# A PSYCHOBIOLOGICAL APPROACH TO MAPPING TEMPERAMENT AND PSYCHOPHYSIOLOGICAL ACTIVITY EARLY IN DEVELOPMENT

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# A THESIS

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

Psychology - Master of Arts

## ABSTRACT

# A PSYCHOBIOLOGICAL APPROACH TO MAPPING TEMPERAMENT AND PSYCHOPHYSIOLOGICAL ACTIVITY EARLY IN DEVELOPMENT

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A growing literature indicates that early emerging differences in emotional reactivity and regulation, known as temperament, may relate to an increased risk for developing internalizing psychopathology. Much of the evidence for these claims has relied on temperament models derived from parent-report. Few studies have drawn upon basic findings in affective, cognitive, and developmental science to measure temperament using a multi-method approach. The aim of this study is to draw upon these perspectives to map associations between behavioral and psychophysiological measures of effortful control (EC) and fear-proneness (FP) dimensions of temperament, and to explore their relationship with maternal-reported internalizing problems in children. Children between the ages of 3 and 7 years (N = 275) completed laboratory assessments of temperament, and a subset (N = 55) completed psychophysiological tasks designed to measure responses to error-induced demands on EC (the error-related negativity, or ERN) and to aversive stimuli (fear-potentiated startle). Results revealed a relationship between higher ratings of laboratory-assessed FP and smaller ERN amplitude. Additionally, higher ratings of laboratory-assessed FP were associated with larger startle magnitude responses to negatively-valenced stimuli. Results also indicated a moderate effect in association between larger ERN amplitude and higher maternal-reported internalizing symptoms. The present findings support the use of psychophysiological measures as a useful complement to behavioral and informant measures to better understand the relationship between temperament and risk for internalizing problems in children.

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# **INTRODUCTION**

Since Ancient Roman times, individual differences in emotional reactivity and selfregulation, known as temperament, have been identified as among the earliest emerging biological differences in children. Galen, famously known for his theory of the four humors, argued that these early behavioral differences in children serve as a foundation for understanding "the faculties of the soul" (as cited in Rothbart, 1989). Modern temperament research has primarily focused on how early differences in emotional behavior may be related concurrently and prospectively to risk for psychopathology (Caspi et al., 1996; Shiner, 2000; Clark, 2005; Durbin, Klein, Hayden, Buckley, & Moerk, 2005; Eisenberg, et al., 2000). While most of the empirical support for contemporary models of temperament has relied primarily on parent-report methods, individual differences in temperament can also be understood from a behavioral and psychophysiological perspective (Gunnar, 1990; Rothbart, 1989). Connections between affective and cognitive neuroscience and dimensions of temperament (e.g., Caspi et al., 2002; Rothbart, 2004; Schwartz, Wright, Shin, Kagan, & Rauch, 2003) have allowed the pursuit of mapping temperament dimensions to basic neural systems related to attention and affective-motivational systems, including effortful control (EC) and fear-proneness (FP). The present study drew upon this approach to achieve two aims: (1) explore the relationship between behavioral and psychophysiological measures of EC and FP, and (2) quantify associations between these measures and internalizing problems in children.

# **Fear-Proneness and Internalizing Problems**

FP is defined as the propensity for having a fearful response style, as evidenced by reticence, behavioral withdrawal, and expressions of fear and anxiety, in both novel and non-novel contexts (Goldsmith & Lemery, 2000), and is considered a marker of risk for internalizing

symptoms (Blackford & Pine, 2012). FP is distinct from behavioral inhibition (BI), a temperament construct that is characterized by low engagement and fear in response to novel stimuli only (Kagan, 1997). Studies using parent-report measures indicate that while FP loads on a higher order negative affect (NA) factor along with distress and anger/frustration, FP is distinguishable from anger/frustration (Rothbart, Ahadi, Hershey, & Fisher, 2001). Similar structural findings have been reported for laboratory-assessed measures of temperament (Dyson, Olino, Durbin, Goldsmith, & Klein, 2012).

Individual differences in FP are suggested to act as sources of vulnerability for developing internalizing problems (e.g., Goldsmith & Lemery, 2000). For example, measures of fearfulness and related constructs in early childhood have been shown to reliably predict concurrent and later subthreshold and clinically significant internalizing problems (e.g., Caspi, Henry, McGee, Moffitt, Silva, 1995; Colder, Mott, & Berman, 2002; Kagan, Snidman, Zentner, & Peterson, 1999), which may manifest in a number of ways, such as increased social reticence as a toddler (Fox, Henderson, Marshall, Nichols, & Ghera, 2005) or elevated risk for developing social anxiety disorder in adolescence (Chronis-Tuscano et al., 2009). There is some evidence that anxiety disorders may be part of developmental pathways to later depressive disorders, and some have argued that individual differences in FP are markers of the genetic component of this pathway (e.g., Silberg, Rutter, & Eaves, 2001; Warner, Wickramaratne, & Weissman, 2008). However, the expression of FP over time is as discontinuous as it is continuous, reflecting the multifinality of development. While children with high FP are at increased risk for developing internalizing psychopathology (e.g., Caspi et al., 1995, Chronis-Tuscano et al., 2009, Kagan et al., 1999), high FP in early childhood is not uniformly associated with these outcomes, given that some children with high FP display less withdrawn social behavior in late childhood as

compared to their presentation at earlier assessments (Schwartz, Snidman, & Kagan, 1999), and do not evidence any anxiety disorders in adolescence or adulthood (see review by Degnan & Fox, 2007). This suggests that other factors might moderate the association between FP and internalizing problems; however, research has yet to identify the role of other endogenous or exogenous factors in elucidating the variability in developmental trajectories associated with elevated FP in childhood.

