Behavioral inhibition (BI), a temperament characterized in early childhood by wariness and avoidance of novelty, is a risk factor for anxiety disorders. An enhanced startle response has been observed in adolescents characterized with BI in childhood, particularly when they also manifest concurrent symptoms of anxiety. However, no prior study has examined relations among BI, startle responsivity, and anxiety in a prospective manner. Data for the present study were from a longitudinal study of infant temperament. Maternal reports and observations of BI were assessed at ages 2 and 3. At age 7, participants completed a startle procedure, while electromyography was collected, where participants viewed different colors on a screen that were associated with either the delivery of an aversive stimulus (i.e., puff of air to the larynx; threat cue) or the absence of the aversive stimulus (i.e., safety cue). Parental reports of child anxiety were collected when children were 7 and 9 years of age. Results revealed that startle responses at age 7 moderated the relation between early BI and 9-year anxiety. These findings provide insight into one potential mechanism that may place behaviorally inhibited children at risk for anxiety.

Descriptors: Temperament, Risk factors, Startle, Behavioral inhibition, Anxiety

The startle response is elicited by sudden and intense sensory stimuli (Landis & Hunt, 1939). When anticipating threats, the startle response is potentiated, providing a measure of threat processing (Grillon, 2002). Compared to healthy controls, individuals with anxiety disorders display typical startle potentiation to explicit threat cues but elevated startle responses to cues signaling safety, which has been suggested to reflect an overgeneralization of threat cues (Lissek et al., 2005). Generalized fear responses may also result in elevated anxious responding to other stimuli, such as contextual cues in the environment (Grillon, 2002). Anxious adults demonstrate elevated contextual startle responses (during the intertrial interval [ITI] between threat and safety cue presentations), particularly in anxiogenic environments (Grillon et al., 2009). Thus, enhanced startle responses to both contextual cues (i.e., startle during the ITI) and safety cues are possible mechanisms in the development of anxiety. However, little research has examined how generalized startle responsivity predicts the emergence of anxiety over the course of development.

Behavioral inhibition (BI) is a temperament identified early in toddlerhood that is characterized by a heightened state of vigilance in response to novelty (Fox, Henderson, Rubin, Calkins, & Schmidt, 2001). The construct of BI is typically measured by laboratory observations (e.g., Kagan & Snidman, 1991), parent report (e.g., Goldsmith, 1996), or a combination of both methods (e.g., Fox et al., 2001). BI is a relatively stable temperament over childhood and has been linked to a number of later internalizing and anxiety problems. For example, behavioral inhibition at age 2 predicts internalizing problems and social reticence at 4 years of age (Rubin, Burgess, & Hastings, 2002), as well as shyness at 7 years of age (Kagan, Reznick, & Snidman, 1988; Kagan, Reznick, Snidman, Gibbons, & Johnson, 1988). Children who are characterized with a stable BI profile over childhood are at an increased risk of developing social anxiety disorder (SAD) in childhood and adolescence (see Clauss & Blackford, 2012, for review). The link between BI and later risk for SAD has been found utilizing both observation measures of laboratory-assessed BI (Biederman et al., 1993, 2001; Schwartz, Snidman, & Kagan, 1999), and maternal report of BI (Chronis-Tuscano et al., 2009).
However, not all behaviorally inhibited children develop anxiety, suggesting that other mechanisms, such as altered startle responses, may influence individual trajectories. Studies of BI and startle have found that adolescents with a stable history of heightened laboratory-observed BI and a lifetime history of anxiety display increased startle potentiation to safety cues compared to adolescents without a stable history of BI and no lifetime history of anxiety (Reeb-Sutherland et al., 2009). Additionally, 7-year-old children characterized in toddlerhood with high laboratory-observed BI exhibit generalized startle responses to safety cues (Barker, Reeb-Sutherland, & Fox, 2013). However, in both of these studies, startle responses and anxiety were measured concurrently, making it difficult to discern whether altered startle responses predict the later development of anxiety.

To date, no studies have prospectively examined the startle response as a predictor of later anxiety in behaviorally inhibited children. In a sample of older adolescents characterized as high in neuroticism, startle responses to contextual cues were concurrently related to neuroticism only among males (Craske et al., 2009). In a follow-up study using the same sample, startle potentiation to safety cues at 16 years of age prospectively predicted the emergence of anxiety disorders 3 to 4 years later (Craske et al., 2012). However, few prospective studies have examined startle patterns as predictors earlier in development (i.e., childhood).

