Measuring anxious responses to predictable and unpredictable threat in children and adolescents

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Abstract

Research has highlighted the need for new methods to assess emotions in children on multiple levels in order to gain better insight into the complex processes of emotional development. The startle reflex is a unique translational tool that has been utilized to study physiological processes during fear and anxiety in rodents and in human subjects. However, it has been challenging to implement developmentally-appropriate startle experiments in children. This paper describes a procedure that uses predictable and unpredictable aversive events to distinguish between phasic fear and sustained anxiety in children and adolescents. We investigated anxious responses, as measured with the startle reflex, in youth ($N = 36$, mean age[range] = 12.63 [7–17]) across three conditions: no aversive events (N), predictable aversive events (P), and unpredictable aversive events (U). Short-duration cues were presented several times in each condition. Aversive events were signaled by the cues in P, but were presented randomly in U. Participants showed fear-potentiated startle to the threat cue in P. Startle responses were also elevated between cues in U compared to N, suggesting that unpredictable aversive events can evoke a sustained state of anxiety in youth. This latter effect was influenced by sex, being greater in girls compared to boys.

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These findings indicate the feasibility of this experimental induction of the startle reflex in response to predictable and unpredictable events in children and adolescents, enabling future research on inter-individual differences in fear and anxiety and their development in youth.

Keywords

fear; anxiety; unpredictability; psychophysiology; startle reflex; sex differences

In order to understand the development of normal and pathological emotional processing, research on all components of emotional experiences in children and adolescents is crucial (Zeman, Klimes-Dougan, Cassano, & Adrian, 2007). Research on emotion faces at least two major methodological challenges, the induction of emotions and the measurement of emotion. Emotion induction in children can be problematic due to ethical considerations, especially if negative emotions are investigated. Regarding measurement, it has been argued that emotions should be assessed across several domains including verbal report, physiological activity, and overt behavior. Currently, the majority of studies utilize only a single measurement instrument and many studies assessing emotions are still relying soley on self-report or other-reports (e.g., parent report, teacher report) as sole source of information on the emotional experiences of a child (Zeman et al., 2007). This is problematic, because these different response systems (i.e. verbal-cognitive, behavioral, physiological) can be activated independent from each other (Lang, 1993). Furthermore, self-report measures are vulnerable to voluntary and involuntary distortions such as cognitive appraisal, social desirability, or recall bias (Dadds, Perrin, & Yule, 1998; Matt, Vazquez, & Campbell, 1992). Measuring physiological responses to emotional experiences can overcome some of these problems and add additional information that may not be consciously accessible. The goal of this study was to develop an experimental procedure to measure physiological responses in children and adolescents during two different emotional states: fear and anxiety.

Fear and anxiety are frequently used interchangeably, but although they are closely related concepts several lines of research suggest that they are two functionally different defense mechanism mediated by distinct brain structures (Davis et al., 2010). Fear is elicited by an imminent threat and leads to a phasic fight or flight reaction whereas anxiety is characterized by a sustained state of heightened vigilance and apprehension due to temporally uncertain danger. Research in rodents, healthy adults, and clinical populations shows that these differences are apparent across several levels of analysis. For instance, the structure of internalizing disorders has been described as consisting of a fear and an anxious misery factor (Cox, Clara & Enns, 2002; Krueger, 1999). The fear factor usually consists of phobias such as social phobia, specific phobias and agoraphobia as well as panic disorder, whereas the anxious misery factor is comprised of generalized anxiety disorder, posttraumatic stress disorder, dysphoria and major depression. Parallel to the distinction between human fear and anxiety, these emotions can also be distinguished in rodent defensive behaviors and their alteration by anxiolytic drugs. In a series of very elegant experiments Blanchard and colleagues (Blanchard, Yudko, Rodgers & Blanchard, 1993) distinguished between defensive behaviors evoked by a Fear/Defense Test Battery that involved the actual presence of a threat (e.g., a cat) and defensive behaviors provoked by an Anxiety/Defense Test Battery, in which rodents are presented with a potential threat (e.g., cat odor). Blanchard et al. showed that defensive behaviors to a potential threat were systematically altered by anxiolytic drugs, but defensive behaviors to a clearly identifiable and imminent threat were not. Distinct anatomical structures have been implicated in the mediation of these responses (Davis et al., 2010). Although the amygdala plays a crucial role in fear processing, the bed nucleus of the stria terminalis mediates responses to sustained

states of anxiety, suggesting a functional differentiation between fear and anxiety. Research on this topic has also been conducted in humans. Specifically, clinical and psychopharmacological observations have provided support for an empirical distinction between fear and anxiety (Davis et al., 2010).

