutral Faces	Percent of Neutral Faces Judged as Happy	Percent of Neutral Faces Judged as Sad	Percent of Neutral Faces Judged as Angry
Non- Anxious Control (n = 10)	100 (00.00)	66.66 (38.49)	14.66 (21.08)	15.00 (16.57)	3.33 (2.22)
High Anxious (n = 12)	94.44 (14.97)	62.5 (37.01)	20.83 (36.32)	12.5 (20.26)	4.16 (2.99)

Figure Caption

Figure 1 Mean EMG (left panel) and SCR (right panel) magnitudes in response to auditory startle-eliciting sounds for high anxious ($n = 12$) and non-anxious control children ($n = 10$) during the four blocks of trials in the baseline phase and during angry and neutral faces in the affective modulation phase.