Startle Response in Behaviorally Inhibited Adolescents With a Lifetime Occurrence of Anxiety Disorders

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ABSTRACT

Objective: Behaviorally inhibited children face increased risk for anxiety disorders, although factors that predict which children develop a disorder remain poorly specified. The current study examines whether the startle reflex response may be used to differentiate between behaviorally inhibited adolescents with and without a history of anxiety. **Method:** Participants were assessed for behavioral inhibition during toddlerhood and early childhood. They returned to the laboratory as adolescents and completed a fear-potentiated startle paradigm and a clinical diagnostic interview (Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version). Magnitude of the startle reflex was examined at baseline and during cues associated with safety and threat. **Results:** Only adolescents who showed high levels of behavioral inhibition and had a lifetime occurrence of anxiety disorders showed increased startle reactivity in the presence of safety cues. Neither behavioral inhibition nor diagnosis was related to startle reactivity during threat cues. **Conclusions:** These results suggest that neurobiological measures, such as the startle reflex, may be a potential risk marker for the development of anxiety disorders among behaviorally inhibited adolescents. These methods may enhance our ability to identify vulnerable individuals before the development of anxious psychopathology. *J. Am. Acad. Child Adolesc. Psychiatry,* 2009;48(6):610–617. **Key Words:** anxiety, temperament, startle, risk factors, adolescence.

Behavioral inhibition (BI) is a temperamental disposition that is identified early during toddlerhood and remains relatively stable throughout childhood.[1,2] Behaviorally inhibited children tend to withdraw in the face of novelty and show general fearfulness of unfamiliar situations.[1,2] In addition, BI has been identified as a risk marker for the development of anxiety disorders, particularly social anxiety, during childhood and adolescence.[3–6] However, a number of these children do not develop such disorders. Therefore, it is important to identify behavioral or physiological markers that may be used to differentiate between behaviorally inhibited children who develop anxiety disorders and those who do not.

Fox and colleagues[7] have suggested that the association between BI and risk for anxiety disorders is moderated by attention processes, particularly attention to threat. An individual's tendency to exhibit potentiation of the startle reflex in the presence of cues that predict aversive events represents a particularly important marker for heightened attention to threat.[8] To date, few studies have investigated individual differences in this tendency, known as "fear-potentiated startle," among behaviorally inhibited infants or children.[9,10] Infants who were selected for their heightened motor and negative affect responses to novelty at 4 months of age (a precursor of BI[11]) showed both increased baseline and fear-potentiated startle amplitude

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during the approach of a stranger compared with positively reactive infants.[9] In contrast, 7-year-old children selected for shyness did not show heightened baseline or fear-potentiated startle compared with socially outgoing children.[10] The age of the subjects in these studies precludes examining the effects of anxiety on fear-potentiated startle within this risk group. Studying a sample of behaviorally inhibited participants at an age where anxiety disorders may be identified would help clarify the role of anxiety in shaping this physiological response.

In a relatively large series of studies among adults, startle potentiation has been shown to differ between individuals with anxiety disorders and controls.[12–17] Interestingly, both anxious and control individuals show the same magnitude of relatively large startle potentiation during presentations of overtly threatening stimuli in comparison to pleasant or nonthreatening stimuli.[12–20] However, anxious individuals and controls tend to most consistently differ in responses to safety cues that signal that no aversive stimuli will be presented.[12–17] Specifically, anxious but not control individuals show an increased startle during safety cues relative to a no-cue baseline condition. Therefore, failing to discriminate between safety and threat cues represents the most consistent between-group difference, a difference that may reflect perturbations in underlying inhibitory fear processes.[17] As such, anxious individuals are unable to inhibit their fear response in a safe environment. Examining an individual's discrimination of threat and safety within the context of a fear-potentiated startle paradigm may be important to further understand why some behaviorally inhibited children develop anxiety, whereas others do not.

