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The N170 to Angry Faces Predicts Anxiety in Typically Developing Children Over a Two-Year Period

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Enhanced threat processing has been associated with elevated anxiety in adults, but less is known about how threat processing influences the developmental trajectory of anxiety in children. We used the N170 to measure threat (angry faces) processing in relation to child anxiety over a 2-year period. Participants were 27 typically developing 5-to-7-year-olds (13 females). Higher anxiety when children were aged 5 to 7 was associated with higher anxiety 2 years later, but only for children showing larger N170 amplitudes to angry versus happy faces. The N170 captures individual differences in threat processing that may characterize children at enhanced risk for anxiety.

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Stimuli signifying threat are more rapidly and accurately detected than neutral stimuli (for a review see Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007). Within normative levels, this attentional bias is adaptive because such facilitated attention fosters an individual's ability to detect and process potential danger in the environment, which in turn may serve to prepare the individual to respond to the threatening situation. On the other hand, enhanced attention to threat stimuli, such as human faces depicting anger, has been uniquely associated with elevated anxiety in adults (e.g., Fox, Russo, Bowles, & Dutton, 2001) and children (Roy et al., 2008; Waters, Henry, Mogg, Bradley, & Pine, 2010) and has been implicated in the etiology and maintenance of anxiety disorders (Bar-Haim et al., 2007; Hakamata et al., 2010). Selective attention to threat may contribute to the continuity of anxiety, even in a normative range, by facilitating preferential processing of threat at the expense of pleasant cues or cues for safety (e.g., Hofmann, 2007; Mathews & Mackintosh, 1998).

Almost the entirety of the literature on anxiety and the attentional threat bias relies upon behavioral measures of threat processing, which likely reflect a number of performance-related factors in addition to attention. That is, reaction time-based behavioral measures provide an endpoint assessment of many processes that intervene between the onset of threat stimuli and the execution of the response, rather than an assessment of biased attention alone. In contrast, scalp-recorded event-related potentials (ERPs) can index attention to threat even in the absence of a behavioral response, and due to their excellent temporal resolution, can be used to examine the earliest points at which facilitated attention to threat may appear (Banaschewski & Brandeis, 2007; O'Toole & Dennis, 2012).

Using ERPs to measure emotional face processing may provide important information about some of the most rapid and automatic stages of threat processing (Eimer & Holmes, 2002; Righart & de Gelder, 2006). In the present study, we examined an ERP with particular relevance for threat processing, the face-sensitive N170. The N170 is larger in amplitude in response to faces as compared to other objects, and is maximal in posterior electrodes around 170 msec (Batty & Taylor, 2003; Bentin, Allison, Puce, Perez, & McCarthy, 1996). The N170 is typically thought to reflect the structural encoding of faces (Eimer, 2011; Wronka & Walentowska, 2011).

The N170 is also sensitive to emotion, such that N170 amplitudes are larger to emotional versus neutral faces (Ashley, Vuilleumier, & Swick, 2004; Batty & Taylor, 2003, 2006). However, research has also shown that the N170 is *not* differentially sensitive to emotional face type (Batty & Taylor, 2006; Eimer & Holmes, 2002). The inconsistent support for emotional sensitivity of the N170 may be due to methodological differences among studies, such as stimulus or task parameters. These studies have presented faces for varying durations, ranging from 100 msec to 750 msec, in the context of tasks that require attention to the stimuli (e.g., responding to non-face targets or repeated faces) or tasks where the faces were incidental. Recent findings suggest that the N170 is sensitive to emotion when participants are instructed to monitor the emotion of the stimulus but not when they are instructed to monitor the gender of the stimulus (Wronka & Walentowska, 2011). Additionally, differential emotional responding of the N170 is reduced under heavy cognitive load (Pessoa, McKenna, Gutierrez, & Ungerleider, 2002). Taken together, these studies suggest that the emotional sensitivity of the N170 is dependent on task parameters. Additionally, only one study reported above investigated the N170 in participants across a range of ages (Batty & Taylor, 2006), in which older but not younger children showed differential emotional responding of the N170. These differences may be due to ongoing maturation of neural systems involved in emotional processing (Thomas et al., 2001). Thus, in the present study, we will explore whether the N170 is larger to task-irrelevant emotional versus neutral faces in a group of early-school-aged children.