Prior studies have been conducted solely on adults so it has been difficult to identify factors related to the emergence of this distinction. An extension of such a work to youth would enhance our understanding of the normal and pathological development of these emotions. Therefore, the primary goal of the present study was to examine the feasibility of a psychophysiological experiment designed to distinguish fear and anxiety in adults for use in children and adolescents.

The startle reflex, a protective response to abrupt and intense stimuli (Landis & Hunt, 1939), is an attractive tool to examine fear and anxiety mechanisms for various reasons. Because startle is an automatic/reflexive response, it is not primarily influenced by intentional control and is resistant to demand effects and response biases that can interfere with verbal reports and voluntary motor responses (e.g., reaction time). Furthermore, the amplitude of the startle reflex can be modulated by emotional states. In contrast to many other psychophysiological measures like skin conductance, the modulation of the startle reflex depends on the valence of the participant's emotional state and is not a mere index of emotional arousal. Finally, similar experiments can be conducted in animals and in humans, which make this methodology especially valuable for a translational approach (Davis et al., 2010).

The startle reflex is strongly potentiated (fear-potentiated startle) by a short-duration threat cue that predicts an imminent aversive event (e.g., shock). This effect is mediated by the central nucleus of the amygdala (Hitchcock & Davis, 1986). More recently, it has been shown that lesions of the amygdala fail to eliminate startle potentiation that is evoked in a more sustained manner (e.g., by administering shocks unpredictably). Rather, startle potentiation is suppressed by lesions of the bed nucleus of the stria terminalis (BNST). In fact, there is a double dissociation between the effect of lesions of the amygdala and BNST on startle potentiation to short- and long-duration threats, with lesions of the former affecting response to short-duration threat and lesions of the latter affecting response to long-duration threat (Davis, 1998). Given that a short-duration threat cue is associated with a highly predictive danger that evokes a phasic aversive response and that long-duration threat cues are associated with a sustained aversive state, it has been proposed that these two manipulations provide laboratory analogues of fear and anxiety, respectively (Davis et al., 2010).

In order to extend research on phasic fear and sustained anxiety to humans, Grillon and colleagues (2004) developed an experiment that assesses startle potentiation during alternating periods of predictable and unpredictable aversive events. A typical experiment consists of three conditions: neutral (N) in which participants are informed that they will not receive any unpleasant events, predictable (P) in which unpleasant events only occur during a specific cue, and unpredictable (U) where unpleasant events occur randomly. In a series of studies, Grillon and colleagues showed that adult patients with panic disorder or PTSD were selectively more sensitive to unpredictability than healthy controls free of any past or current psychiatric disorder. Patients showed stronger differences in startle potentiation between no-cue phases of the U and the N condition, but similar potentiation as healthy controls to predictable threat (Grillon et al., 2008; Grillon et al., 2009). These results support theories that unpredictability of potential threat is a key element in the development of anxiety disorders (Mineka & Zinbarg, 2006).

Applying this experiment in youth would be a significant methodological advance because it enables us to study normal and pathological developmental trajectories of fear and anxiety, which might be distinct from each other. The cross-species nature of the experiment and the increasing data available in animals will then allow us to draw inference on the neurobiological mechanisms behind these trajectories. From a clinical perspective, a stronger sensitivity for unpredictability may serve as a vulnerability factor for the development of anxiety disorders in children and adolescents. Therefore, identifying these differences in youth provides a developmental approach to fear and anxiety in order to uncover possible pathological mechanisms, risk factors, and potential targets for prevention.

To date, no age-appropriate startle experiment has been developed to assess these fear- and anxiety-related processes in youth. Adapting this experiment for use with children and adolescents is especially challenging because the applied aversive stimulus has to be sufficiently unpleasant to elicit enhanced startle potentiation due to unpredictability (Grillon et al., 2004). For example, we found that in adults while startle is reliably potentiated by a threat cue predicting a shock or an airblast to the neck, startle is potentiated in a sustained manner only during long periods of unpredictable shocks but not unpredictable airblasts (Grillon et al., 2004). Because electric shocks could not be employed in children due to ethical considerations, we investigated whether less aversive stimuli that were developed in our laboratory and have been used in earlier studies in adults and youth (Grillon et al., 1999; Lau et al., 2008; Lissek et al., 2005) would be adequate to potentiate startle during unpredictability.

