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Developmental investigation of fear-potentiated startle across puberty

Anja Schmitz,

Genetic Epidemiology Research Branch, National Institute of Mental Health (NIMH), Bethesda, MD, USA

Christian Grillon,

Section on Neurobiology of Fear and Anxiety, National Institute of Mental Health (NIMH), Bethesda, MD, USA

Shelli Avenevoli,

Division of Developmental Translational Research, National Institute of Mental Health (NIMH), Bethesda, MD, USA

Lihong Cui,

Genetic Epidemiology Research Branch, National Institute of Mental Health (NIMH), Bethesda, MD, USA

Kathleen R. Merikangas

Genetic Epidemiology Research Branch, National Institute of Mental Health (NIMH), Bethesda, MD, USA

Abstract

The goal of this study was to examine the association between affective development, puberty, and gender using the startle reflex as a marker of defensive mechanisms. Thirty-one male and thirty-five female adolescents aged ten to thirteen participated in a prospective study with up to five assessments. Longitudinal analyses revealed a significant effect of sex, with girls showing stronger fear-potentiation at all pubertal stages. Post hoc tests revealed that fear-potentiation increased in girls but not boys over the course of puberty. Furthermore, baseline startle decreased over the course of puberty. Because age was included as a covariate in all analyses, the puberty effect cannot be accounted for by age. To the best of our knowledge, this study provides the first evidence for a significant increase in fear-potentiated startle across the pubertal transition. Attribution of these changes to pubertal status rather than age has important implications for our understanding of the neurobiology of anxiety and affect regulation.

Correspondence concerning this paper should be addressed to: Kathleen R. Merikangas, Intramural Research Program, National Institute of Mental Health, 35 Convent Drive, MSC#3720, Bethesda, MD 20892, USA, Phone: 301-406-1172, Fax: 301-480-2915, Kathleen.Merikangas@nih.gov.

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Keywords

Fear-potentiated startle; adolescence; puberty; sex differences; anxiety; development

Puberty is a developmental time-period characterized by acute changes in physiological and neural systems as well as behavior (Dahl, 2004). It is associated with intense emotional experiences, which are part of normal development, but may also comprise risk factors for emerging psychopathology during adolescence. It has been proposed that the relative maturity of limbic structures in adolescence, most notably the amygdala, as compared to not yet fully developed prefrontal regions involved in emotional control, might lead to more pronounced reactivity to emotional cues in general during this period (Somerville, Jones, & Casey, 2010). Furthermore, some studies in rodents suggest that although simple forms of fear-learning are already present in very young animals, more complex forms develop during adolescence (Kim & Richardson, 2010; Rudy, 1993). In addition to enhanced reactivity, puberty is marked by an increased incidence of depression and symptoms of anxiety (Angold & Costello, 2006; Reardon, Leen-Feldner, & Hayward, 2009), and there is now compelling evidence that advancing pubertal stage rather than chronological age accounts for the early adolescent rise in female emotional symptoms (Angold & Costello, 2006; Patton et al., 1996; Patton & Viner, 2007; Reardon, et al., 2009). However, the relationship between pubertal status and sex differences in depression and anxiety is complex since there appears to be greater reactivity to stress among females compared to males prior to the onset of puberty (Hillman, Rosengren, & Smith, 2004; McManis, Bradley, Berg, Cuthbert, & Lang, 2001). Although sex differences in reactivity to threat during adolescence have been studied extensively in cross-sectional designs (for review, see Ordaz & Luna, 2012), there is currently limited knowledge of the mechanisms that underlie the impact of puberty on mood and emotional regulation across stages of pubertal development based on prospective studies that simultaneously evaluate the impact of pubertal status and age.

One tool that has been used as a potential objective index of defensive reactivity and propensity to anxiety is the startle reflex, a reflex response to an abrupt and unexpected stimulus that is potentiated by fear and anxiety. Extensively studied in both animal models and human populations, the potentiated startle reflex is a compelling tool because of abundant basic research that informs its underlying anatomic and functional basis, and potentially sheds light on the neural circuits involved in fear and anxiety (Davis, Walker, Miles, & Grillon, 2010; Grillon, Dierker, & Merikangas, 1998; Lang & Davis, 2006). With its grounding in basic, behavioral and neurophysiological research, and consistent pattern across human and animal studies (Davis, et al., 2010; Koch, 1999; Lang & Davis, 2006), the startle reflex provides a powerful tool to investigate developmental changes in defensive reactivity across species.