## **Effortful Control and Internalizing Problems**

EC is defined as the capacity to regulate reactive processes, such as fear, and to purposefully suppress a predominant response and execute a subordinate response (Rothbart, Ellis, Rueda, & Posner, 2003). Increasing evidence suggests that individual differences in EC play a role in moderating the relationship between other temperament traits and internalizing problems (e.g., Eisenberg et al., 2009; White, McDermott, Degnan, Henderson, & Fox, 2011). Studies of EC in adults indicate that low EC is associated with both internalizing and externalizing problems (Carver, Johnson, & Joormann, 2008), but this relationship is not as well understood in children. While there is evidence from both parent-reported and laboratoryassessed temperament measures to suggest that high EC, or the ability to restrain predominant urges, may be related to fewer concurrent and later externalizing behaviors (e.g., Kochanska & Knaack, 2004; Wachs & Bates, 2001), its relationship with internalizing problems is less apparent. Some studies have found that high EC is associated with self-regulatory behaviors that allow the child to adapt and cope with frustration (Shoda, Mischel, & Peake, 1990), whereas other studies suggest that children with high EC are also high in guilt and shame, a predisposition for developing later depression and anxiety disorders (Rothbart, Ahadi, & Hershey, 1994). These differing associations hark back to the Blocks' constructs of ego control

and ego resiliency (Block & Block, 1980), where ego control is defined as a person's typical tendency to restrain behavioral impulses, and ego resiliency is defined as a person's ability to flexibly engage ego control (i.e., reduce or increase such control) when called upon by differing contexts. Based on the literature reviewed above on EC, one could expect that ego control has curvilinear relationship with internalizing problems such that low and high levels of ego control relate to internalizing problems, whereas ego resiliency would have a linear, negative relationship where low ego resiliency is associated with more internalizing problems.

Current research methods are insufficient for attempting to assess the biobehavioral process of EC and FP as they were originally defined by Rothbart and Derryberry (1981). Current methods primarily rely on one measure such as parent-reported questionnaires or on a single behavioral lab task. Therefore, it seems critical to capitalize on knowledge from between basic neuroscience and emotional development, which is grounded in the ability to relate behavioral observations of temperament in humans to specific responses (e.g., avoidance, freezing) observed in animals (Fox et al., 2005). Elucidating this relationship between temperament and internalizing problems in children using both behavioral and psychophysiological methods parallels the psychobiological approach proposed by Rothbart and Derryberry (1981) in which temperament is defined by biologically based differences in reactivity and self-regulation. Temperament dimensions related to defensive reactions, approach behavior, and attention have shown strong similarities to the temperament structure of other animals (Rothbart, 2007). Moreover, cognitive processes and temperament are intimately intertwined given that successful execution of self-regulatory and reactive behaviors requires efficient recruitment of higher order cognitive processes (Posner & Rothbart, 2000), which can now be investigated at the neural level due to technological advances that allow a more direct

measure of brain activity related to cognitive control and therefore a more distinct test of how EC is related to the expression of internalizing problems.

Identifying psychophysiological correlates of EC and FP that emerge early in life may act as a tool for assessing psychobiological risk, allowing for more targeted intervention and prevention strategies. Given that very little is known about the normal development of brain mechanisms implicated in risk for internalizing disorders, one approach is to examine psychophysiological correlates in young children that have previously been associated with internalizing in adults (Pine, 2007). The present study thus has two aims: (1) assess the validity of psychophysiological markers previously identified as associated with risk for internalizing psychopathology in adults in a sample of young children prior to the age of risk for internalizing disorders (in order to better understand if these markers represent risk factors for or correlates of internalizing symptomatology) by using laboratory-assessed and maternal-reported temperament traits as external criterion validators; and (2) further validate these markers by exploring their associations with frank internalizing problems.

## The ERN and Internalizing Problems

Existing research provides a strong foundation for examining the error-related negativity (ERN) and fear-potentiated startle (FPS) as psychophysiological correlates of EC and FP, respectively. EC is intimately tied to frontal lobe processes involving the anterior cingulate cortex (ACC), which helps monitor and signal other regions to implement control and active regulation (Botvinick, 2007; Ridderinkhof, Ullsperger, Crone, & Nieuwenhuis, 2004). EEG studies examining performance errors have identified a robust marker of ACC-mediated EC functions that appears as a waveform with a negative deflection and frontocentral peak approximately 50 ms following an erroneous response called the ERN (Gehring, Gross, Coles,

Meyer, & Donchin, 1993). Earlier studies once suggested that the ERN could only be reliably elicited in adolescents and adults (Davies et al., 2004), but more recently several studies have observed the ERN in children as young as 3 years old (Grammer, Carrasco, Gehring, & Morrison, 2014) with a notable developmental difference wherein the ERN amplitude is smaller in younger children as compared with adults (i.e., Kim, Iwaki, Imashioya, Uno, & Fugita, 2007; Wiersema, van der Meere, & Roeyers, 2007).