As a secondary aim, we explored potential sex differences in fear-potentiated and anxiety-potentiated startle. It is well established that normal as well as pathological anxiety is generally higher in girls compared to boys (Costa, Terracciano, & McCrae, 2001; Kessler et al., 2005; Mount, Crockenberg, Jo, and Wagar, 2010; Van Oort, Greaves-Lord, Verhulst, Ormel, & Huizink, 2009). Sex differences in anxious behavior have been observed starting at a very young age. Mount et al. (2010), for example, report that mothers observed more anxious behavior in 2.5 year old daughters than sons, and higher levels of trait anxiety among girls have been found to persist into adulthood (Costa et al., 2001). The higher prevalence of anxiety disorders in women and girls (Kessler et al., 2005; Van Oort et al. 2009) suggests that sex may be a key factor in the development of pathological fear and anxiety. Previous analogue studies of humans and rodents using startle modulation have reported increased anxiety to uncertain threat but equivalent levels of fear to imminent threat in adult females compared to males (Grillon, 2008; Toufexis, 2007), consistent with the observation that the bed nucleus of the stria terminalis shows a strong sexual dismorphism (Allen & Gorski, 1990). We therefore hypothesized greater anxiety but not fear in girls compared to boys.

In summary, the main goal of the present study was to investigate the feasibility of the proposed experimental modulation of the startle reflex in response to predictable and unpredictable events in children and adolescents, and to explore whether the sex differences found in adults can be replicated in children. A successful adaptation would enable future psychophysiological research on inter-individual differences in fear and anxiety and their development in youth.

Methods

Participants

Participants included 48 children and adolescents (27 boys and 21 girls) recruited from the general population in the greater Washington DC area. All subjects were of Caucasian descent and lived in middle-class households. Participants were screened for psychiatric

disorders and all were free of any current diagnosis. The age of the participants ranged from 7 to 17 years (M = 12.63, SD = 3.05) and did not differ between boys and girls (t[46] = .46, p = .73). The study was approved by the National Institute of Mental Health (NIMH) Institutional Review Board and parental informed consent and child assent was obtained prior to participation.

Seven out of 48 participants refused to participate in the study after initial presentation of all stimuli (2 boys, 5 girls), and two subjects were excluded because of excessive movement and inattention during the experiment (1 boy, 1 girl). Three participants were not included in the analysis of the EMG data, due to an excess number of 0 startle responses throughout the experiment (2 boys, 1 girl). The final sample consisted of 36 participants (22 boys, 14 girls) with a mean age of 13.44 years (SD = 2.96). Age did not differ between boys and girls (t[34] = 1.13, p = .76). This sample was significantly older than the initial sample (t[46] = 3.61, p = .04), but groups did not differ in trait (t[37] = .46, p = .57) nor state anxiety (t[39] = .46, p = .65).

Procedure

We modified the original 'NPU' experiment (Grillon et al., 2004) to be developmentally appropriate by replacing electric shocks by an intense blast of air (80 psi) directed to the neck at the level of the larynx. Such stimuli are an effective means of potentiating startle and are well tolerated by adolescents (Grillon et al., 1999). Previous research has shown that the anticipation of the blast of air alone was not aversive enough to reliably elicit a potentiation of the startle reflex by unpredictable aversive events in adults (Grillon et al., 2004). We therefore added a second aversive stimulus: a briefly-presented picture of a fearful female face accompanied by a piercing loud scream. This stimulus-pair has previously been successfully used in adult fear-potentiated startle experiments (Lissek et al., 2005) and fear conditioning studies in children (Lau et al., 2008).

The experiment consisted of two recording blocks separated by a short 5-min rest period. Each block consisted of an initial habituation phase consisting of 6 startle stimuli, followed by three N, two P, and two U conditions. Each participant was presented with two condition sequences: P N U N U N P and U N P N P N U. During each condition, a colored geometric shape was displayed as a cue twice for eight seconds on a computer screen (a red square in the P condition, a green circle in the N condition and a blue triangle in the U condition). In the N condition, no aversive event was administered. In the P condition, an aversive event only occurred during the cue and in the U condition aversive events were administered randomly. A sentence at the top of the computer screen indicated the present condition at all times ("no unpleasant event" in the N condition, "unpleasant event only during red square" in the P condition, and "unpleasant event at any time" in the U condition). Startle responses were elicited during and between cues by a burst of white noise at 103dB (A) for 40ms with a near instantaneous rise time. For a schematic draft of a typical N, P, U condition see Figure 1.

Prior to the beginning of the experiment, the procedure was carefully explained to the participants and each stimulus was presented once. Participants were informed that they could withdraw from the study after this initial presentation of the stimuli as well as at any time during the experiment. Participants were instructed to avoid voluntary movement during the recording and to stay focused on the computer screen. They were monitored via a camera throughout the procedure.

The eyeblink component of the startle reflex was measured by an electromyography (EMG) with two electrodes placed under the left eye. Amplifier bandwidth was set to 30–500 Hz.

Recording and stimulation were controlled by a commercial system (Contact Precision Instruments, Cambridge, MA).