In the current study, we examined whether measures of BI affect baseline and fear-potentiated startle magnitude and, more specifically, whether these startle responses differ between behaviorally inhibited adolescents with a lifetime history of anxiety disorders from those who do not. Previous research suggests that individuals with anxiety disorders have normal startle during conditions of explicit threat but enhanced startle in conditions of safety compared with controls.[12–17] We hypothesized that the startle reflex response would differentiate between behaviorally inhibited adolescents with and without anxiety. Specifically, behaviorally inhibited adolescents with a diagnosis of anxiety disorder were expected to show increased startle magnitude when processing safety cues, relative to behaviorally inhibited adolescents without anxiety. No such anxiety-related differences were expected in the startle response to threat cues.

METHOD

Participants

One hundred three adolescents participated in the current study. The adolescents were recruited from two cohorts participating in an ongoing longitudinal study of temperament and emotional reactivity.[1,11] The original cohorts consisted of 166 children (81 male) who were selected at 4 months of age. A broad range of behavioral and psychophysiological measures were assessed in the laboratory at 9, 14, 24, 48, and 84 months of age.[1]

At 14 and 24 months of age, BI was assessed via responses to novel objects and unfamiliar adults using standard laboratory procedures.[1,11] Behaviors coded include proximity to the caregiver and latency to approach novel stimuli. At 48 and 84 months of age, social reticence was assessed during play with an unfamiliar peer, and unoccupied onlooking behavior was coded using the Play Observation Scale (POS; see references 21 and 22 for paradigm details). Interrater reliability was calculated from 30% of the sample and Cohen κ scores ranged from 0.81 to 0.94.

Participants returned to the laboratory during mid-adolescence to complete a battery of social, emotional, and cognitive tasks. A total of 103 adolescents (48 male) participated in the startle experiment. There were no significant differences between the participants who completed the startle task and those who did not on measures of BI scores, psychopathology, age, sex, and ethnicity. The mean age of the participants for this study was 15.5 years (range 13.1–17.3 years). Three subjects were excluded from analysis because of excessive artifact found in electromyography (EMG) activity before onset of the startle probe on more than 50% of startle trials. An additional 20 subjects were excluded for being startle nonresponders, therefore showing no perceptible eyeblink reflex on more than 70% of the trials during any one condition. Finally, four participants were excluded because the Schedule for Affective Disorders and Schizophrenia for School-Age Children assessment was not completed. The excluded adolescents did not differ from the final sample ($N = 76$, 36 male subjects) in regard to age, sex, BI scores, or psychopathology.

This study was approved by the University of Maryland institutional review board. All children and their parents provided written informed assent/consent to participate in the study.

Startle Testing Procedure

Methods used for emotional potentiation of startle in adults have typically used the use of aversive stimuli, such as unpleasant electric shocks,[23–25] or the viewing of affective slides.[18,20] However, these methods are not necessarily appropriate for testing children and adolescents for either ethical or practical reasons. Therefore, in the current study, we used a startle paradigm that has previously been shown to potentiate startle responses in adolescents in an ethical and reliable manner by presenting an air blast to the neck rather than a shock as the threatening stimuli.[26,27]

During the testing, adolescents sat in a comfortable chair while facing a 15-in computer monitor placed at a distance of 82 cm. Two 6-mm miniature EMG electrodes were placed under the left eye to record startle activity from the orbicularis oculi muscle, and a ground electrode was placed on the back of the neck. A cloth collar attached to nylon tubing (3-m long, 3.175-mm internal diameter)

was placed around the participant's neck with the tubing aimed at the larynx. The tubing was attached to equipment that included a compressed air cylinder, regulator, and pneumatic stimulator containing a solenoid valve controlled by an AC switch (James Long Company, Caroga Lake, NY) that produced an air blast with a peak flow rate of 250 cm^3/s at 700-kPa input pressure. Participants were told that they would see either a blue or a green monitor screen (12 cm^2) while hearing bursts of white noise (startle probe; 50-millisecond 105-dB peak sound pressure level with instantaneous rise time presented binaurally through EAR-3A earplugs). Participants were also told that one of the colors (e.g., blue) indicated that there was a possibility of receiving an air blast to the neck (threat cue) and that the other color (e.g., green) indicated that there was no possibility of receiving an air blast (safety cue). The association between color and threat/safety cues was counterbalanced between participants. Before starting the experiment, participants received a sample air blast to the neck. The participants were told that the air blast was to be unpleasant but not painful and were asked to confirm this perception.