There is strong evidence to suggest that enhanced ERN in adults is associated with anxiety-related problems (Vaidyanathan, Nelson, & Patrick, 2011; Weinberg, Riesel, & Hajcak, 2012), indicating an overinvestment in frontally mediated inhibitory behaviors (Eysenck & Derakshan, 2011; Moser, Moran, & Jendrusina, 2012). While enhanced ERN is also reported in pediatric anxiety disorders for children 8 years or older (i.e., Hajcak, Franklin, Foa, & Simons, 2008; Ladouceur, Dahl, Birmaher, Axelson, & Ryan, 2006) and in adolescents who were behaviorally inhibited as children, a recent study found that somewhat smaller ERNs are common in younger children high in FP (Meyer et al., 2012). Taken together, the reviewed literature highlight the importance of, but substantial lack of research on the ERN, as a psychophysiological indicator of internalizing problems in very young children.

#### **FPS and Internalizing Problems**

FPS, or the potentiation of the startle eyeblink reflex in response to aversive compared to nonemotional stimuli, is interpreted as an index of defensive reactivity (e.g., Lang, Bradley, & Cuthberg, 1990). Research in both animals (e.g., Davis, Antoniadis, Amaral, & Winslow, 2008; LeDoux & Schiller, 2009) and humans (e.g., Sabatinelli, Bradley, Fitzsimmons, & Lang, 2005) has consistently demonstrated that FPS activates the brain's fear-defense circuit, centered in the amygdala. There is evidence from the adult literature that elevated FPS is associated with

elevated trait anxiety and fearfulness (Temple & Cook, 2007). While most studies have relied on using images of life threatening situations or aversive scenes to elicit FPS in adults (e.g., Bradley, Codispoti, Cuthbert, & Lang, 2001), the content of these images is not appropriate for use with younger children. Therefore, literature on FPS in younger children is inconsistent, primarily due to methodological challenges in selecting developmentally appropriate stimuli that can also successfully elicit fearful responses. However, FPS has been observed in infants as young as 5-months using images of angry faces (Balaban, 1995), and one recent study using ageappropriate film clips demonstrated robust FPS across a range of age groups from 3 years to adulthood (Quevedo, Smith, Donzella, Schunk, & Gunnar, 2010). Even though there is evidence from the adult literature showing elevated FPS in adults high in trait FP and reduced FPS in chronically distressed adults (Lang & McTeague, 2009; McTeague & Lang, 2012), the lack of research on FPS in young children precludes the investigation of the association between individual differences in FPS and FP from a developmental perspective. Together, both the adult literature and the limited youth literature highlight the importance of further examining FPS as a psychophysiological correlate of FP and its associations with internalizing symptoms in young children.

Research suggests that the structure of EC and FP is consistent with evidence that their psychophysiological correlates (ERN and FPS, respectively) reflect distinct brain circuits that are susceptible to different developmental pressures (Casey et al., 2010; Ernst & Fudge, 2009). A recent analysis found that psychometric indices of EC and FP assessed using laboratory measures in two separate samples of young children are uncorrelated with one another, suggesting that these constructs are orthogonal dimensions (Durbin, Mendelsohn, & Wilson, 2013). This structure is maintained across the lifespan and demonstrates the advantage of taking

a psychobiological approach in investigating EC and FP from a temperament and psychophysiological perspective, where their associations may be more clearly separated and interpreted. The hypotheses tested in the current study include: (1) psychohysiological markers examined in children that are implicated in risk for internalizing psychopathology in adults will be associated with laboratory-assessed EC and FP; and (2) laboratory-assessed EC and FP will be unrelated to maternal-reported internalizing problems whereas psychophysiological markers of EC and FP will be related to maternal-reported internalizing problems.

## **METHODS**

# **Participants**

Subjects consisted of 275 children from the ages of 3 to 7 from the surrounding Lansing, MI area who did not have any significant medical conditions or developmental disabilities and lived with at least one English-speaking parent. Participants were recruited using commercial mailing lists and postings on classified advertisement websites. Eligible families were provided a detailed description of the study and invited to complete child questionnaires and a laboratory temperament assessment, for which they were financially compensated. Following the lab visit, a subset of 55 participants was selected to complete the psychophysiological portion of the study. A total of 8 participants were excluded from analyses due to poor quality EEG recordings. Participants who committed errors on more than 35% of trials and/or committed fewer than 6 errors on both ERN tasks (Olvet and Hajcak, 2009) were excluded from the final sample (2 subjects excluded from Go/No-Go task, and 21 subjects excluded from flanker task). In total, 26 participants (female = 10, male = 16) were included in analyses for the Go/No-Go task, 28 participants (female = 15, male = 13) were included in analyses for the flanker task and 19(female = 7, male = 12) were included in analyses for the startle paradigm. On average, children used in analyses were 5.78 years old (SD = 1.17, range = 3.61-7.99 years). See Table 1 for a summary of multimethod assessments used in the present study.

## **Measures of EC and FP dimensions**

**Maternal report of temperament.** Maternal report on child temperament was collected using the Children's Behavior Questionnaire (CBQ; Rothbart, et al., 2001). The CBQ is a widely used caregiver report measure designed to assess temperament traits in children ages 3 to 7. The 195-item questionnaire has scales with adequate internal consistency (Rothbart et al., 2001) that

yield scores on higher order dimensions of extraversion, NA, and EC. In the present study, scales tapping EC (Low Intensity Pleasure, Inhibitory Control, Attentional Shifting, Attentional Focus, and Impulsivity) and FP (Fear) dimensions were examined.