Additional measures

State and trait anxiety were measured before the experimental procedure with the State Trait Anxiety Questionnaire for children (STAIC; Spielberger, Edwards, Lushene, Montuori, & Platzek, 1973), which consists of 20 items for state and trait anxiety, respectively. Items measuring state anxiety assess how the child is feeling at the time of the assessment. All items begin with *"I feel…"* followed by three possible answers containing the same adverb (e.g. *"very happy"*, *"happy"*, *"not happy"*). Items measuring trait anxiety assess how often children and adolescents experiences certain feelings, thoughts or situations (e.g. "*I worry about making mistakes"*). These items are rated on a three point scale indicating frequency (e.g. *"hardly ever"*, *"sometimes"*, *"often"*). Both scales showed good internal consistency (trait anxiety α = .88; state anxiety: α = .84).

Immediately after the startle test, participants rated their subjective anxiety level during the cue and no cue components in the N, P, and U conditions. On a visual analog scale, participants were asked to rate their level of anxiety (i.e., "*how anxious were you while seeing this?*") using a response scale that ranged from 0 ("*not anxious at all*") to 10 ("*extremely anxious*").

Data analysis

EMG data was rectified and smoothed and the peak amplitude of the blink reflex was determined in the 20–100-ms time frame following stimulus onset relative to baseline. Baseline was defined as the average EMG level for the 50 ms immediately preceding stimulus onset. Amplitudes were averaged for startle responses during cue vs. no cue in each condition over the two blocks. Eyeblink/startle scores were converted in *t* scores, after standardization within subjects. Since similar results were obtained with the raw scores and with the *t* scores, only results of the raw scores are presented.

Fear was operationally defined as the difference between startle amplitudes during cue and no cue in the P condition (fear-potentiated startle). Anxiety was operationally defined as the difference in startle magnitude during the absence of a cue in the N and the U condition (anxiety-potentiated startle) (see Figure 1). The data was analyzed with repeated measures analysis of variance (ANOVA) and sex was entered as a between-subject factor.

We also explored possible age effects because of the participants' wide age range. Therefore, based on their age participants were placed into a child (7–11 years old) or an adolescent group (12–17 years old). There were no main or interaction effects of age on the primary outcome measures. Additionally, we calculated correlations between age and the difference scores of *"P cue"* minus *"no cue P"* (fear-potentiated startle) and *"no cue U"* minus *"no cue N"* (anxiety-potentiated startle). Because there were no significant correlations, age was removed from all subsequent analyses.

In order to assess associations of state/trait anxiety and startle amplitudes, we calculated correlations between state and trait anxiety and the difference scores for anxiety- and fear-potentiated startle described above. We also assessed correlations between these difference scores and subjective anxiety reports for every part of the experiment.

Results

STAIC scores

The mean observed state anxiety score was $M = 31.56$ ($SD = 5.47$) with a minimum of 24 and a maximum of 47. The mean observed trait anxiety score was $M = 31.07$ ($SD = 6.50$) with a minimum of 21 and a maximum of 43. Girls reported slightly more state anxiety ($M = 31.50$; $SD = 7.42$) compared to boys ($M = 30.20$; $SD = 3.62$), ($F[1, 32] = 4.46$, $p = .04$, $\eta^2 = .03$). Girls and boys did not differ in terms of self-reported trait anxiety ($F[1, 30] = 1.13$, $p = .30$; $M_{girls} = 31.00$ [$SD = 6.15$], $M_{boys} = 30.95$ [$SD = 7.29$]).

EMG amplitudes

Fear-potentiated startle—Startle amplitudes during the cue in the P condition ("*P cue*") and in the absence of the cue during the P condition ("*no cue P*") were entered into the analysis as the dependent variable ("*stimulus type*") and sex as the between-subject factor. There was a significant fear-potentiation (main effect *stimulus type*: $F[1, 34] = 40.46$, $p < .001$, $\eta^2 = .54$) and no significant main effect of sex ($F[1, 34] = 2.05$, $p = .16$). Boys and girls also did not differ in the amount of fear-potentiation (*stimulus type* x *sex*: $F[1, 34] = 2.04$, $p = .16$) (see Figure 2).

Anxiety-potentiated startle—Startle amplitudes in the absence of the cue during the N condition ("*no cue N*") and in the absence of the cue during the U condition ("*no cue U*") were entered into the analysis as a dependent variable ("*stimulus type*") and sex as the between-subject factor. Results showed a significant anxiety-potentiation (main effect *stimulus type*: $F[1, 34] = 42.56$, $p < .001$, $\eta^2 = .56$; see Figure 3) and no significant main effect of sex ($F[1, 34] = 1.99$, $p = .17$, $\eta^2 = .19$). However, boys and girls differed in the amount of anxiety-potentiation (*stimulus type* x *sex*: $F[1, 34] = 7.99$, $p = .008$) with girls showing a stronger potentiation than boys (see Figure 3).