Studies of defensive reactivity using startle paradigms have largely focused on adults, although studies in children and adolescents have been emerging over the past decade (Barker, Reeb-Sutherland, & Fox, 2013; Borelli, Sbarra, Crowley, & Mayes, 2011; Glenn, Klein, Lissek, Britton, Pine, & Hajcak, 2012; McManis, et al., 2001; Quevedo, Benning, Gunnar, & Dahl, 2009; Reeb-Sutherland et al., 2009; Waters & Ornitz, 2005). The relative

lack of studies in children and adolescents is partly due to difficulties in inducing aversive affective states that are developmentally and ethically appropriate. For example, shocks, which are frequently used in adult human and animal fear-potentiated startle studies, cannot be employed in children because of Investigation Review Board and parental concerns. Similarly presenting unpleasant pictures in children has not consistently yielded reliable startle potentiation (Armbruster et al., 2010; McManis, et al., 2001), probably because only mildly aversive stimuli can be used. We have shown that a blast of air to the larynx is one of the few aversive stimuli that potentiates startle in an efficient, replicable, and robust manner in children (Grillon et al., 1999; Schmitz et al., 2011). Using an instructed fear paradigm with this aversive stimulus, we found marked differences in startle reactivity between children with high and low vulnerability for anxiety disorders (Grillon, et al., 1998; Waters et al., 2008) and for major depression (Grillon, Dierker, & Merikangas, 1997; Grillon, et al., 1998). However, even though our findings in children and adolescents are compelling, we could not investigate age or sex differences, as interpretation of these findings is difficult in the absence of normative developmental data upon which to build theory and research.

The specific aim of the present study was to examine the association between affective development, puberty, and gender using the startle reflex as a marker of activation of defensive brain mechanisms. Cross-sectional and longitudinal associations between age, gender, and pubertal status with baseline and fear-potentiated startle were examined using the threat of an airblast paradigm in healthy children. Based on earlier findings of heightened defensive mechanisms in women and girls using the emotional modulation of the startle reflex (Bradley, 2001; McManis, et al., 2001; Schmitz, et al., 2011) we predicted that there would be greater startle reactivity among females than males. On the basis of earlier studies that demonstrated increased fear-potentiated startle with age (Glenn, et al., 2012), and larger startle amplitudes in later pubertal stages assessed with a cross-sectional picture viewing paradigm (Quevedo et al., 2009), we further predicted that there would be increased startle reactivity with pubertal progression.

Methods

Sample

The sample for this study included 31 male and 35 female adolescents, recruited from a normal birth cohort of children from a large university hospital. Parents of eligible children were mailed a letter describing developmental research underway, and those who granted permission by return post card were then enrolled in the data base. For the present study, all families in the data base with female children in the age range of 10 years 0 months to 12 years 12 months and male children ages 11 years 0 months to 13 years 12 months were contacted via telephone by a research assistant. After a description of the topic and data collection procedures, preliminary verbal consent was obtained and the initial laboratory session was scheduled.

Procedure and Measures

Upon arrival to the first laboratory session, the child and parent received a detailed description of the procedure and informed consent was obtained from the parent, and assent

was obtained from the child. A total of 2-5 sessions were conducted with each participant. All participants were evaluated every three to four months for a total of up to five sessions, with 7 participants completing 2 sessions, 9 youth participating in 3 sessions, 12 participants completing 4 sessions, and 32 participating in 5 sessions. The mean time interval between sessions was 5.52 months (3.0 – 8.16 months). Larger time intervals than 4 months between sessions were a result of missing data for one or more of the assessments.

The threat experiment examined startle potentiation during the anticipation of an airblast directed to the larynx. The experiment consisted of three conditions: safe, threat, and intertrial interval (ITI), with safe and threat conditions signaled by visual cues presented for 8 seconds on a monitor. The visual cues consisted of a blue circle for the safe condition and a green square for the threat conditions, with the associational cues reversed from one participant to the next. Participants were informed that the threat cue signaled the possibility of receiving an airblast and that no airblast was administered during the safe cue. Before the beginning of the experiment, participants were presented with a sample airblast.