Behavioral assessment of temperament. Child temperament was also assessed using a 2-hour battery of 15 structured tasks composed of episodes from the Laboratory Temperament Assessment Battery-Preschool Version (Lab-TAB; Goldsmith, Reilly, Lemery, Longley, & Prescott, 1995), earlier studies assessing temperament in this age range (Durbin, 2010), or newly developed for this study. The structured tasks were designed to elicit behaviors and emotional responses indicative of individual differences in EC and FP traits. Prior to the assessment, parents were instructed to respond neutrally to their child's advances and limit the amount of interaction with their child. One parent accompanied the child into the assessment room, with the exception of four tasks (Stranger Approach, Picture Tearing, Pop-Up Snakes, and Box Empty), for which the parent was directed to the camera room and watched the child through a one-way mirror. All lab episodes were videotaped and used later for coding. In between each task, a short play break was used to allow children to return to a baseline affective state. Episodes were ordered such that ones eliciting similar emotions were not presented consecutively. Each lab task and its corresponding emotional or behavioral responses are described below in the order that the episode was conducted during assessment.

*Exploring New Objects (fear, happiness)*. The child was instructed to explore a room alone for 5 minutes that contained novel objects such as a large plastic skull hidden beneath a red cape, a remote-controlled spider, and a box of gooey water-filled gel balls.

*Making a T-Shirt (engagement, happiness).* The experimenter presented a blank white t-shirt to the child and provided instruction on how to use stamps to decorate the t-shirt. The child

was then allowed to independently decorate the t-shirt for 2 minutes.

*Dimensional Change Card Sort (DCCS; attentional control).* The DCCS is a common measure used to assess executive functioning in children. The child was instructed to sort two bivalent target cards (e.g., blue rabbit and red boat) based on one dimension (e.g., color). After 6 pre-switch trials, the experimenter instructed the child to sort the target cards based on the second dimension (e.g., shape). If the child correctly identified 5 out of the 6 post-switch trials, the experimenter administered an additional version of the DCCS where target cards with a black border followed sorting rules on one dimension and target cards without a black border followed sorting rules on the second dimension.

*Stranger Approach (fear).* The child was told to wait alone in the room for a moment. During this time, a male research assistant who the child had not seen before entered the room and had a brief interaction with the child based on a neutral scripted conversation.

*Green Circles (anger, sadness)*. The experimenter asked the child to draw a perfect green circle. The experimenter mildly criticized the child's green circle for little imperfections such as its size or shape and repeatedly asked the child to draw another green circle. After 2 minutes, the experimenter made a positive comment about all of the child's drawings.

*Popping Bubbles (activity level, happiness).* The experimenter and child took turns playing with a bubble-making toy. The experimenter instructed the child to try popping the bubbles with different body parts (e.g., hands, feet).

*Diorama Snakes (fear, surprise)*. The experimenter asked the child to explore a sand tray containing two remote-controlled snakes. When the child approached the sand tray, a second experimenter standing in the back of the room used hidden remote controls to make the snakes move back and forth. The child was encouraged to try touching the toy snakes.

*Snack Delay (inhibitory control).* The child was instructed to wait until the experimenter rang a bell to eat a piece of candy. The experimenter followed a systematic series of 8 delay trials that ranged from 10 to 30 seconds.

*Picture Tearing (anger, fear, sadness, surprise).* A second experimenter showed the child a photo album, specifically emphasizing the last photo as their favorite. The second experimenter left the room and the main experimenter instructed the child to tear the second experimenter's favorite picture.

*Balloon Bop (activity level, happiness, inhibitory control).* The child and experimenter hit a balloon back and forth for three minutes. The child was instructed to remain inside a circle outlined on the ground while hitting the balloon.

*Transparent Box (anger, sadness).* The experimenter presented two appealing toys and the child picked their favorite to lock inside a transparent box. The experimenter left the child with a set of nonfunctional keys. After 3 minutes, the experimenter returned explaining that she made a mistake and gave the child the right set of keys.

*Tell a Story (fear).* The child was given a book without words and was instructed to tell a story from the pictures. The child was informed that the second experimenter, a "story expert", would provide a grade at the end of the story. The experimenter left the room for 4 minutes while the child told a story in front of the second experimenter. The second experimenter then praised the child for his or her story.

*Pop-Up Snakes (anticipatory PA, happiness, surprise).* The experimenter pretended to struggle with opening a can of chips and asked the child for help. The child opened the can of chips to find two coiled-spring snakes that flew out of the can. The child was then encouraged to also scare his or her parent with the pop-up snakes.

*Walk a Line Slowly (activity level, inhibitory control).* The child was asked to walk as slowly as possible on a line taped on the floor, and then as quickly as possible. After walking on the taped line, the child was asked to walk as slowly as possible on a balance beam.

*Box Empty (anger, anticipatory PA, sadness).* The child was given a brightly colored gift bag under the impression there was an appealing toy inside. After a period of 2.5 minutes when the child was left alone to discover the gift bag is empty, the experimenter returned with several toys for the child to take home explaining that she forgot to place them in the gift bag.

**Temperament coding procedures.** Laboratory episodes were coded based on a global coding system validated in earlier studies (Durbin et al., 2005). To assess FP, coders watched an entire task and rated discrete instances of facial, vocal, and bodily expressions of fear by their intensity. Low, moderate, or high intensity ratings were determined based on the degree of fear demonstrated by the behavior. For example, for facial expressions, high intensity expressions were indicated by definite movement in both facial regions (mouth, eyes) indicative of fear, whereas moderate expressions were defined by definite movement in only one facial region. Counts were weighted by intensity (low = 1, moderate = 2, high = 3), then summed across channels (facial, vocal, bodily). Weighted counts were averaged across separate lab tasks, but tasks for which fear scores did not exhibit significant variance or which did not correlate with fear scores from other tasks were not included in this composite. Thus, the fear composite consisted of weighted counts of fear expressions in the following episodes (Exploring New Objects, Diorama Snake, Pop up Snakes, Stranger Approach, Tell a Story, Green Circles; alpha = .51). Other emotionality traits such as sadness, anger, and positive affect were also coded using these procedures, but are not mentioned further as these traits were not the focus of the present study.