Anxiety ratings

Fear-potentiation—Subjective anxiety ratings during the cue in the P condition ("*P cue*") and in the absence of the cue during the P condition ("*no cue P*") were entered into the analysis as a dependent variable ("*stimulus type*") and sex as the between-subject factor. Children and adolescents rated their anxiety level significantly higher during *P cue* vs. during *no cue P* (main effect *stimulus type*: $F[1, 38] = 25.26$, $p < .001$, $\eta^2 = .48$). There was no main effect of sex ($F[1, 38] = .02$, $p = .90$) and no difference in potentiation of anxiety ratings between boys and girls (*stimulus type* x *sex*: $F[1, 38] = .01$, $p = .94$) (see Figure 4).

Anxiety-potentiation—Subjective anxiety ratings in the absence of the cue during the N condition ("*no cue N*") and in the absence of the cue during the U condition ("*no cue U*") were entered into the analysis as the dependent variable ("*stimulus type*") and sex as the between subject factor. There was a significant difference in the ratings of anxiety levels during the *no cue N* vs. the *no cue U* (main effect *stimulus type*: $F[1, 38] = 83.87$, $p < .001$, $\eta^2 = .71$) and there was no main effect of sex ($F[1, 38] = .48$, $p = .50$), but there was a trend for an interaction with sex (*stimulus type* x *sex*: $F[1, 38] = 3.75$, $p = .06$, $\eta^2 = .09$) (see Figure 5).

EMG amplitudes, anxiety ratings and state/trait anxiety

EMG amplitudes and state/trait anxiety—None of the correlations between fear-potentiated startle or anxiety-potentiated startle and the trait or state anxiety reached significance. Correlations remained nonsignificant when the sample was stratified by sex.

EMG amplitudes and anxiety ratings—Fear-potentiated startle was not correlated with any of the anxiety ratings but anxiety-potentiated startle was correlated with subjective anxiety ratings throughout the U condition (anxiety during "no cue U": $r = .37$, $p = .03$; anxiety during "U cue": $r = .39$, $p = .02$). None of the correlations reached significance when the sample was stratified by sex.

Discussion

Previous research has highlighted the need to develop and refine methods to assess emotions in children using multiple levels of analysis in order to gain better insight into and more complete understanding of the complex processes of emotional development (Zeman et al., 2007). The main goal of the presented study was to develop an experimental procedure to assess and differentiate between fear and anxiety in children and adolescents by measuring the startle response during anticipation of predictable and unpredictable aversive events that are developmentally-appropriate (i.e., scream, strong air puff). Consistent with adult data (Grillon et al., 2004) children and adolescents show significant fear-potentiated startle to a cue that predicts an unpleasant event as well as anxiety-potentiated startle caused by anticipation of unpredictable unpleasant events. These results are corroborated by similar differences in self-reported anxiety levels. Consequently, this experiment adds incrementally to the assessment base by providing a non-invasive and feasible tool for studying fear and anxiety in youth.

The present study found sex differences in anxiety-potentiated startle but not fear-potentiated startle; relative to boys, girls showed stronger potentiation during the unpredictable condition, suggesting a higher level of vulnerability to sustained anxiety. Although these results are based on a small sample size, especially in the female group ($N = 14$), they are in line with a previous study using the same experimental procedure in adults (Grillon, 2008), and with neuroanatomical and physiological data from other human and animal studies (Allen & Gorski, 1990; Toufexis 2007). In a review on sex-specific modulations of anxiety behaviors in rats, Toufexis (2007) concluded that females display increased potentiation of the startle reflex due to sustained states of anxiety, but not short-duration cued fear. In human research, a sexual dimorphism in the BNST has been described (Allen & Gorski, 1990) that may be related to the observed sex differences. Specific sex differences in startle potentiation due to anxiety (and not to cued fear) could also be an indicator of a sex-specific vulnerability factor for anxiety disorders. Prevalence rates of anxiety disorders are higher in women and girls (Kessler et al., 2005; Van Oort et al., 2009) and findings in adult patients suffering from anxiety disorders show a very similar startle pattern: a selective increase in startle potentiation due to unpredictability (Grillon et al., 2008; 2009).