The experiment consisted of a startle habituation period followed by the fear-potentiated startle task (Fig. 1). The startle habituation period consisted of six startle probes with an interprobe interval of either 10 or 20 seconds. After the habituation period, the experimenter reminded the adolescent of which colors were associated with the threat and safety cues. Next, the fear-potentiated startle task began. The task consisted of randomly presented eight threat, eight safe, and eight intertrial interval (ITI) trials that were presented randomly. During ITI trials, startle probes were delivered randomly without presentation of either threat or safety cues. Duration of the threat and safety cues was 12 seconds. The time interval between the onset of two successive signals varied from 17 to 42 seconds. For each of the safe and threat cues, half of the startle probes were delivered 4 seconds after cue onset, and the other half were presented 7 seconds after cue onset (Fig. 1). The air blast was administered randomly during half of the threat trials.

Data Collection and Reduction

The raw EMG signal was amplified using a custom bioelectric amplifier (SA Instruments, San Diego, CA) with a gain of 1,000 Hz and filtered using high- and low-pass filters of 1 and 100 Hz, respectively. The amplified signal was digitized at a sampling rate of 512 Hz using a 12-bit A/D converter (±2.5 V input range) and SnapMaster data acquisition software (HEM Data Corporation, Southfield, MI). Before recording EMG from each participant, a 50-μV, 10-Hz signal was input into the channel, and the amplified signal was recorded for calibration purposes.

The raw EMG signal was processed and analyzed offline using the EMG Analysis System from James Long Company (Caroga Lake, NY). The signal was digitally filtered offline with a high-pass filter of 28 Hz, and a digital band-stop filter (50–70 Hz) was used to remove 60-Hz noise. The signal was rectified and smoothed by using moving averages with a 20-millisecond window. Because the 100-Hz low-pass filter setting reduced the power of the startle response signal,[28] peak amplitude of the blink reflex was determined in the 20- to 80-millisecond time window after stimulus onset relative to the baseline. This relatively narrow time window was used to decrease the possibility of including involuntary blinks in the analysis (Blumenthal, personal communication). Baseline EMG value was defined as the average activity recorded during the 20 milliseconds before stimulus onset. Electromyography responses were visually inspected by two independent coders to verify that a blink occurred within the 20- to 80-millisecond time window. Twenty-five percent of the sample was used to compute interrater reliability, and a Pearson correlation of 0.99 was obtained. Approximately 5.3% of startle responses were excluded because reflex onset occurred before the 20-millisecond window after the probe onset or the eyeblink was indistinguishable from baseline noise. A magnitude of zero was assigned for nonresponse trials and included in the analysis for participants who had nonresponse trials but did not meet exclusionary criteria as a nonresponder.

Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version

Adolescents and their parent (most often mothers) were separately administered the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version,[29] a semistructured diagnostic interview designed to assess current and lifetime episodes of psychopathology in children and adolescents according to *DSM-IV* criteria. Probes and objective criteria are provided to rate individual symptoms. The present study focused on the prevalence of anxiety disorders that included separation anxiety disorder, generalized anxiety disorder, social phobia, posttraumatic stress disorder, specific phobia, and obsessive-compulsive disorder. Interviews were conducted by advanced clinical psychology doctoral students under the close supervision of a board-certified psychiatrist and a licensed clinical psychologist, all of whom were blind to the subject's BI classification. Discrepancies between parent and adolescent reports on the Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version were resolved by bringing the parent and child together to discuss discrepant perspectives, and the interviewer made a final