The current longitudinal study provided an opportunity to examine whether startle responses to safety cues and contextual cues (during the ITI) at age 7 predicted the emergence of later anxiety at age 9 in children characterized as behaviorally inhibited in early childhood. To measure BI, we utilized two independent assessments (parent report and observed behavior) at ages 2 and 3. We hypothesized that startle responses would moderate the relation between BI and anxiety such that BI would predict anxiety only among children with elevated startle responses to safety and contextual cues.

**Method**

**Participants**

Children were selected for participation at 4 months of age based on their degree of observed emotional and motor reactivity in the laboratory (N = 291; Hane, Fox, Henderson, & Marshall, 2008). At age 2 and age 3, children and parents returned to the laboratory where children participated in a structured observation of behavior and parents completed questionnaires on child behavior (N = 268; Fox et al., 2001). Children returned to the laboratory at age 7 and completed the potentiated startle procedure (N = 195; M = 91.73 months, SD = 2.88). Participants were excluded due to various exclusion criteria (e.g., refused to participate, excessive electromyography artifact; see Barker et al., 2013) resulting in 95 participants (50 females) with usable startle data. At 9 years of age (M = 121.79 months, SD = 4.88), children and their mothers returned to the laboratory and completed questionnaires assessing anxiety symptoms (N = 194). Due to differences in available data across measures, sample size for analyses varied from 114–184 participants.

**Measures**

**Behavioral inhibition.** At age 2 and 3, individual differences in BI were assessed based on children’s proximity to their caregiver and their latency to approach and vocalize in a variety of novel contexts in the laboratory (see Fox et al., 2001). Additionally, parents completed the social fear scale of the Toddler Behavior Assessment Questionnaire (Goldsmith, 1996). Separate measures of BI were created by combining all available data for each method (i.e., laboratory-observed BI, parent report of BI). Additionally, a global measure of BI was created by averaging all available data across both methods.

**7-year startle.** Prior to the startle procedure, children were told they would be hearing sounds and seeing green- or blue-colored screens. On one of the screens, there was a possibility of receiving an unpleasant puff of air to the neck (threat cue), and the other color (e.g., green) indicated that there was no possibility of receiving a puff (safety cue). Children were randomly presented eight threat, eight safety, and eight ITI trials. During the safe and threat conditions, children were presented with the safety or threat cue, respectively, followed by a startle probe occurring either 4 or 7 s after cue onset. On 50% of the threat trials, a puff of air was presented to the neck following the presentation of the startle probe. No air puff was presented on safety or ITI trials. Threat and safety cues were presented for a total duration of 12 s. During ITI trials, startle probes were delivered randomly without presentation of either threat or safety cues (i.e., children were presented a black screen). The time interval between the onsets of two successive startle probes varied between 17 and 42 s. Raw startle magnitude for each trial was converted to a T score, then all startle probes within each condition were averaged. Startle to contextual cues was evaluated as the startle magnitude during cue-free periods (i.e., ITI condition). Startle to safety cues and threat cues were evaluated as the average startle magnitude during each cue, respectively. Additional details regarding psychophysiological collection and processing are described in full elsewhere (Barker et al., 2013).

**9-year anxiety and internalizing problems.** At 9 years, mothers completed the Screen for Child Anxiety Related Emotional Disorders (SCARED; Birmaher et al., 1999) and the Child Behavior Checklist (CBCL; Achenbach & Rescorla, 2001) to assess children’s anxiety problems. Analysis of the SCARED focused on the social anxiety scale. For the CBCL, the current analysis focused on the internalizing broadband T score. Among participants with available 7-year startle data, males demonstrated higher internalizing problems (M = 51.65) than females (M = 46.39) on the CBCL, t(79) = 2.83, p = .006. No differences between genders were observed for the SCARED (p < .20).