State and trait anxiety were not related to fear- and anxiety-potentiated startle, but the subjective anxiety ratings during the unpredictable condition correlated with anxiety-potentiated startle. These correlations were not detectable within each sex group, which is most likely attributable to a lack of power due to the small sample size in these subgroups. The lack of correlation between state and trait anxiety and potentiated startle is not uncommon; potentiated startle abnormalities are more consistently related to pathological states of anxiety (Grillon & Baas, 2003). Our exclusion of participants with mood and anxiety disorders may also have led to a restricted range of self-reported anxiety. Studies with larger variability or extreme groups might be better suited to address the association between self-reported anxiety and fear- and anxiety-potentiated startle. However, the association between anxiety-potentiated startle and the anxiety ratings during the unpredictable condition show that startle was sensitive to the self-reported anxiety level during the experiment. Therefore, despite the absence of an association with trait and state

anxiety, anxiety-potentiated startle contributes to our understanding of the reactivity of the defensive system. This measure provides a psychophysiological complement to self-reports of anxiety that may be vulnerable to intentional distortion and individual perception. Although studies have shown that self reports of anxiety in children have good reliability and validity in general (e.g., Muris, Merckelbach, Ollendick, King, & Bogie, 2002), some research suggests these measures might be biased, for example, by social desirability (Dadds et al., 1998; Pina, Silverman, Saavedrea, & Weems, 2001). Dadds and colleagues (1998) found that young girls have higher scores on the lie scale of the Revised Children's Manifest Anxiety Scale (RCMAS) than young boys, which suggests that their responses are influenced by social desirability. Lie scores of girls were also correlated with teacher ratings of anxiety, but lie scores were not associated with self report of anxiety. These results indicate that girls that are perceived as anxious by others are under-reporting their anxiety. Similarly, younger children exhibited higher lie scores than older children (Dadds et al., 1998; Pina et al., 2001). The authors argue that this might reflect a normal developmental process. Younger children might not be able to distinguish between their actual and ideal behavior, which points out another limitation of self reports in younger children.

The results of the present study should be considered within the context of its strengths and limitations. This study had several strengths. First, all participants were evaluated clinically. Subjects with psychiatric disorders, including mood and anxiety disorders, were excluded, providing a normative sample for future comparison. Second, the experiment was based on a robust paradigm that has provided clinically- and psychopharmacologically-relevant data in adults. Further, the paradigm was designed based on animal models that have provided evidence for a neural differentiation between fear and anxiety. Third, unpredictability is a key component of several theoretical accounts of anxiety disorders. Unpredictability has been linked to PTSD (Foa, Zinbarg, & Rothbaum, 1992) and panic disorder (e.g., unpredictable panic attacks) (Craske, Clover, & DeCola, 1995). It is also linked to the concept of intolerance to uncertainty (i.e., the tendency to react negatively to uncertain events and stimuli) -- a cognitive vulnerability factor for excessive worry and generalized anxiety disorder (Koerner & Dugas 2008). Research on this topic in children is scant (cf. Comer et al., 2009). It will be informative to examine the extent to which psychometric measures of intolerance to uncertainty in children correlate with anxiety-potentiated startle, potentially providing cues to physiological pathways underlying individual differences in intolerance of uncertainty. Lastly, verbal threat procedures have good face validity to study fear and anxiety mechanisms in children. Indeed, verbal threat information is a major pathway to fear in children (Field & Lawson, 2008). Reactivity to threat measured during the experiment may therefore be similar to children's experience when they are facing a threat outside of the laboratory.

Among the limitations, the observed sex difference should be interpreted with caution due to the small group sizes. Nevertheless, the concordance of our findings with previous studies in adults (Grillon et al., 2008) is encouraging and further research will determine the extent to which this is a stable characteristic in children and adolescents. A second limitation was the relatively wide age range of participants. Although no significant age effect was detected, subtle differences may still have been present but not apparent in this relatively small sample size, particularly in the presence of sex differences. A third limitation is the selective dropout of very young children. Although this is a limitation of the present study, it underscores the importance of the development of age appropriate measures to assess emotions over the full developmental span. Procedures with milder aversive stimuli need to be developed to assess reactions to predictable and unpredictable events in younger children. Nevertheless, it is important to note that the final sample did not differ from the initial sample in terms of self-reported state/trait anxiety.