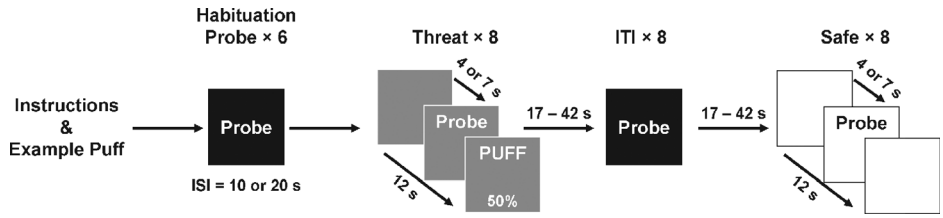

Fig. 1 Startle procedure. Participants were first habituated to a startle probe (50-millisecond 105-dB burst of white noise with a 10- or 20-second interstimulus interval. During the startle task, participants were presented with a colored screen (blue or green during actual task) indicating threat or safety. During threat trials (gray squares), the colored screen was presented. After 4 or 7 seconds, the startle probe was presented followed by an air blast to the larynx (PUFF) on 50% of the trials. During safe trials (white squares), the colored screen was presented followed by the startle probe. There was no presentation of an air blast during safe trials. Intertrial interval trials consisted of randomly presenting startle probes between presentations of the threat and safe trials. Intertrial interval, threat, and safety cues were randomly presented during the task. ISI = interstimulus interval; ITI = intertrial interval.

determination based on clear evidence of functional impairment. Final clinical diagnoses were discussed by the team and made by expert consensus. Reliability was computed from 39% of the interviews ($\kappa = 0.92$).

BI Profiles

Measures of BI during early childhood have been shown to relate to the development of anxiety during later childhood and adolescence.[4–6] Therefore, preliminary analyses investigating the effect of early BI and anxiety on outcome measures of startle magnitude were conducted using both the 14- and 24-month behavioral measures. These BI measures alone did not yield any significant main effects or interaction effects with anxiety measures or startle condition. However, significant results (reported below) were evident when examining stable temperamental profiles over the course of childhood by including measures of BI in toddlerhood and social reticence at ages 4 and 7 years in the model. Longitudinal profiles of BI were obtained by performing latent class analysis on behavioral composites of BI at 14 and 24 months and social reticence at 4 and 7 years using Mplus version 4.1.[30] Because of the skewness of the data, behavioral measures for 14, 24, 48, and 84 months were individually dichotomized where 0 denoted a child rated in the lower half of the sample at a particular time point and 1 denoted a child rated in the top half of the sample at that time point. Models with two through four profiles were estimated. Best model fit was assessed using Bayesian information criteria (BIC), where the smallest negative number indicates best fit. The Lo-Mendell-Rubin Likelihood ratio test was also used to test the significance of the -2 log likelihood difference between models with k and k-1 profiles.[31]

Model fit (BIC) for the current sample was -766.12 for one profile, -768.92 for two profiles, -792.71 for three profiles, and -817.55 for four profiles. The two-profile model was selected as the best fitting model, given the low BIC value and significant Lo-Mendell-Rubin Likelihood ratio test. The average posterior probabilities of membership ranged from 0.78 to 0.86 across the two profiles, reflecting a moderately high degree of confidence in profile assignment. The "high" BI profile ($n = 69$) showed high average levels of BI at all four time points, and 42% of the sample had a higher probability of membership in this profile than the other profile. The "low" BI profile ($n = 97$) showed lower levels of BI at all four time points, and 58% of the sample had a higher probability of membership in this profile than the other profile.