There were eight threat and eight safe cues presented alternatively. Three out of the eight threat cues were reinforced with an airblast delivered at the end of the cue. The first cue was not paired with an airblast. The time interval between threat/safe cues varied from 18 to 40 seconds. In approximately half the participants the threat cue appeared first, whereas the safe cue appeared first in the remaining half. Thirty startle stimuli were delivered. Six were delivered before presentation of the first safe/threat cue to reduce initial startle reactivity. One startle stimulus was delivered during each safe and threat cue (5-7 seconds following cue onset), and the 8 remaining startle stimuli were delivered during ITI between safe/threat cues.

To prepare each participant for testing, miniature Ag/AgCl electrodes were placed under each eye and an airblast-delivery system was attached at the level of the larynx (Blumenthal et al., 2005; Grillon, et al., 1999). The participants were informed that the electrodes would measure their physiological reaction to various types of external stimuli. The acoustic startle stimulus consisted of a 40-ms duration 102-dB (A) burst of white noise with a near instantaneous rise time presented binaurally through headphones. The airblast (60 psi, 100-ms duration) was delivered by plastic tubing taped to the neck of the participant and directed at the larynx. The electrodes placed underneath each eye recorded the left and right orbicularis oculi EMG eyeblink reflex for which impedance levels were kept below 5 Kohms. The presentation of the acoustic startle stimuli and the recording of the EMG signal measured by the electrodes were controlled by a commercial system (Contact Precision Instrument, London, Great Britain). The EMG signal was amplified, filtered (30-500 Hz), rectified and integrated (100 ms time constant), digitized (1000 Hz), and stored on a PC for later analysis.

Upon completion of the threat experiment, participants were asked to rate how unpleasant, painful, and irritating the airblast stimuli were using analog scales ranging from 1 (not at all) to 5 (extremely). These ratings were combined into a summary score of aversiveness by adding up all ratings of at one time point. They also rated their level of subjective anxiety (calm, nervous, scared, relaxed) during the safe and threat conditions on the same 1

- 5 analog scale. In addition, trait anxiety was assessed by self-report (State Trait Anxiety Inventory, Spielberger, 1983).

Pubertal status of the participants was assessed at each visit using a widely-used, standardized questionnaire that uses sex-specific anatomic drawings of physical changes characteristic of each of the Tanner stages of pubertal development, which have been shown to be assessed more accurately through drawing than through verbal descriptions of physical development (Morris, 1980). The adolescent was told that the explicit drawings were the most accurate method to depict the changes that they were undergoing as they moved to adulthood. The form was completed in a private room and adolescents returned completed forms in sealed envelopes to minimize potential embarrassment due to the personal nature of this information. Pubertal status was classified according to the four Tanner stages, and collapsed into pre/early (Stages I, II) and mid/late (Stages III, IV) for the analyses.

Data Analysis

Differences in trait anxiety between pubertal groups and sex were evaluated by univariate ANCOVA using age as a covariate. Peak amplitude of the blink reflex was determined in the 20-120-ms time frame following stimulus onset relative to the average EMG level for the 50 ms immediately preceding stimulus onset. Peak magnitudes were averaged separately for the threat and safe conditions over blocks. An initial analysis revealed no difference in startle magnitudes between the two eyes, and were averaged over the two eyes. Baseline startle was defined as the mean startle magnitude during ITI. To assess fear-potentiated startle, t-scores were calculated in order to control for baseline startle differences. Fear-potentiated startle was defined as difference score between mean startle magnitudes (t-scores) during safe and threat conditions.

Cross-sectional Analysis—To assess cross-sectional differences in baseline and fear-potentiated startle, univariate ANCOVAs were calculated (GLM Univariate Model, SPSS 19), with either baseline startle or fear-potentiated startle of the first assessment as dependent variable, Sex (1 = male, 2 = female) and Pubertal Status (0= pre/early, 1= mid/late) as independent variables, and Age as covariate. Cross-sectional difference in anxiety ratings of the safe and threat conditions of the first assessment were assessed using ANCOVA for repeated measures (GLM Repeated Measures Model, SPSS 19) to evaluate main effects and interactions between Condition (Safe, Threat), Sex (Male, Female) and Pubertal Status (0= pre/early, 1= mid/late), with Age as covariate.