Future research replicating and extending these findings will have significant developmental implications in several areas. The present experiment can be applied to investigate the development of fear, anxiety and related concepts such as intolerance of uncertainty over time. This will add critical information about individual differences in physiological processes during these emotional experiences in children. Fear and anxiety are often used interchangeably in everyday language, but have been shown to represent distinct emotional processes (Davis et al., 2010). It is very likely that children and adolescents would have great difficulties to distinguish between fear and anxiety in self-reports. The newly developed experiment is therefore unique in its potential to distinguish between fear and anxiety in children. Another key issue that can be investigated with the presented experiment is whether sex differences in threat processing are present early in development (Silverman, La Greca, & Wasserstein, 1995; Van Oort et al., 2009) or emerge during puberty (Craske, 2003). Future studies should examine the effect of puberty on sex differences in the development of fear and anxiety. In clinical research, the experiment can be implemented in children and adolescents with anxiety disorders to investigate whether startle modulation identified in adults with anxiety disorders (Grillon et al., 2008, 2009) can be replicated in youth with these conditions. This will provide valuable insights into whether the underlying mechanisms are already altered at a young age in contrast to being a consequence of a lifetime history of heightened anxiety. Furthermore, longitudinal studies will help to clarify whether abnormalities in fear and/or anxiety pre-date the development of anxiety disorders. Such studies could be conducted in children at risk for anxiety disorders (e.g., children of parents with an anxiety disorder or children with high levels of behavioral inhibition). Earlier work in our group showed that children of parents with anxiety disorders exhibited overall elevated startle responses in a threatening context (Grillon, Dierker, & Merikangas, 1997). They also show differential fear-potentiated startle modulated by sex (Grillon, Dierker, & Merikangas, 1998). Past studies were not able to distinguish whether these abnormalities mapped onto the fear/anxiety distinction. The newly developed experiment will help us further identify specific risk mechanisms for anxiety disorders.

In conclusion, the developed experiment combines an efficient emotion induction with a powerful measurement technique to study fear and anxiety in youth. The cross-species nature of this methodology provides a novel translational approach to study the development of normal and pathological processing of fear and anxiety.