Data Analysis

Peak amplitude of the blink reflex was measured for each individual trial. For the habituation period, peak amplitude of the six startle responses were averaged into two blocks of three eyeblinks each (trials 1–3 and trials 4–6). Peak amplitude of the eight startle responses for each condition (ITI, safe, threat) was averaged. Standardized *T* scores for eyeblink amplitude were also analyzed. Because similar results were obtained with the raw scores and *T* scores, only results of the raw scores are presented. Univariate and repeated-measures analysis of variance (ANOVA) was used to examine the interaction between BI profiles and anxiety on measures of startle magnitude. Previous studies of anxiety disorders using variants of the current paradigm find consistent between-group differences specifically for the "safe" condition. Given the consistency of these findings, we also examined between-group differences and BI-by-diagnosis interactions, specifically, in the "safe" condition.

Sex has been shown to significantly affect startle magnitude in individuals who are at high risk for anxiety.[27,32] Therefore, preliminary analyses were conducted to examine the effect of sex on startle response during both habituation and the fear-potentiated startle task, and no significant effects of sex, sex-by-temperament, sex-by-lifetime diagnosis, or sex-by-condition were found. Therefore, sex was excluded from any further analyses.

RESULTS

BI and Anxiety

Twenty (8 male subjects) and 31 (17 male subjects) participants from the high BI and low BI groups, respectively, did not have an anxiety diagnoses. Twelve (5 male subjects) adolescents in the high BI group (mean number of anxiety diagnoses 1.50, SD 1.0) and 13 (6 male subjects) adolescents in the low BI group (mean number of anxiety diagnoses 1.54, SD 0.66) had a lifetime diagnosis of anxiety. Of these children, 6 (1 male subject) in the high BI group and 9 (4 male subjects) in the low BI group had current anxiety diagnoses.

Chi-square analysis was used to determine whether the occurrence of having either a lifetime anxiety diagnosis or a current anxiety disorder differed between the high and low BI groups. High BI and low BI adolescents did not differ in the likelihood of having either lifetime or current diagnosis of an anxiety disorder ($p > .2$). Previous studies have shown that behaviorally inhibited children and adolescents are at increased risk for the development of social anxiety compared with other anxiety diagnoses.[3,4,6] Therefore, high BI adolescents may have increased rates of social anxiety disorder compared with low BI adolescents. However, χ^2 analysis revealed that neither current nor lifetime social anxiety diagnosis differed between high and low BI adolescents ($p > .2$).

Startle Habituation

Repeated-measures ANOVA with block (trials 1–3 and trials 4–6) as a within-subjects factor and temperament (high BI and low BI) and lifetime diagnosis (control and anxious) as between-subjects factors was computed for startle magnitude during the habituation period. No significant main or interaction effects were found (Table 1). Similar analyses were conducted examining whether having a current anxiety disorder interacted with BI to affect startle habituation, and no significant effects were found ($p > .2$). In addition, social anxiety did not interact with BI or significantly influence startle habituation ($p > .2$).

TABLE 1
Mean (SE) Startle Magnitude (Microvolts) During Habituation

Participants	Block 1[a]	Block 2[a]
Low BI–control (n = 31)	33.33 (4.4)	30.36 (4.3)
Low BI–anxious[b] (n = 13)	29.22 (7.0)	31.78 (7.0)
High BI–control (n = 20)	30.10 (5.6)	24.29 (5.5)
High BI–anxious[b] (n = 12)	36.71 (7.0)	31.78 (7.0)

Note: BI = behavioral inhibition.
[a] Average of three trials.
[b] Lifetime anxiety diagnosis.

Fear-Potentiated Startle

Table 2 presents the startle data during the ITI, safe, and threat conditions for individuals with a lifetime anxiety diagnosis, current anxiety diagnosis, and no anxiety diagnosis. Repeated-measures ANOVA with condition (ITI, safe, and threat) as the within-subjects factor and temperament (high BI and low BI) and lifetime diagnosis (control and anxious) as between-subjects factors was computed for startle magnitude during the fear-potentiated startle task. As expected, startle varied as a function of the different conditions ($F_{2,144}$ = 70.180, p < .001, f = 0.987) with significantly greater startle magnitude during the threat condition compared with safe (t_{75} = 9.746, p < .001, d = 2.251) and ITI (t_{75} = 9.387, p < .001, d = 2.168). There was no significant difference between the safe and the ITI conditions (t_{75} = 0.559, p > .20). There were no other significant main or interaction effects.