Longitudinal Analysis—To assess longitudinal changes, the sample was restricted to youth changing pubertal status from pre/early to mid/late pubertal stage in between experimental session (N = 26). A linear mixed models procedure (Linear mixed models, SPSS 19) was applied to analyze differences in baseline startle and fear-potentiated startle including all available sessions, respectively. The effects of Pubertal Status (0= pre/early, 1= mid/late), Sex (1 = male, 2 = female), and the interactions of Sex and Pubertal Status were evaluated. Session was entered as repeated factor (1, 2, 3, 4, 5), Participant was controlled for as random factor, and Age was entered as covariate. Identical analyses were conducted to

assess longitudinal changes of anxiety ratings of the safe and threat conditions respectively, and for the difference score of the safe and threat ratings.

Results

There were neither significant main effects of pubertal group ($F_{1,61}=1.42$, $p=0.24$, $\eta^2 = .02$) or sex ($F_{1,61}=2.78$, $p=0.10$, $\eta^2 = .04$) on trait anxiety, nor a significant interaction between sex and pubertal status on Trait anxiety ($F_{1,61}=70$, $p=0.41$, $\eta^2 = .01$).

Cross-sectional Analysis

Participants' mean age stratified by puberty stage and sex is presented in Table 1. There were no differences in aversiveness ratings of the blast by pubertal status or sex at the first assessment. Age was significantly associated with pubertal status in the cross-sectional sample ($r = .59$, $p < .001$), and baseline startle and fear-potentiated startle did not correlate ($r = -.14$, $p = .26$).

Baseline Startle—The mean baseline startle is presented by sex and pubertal status in Table 2. Baseline startle was not associated with pubertal status ($F_{1,61}=1.56$, $p=0.22$, $\eta^2 = .03$), but was associated with age ($F_{1,61}=10.94$, $p=0.002$, $\eta^2 = .15$). The scatterplot indicates that older age was associated with larger baseline startle. There was also a trend for larger baseline startle in females compared to males ($F_{1,61}=2.91$, $p=0.09$, $\eta^2 = .05$).

Fear-potentiated Startle—The mean fear-potentiated startle by pubertal stage and sex is shown in Table 2. Fear-potentiated startle was significantly greater in the mid/late pubertal stage group compared to the pre/early pubertal stage group ($F_{1,61}=4.61$, $p=0.04$, $\eta^2 = .07$). There were no significant differences between boys and girls, and no significant interaction between sex and pubertal status.

Self-rated anxiety—Anxiety ratings were available for 38 participants (19 boys). After the startle procedures were completed, participants rated their level of anxiety during the safe and threat conditions. Main effects and interactions between pubertal status (pre/early, mid/late), Sex (male, female) and condition (safe, threat) on ratings of anxiety were evaluated using ANCOVA (with age as a covariate). This analysis revealed a main effect for condition ($F_{1,33} = 4.2$, $p = .048$, $\eta^2 = .11$). As expected, subjective report indicated higher anxiety during the threat (Mean = 2.9, S.E. = 2) compared to the safe (Mean = 1.6, S.E. = .1) condition. There was no other significant main effect or interaction involving the factors pubertal status or sex.

Longitudinal Analysis

Of the participants who transitioned across pubertal status during the study sessions, 4 participants completed 2 sessions, 2 youth participated in 3 sessions, 5 participants completed 4 sessions, and 14 participated in 5 sessions. Data were missing completely at random as determined by Little's MCRA test ($\chi^2 = 1.45$, $p = .695$). There were no differences in ratings of aversiveness of the blast of air between pubertal stages; however, girls perceived the blast of air as more aversive than boys ($F_{1,69.7}=23.56$, $p<001$).

Participants' mean age stratified by pubertal stage and sex is presented in Table 1. Age was significantly associated with pubertal status in the longitudinal analysis ($r = .45$, $p < .001$).

Baseline Startle—Baseline startle was not influenced by age ($F_{1,73.2} = .69$, $p = 0.41$) or sex ($F_{1,92.3} = 2.41$, $p = .12$), but it decreased with advanced pubertal status ($F_{1,89.9} = 4.90$, $p = .03$) (see Figure 1 and Table 2).

Fear-potentiated Startle—The magnitude of fear-potentiated startle during the threat experiment in the longitudinal study is shown in Figure 2 (also see Table 2). There was a significant main effect of sex with girls showing stronger fear-potentiation than boys ($F_{1,88.3} = 10.46$, $p = .002$), but no significant effect of puberty ($F_{1,86} = .31$, $p = .58$) or age ($F_{1,85.7} = .85$, $p = .42$). There was no interaction observed between sex and puberty ($F_{1,88} = 1.81$, $p = .18$). However, because of our previous findings in the cross-sectional analysis on pubertal status and fear-potentiated startle, we conducted follow-up analyses stratified by sex. These analyses revealed that girls showed significantly higher fear potentiation in the mid/late pubertal stage compared to the pre/early stage ($F_{1,55.7} = 6.76$, $p = .01$), and boys showed a trend towards lower fear-potentiated startle in the mid/late pubertal stage ($F_{1,13.2} = 3.50$, $p = .08$).