To assess EC, coders watched an entire task and assigned a single rating for each of several variables relevant to EC, based on all behaviors observed during the task. The following variables relevant to EC were rated on a four-point Likert scale (0 = low, 1 = moderate, 2 = moderate to high, and 3 = very high): activity level, compliance, attentional control, and behavioral control/impulsivity. Activity level was based on the child's overall movement around the room and vigor in manipulating stimuli. Compliance was based on the severity of the child's deliberate unwillingness to comply with the experimenter's or parent's demands or suggestions. Attentional control was based on the child's ability to effectively allocate his or her attention in a flexible manner. Behavioral control was based on the child's tendency toward planfulness and adaptive regulation of behavior, as opposed to impatience and impulsivity. Global behavior ratings were averaged across all 15 episodes to yield composite scores of activity level (alpha = .89), compliance (.79), attentional control (.70) and behavioral control (.85). The primary lab measure of EC was a standardized average of these 4 ratings (alpha = .88).

**Psychophysiological assessment of temperament.** The psychophysiological assessment of temperament consisted of one index of EC and one index of FP (ERN and FPS, respectively) taking a total of 2 hours to complete. Parents of child participants who were selected to complete the psychophysiological portion of the study were contacted following the first laboratory visit. After providing the participants with more detailed information regarding the second laboratory visit, participants who agreed (99%) to participate were scheduled for appointments at the same location where the first laboratory visit was completed. An experimenter guided the child through each step of the EEG set up and electrode application. The parent was permitted to stay in the room to observe EEG electrode application and set up. After set up, parents waited outside of the testing room in an observation room where they could view their child completing tasks

through a ceiling camera. The experimenter was present throughout testing, but sat behind the child out of their view and either provided encouragement to complete the task or performance feedback in between task blocks depending on the child's accuracy (see below for description). The children completed a total of three tasks on the computer. The child was positioned 17 inches from a 21-inch computer monitor for each task. The first two tasks were used to assess the ERN and the last task was used to assess FPS.

*Go/No-Go Zoo Game (ERN).* First, children completed a picture version of the Go/No-Go task called the Zoo Game, which was developed by McDermott, Henderson, Degnan, & Fox (2014) and used on similar samples as the present study (Grammer, Carrasco, Gehring, & Morrison, 2014). Children were asked to help a zookeeper capture zoo animals that had escaped from their cages. Children were presented with images of three orangutans who were helping the zookeeper and therefore did not need to be put back in their cages. The child was instructed to press the spacebar quickly and accurately to each animal (Go stimuli) except when the animal was an orangutan (No-Go stimuli), in which case the child was to withhold pressing the spacebar. On each trial, a stimulus of a colorful zoo animal was presented at a central location on the computer monitor (see Figure 1). A fixation cross appeared before the stimulus, which remained on the screen for 750 ms. Intertrial intervals (ITI) were set to 500 ms.

The task began with a brief practice block, which consisted of 12 trials (9 Go trials and 3 No-Go trials). The practice block was repeated until the child demonstrated an understanding of the task. After the practice block, children completed 8 blocks that consisted of 40 trials (30 Go trials and 10 No-Go trials), totaling to 320 trials and lasting approximately 20 minutes. Novel sets of animal images balanced for animal size, color, and type were used in each block. Children were given performance feedback after each block of the task that was either related to making

too many errors such as, "Remember to watch out for the orangutan friends", or not enough errors such as, "Remember to try and catch the animals even faster next time!" The performance feedback prompts provided for the children was determined by their accuracy in the preceding block, which was automatically calculated to help yield error rates higher than 10% but lower than 35% to ensure adequate accuracy rates and number of useable error trials for stable error-related waveforms. Before the beginning of the task and after blocks 2, 4, 6, 7, and 8, feedback was also provided using a "Zoo Map" (see Figure 2), which allowed children to track their progress in the task. The ERN was defined as the average amplitude in the 0-100 ms post-response time window relative to a -200 to 0 ms pre-response baseline.

*Flanker Fish Game (ERN).* Following the Go/No-Go task, a developmentally appropriate adaptation of the Eriksen flanker task (Eriksen & Eriksen, 1974) called the Fish Game was administered to subjects. A subset of participants (n = 16) completed a version of the flanker task before it was modified to be more captivating for children. In the unmodified version, the flanker task stimuli consisted of 5 yellow cartoon fish swimming to the left or right on a blue background. The child was instructed to focus on responding to the swimming direction of middle fish or central target stimulus while ignoring the flanking fish stimuli. In order to account for developmental differences in cognitive processing, two versions of the flanker task were used based on procedures outlined by McDermott, Perez-Edgar, and Fox (2007). Inter-trial intervals (ITI) for 4-year old subjects varied randomly between 5,900 and 6,400 ms while ITI for subjects older than 5 years of age varied randomly between 3,900 to 4,400 ms. During each trial, a fixation cross was presented during the ITI. Children completed a total of 288 trials, grouped into 12 blocks of 24 trials, during which speed and accuracy were equally emphasized.