Despite the debate concerning whether the N170 is enhanced to emotional versus neutral faces, the N170 likely represents a useful measure of preferential processing of threat for several reasons. First, faces are both socially salient and have high evolutionary relevance. Indeed, for both anxious and non-anxious individuals, threatening faces—angry faces in particular—rapidly and automatically heighten awareness and recruit attentional resources (Eysenck & Calvo, 1992; Hadwin, Donnelly, Richards, French, & Patel, 2009). Moreover, the majority of research on the attentional threat bias in anxiety uses angry faces as threat stimuli (e.g., Bar-Haim et al., 2007; Fox, Russo, & Georgiou, 2005). In addition, facial processing is a core capacity underlying the ability to learn and recognize emotions, which play an important role in the development of successful emotion regulation (Lang & Bradley, 2010; Thompson & Goodman, 2010) and disruptions in emotion such as anxiety (McLaughlin, Hatzenbuehler, Mennin, & Nolen-Hoeksema, 2011; Pollak, Cicchetti, Homung, & Reed, 2000). In the present study, we used the N170 to angry compared to neutral and happy faces as a measure of preferential processing of threat—or an attentional threat bias. We reasoned that, whether or not children in this study overall show larger N170 amplitudes to angry compared to other face types, the degree to which the N170 is larger to angry faces reflects facilitated threat processing. Research on attention training suggests that inducing an attentional bias toward threat leads to greater stress reactivity (Eldar, Ricon, & Bar-Haim, 2008); thus, individual differences in attention to threat may reflect a propensity to maintain or develop symptoms of anxiety in children. Identifying a biomarker for such biased processing, such as the N170, could contribute toward a better understanding of the development of anxiety and how to identify those at risk. We included both neutral and happy faces as a non-threat comparison because developmental affective neuroscience research shows that neutral faces elicit amygdala activity similar to that for fearful faces, perhaps because neutral faces provide ambiguous information about emotional state (Thomas et al., 2001).

The goal of the present longitudinal study was to examine whether, in a typically developing group of children, the N170 is a sensitive measure of facilitated processing of threat-relevant (angry) faces, such that it can be used to predict (a) concurrent attention performance following presentation of threatening versus non-threatening faces; (b) fear and anxiety-related behaviors; and (c) whether children manifest signs of anxiety over time. The present study targets a typically developing group of children, allowing us to examine whether the N170 is sensitive to even relatively non-extreme fluctuations in threat processing that could have implications for the emergence of anxiety over time. Such findings would suggest that the N170 has the potential to be a clinically relevant biomarker for threat processing, one that could be integrated into future studies of clinical anxiety. Furthermore, this is among the youngest child sample to be examined using a combination of questionnaire and physiological data, and the first to use the N170 as a marker for the attention bias to threat.

At ages 5 to 7, we measured how attention performance was influenced by the presence of threat (angry) and non-threat (happy and neutral) human faces. The N170 was generated to the angry, happy, and neutral faces during this attention task. We also measured signs of child anxiety via maternal report at 5 to 7 years of age (Time 1) and then at seven to nine years of age (Time 2).

We tested three hypotheses. First, we explored whether N170 amplitudes were enhanced to angry faces as compared to neutral or happy faces, reflecting an attentional bias to threat, and that this attentional bias would be related to subsequent disruptions in attention performance.

These analyses were exploratory because of inconsistent findings in the literature concerning the sensitivity of the N170 to facial emotion type. Second, we predicted that an enhanced N170 to angry as compared to neutral or happy faces will correlate concurrently with greater maternal report of anxiety in 5- to 7-year-olds. Third, we predicted that those 5- to 7-year-old children showing heightened signs of maternal report of anxiety *and* enhanced N170 to angry faces will go on to show sustained or increased signs of anxiety 2 years later. That is, an enhanced N170 to angry faces will moderate the association between Time 1 and Time 2 anxiety symptoms.