**Data Analysis**

Hierarchical regression analyses were conducted to examine whether 7-year startle responses moderated the relation between early BI and 9-year internalizing and anxiety outcomes. To account for missing data, maximum likelihood estimation was utilized in all analyses for participants who had available data on at least one independent measure (i.e., BI or startle data). Patterns of missing data were not associated with BI, startle, or anxiety measures (ps > .20). All analyses were first conducted using the BI composite as the predictor, comprised of both laboratory observation and parent report. Next, to explore how different measures of BI influence later outcomes, separate regression analyses were conducted using laboratory-observed BI and then parent report of BI. Gender was included as a predictor for 9-year outcomes because preliminary analysis found that males exhibited greater internalizing problems than females. An interaction variable was computed as the
product of the mean-centered startle and BI variables and was included in all regression analyses. Significant interactions were

among children with higher startle potentiation during the ITI, early behavioral inhibition (BI) predicted later internalizing problems (top) and social anxiety symptoms (bottom). However, BI did not predict later internalizing or anxiety problems among children with lower ITI startle.

Results

Composite BI (Parent Report and Laboratory-Observed BI)

CBCL internalizing problems were regressed onto composite BI, ITI startle, gender, and the BI \( \times \) ITI Startle interaction. BI was positively related to 9-year internalizing problems, \( \beta = 1.41 \), \( t(180) = 2.79 \), \( p = .005 \), and males demonstrated more internalizing problems than females, \( \beta = -5.64 \), \( t(180) = 3.40 \); \( p = .001 \). However, the BI \( \times \) ITI interaction was not significant, \( \beta = .17 \), \( t(180) = 1.01 \); \( p = .31 \). Next, SCARED social anxiety was regressed onto composite BI, ITI startle, gender, and BI \( \times \) ITI Startle. No main or interaction effects were significant (\( ps > .20 \)). Next, multiple hierarchical regression analyses were conducted with safe startle as the moderator between BI and CBCL internalizing problems and then between BI and SCARED social anxiety. Neither regression analysis revealed any main or interaction effects (\( ps > .20 \)).

Laboratory-Observed BI

CBCL internalizing problems were regressed onto laboratory-assessed BI, ITI startle, gender, and the BI \( \times \) ITI Startle interaction. Boys exhibited more internalizing problems than girls, \( \beta = -6.87 \), \( t(118) = 3.70 \); \( p < .001 \).1 In addition, the BI \( \times \) Startle interaction was significant, \( \beta = 2.23 \), \( t(118) = 2.29 \); \( p = .022 \).2 Among participants with higher ITI startle, there was a positive association between BI and later internalizing problems, \( \beta = 8.09 \), \( t(118) = 1.99 \); \( p = .046 \) (see Figure 1, bottom panel). However, there was no relation between BI and internalizing among participants with lower ITI startle, \( \beta = -4.48 \), \( t(118) = 1.49 \); \( p = .14 \). Next, SCARED social anxiety was regressed onto laboratory-assessed BI, ITI startle, gender, and the BI \( \times \) ITI Startle interaction. There were no main effects of the predictors (\( ps > .20 \)). However, the BI \( \times \) ITI Startle interaction was significant, \( \beta = 0.95 \), \( t(110) = 2.54 \); \( p = .011 \).3 Among participants with higher ITI startle, there was a positive association between observed BI and social anxiety, \( \beta = 3.80 \), \( t(110) = 2.31 \); \( p = .021 \) (see Figure 1, top panel). However, there was no relation among participants with lower ITI startle, \( \beta = -1.53 \), \( t(110) = 1.29 \); \( p = .20 \). Next, multiple hierarchical regression analyses were conducted with safe startle as the moderator for CBCL internalizing problems and then for SCARED social anxiety. Neither regression analysis revealed any main or interaction effects (\( ps > .20 \)). Last, multiple hierarchical regression analyses for threat startle as the moderator for CBCL internalizing problems and for SCARED social anxiety revealed no main or interaction effects (\( ps > .20 \)).

Parent Report of BI

CBCL internalizing problems were regressed onto parent report of BI, ITI startle, gender, and the BI \( \times \) ITI Startle interaction. Boys exhibited more internalizing problems than girls, \( \beta = -4.30 \), \( t(151) = 2.45 \); \( p = .014 \). No other main or interaction effects were significant (\( ps > .20 \)). Next, SCARED social anxiety was regressed onto parent report of BI, ITI startle, gender, and the BI \( \times \) ITI Startle interaction. No main or interaction effects were significant

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1. To explore the influence of gender on startle and BI in predicting internalizing problems, a regression analysis was conducted including a BI \( \times \) Startle \( \times \) Gender three-way interaction variable. Results revealed similar results as observed for the two-way interaction (\( p < .05 \)). However, the three-way interaction variable did not reach significance (\( p > .20 \)).