The modified version was completed by 12 participants and is described below. Analyses suggested that there were no significant differences between the two versions of the flanker task in participant characteristics, including age (t(1, 26) = 1.10, p = 0.28, d = 0.42) and gender (t(1, 26) = -0.32, p = 0.75, d = 0.12). However, there were several moderate to large effect size mean differences in voltage amplitude at frontal and central electrode sites on error and correct trials, respectively, Fz: t(1, 26) = 2.14, p = 0.04, d = 0.77; t(1, 26) = -1.94, p = 0.06, d = 0.70; FCz: t(1, 26) = 1.79, p = 0.09, d = 0.66; t(1, 26) = -1.49, p = 0.15, d = 0.56; Cz: t(1, 26) = 2.79, p = 0.01, d = 0.95; t(1, 26) = -0.18, p = 0.86, d = 0.07; CPz: t(1, 26) = 2.04, p = 0.05, d = 0.74; t(1, 26) = 0.67, p = 0.51, 0.26; Pz: t(1, 26) = 0.71, p = 0.49, d = 0.27; t(1, 26) = -0.73, p = 0.48, d = 0.28Both subsets were combined in analyses for the present study.

The modified flanker task used the same stimuli, which consisted of 5 yellow cartoon fish swimming to the left or right on a blue background (see Figure 3). The child was informed that "Goldie", the middle fish, needed the child's help to find hidden treasure by telling Goldie which direction to swim. The child was instructed to focus on responding to the swimming direction of the middle fish or central target stimulus while ignoring the flanking fish stimuli. The task began with a practice block consisting of 20 trials, which consisted of 5 congruent left trials (all fish facing to the left), 5 congruent right trials (all fish facing to the right), 5 incongruent left trials (middle fish facing left and flanking fish facing right), and 5 incongruent right trials (middle fish facing right and flanking fish facing left). The practice block was completed until the child understood the task. After the practice block, children completed 7 blocks that consisted of 20 trials (5 of each trial type as described in the practice block), for a total of 140 trials and lasting approximately 15 minutes. A fixation cross appeared before the stimulus, which remained on the screen for 750 ms. ITI varied randomly between 700-1200 ms. Similar to the Go/No-Go task,

children were provided performance feedback after each block using the accuracy calculations described in the Zoo Game. Children were either prompted to "Remember to pay attention to Goldie, he's always in the middle" or "Try to tell Goldie which way to go even faster next time". Similar to the Zoo Game, before the beginning of the task and after blocks 1, 3, 4, 5, 7, feedback was also provided using a "Treasure Map" (see Figure 4), which allowed children to track their progress in the task. The ERN in the flanker task was observed to occur shortly after the response (ERN peak identified at 11 ms), so it was defined as the average amplitude in the time window 50 ms before and after the peak (-39 to 61 ms) relative to a -150 to -50 ms pre-response baseline.

*Video startle paradigm (FPS).* Following the flanker Fish Game, the EEG cap and electrodes were removed and electrodes were applied to measure EMG activity during the startle paradigm (see below for description of electrode placement). FPS, or difference in startle response elicited by an unpleasant versus neutral video clip, served as another primary psychophysiological measure of FP (Quevedo, Smith, Donzella, Schunk, & Gunnar, 2010). Experimenters adjusted and placed headphones on the child, ensuring that the ear cushions completely encircled the ear. Children viewed 12 age-appropriate video clips (4 pleasant, 4 unpleasant, and 4 neutral), each 1-minute long with 10-second intervals between each video during which a blue screen was presented. The soundtrack of the video clips were delivered binaurally and kept at approximately 65dB. Preceding the set of 12 video clips, a neutral film clip with a nature scene lasting about 1-minute was used for habituation. Children viewed one of three possible orders of video clip presentation following the habituation clip. The order of the video clips was systematically varied such that video clips with the same emotional valence were not presented sequentially.

White noise bursts (set at 95dB) were presented binaurally at varying points throughout

the task including the habituation clip, during video viewing, and 10-second rests in between videos, to elicit a startle eyeblink response recorded from two electrodes under the left eye. A total of three noise probes were presented during video viewing. After the video played for 13 seconds to allow children to orient to the nature and affective material of the video clip, the presentation of first noise probe varied randomly between 7-12 seconds, the second noise probe varied randomly between 7-12 seconds after the first probe, and the third noise probe varied randomly between 7-12 seconds after the second probe. One noise burst was presented during the 10-second rest and was delivered at 2, 4, or 5 seconds following the end of the previous video clip. Throughout the experiment, 52 noise probes were delivered: 39 during each video clip (3 per video clip) and 13 in between video clips (1 per 10-second rest).

*Psychophysiological recording and data reduction.* All EEG recordings were taken from 64 Ag-AgCl electrodes using the Active Two Biosemi System (BioSemi, Amsterdam, The Netherlands). For EEG data acquisition, electrodes were placed in a stretch-lycra cap according to the 10/20 system with two additional electrodes placed on the left and right mastoids. Electrooculogram activity from eye movements and blinks were recorded at FP1 and three additional electrodes placed 1 cm from the pupil, one placed directly beneath the left pupil and the remaining two placed on the left and right outer corner of the eye. In accordance with BioSemi's design specifications, the Common Mode Sense active electrode and Driven Right Leg passive electrode served as a grounding device during data acquisition. All EEG signals were digitized with a sampling rate of 512 Hz using ActiView software (BioSemi). EMG activity was recorded from Offline analyses, described for each task below, were performed using BrainVision Analyzer 2 (BrainProducts, Gilching, Germany).