METHOD

Participants

A total of 51 children aged 5 to 7 years old were recruited from areas in and around an urban area. Of these 51 children, seven were excluded due to excessive electroencephalogram (EEG) artifacts. Of the remaining 44 children, data was collected at both Time 1 and Time 2 for 27 children (13 females). The race and ethnicity breakdown was as follows: 11 Caucasian, eight African American, six Hispanic/Latino, one Pacific Islander, and one other race. Handedness information was obtained about the children from their parent using the Edinburgh Handedness battery.

The majority of children were right-handed: three children (11%) had a score of –100 (left-handed), one child (4%) had a score of 67 (right-handed), one child (4%) had a score of 88 (right-handed), and 22 children (81%) had a score of 100 (right-handed).[1] Children first visited the lab (Time 1) when they were 5 to 7 years of age ($M = 74.30$, $SD = 6.34$, in months) and then again approximately 2.5 years later (Time 2) at 7 to 9 years of age ($M = 97.70$, $SD = 5.61$, in months).

Materials and Procedure

After informed consent and assent, the child was fitted with an elasticized nylon cap and scalp electrodes were applied. Children were seated 65 cm from the computer screen during the computer task while EEG was recorded.[2] Following this task, children and their parent were given time to clean up before debriefing. The visit to the lab lasted approximately 2 hours. Children were given astronaut ice cream as a gift at the end of the visit and families were compensated $100 for their time.

Child Behavior Checklist (CBCL). Parents completed the Child Behavior Checklist (CBCL; Achenbach & Rescorla, 2000, 2001), which measures symptoms of emotional and behavioral problems using a three-point Likert scale. There are different versions for children 1.5 years old to 5 years old (100 items) and ages 6 years and older (112 items). DSM anxiety T-scores were computed, using items such as “clings to adults or too dependent” or “doesn’t want to go out of home.” Approximately 2 years later parents filled out the CBCL a second time (Time 1 $\lambda 2 = .62$, based on 10 items for 5-year-olds and six items for 6- and 7-year-olds; Time 2 $\lambda 2 = .69$, based on 6 items; Sijtsma & Molenaar, 1987).

[1] Results did not change when handedness was entered as a covariate in analyses.

[2] While all children were seated 65 cm from the computer screen, variations in height and other factors that would affect the visual angle were not documented. Visual acuity was assumed to be normal or corrected-to-normal.

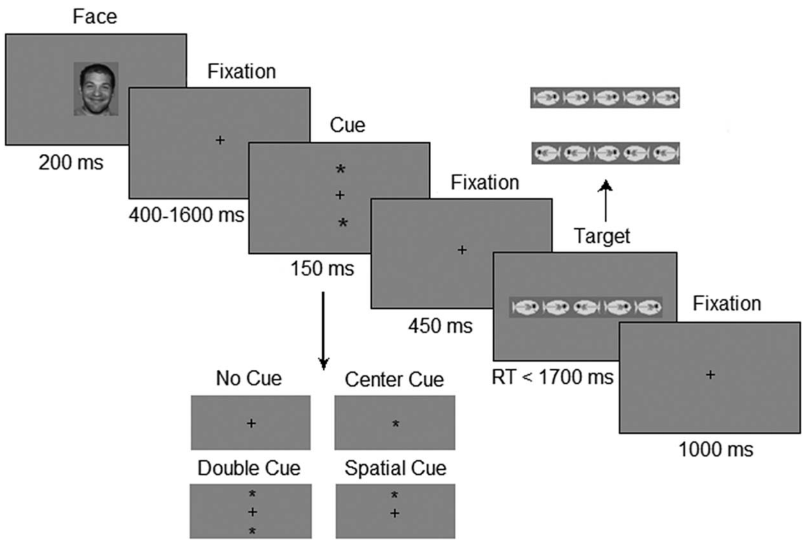

FIGURE 1 Sequence of events in the emotional Attention Network Test (E-ANT).