As previously noted, previous research has consistently shown that individuals with anxiety disorders show a heightened startle response to safety cues compared with controls, although the startle response to threat cues does not differ.[12–17] Therefore, it was hypothesized that group differences on startle magnitude would be observed during presentation of safety cues rather than threat cues.

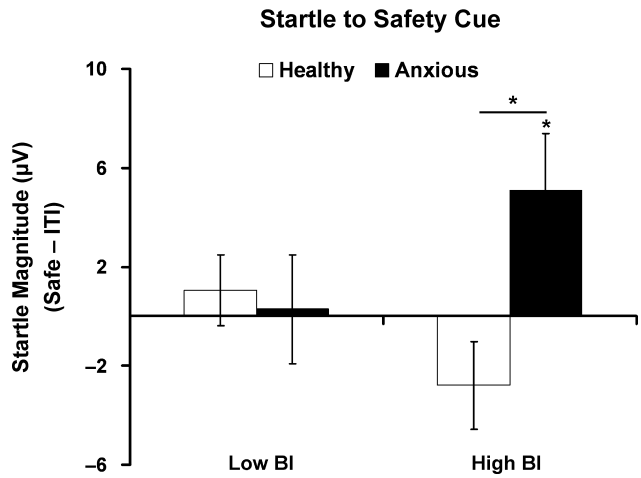

Fig. 2 Startle magnitude to the safety cue in low (low BI) and high (high BI) behaviorally inhibited adolescents with and without a lifetime diagnosis of anxiety. Magnitude is calculated as the difference between the mean startle response to the startle probe during presentation of the safety cue and the mean startle response to the startle probe during the ITI trials. Low BI anxious: n = 13; high BI anxious: n = 12; low BI healthy: n = 31; high BI healthy: n = 20. Errors bars represent SE of the mean. *p < .05. BI = behavioral inhibition; ITI = intertrial interval.

Thus, univariate analysis was used to examine startle magnitude during safe and threat trials separately. To control for individual differences in the baseline (ITI) startle response, difference scores were computed between safe and ITI startle magnitude (safe-ITI) and between threat and ITI startle magnitude (threat-ITI).

A significant temperament-by-lifetime diagnosis interaction effect was found for the safe-ITI difference score ($F_{1,72}$ = 4.892, p < .05, f = 0.261; Fig. 2). Post hoc analyses revealed a significant difference between the control and anxious adolescents within the high BI group (t_{30} = 2.512, p < .05, d = 0.917). No other comparisons were significant. To determine whether a significant difference exists between safe and ITI magnitude, one-sample t tests were computed for low and high BI control and anxious

TABLE 2
Mean (SE) Startle Magnitude (Microvolts) During Startle Task

	Lifetime Anxiety[a]		Current Anxiety[a]		No Anxiety	
Condition	Low BI (n = 13)	High BI (n = 12)	Low BI (n = 9)	High BI (n = 6)	Low BI (n = 31)	High BI (n = 20)
ITI	17.92 (4.4)	18.88 (4.6)	18.71 (5.3)	12.77 (6.5)	19.24 (2.9)	21.69 (3.6)
Safe	18.20 (4.9)	23.98 (5.1)	19.20 (5.8)	13.70 (7.2)	20.29 (3.2)	18.90 (3.9)
Threat	37.69 (6.1)	34.16 (6.3)	36.61 (7.3)	33.07 (8.9)	35.43 (3.9)	30.39 (4.9)

Note: BI = behavioral inhibition; ITI = intertrial interval.
[a] Categories are not mutually exclusive.