2. Regression analyses were also conducted using the subscales that partially compose the CBCL broadband internalizing scale (i.e., anxiety problems scale and the affective problems scale). Neither scale was significantly predicted by any BI or startle measure (\( ps > .20 \)), likely reflecting limited variability within each scale.

3. Regression analyses were also conducted controlling for 7-year internalizing symptoms when predicting 9-year internalizing symptoms. Results revealed that 7-year CBCL internalizing positively predicted 9-year CBCL internalizing symptoms, \( \beta = .48 \), \( t(117) = 5.45 \); \( p < .001 \). However, the BI \( \times \) ITI Startle interaction did not significantly predict 9-year internalizing symptoms above and beyond that at 7 years, \( t(117) = 1.25 \); \( p = .18 \).

4. Regression analyses were also conducted controlling for 7-year internalizing problems when predicting 9-year social anxiety. Results revealed that 7-year CBCL internalizing problems positively predicted 9-year social anxiety symptoms, \( \beta = .99 \), \( t(109) = 2.57 \); \( p = .01 \). In addition, the Startle \( \times \) BI interaction variable positively predicted 9-year social anxiety symptoms, \( \beta = .78 \), \( t(109) = 2.41 \); \( p = .016 \).
contextual startle and behavioral inhibition

1547

Kagan, Reznick, and Snidman (1987) suggest that individual differences in BI are in part due to biological differences in the threshold of reactivity in the amygdala. The startle response, a physiological measure of reactivity and arousal, has been localized to regions of the amygdala (Davis, 1998), suggesting that fine-tuned measurement of BI in the laboratory may specifically tap these physiological responses. Observed negative reactivity in infancy predicts observed BI in early childhood (Fox et al., 2001), suggesting that observed reactivity to the environment is a stable measure of infant and child temperament, which may reflect early biological disposition. However, these findings do not negate the importance of parent report of BI as a valid measure of child temperament. Parent report of infant temperament is relatively stable across the first year of life (Rothbart, 1981, 1986), and moderate to high internal reliability has been observed for parent report measures (Worobey & Blajda, 1989). However, few studies have compared parent report and observed BI with different outcome measures (e.g., social, psychophysiological). Future research should examine how different measures of BI differentially predict physiological and behavioral outcomes.

Across the entire sample, boys exhibited more internalizing symptoms than girls. This finding warrants attention because studies typically find that, compared to boys, girls exhibit higher rates of internalizing problems and are at a greater risk for developing affective disorders in childhood and adolescence (Angold, Costello, Erkanli, & Worthman, 1999; Leadbeater, Kuperminc, Blatt, & Hertzig, 1999). However, there is evidence to suggest that the relation between temperament and later behavioral problems is stronger among boys than girls (Fagan, 1990; Stevenson-Hinde & Glover, 1996). It has been reported that infant boys identified as high in negative reactivity (a precursor to behavioral inhibition) are more likely to be identified as behaviorally inhibited in childhood than girls (Fox, Snidman, Haas, Degnan, & Kagan, 2015), and infant negative reactivity predicts social wariness at 4 years of age only among boys (Henderson, Fox, & Rubin, 2001). Furthermore, stable maternal report of BI predicts social anxiety symptoms only among males with insecure attachment (Lewis-Morrarty et al., 2015). One possibility for such findings is due to qualitative differences in caregiving between highly reactive boys versus highly reactive girls (MacDonald & Parke, 1984; Rubin, Cheah, & Fox, 2001; Stevenson-Hinde & Glover, 1996). Future studies should further examine the moderating role of gender in the relation between startle and later social behavior.

There are a number of limitations in the present study. One is that a large percentage of the sample was excluded due to a high number of noisy and/or nonresponse trials. Although the current startle procedure did potentiate the startle response, it may have been too aversive for a number of the participants. Alternative, less aversive procedures should be explored that can elicit a potentiated startle response (e.g., Quevedo, Smith, Donzella, Schunk, & Gunnar, 2009). Similarly, a limited sample size in the present study did not allow for the examination of whether early startle patterns predicted later psychiatric diagnosis. Future studies should examine startle patterns using a larger sample size into adolescence, a developmental period in which anxiety disorders are increasingly diagnosed (Kessler et al., 2005).