EEG data were re-referenced to the numeric mean of the mastoids and band-pass filtered

with cutoffs of 0.1 and 30 Hz (12 dB/oct rolloff). All trials were also corrected for eye movements and blinks according to methods outlined by Gratton, Boles, and Donchin (1983). A computer-based algorithm was used to detect physiological artifacts such that individual trials were rejected if there was a voltage step greater than 50  $\mu$ V between sample points, a voltage difference of more than 200  $\mu$ V within a trial, or a maximum voltage difference less than 0.5  $\mu$ V within a trial. Trials with reaction times occurring outside of a 200-1,300 millisecond window were also removed from subsequent analyses.

EMG activity was recorded from two Ag-AgCl electrodes placed over the orbicularis oculi (one electrode directly under the left pupil and the second electrode placed to the right of the electrode beneath the pupil). EMG signals were digitized with a sampling rate of 1000 Hz, bandpass filtered from 30-300 KHz, and amplified 20K. Startle responses were coded based on a set of criteria outlined by Blumenthal et al. (2005) and used on a similar sample population by Quevedo and colleagues (2010). Coding parameters included a sharp increase in EMG signal amplitude between 20-175 ms following the onset of the noise burst, and a quiet baseline period from 0-20 ms. Trials that did not meet such criteria were eliminated. Trials with eye-blinks occurring 50 ms before the noise burst were discarded. Eliminated trials did not differ by age, gender, or condition. FPS was computed as the averaged magnitude of coded startle responses for each video clip valence. As is common for FPS, magnitude values included startle responses that had near-zero amplitudes and a positively skewed distribution, with skewness coefficients ranging from 1.37 to 2.19, and kurtosis coefficients ranging from 0.63 to 5.67. Startle magnitude values were log-transformed in order to account for the non-normal distribution.

## **Measure of Child Internalizing Problems**

Maternal report of child internalizing problems. Maternal report of their child's

current internalizing symptoms was collected during the first laboratory visit using the Child Behavior Checklist (CBCL; Achenbach, 1991). The CBCL provided two scales relevant to general internalizing problems (anxious/depressed and withdrawn/depressed), in addition to scales keyed to DSM-IV definitions of anxiety and mood problems.

#### RESULTS

## Psychophysiological Assessment of EC and FP

**Behavioral performance.** Children's behavioral performances on the Go/No-Go task were observed and are described in Table 2. On average, children committed 30.77 errors (SD = 10.45; range = 13-54), which contributed to the analyses of error-related waveforms. As expected, children were significantly faster in responding on error No-Go trials (M = 439.86 ms, SD = 66.42) relative to correct Go trials (M = 557.05 ms, SD = 71.94; t(1, 25) = 11.03; d = 2.16). Children's overall accuracy on both Go and No-Go trials was 86.01%, with an average of 5.83% inaccurate responses on Go trials and 38.46% inaccurate responses on No-Go trials.

Children's behavioral performances on the flanker task were observed and are described in Table 3. Due to a computer programming error, responses occurring after 750 ms post stimulus presentation were not collected and therefore, the number of correct responses is an underestimate. Children included in analyses committed an average of 30.18 errors (SD = 18.62; range = 8-80), and had an overall accuracy rate of 80.20%. As expected, children were significantly faster in responding on error trials (M = 550.26 ms, SD = 173.36) relative to correct trials (M = 648.77 ms, SD = 121.71; t(1, 27) = 6.36, p < .001; d = 1.20). Behavioral performance in both Go/No-Go and flanker tasks did not differ by gender.

**ERN.** The presence of the ERN in both Go/No-Go and flanker tasks was assessed with a five (Site: Fz, FCz, Cz, CPz, and Pz) by two (Trial Type: error and correct) repeated measures ANOVA. The response-locked waveforms at the frontal and central midline electrode sites (Fz, FCz, and Cz) from the Go/No-Go task can be seen in Figure 5. In the Go/No-Go task, results indicated that there was greater negativity on error trials compared to correct trials (F(1, 25) = 23.67, p < .001, partial  $\eta^2 = 0.49$ ). While no differences across electrode sites were observed

 $(F(4, 100) = 2.04, p = 0.09, \text{ partial } \eta^2 = 0.08)$ , the significant interaction between Site and Trial Type  $(F(4, 100) = 12.72, p < .001, \text{ partial } \eta^2 = 0.34)$  provided further support for the presence of the ERN. This interaction suggested that amplitude differences between error and correct trials varied as a function of electrode site, with frontal sites showing greater negativity relative to posterior sites on error trials. Follow-up paired-samples *t*-tests were conducted to analyze amplitude differences between error and correct trials at different electrode sites.