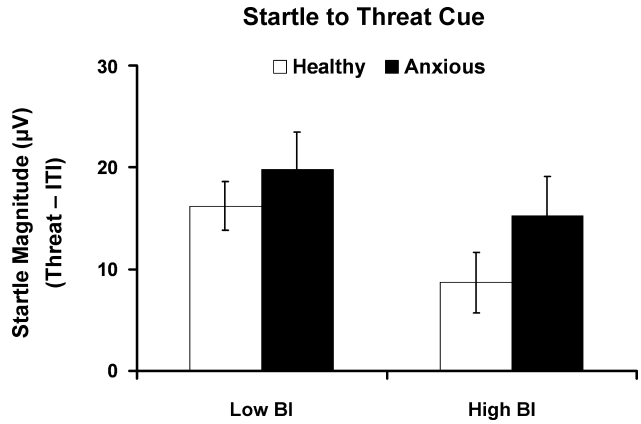

Fig. 3 Startle magnitude to the threat cue in low (low BI) and high behaviorally inhibited (high BI) adolescents with and without a lifetime diagnosis of anxiety. Magnitude is calculated as the difference between the mean startle response to the startle probe during presentation of the threat cue and the mean startle response to the startle probe during the ITI trials. Low BI anxious: $n = 13$; high BI anxious: $n = 12$; low BI healthy: $n = 31$; high BI healthy: $n = 20$. Errors bars represent SE of the mean. BI = behavioral inhibition; ITI = intertrial interval.

adolescents separately. Only the high BI adolescents with a lifetime anxiety diagnosis had a difference score that was significantly greater than 0 ($t_{11} = 1.483$, $p < .05$, $d = 0.894$, one tailed; Fig. 2), suggesting that high BI adolescents with lifetime anxiety have a significant increase in startle magnitude during safe trials compared with baseline (ITI) trials.

Similar univariate analyses were conducted using the threat-ITI difference score as a dependent measure. No significant interaction or main effects were found ($p > .20$), suggesting that the startle response to threat cues did not differentiate between behaviorally inhibited adolescents with and without lifetime anxiety. All groups showed a significant increase in response to the threat cue compared with ITI (one-sample t tests: all p's $< .05$; Fig. 3).

Separate analyses using current anxiety diagnosis and social anxiety diagnosis as independent between-subjects measures found no significant interactions or main effects on startle outcomes measures (p's $> .2$), suggesting that these findings were not the result of having a current anxiety diagnosis or social anxiety.

DISCUSSION

In the present study, we found that startle magnitude differed between adolescents with a stable history of heightened BI during childhood who went on to develop an anxiety disorder versus those BI adolescents with no anxiety disorder. This study is the first to investigate BI along with the startle reflex to differentiate between adolescents with and without anxiety. Although startle was significantly potentiated by the threat cue, the magnitude of startle did not differentiate between high- and low-BI adolescents, regardless of anxiety history. In contrast, the startle response to the safety cue differentiated adolescents characterized both as highly behaviorally inhibited as children and as having a lifetime diagnosis of anxiety from the other three groups of participants. Specifically, high-BI anxious adolescents were the only group to exhibit significantly elevated startle response to safety cues compared with ITI. Neither adolescents who were high on BI but free of an anxiety disorder nor those low on BI (including those with an anxiety disorder diagnosis) exhibited an increased startle response to the safety cue. These findings suggest that the startle response may be an important psychophysiological marker that may be used to differentiate anxious adolescents with an underlying temperamental predisposition (i.e., BI) from adolescents who do not share this risk factor. This, in turn, may help clarify current questions surrounding the multiple etiologies and trajectories that may lead to clinical anxiety.[33]

Fear-potentiated startle paradigms have been used to examine psychophysiological differences between clinically anxious and control individuals.[12–17] One of the most robust and consistent findings reported in this literature is that anxious patients show greater startle potentiation to safety cues but normal startle responses to threat cues compared with the controls.[17] It has been suggested that stimulus generalization between threat and safety signals reflects perturbations in inhibitory fear mechanisms in which there is a specific problem distinguishing between cues of safety and cues of threat.[17,34] In contrast to these previous studies, we did not find a heightened startle response to safety cues among all individuals with a lifetime anxiety disorder, in general. Rather, we found that only high BI adolescents who also had an anxiety disorder showed an increased startle response to safety cues compared with ITI. Low-BI adolescents with a lifetime anxiety disorder did not differ from nonanxious adolescents on their startle response between safety cues and ITI. These findings suggest that behaviorally inhibited individuals who show generalized startle between threat and safety may do so as a result of perturbed inhibitory fear mechanisms. Such

perturbations may be a possible mechanism that is specific to the phenotypic expression of anxiety among individuals with a history of BI.