Self-rated Anxiety—Girls were more anxious than boys during the threat condition ($F_{1,67.8} = 14.62$, $p < .001$) but girls and boys did not differ in terms of anxiety ratings during the safe condition ($F_{1,66.65} = 1.84$, $p = .179$). There were no significant differences in anxiety ratings between pubertal stages, or interaction effects of sex and pubertal status on anxiety ratings. There were also neither significant main effects of sex or puberty, nor a significant interaction between these factors on the potentiation of anxiety ratings from safe to threat (i.e. the difference score between threat and safe ratings).

Discussion

The present study provides the first evidence to our knowledge of increase in fear-potentiated startle and overall decreased levels of baseline startle across the course of puberty. These associations can be attributed to pubertal status rather than age, because age has been included as covariate in all models. This distinction has important implications for our understanding of the neurobiology of human anxiety and affect regulation. The increase in threat reactivity associated with pubertal status, especially in girls, confirms both human and animal studies of the role of pubertal development in increased reactivity to stress (Hayward & Sanborn, 2002; Hodes & Shors, 2005; Hyde, Mezulis, & Abramson, 2008; LeResche, Mancl, Drangsholt, Saunders, & Korff, 2005; McEwen, 2003; Sisk & Zehr, 2005; Spear, 2009). Prospective studies of adolescents have established direct links between increased rates of mood disorders (Angold, Costello, Erkanli, & Worthman, 1999) and panic attacks (Hayward & Sanborn, 2002) with changes in reproductive hormones across pubertal development. However, identification of the mechanisms underlying the association between pubertal development and clinical disorders is highly complex, because of the convergence of changes in multiple processes underlying pubertal development (Sisk & Zehr, 2005). Findings of the present study suggest that the changes in the fear circuit involved in fear-

potentiated startle might be one mechanism by which reactivity to threatening stimuli is altered over the course of puberty.

Few studies have investigated the association between pubertal development and startle reactivity. Although some studies examined age effects on startle (Glenn et al., 2012; Quevedo, Smith, Donzella, Schunk, & Gunnar, 2010), only one previous cross-sectional study directly investigated the effects of pubertal status on startle reactivity (Quevedo, Benning, Gunnar, & Dahl, 2009). Moreover, most prior studies of children and adolescents did not report age or pubertal status (Fairchild, Stobbe, van Goozen, Calder, & Goodyer, 2010; Fairchild, Van Goozen, Stollery, & Goodyer, 2008; Syngelaki, Fairchild, Moore, Savage, & van Goozen, 2013) or assessed a very narrow age range of children. The present study is the first to date investigating the association of pubertal status with changes in fear-potentiated startle using an instructed fear paradigm.

We found a direct association between increases in fear-potentiated startle and advances in pubertal stage, particularly among girls. The greater subjective anxiety ratings of girls compared to boys in the threat condition suggest that the increase in fear potentiation may reflect increased anxiety reactivity among girls. These findings may reflect the earlier pubertal maturation of girls than boys, who may develop increased reactivity at later ages (Palanza, 2001; Toufexis, 2007). The lack of a sex difference in adults would support this potential explanation. Follow up of this sample is necessary to examine whether such sex differences persist in later adolescence.