Modified Attention Network Test (ANT; Fan, McCandliss, Sommer, Raz, & Posner, 2002). E-Prime software (Version 1.1, Psychological Software) was used to program the modified Attention Network Task. Children were seated 65 cm from the 17 in computer screen during the experiment. The task combines a cued reaction time (RT) with a flanker task that requires the subject to identify the direction (right or left) of the central arrow that is flanked by either four arrows facing the same direction (congruent trial) or the opposite direction (incongruent trial). Comparison of RTs to the two flanker types provides a measure of conflict interference (executive attention). Prior to the flankers, one of four cue conditions are presented: no cue, double cue (appearing above and below fixation), center cue (superimposed on fixation), and spatial cue (appearing either above or below fixation). The cues signal the impending presentation (alerting) or location (orienting) of the flanker task.

The modified version used in the present study included task-irrelevant emotional face (angry, happy, or neutral) preceding each trial and developmentally appropriate fish instead of arrows. There were 64 trials per face type. Figure 1 shows components of the task: face types presented before each trial of the task (1a), cue conditions (1b), flanker conditions (1c), and the sequence of events for a single trial (1d).

Emotional face stimuli were taken from the NimStim Set of Facial Expressions (Tottenham et al., 2009). Forty-eight gray scale images of faces portraying either angry, happy, or neutral expressions were used, with an equal number of male and female actors.[3] Faces were presented for 200 msec prior to each ANT trial.

Three attention efficiency scores are calculated from RTs on different types of trials: *alerting* (RT no cues – RT double cues), *orienting* (RT center cues – RT spatial cues), and *executive*

[3]Actors: 01, 06, 07, 08, 10, 12, 13, 14, 20, 21, 23, 30, 33, 38, 39, 43.

attention (RT incongruent – RT congruent flankers). A higher score indicates greater alerting and orienting efficiency while higher executive attention scores indicate *decreased* executive attention efficiency (greater conflict interference). Attention scores were calculated separately for each task-irrelevant emotional stimulus type.

Electrophysiological recording and data reduction. EEG activity was recorded continuously via 64 Ag/AgCl scalp electrodes using a 10/20 system at a sampling rate of 512 Hz (BioSemi; Amsterdam, NL). The Biosemi system forms the ground electrode using Common Mode Sense active electrode and Driven Right Leg passive electrode during EEG acquisition; data were re-referenced offline to an average reference. Eye movements were monitored by electrooculogram (EOG) signals from electrodes placed 1 cm above and below the left eye (to measure vertical eye movements) and 1 cm on the outer edge of each eye (to measure horizontal eye movements). All data preparation after recording was conducted using Brain Vision Analyzer (Version 2.2, GmbH; Munich, DE). The continuous EEG was filtered with a low cutoff frequency of .1 Hz and a high cutoff frequency of 30 Hz.

Stimulus-locked data were baseline corrected, and segmented into epochs from 400 msec before to 600 msec after stimulus onset. EEG was corrected for blinks using independent components analysis. Artifacts were identified using the following criteria: voltage steps greater than 75 μV, differences in amplitudes more than $\pm$120 μV, voltage changes in a segment greater than 200 μV, and activity lower than .2 μV per 100 msec were considered artifacts and excluded from analyses.

N170 amplitudes were generated separately in response to the angry, happy, and neutral faces. Peak amplitudes were identified for the N170 between 170 and 270 msec (maximal at approximately 200 msec) over P5/P7/P6/P8. A 4(Electrode: P5, P6, P7, P8) $\times$ 3(Emotion: angry, happy, neutral) repeated measures ANOVA revealed no effects of electrode on amplitudes; thus, amplitudes were averaged across the four electrodes for all subsequent analyses. Of the possible 64 trials per face type, the average number of trials with good EEG data across all participants was: angry ($M = 61.49$ $SD = 4.32$), happy ($M = 60.01$, $SD = 5.83$), neutral ($M = 59.50$, $SD = 6.78$). There were two participants who had unusable data for one electrode from the cluster of four. Findings did not change when these children were excluded from analyses.

