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Developmental investigation of fear-potentiated startle across puberty☆

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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.

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1. Introduction

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 et al., 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

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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 et al., 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, Codispoti, Sabatinelli, & Lang, 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.

2. Methods

2.1. 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 database. For the present study, all families in the database 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.

2.2. 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 3–4 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 s 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 s. 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 s 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, and 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, Gorssuch, Lushene, Vagg, & Jacobs, 1983).

Pubertal status of the participants was assessed at each visit using a widely-used, standardized questionnaire that uses