Acknowledgments

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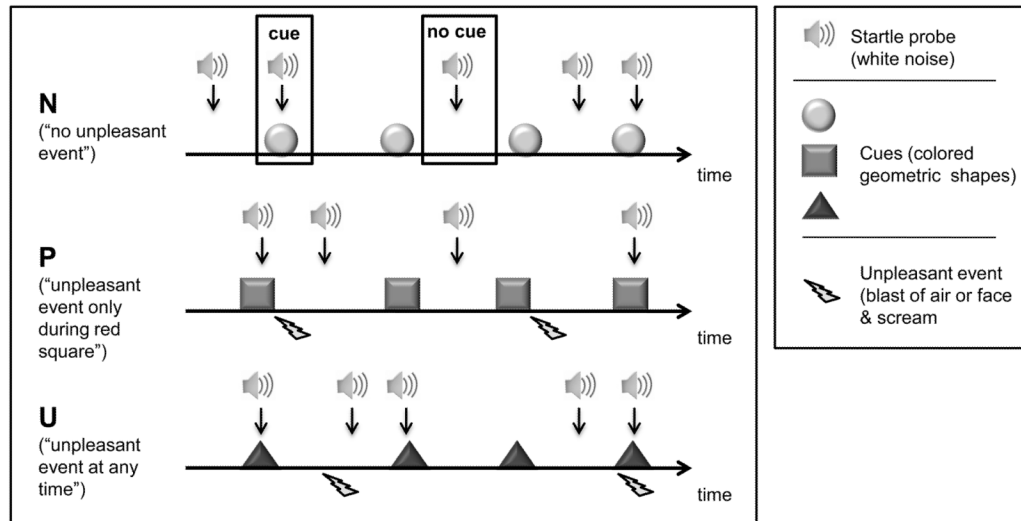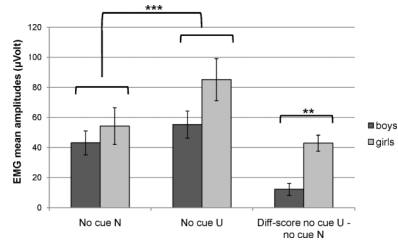
Figure 1.
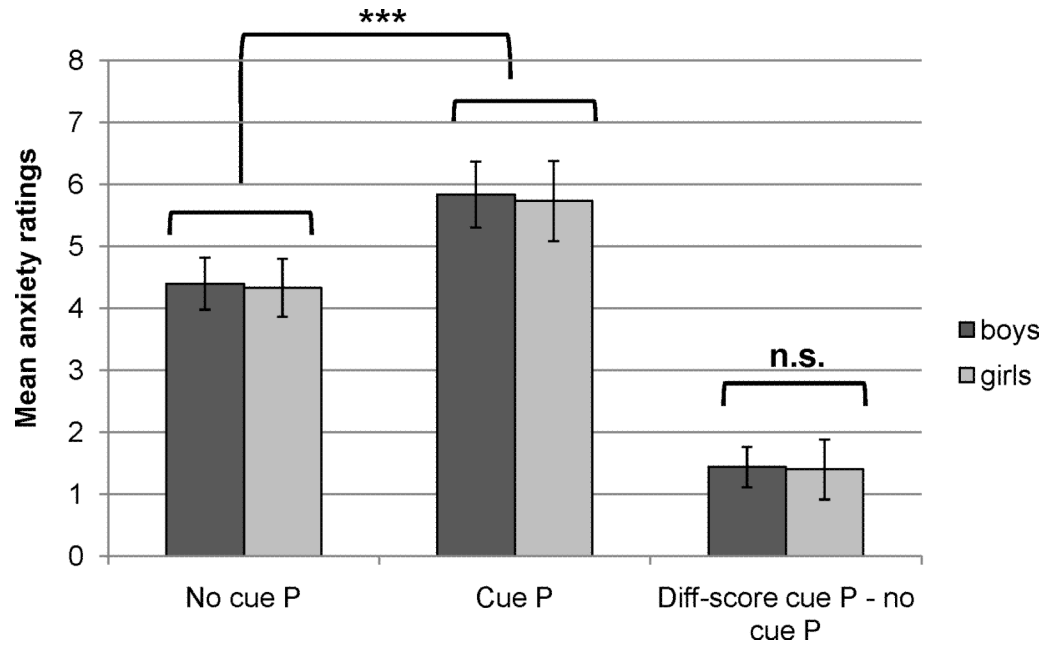Schematic draft of typical N, P, and U condition
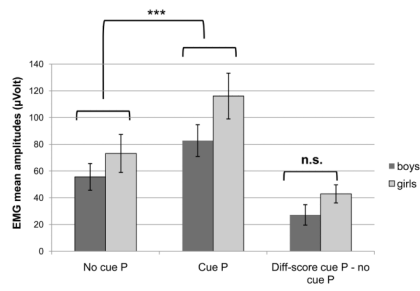
Figure 2.
Fear-potentiation: Mean EMG amplitudes during the cue and the absence of the cue in the P condition stratified by sex. Difference scores (Diff-score) illustrate the amount of potentiation. Error bars represent standard errors.
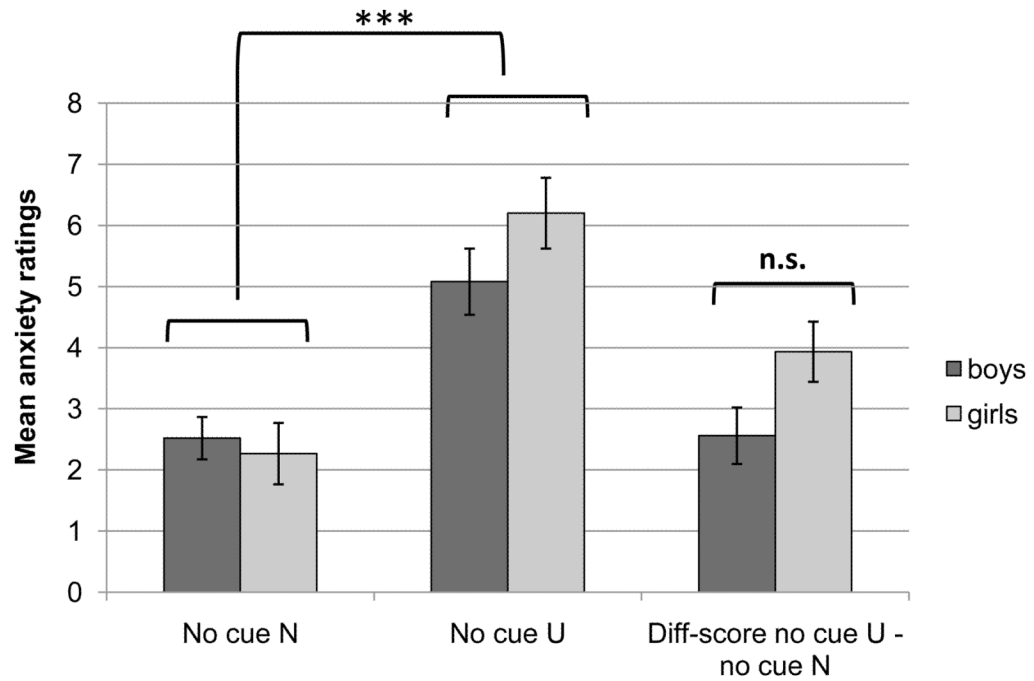
Figure 3.
Anxiety-potentiation: Mean EMG amplitudes in the absence of the cue in the N and U condition stratified by sex. Difference scores (Diff-score) illustrate the amount of potentiation Error bars represent standard errors.

Figure 4.
Fear-potentiation: Mean anxiety ratings during the cue and the absence of the cue in the P condition stratified by sex. Difference scores (Diff-score) illustrate the amount of potentiation. Error bars represent standard errors.

Figure 5.
Anxiety-potentiation: Mean anxiety ratings in the absence of the cue in the N and U condition stratified by sex. Difference scores (Diff-score) illustrate the amount of potentiation. Error bars represent standard errors.