In sum, the current study is among the first to prospectively examine contextual startle as an early biological marker of later anxiety in childhood. Contextual startle responses moderated the relation between early BI and later anxiety problems, such that early BI predicted later anxiety only among children with high contextual startle responses. These findings suggest that anxiety in middle childhood may result from the combination of early behavioral inhibition and enhanced contextual startle.

Discussion

The goal of the present study was to examine whether startle responses measured at age 7 moderated the relations between early BI (i.e., at 2 and 3 years of age) and 9-year anxiety. Previous studies have found that startle patterns in adolescence predict the emergence of later anxiety disorders among risk populations (Craske et al., 2009, 2012). However, little research has examined if startle patterns in childhood predict later anxiety. This study was the first to prospectively examine whether altered startle patterns predict later anxiety symptoms in childhood among behaviorally inhibited children. Results of this study revealed that startle during the ITI, but not startle to safety cues or threat cues, moderated the relations between laboratory-assessed BI and anxiety. Specifically, BI in toddlerhood positively predicted anxiety at 9 years only among children with elevated ITI startle responses at age 7. However, there was no relation between BI and anxiety in children with lower levels of ITI startle.

Abnormal startle patterns have been observed in behaviorally inhibited adolescents with anxiety (Reeb-Sutherland et al., 2009) and behaviorally inhibited children (Barker et al., 2013). Anxious individuals demonstrate elevated startle responses during ITI when no explicit cues of threat or safety are present (Grillon et al., 2009), suggesting that anxiety is characterized by an overgeneralization of threat to contexts and cues that pose no threat (Lissek et al., 2005). Heightened startle responses during these cue-free periods reflect enhanced contextual anxiety to a perceived threatening environment (Grillon, 2002), and sustained responding to contextual threat may lead to the development of anxiety disorders (Grillon & Baas, 2003). Enhanced startle to contextual cues have also been observed in children with an anxiety disorder (Waters, Neumann, Henry, Craske, & Ornitz, 2008), and children and adolescents with a family history of anxiety (Grillon, Dierker, & Merikangas, 1998; Waters, Craske et al., 2008). The present study found that there was a positive prospective relation between BI and anxiety among individuals with elevated contextual startle. We previously observed elevated startle responding to safety cues in behaviorally inhibited children at 7 years of age (Barker et al., 2013). Furthermore, similar to the present findings, startle to safe cues was unrelated to anxiety symptoms. Taken together, these findings suggest that, although behaviorally inhibited children are characterized by enhanced startle to safe cues, such startle patterns do not predict risk for anxiety. In contrast, Barker and colleagues (2013) observed no differences in ITI startle between behaviorally inhibited and less inhibited children. Thus, startle during ITI may independently modulate risk for anxiety among behaviorally inhibited children. Future studies should further explore differences in startle to safe cues and contextual startle in predicting later anxiety.

Regression analyses of the different measures of BI demonstrated that only laboratory-assessed BI interacted with startle measures in predicting anxiety. These findings are consistent with previous findings that have found an association between observed BI and startle in childhood (Barker et al., 2013) and adolescence (Reeb-Sutherland et al., 2009). Kagan, Reznick, and Snidman (1987) suggest that individual differences in BI are in part due to biological differences in the threshold of reactivity in the amygdala. The startle response, a physiological measure of reactivity and arousal, has been localized to regions of the amygdala (Davis, 1998), suggesting that fine-tuned measurement of BI in the laboratory may specifically tap these physiological responses. Observed negative reactivity in infancy predicts observed BI in early childhood (Fox et al., 2001), suggesting that observed reactivity to the environment is a stable measure of infant and child temperament, which may reflect early biological disposition. However, these findings do not negate the importance of parent report of BI as a valid measure of child temperament. Parent report of infant temperament is relatively stable across the first year of life (Rothbart, 1981, 1986), and moderate to high internal reliability has been observed for parent report measures (Worobey & Blajda, 1989). However, few studies have compared parent report and observed BI with different outcome measures (e.g., social, psychophysiological). Future research should examine how different measures of BI differentially predict physiological and behavioral outcomes.

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(ps > .20). Next, multiple hierarchical regression analyses were conducted with safe startle as the moderator for CBCL internalizing problems and then for SCARED social anxiety. Neither regression analysis revealed any main or interaction effects (ps > .20). Last, multiple hierarchical regression analyses for threat startle as the moderator for CBCL internalizing problems and for SCARED social anxiety revealed no main or interaction effects (ps > .30).


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