RESULTS

Descriptive Statistics

See Table 1 for descriptive statistics of CBCL anxiety *t*-scores at Time 1 and Time 2. At Time 1, two children had CBCL scores in the borderline clinical range (64–69) and one in the clinical range (69+). At Time 2, two children had CBCL scores in the borderline clinical range and two in the clinical range. Thus, this sample showed largely normative levels of anxiety.

Effects of Emotional Faces on N170 Amplitudes and Attention Performance

To test the first hypothesis, whether N170 amplitudes and attention performance were affected by the threatening versus non-threatening faces, we conducted a series of analyses. First to examine

TABLE 1
Descriptive Statistics for CBCL Anxiety *t*-Scores at Time 1 and Time 2

	M	*SD*	*Range*
Time 1	55.79	5.92	50.00–72.00
Time 2	53.82	6.04	50.00– 70.00

Note. At Time 1, two children had Child Behavior Checklist (CBCL) scores in the borderline clinical range (64–69) and one in the clinical range (69+). At Time 2, two children had CBCL scores in the borderline clinical range and two in the clinical range.

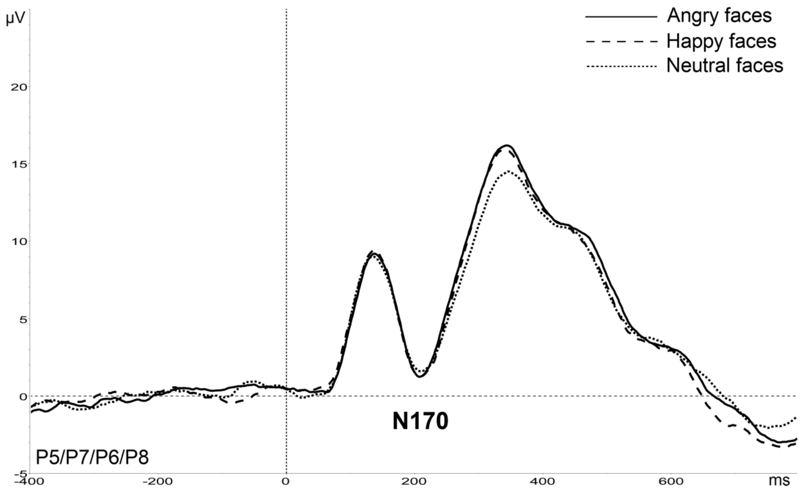

FIGURE 2 There was no effect of emotional face type on N170 amplitudes.

whether the N170 was sensitive to emotional face type, we conducted a 2(Hemisphere: left, right) × 3(Emotion: angry, happy, neutral) repeated-measures ANOVA with N170 amplitudes as the dependent variable. No significant effects emerged (see Figure 2). Because the N170 did not vary by hemisphere, amplitudes were averaged across hemisphere for all subsequent analyses.

Next, to test whether attention performance was influenced by emotional faces presented prior to each trial of the task, we conducted a one-way repeated measures ANOVA with Emotion (angry, happy, neutral) as the within-subjects factor separately for each attention score (alerting, orienting, executive attention). No significant effects emerged suggesting that these task-irrelevant emotional stimuli did not interfere with attention performance.[4]

[4]We also conducted Pearson's correlations between N170 amplitudes to angry as compared to neutral or happy faces and reaction times on the attention performance task. No significant effects emerged.

Associations Between Anxiety and N170 Amplitudes at Time 1

To test the second hypothesis, that enhanced N170 amplitudes to angry as compared to neutral or happy faces will correlate concurrently with greater signs of anxiety in 5- to 7-year-olds, Pearson correlations were conducted with anxiety *t*-scores and N170 amplitudes to angry versus happy and angry versus neutral faces. There were no significant correlations between anxiety and ERP amplitudes. However, greater anxiety at Time 1 was associated with greater anxiety at Time 2, $r = .69$, $p < .001$.