In contrast to the findings of an earlier study (Quevedo et al., 2009), we found a decrease in baseline startle, a potential index of contextual anxiety (for an overview see: Grillon & Davis, 1997; Grillon, 2008; Lang, Bradley, & Cuthbert, 1998; Lang, Davis, & Ohman, 2000), with advances in pubertal status in the longitudinal analyses. In light of the findings that baseline startle levels are an index of trait anxiety in children (e.g. Quevedo et al., 2009) and pathological anxiety in adults (e.g. Grillon, Ameli, Goddard, Woods, & Davis, 1994; Morgan, Grillon, Southwick, Davis, & Charney, 1995), this finding indicates that changes associated with pubertal development may influence contextual anxiety through an increased ability to discriminate between threat cues and context, as shown by earlier work in children demonstrating that fear discrimination improves with age (Glenn et al., 2012). This adaptive process during puberty might be disrupted in those who subsequently develop panic disorder, which has been associated with heightened baseline startle during threat experiments (Grillon, Ameli, Goddard, Woods, & Davis, 1994) that often first emerges during adolescence (Essau, Conradt, & Petermann, 1999; Macaulay & Kleinknecht, 1989; Reed & Wittchen, 1998; Warren & Zgourides, 1988) with increasing prevalence by pubertal stage independently of age (Hayward et al., 1992). Although previous research strongly suggests that baseline startle in the context of a threat experiment is related to contextual anxiety, an alternative explanation for the observed effect of puberty on baseline startle might be a more general influence of puberty on reflex strength. However, animal studies indicate that startle magnitudes measured within an emotionally neutral context, a closer reflection of basic reflex strength, show a directly opposing pattern with increasing magnitudes over the course of puberty (Pietropaolo & Cruiso, 2009). Future research

should investigate both fear-potentiated startle paradigm and emotionally neutral reflexes, to further clarify this question.

Differences in findings between the cross-sectional and longitudinal analyses underline the importance of prospective studies to investigate changes of emotional reactivity throughout puberty. While the cross-sectional analysis indicates that fear-potentiated startle increases uniformly from pre/early to mid/late pubertal stages, the longitudinal analysis suggests that this pattern might only be observed in girls at this pubertal stage, but not (yet) in boys. In the cross-sectional analysis, we also observed somewhat opposing results to the ones obtained by the longitudinal analysis concerning baseline startle, with increasing levels of baseline startle by age, but no association with pubertal status. A possible explanation for these conflicting findings is the difference in samples and analyses. While the cross-sectional analysis compares a pre/early pubertal group of adolescents to youth in mid/late puberty, the longitudinal within subject data provide a more valid index of changes of startle reactivity over time than cross sectional across subject designs. It is possible that between group differences that were not assessed in this study might have influenced findings in the cross-sectional analysis. These inconsistencies emphasize the importance of future research on this topic and the necessity of 1) employing a longitudinal design to assess changes in startle over time and 2) assessing both age and puberty, as there might be independent effects of these factors.

The major strengths of this study include that 1) this is the first prospective study to our knowledge on startle reactivity and pubertal status, which enabled us to distinguish temporal changes in startle as a function of changes in pubertal development, and 2) the use of a population based cohort sample, which enhances the generalizability of these findings to the general population of U.S. youth. Limitations in the conclusions of our study should also be considered in interpreting these findings. First, the lack of direct measurement of pubertal hormones, parental report or professional physical evaluation may have led to some misclassification of pubertal status, as well as limited our ability to study the direct associations between pubertal stage and startle. Second, the relatively small sample size especially in the subsample that results from stratification by sex and pubertal status, reduce the statistical power of our analyses, and precluded further inspection of clinical and demographic correlates of anxiety and startle reactivity, or examination of the full spectrum of pupertal stages. Future studies should assess larger samples of children with and without anxiety disorders and mood disorders as well as those at high risk for anxiety and depression to study the association between startle and the expression of anxiety and mood states across the pubertal progression. In addition, direct measurement of the biologic correlates of stress and reproductive hormones would provide more specific information on the biologic correlates of changes in startle reactivity.

This work demonstrates the value of the application of the fear-potentiated startle reflex to gain insight into the developmental factors underlying mood and anxiety states. Integration of knowledge of biologic pathways underlying startle from basic research (Davis, Walker, Miles, & Grillon; Spear, 2000), with those involved in pubertal development could lead to greater understanding of the pathogenesis of anxiety and mood disorders (Davis, Walker, Miles, & Grillon, 2003; Walker, Toufexis, & Davis, 2003). There are numerous areas of