Studies investigating the startle reflex response as a vulnerability marker for anxiety within an adolescent population have shown that adolescents with a family history for anxiety are more likely to show abnormal startle reflex responses compared with adolescents without such family history.[27,32] These results give some insight into which psychophysiological measures may be used to differentiate between adolescents who are at high or low risk for anxiety. However, these studies did not investigate whether these individuals actually developed anxiety in the future. Therefore, these studies could not determine whether differential startle responses predicted an anxiety disorder. Moreover, these studies also found that startle magnitude did not differ between those individuals with no history of anxiety and with a previous or current history of an anxiety disorder at the time of testing. In the present study, we extended these initial studies by using a longitudinal prospective design in which stability in BI, a known risk factor for anxiety,[5] was measured across toddlerhood and childhood. We subsequently assessed measures of anxiety diagnoses and startle reflex response during adolescence. While neither BI nor startle response magnitude was able to independently differentiate between clinical histories, the interaction between these two factors helped target increased risk for anxiety. These results suggest that predicting the development of anxiety disorders among a population of high-risk children may be more accurately assessed when both BI and psychophysiological measures are used.

There are a number of limitations to the current study and associated data. First, although this is the largest longitudinal study to examine individual differences in the startle reflex response among low- and high-BI adolescents with and without anxiety, the overall sample sizes were relatively small. Accordingly, replication in larger samples is needed. Moreover, because of such small sample sizes, we were forced to combine adolescents with any history of an anxiety disorder into a single group. Future studies with larger samples might consider associations with specific anxiety disorders. The small sample size also limited power on statistical tests of interactions. Although we did detect a significant two-way interaction between anxiety and BI status for startle potentiation to safety cues, we did not detect a three-way interaction, among anxiety, BI status, and condition. The failure to detect this three-way interaction may also be related to the small sample size.

Family history of anxiety diagnosis has been shown to affect the relation between BI and anxiety[35] as well as to affect startle response magnitude, particularly among girls.[27,32] It is likely that the results obtained in the current study may have been influenced by such familial risk. However, family history of anxiety diagnosis was not obtained and therefore could not be examined. Future studies examining startle and BI in anxious individuals should include measures of parental diagnosis.

Finally, in contrast to previous studies investigating fear-potentiated startle in at-risk adolescents,[27,32] no sex differences were observed in the current study. Recent research has shown that the menstrual phase can affect startle response magnitude among female subjects.[36] The majority of the adolescent girls in the current study had started menses at the time of startle assessment. Thus, the menstrual phase of the female participants may have influenced the startle response, leading to a lack of sex differences. However, we did not assess the girls' phase of menstrual cycle and therefore are not able to directly assess this possibility.

In sum, this study provides the first evidence that the fear-potentiated startle response can differentiate between behaviorally inhibited adolescents with and without a history of anxiety diagnosis. Although other studies have reported that anxious adults and adolescents at high risk for anxiety show increased fear-potentiated and baseline startle magnitude, this is the first to demonstrate that high-risk individuals who later develop anxiety also show a startle response pattern that is suggestive of abnormal inhibitory fear processes. Because startle and psychiatric diagnosis were assessed concurrently, this study is not able to assess whether generalized startle to the safety cue can be used as a predictor of anxiety or is the result of being both behaviorally inhibited and anxious. Future studies should examine fear-potentiated startle in children at earlier ages to examine whether differences in startle exist before diagnosis.

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