Interaction Between Time 1 Anxiety and N170 Amplitudes Predicting Time 2 Anxiety

A series of hierarchical multiple regressions were conducted to test the third hypothesis, that those 5- to 7-year-old children showing heightened signs of anxiety *and* enhanced N170 to angry faces will go on to show sustained or increased signs of anxiety 2 years later. Anxiety *t*-score and N170 amplitude were entered into the first step of the model, followed by the interaction between the two in the second step. Separate regressions were conducted for angry minus happy and angry minus neutral N170 amplitudes.

The regression model with N170 amplitudes to angry versus happy faces accounted for significant variance in anxiety at Time 2, $F(3, 23) = 13.68$, $p < .001$, $R^2 = .64$. Specifically, above and beyond the strong main effect of T1 anxiety and N170 amplitudes to angry versus happy faces (Step 1) on Time 2 anxiety [$F\Delta(2, 24) = 13.67$, $p < .001$, $R^2\Delta = .53$], the interaction between anxiety at Time 1 and N170 amplitudes to angry versus happy faces (Step 2) accounted for an additional 11% of the variance in anxiety at Time 2, $F\Delta(1, 23) = 6.93$, $p = .02$, $\beta = -3.72$, $t = -2.63$, $p = .02$. A simple slopes analysis revealed that the slope for greater N170 amplitudes to angry versus happy faces was significantly different from zero, $t(23) = 4.49$, $p < .001$. Thus, as predicted, greater anxiety at Time 1 was associated with greater anxiety at Time 2, but only for children who showed greater N170 amplitudes to angry versus happy faces (see Figure 3).

DISCUSSION

This was among the first studies to test whether the face-sensitive N170 could be used to measure anxiety-related threat processing. Consistent with predictions, typically developing 5- to-7-year-old children showing heightened signs of anxiety *and* enhanced N170 to angry faces went on to show sustained or increased signs of anxiety two years later, although N170 amplitudes to threat-relevant facial stimuli were not associated with heightened anxiety symptoms concurrently. The interaction effect emerged even when Time 1 anxiety was accounted for. For the sample as a whole, however, the N170 was not enhanced to emotional versus neutral faces. This suggests that individual differences in the attentional resources devoted to threat (angry faces) compared to safety (happy faces) cues may have a causal impact on the emergence of anxiety even in a typical range.

The temperament literature is highly relevant to the present study's findings. The attention bias to threat has been detected in children with behaviorally inhibited temperament (Pérez-Edgar et al., 2011; Pine, Helfinstein, Bar-Haim, Nelson, & Fox, 2009), who show signs of fear, wariness, and social withdrawal in response to unfamiliar stimuli or novelty and who are at heightened risk

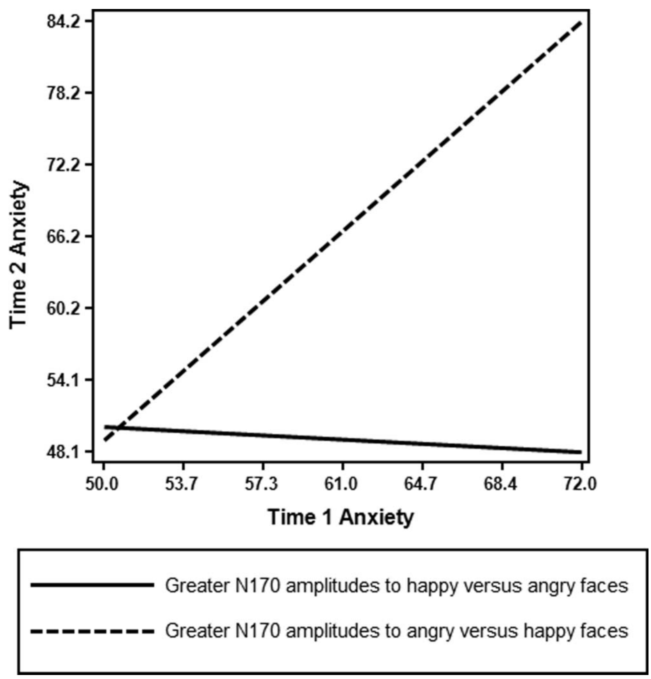

FIGURE 3 Greater anxiety at Time 1 predicted greater anxiety at Time 2, but only for children who showed greater N170 amplitudes to angry versus happy faces.

for clinical anxiety (e.g., Chronis-Tuscano et al., 2009). Given that the evaluation of facial stimuli is particularly salient for social anxiety (Fox et al., 2005), the next critical research step would be to explore the sensitivity of the N170 to threatening facial expressions in a sample of behaviorally inhibited children, and whether the N170 as a measure of threat bias influences the emergence of anxiety over time in this at-risk group of children.