future research that could address potential explanations for the findings of the present study. First, direct studies of pubertal changes in neural pathways underlying stress reactivity in general and the startle reflex in particular using increasingly sensitive neuroimaging approaches could elucidate the role of gonadal hormones and their sex-specific effects on the onset of depression and anxiety (Silk et al., 2009). For example, the earlier peak in the maturation of some brain regions in females (Giedd et al., 2006) and changes in amygdala and hippocampus volume during puberty in girls (Blanton et al., 2012) provides an interesting series of questions regarding neural structures and systems underlying startle and affective reactivity. Second, human studies that map onto animal studies of behaviors associated with anxiety such as social interaction, responses to novel stimuli and predictable and non-predictable stressors could provide insight into the pathways underlying the architecture of anxiety in humans (Cameron, 2004; Sisk & Zehr, 2005). For example, assessing anxiety-potentiated startle, a measure of threat reactivity to unpredictable and longer lasting threat (as opposed to cued fear-potentiated startle) (Grillon, Baas, Lissek, Smith, & Milstein, 2004; Schmitz & Grillon, 2012), in a longitudinal sample of youth undergoing puberty would be helpful to understand the specific neural pathways involved in heightened threat reactivity in later pubertal stages. In animal models, anxiety potentiated startle has been shown to be mediated by a distinct neuronal pathway from fear-potentiated startle (Davis, et al., 2010) and to involve the bed nucleus of the stria terminalis (BNST). This structure has been found to show a sexual dimorphism (Allen & Gorski, 1990) and is likely influenced by gonadal steroids. Toufexis (2007) furthermore concludes in her review of sex-specific modulations of anxiety behaviors in rats that females display increased potentiation of the startle reflex especially due to sustained states of anxiety. Taken together these studies indicate that anxiety potentiated startle might also be sensitive to sex steroid changes over the course of puberty, and could be very valuable in our understanding of the development of threat reactivity during this critical period of life. Third, application of genetically informative approaches to developmental and sex differences in startle reactivity could provide insight into the role of genetic factors in the increased risk of depression that emerges at the pubertal transition as demonstrated by developmental twin studies (Silberg et al., 1999) and preclinical studies that have shown that genetic factors play an important role in changes in startle reactivity between pre- and post-pubertal mice (Pietropaolo & Crusio, 2009).

In conclusion, the present study provides the first evidence for increased fear-potentiated startle and decreased baseline startle over the course of puberty and independently of age. These results highlight the importance of puberty for the development of threat reactivity and have important implications for the emergence of mood and anxiety disorders in adolescence. Further prospective studies in larger samples are needed to elucidate mechanisms by which these changes are mediated.

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Highlights

1. Fear-potentiated startle (FPS) is greater in girls than boys.
2. FPS is elevated in girls in mid/late compared to pre/early puberty.
3. The effect of puberty is independent of chronological age.

Figure 1. Longitudinal changes in baseline startle magnitude (SE) during early and late puberty. The bar graphs shows raw startle magnitudes during ITI.

Figure 2. Longitudinal changes in fear-potentiated startle (SE) during early and late puberty in females and males. The bar graphs show t-scores of difference scores between the threat and safe conditions (fear-potentiated startle).

Table 1

Mean Age (S.D.) and Age Range by Pubertal Stage and Sex in the cross-sectional and longitudinal analyses.

	SEX			
	Male		Female	
	PUBERTAL STAGE			
	Pre/Early	Mid/Late	Pre/Early	Mid/Late
	CROSS-SECTIONAL			
N	n=14	n=17	n=22	n=13
Age	11.62 (.71)	12.42 (.42)	11.44 (.82)	12.45 (.50)
Age Range	10.49 - 12.83	11.70 - 12.92	10.29 - 12.88	11.20 - 12.99
	LONGITUDINAL			
N	n = 9		n = 16	
Age	12.04 (.79)	12.51 (.83)	11.70 (.78)	12.52 (.91)
Age Range	10.52 – 13.20	11.09 - 13.83	10.50 - 13.16	11.08 – 15.07

Table 2

Mean Age (S.D.), mean baseline and fear-potentiated startle by Pubertal Stage and Sex for the cross sectional and longitudinal analyses.

	SEX			
	Male		Female	
	PUBERTAL STAGE			
	Pre/Early	Mid/Late	Pre/Early	Mid/Late
	CROSS-SECTIONAL			
N	n=14	n=17	n=22	n=13
Baseline startle (microV)	9.15 (1.87)	6.03 (1.78)	11.59 (1.59)	9.46 (2.02)
Fear-potentiated startle (Difference score Threat – Safe; t-scores)	7.92 (1.53)	11.96 (1.46)	9.67 (1.31)	12.99 (1.65)
	LONGITUDINAL			
N	n = 9		n = 16	
Baseline startle (microV)	11.38 (1.90)	7.94 (1.46)	9.23 (1.35)	5.64 (1.07)
Fear-potentiated startle (Difference score Threat – Safe; t-scores)	9.38 (1.36)	8.59 (1.21)	11.47 (.99)	13.62 (.91)