Behavioral research demonstrating a stable link between early behavioral inhibition and later social withdrawal for children and adolescents (Pérez-Edgar et al., 2010, 2011) suggests this may be the case. For example, in a study of behavioral inhibition (Pérez-Edgar et al., 2010), adolescents who were previously inhibited as children, compared to those with no such history, showed attention biases to threat. Importantly, it was only those participants who showed attention bias to threat who also showed the link between temperamental inhibition in childhood and social withdrawal in adolescence. Applying the present study's neurophysiological approach, future research should test whether individual differences in the early structural encoding of threatening facial stimuli (measured via the N170) can help explain the developmental trajectory from behavioral inhibition to anxiety. A previous study using another ERP, the error-related negativity (ERN), showed such effects by documenting that the ERN moderated the longitudinal association between behavioral inhibition in childhood and adolescent anxiety, but at the level of a trend (McDermott et al., 2009). Finally, a larger sample, and a focus on more extreme temperamental groups will be crucial for examining the clinical relevance of the N170.

Two null findings provide important information. First, consistent with previous research in adults and children, we found that N170 amplitudes were *not* larger to emotional versus neutral faces (Batty & Taylor, 2006; Eimer & Holmes, 2002). The face stimuli used in this task were brief and were not of high intensity. This might have reduced the sensitivity of the N170 to these particular face stimuli. Findings are mixed in adults regarding the emotional sensitivity of the N170 to briefly presented faces and whether participants must be aware of the stimuli in order for differential responding of the N170 to occur (e.g., Eimer, Kiss, & Holmes, 2008). Additionally, the present study did not instruct children to attend to the emotional faces, which were presented as task-irrelevant stimuli prior to the attention task. Most studies that have found differential N170 responses to emotional versus neutral faces when participants were instructed to attend to the faces in order to complete a task. If, in the present study, children's cognitive resources were directed towards completing the attention task this may have led to reduced processing of the emotional content of the faces (Pessoa et al., 2002). This null finding may also reflect the ongoing maturation of neural systems implicated in face processing (Thomas et al., 2001), consistent with the presence of differential emotional responding in older but not younger children (Batty & Taylor, 2006). Despite this null finding, the present study suggests that the N170 is still useful in measuring emotional face processing in younger children and may be an important tool in tracking the development of anxiety.

A second null finding was that the N170 was not significantly concurrently correlated with child anxiety at Time 1 or Time 2. This is interesting in light of the finding that N170 amplitudes to angry versus happy faces predicted the developmental trajectory of anxiety over time. One possibility is that effects of relatively subtle alterations in the processing of threat compared to pleasant faces has a gradual influence on the expression of anxiety and is not easily detectable at any given time point, particularly with our relatively small sample size. Another possibility is that biased processing of angry faces does not influence the concurrent expression of anxiety, but rather, slowly alters the course of anxiety by facilitating a pattern of vigilance for threat and subsequent behavioral avoidance, which over time, could sustain or increase signs of anxiety (e.g., Hofmann, 2007). Other factors, such as parenting style, may also play a role in the emergence and progression of anxiety in children (Wood, McLeod, Sigman, Hwang, & Chu, 2003). Future research should consider multiple factors that contribute to the emergence of pediatric anxiety, including both neural measures of attention and social context.

In summary, the present study was among the first to suggest that the N170 could prove to be a highly sensitive measure of anxiety-related threat processing in childhood. Future research will clarify whether the N170 can be used as a neural biomarker to predict which children may be at heightened risk for the development of clinical anxiety.

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