Results indicated that amplitudes at Fz (t(25) = 4.60, p < .001; d = 0.90), FCz (t(25) =6.03, p < .001; d = 1.18), Cz (t(25) = 5.16, p < .001; d = 1.01), and Pz (t(25) = 3.03, p = .006; d = 1.010.60) were more negative on error trials relative to correct trials. No difference between error and correct trials at CPz were observed (t(25) = 1.42, p = 0.17; d = 0.28). Although statistically nonsignificant due to sample size, the amplitude of the ERN alone was observed to be larger only at FCz compared to CPz (t(25) = 2.00, p = 0.56; d = 0.40), which was also confirmed by the scalp topography (see Figure 6). The mean difference in amplitude between error and correct trials (i.e.,  $\Delta$ ERN) was larger at FCz compared to Fz (t(25) = 3.72, p = .001; d = 0.73) and Pz (t(25) = 3.72, p = .001; d = 0.73) 4.44, p < .001; d = 0.88), suggesting that maximum difference between error and correct trials occurred at FCz. Therefore, analyses including the Go/No-Go task focused on the ERN at FCz. The ERN amplitude at FCz was negatively associated with age, suggesting that a smaller or more positive ERN was associated with younger age. While there was no relationship between age and the number of errors committed, overall accuracy on the Go/No-Go task was positively associated with age (r = 0.57, p = .002). The ERN amplitude at FCz did not differ for boys (M =-1.63, SD = 4.61) and girls (M = -3.02, SD = 4.89; t(1, 24) = 0.73, p = 0.47, d = 0.30).

The response-locked waveforms at the frontal and central midline electrode sites (Fz, FCz, and Cz) from the flanker task can be seen in Figure 7. While no differences across electrode

sites were observed (F(4, 108) = 0.78, p = 0.54, partial  $\eta^2 = 0.03$ ) in the flanker task, results indicated that there was greater negativity on error trials compared to correct trials flanker (F(1,27) = 5.89, p = 0.02, partial  $\eta^2 = 0.18$ ). Despite the statistically non-significant interaction between Site and Trial Type (F(4, 108) = 2.19, p = 0.08, partial  $\eta^2 = 0.08$ ), the large effect of the amplitude difference between error and correct trials, event-related waveforms, and scalp topography (Figure 8) provided support for the presence of the ERN. Follow-up paired-samples *t*-tests indicated that amplitudes at Fz (t(27) = 2.08, p = 0.05; d = 0.39), FCz (t(27) = 2.28, p = 0.05; d = 0.39), FCz (t(27) = 2.28, p = 0.05; d = 0.39), FCz (t(27) = 2.28, p = 0.05; d = 0.39), FCz (t(27) = 2.28, p = 0.05; d = 0.39), FCz (t(27) = 2.28, p = 0.05; d = 0.39), FCz (t(27) = 2.28, p = 0.05; d = 0.39), FCz (t(27) = 2.28, p = 0.05; d = 0.39), FCz (t(27) = 0.28, p = 0.05; d = 0.39), FCz (t(27) = 0.28, p = 0.05; d = 0.39), FCz (t(27) = 0.28, p = 0.05; d = 0.39), FCz (t(27) = 0.28, p = 0.05; d = 0.39), FCz (t(27) = 0.28, p = 0.05; d = 0.39), FCz (t(27) = 0.28, p = 0.05; d = 0.39), FCZ (t(27) = 0.28, p = 0.05; d = 0.39), FCZ (t(27) = 0.28, p = 0.05; d = 0.39), FCZ (t(27) = 0.28, p = 0.05; d = 0.39), FCZ (t(27) = 0.28, p = 0.05; d = 0.39), FCZ (t(27) = 0.28, p = 0.05; d = 0.39), FCZ (t(27) = 0.28, p = 0.05; d = 0.39), FCZ (t(27) = 0.28, p = 0.05; d = 0.39), FCZ (t(27) = 0.28, p = 0.05; d = 0.39), FCZ (t(27) = 0.28, p = 0.05; d = 0.39), FCZ (t(27) = 0.28, t = 0.39), FCZ (t(27) = 0.39), FCZ (t(27) = 0.39), FCZ (t(27) = 0.39), 0.03; d = 0.43), Cz (t(27) = 2.96, p < 0.01; d = 0.56), and Pz (t(25) = 3.03, p = .006; d = 0.60) were more negative on error trials relative to correct trials. No significant difference between error and correct trials at CPz (t(27) = 1.54, p = 0.14; d = 0.29) or Pz (t(27) = 1.34, p = 0.19; d =0.25) were observed. Analyses including the ERN as measured by the flanker task focused at FCz since the ERN at this site was most negative in amplitude and the difference between error and correct trials at FCz had a moderate effect size. The ERN amplitude at FCz not associated with age, number of errors committed, or overall accuracy. While there was no relationship between age and the number of errors committed, overall accuracy on the flanker task was positively associated with age (r = 0.41, p = 0.03). The ERN amplitude at FCz did not differ for boys (M = -1.34, SD = 4.48) and girls (M = -1.33, SD = 4.67; t(1, 26) = 0.01, p = 0.99, d = .003).

**FPS.** Descriptive statistics of log-transformed startle magnitudes are described in Table 5. Paired-samples *t*-tests were conducted to examine differences between startle magnitude across different valence video clips. Results suggested that startle magnitudes on negative valence video clips was larger compared to positive valence video clips (t(18) = 2.15, p = 0.05; d = 0.50), but not significantly different from neutral valence video clips (t(18) = 0.74, p = 0.50; d = 0.17). Startle magnitudes on neutral valence video clips were not significantly different relative

to the startle magnitudes on positive valence video clips (t(18) = -1.69, p = 0.11; d = 0.39). These findings suggest that while children had a larger startle response to negative relative to positive video clips, this did not necessarily indicate a more heightened startle response to negative video clips or inhibited startle response to positive video clips relative to neutral stimuli, which are prototypical characteristics of fear-potentiated startle. Instead, the baseline startle magnitude on negative valence video clips was larger compared to positive valence video clips and was therefore used as the primary marker of startle response in analyses described below.

#### Validity of Psychophysiological Markers of EC and FP

In order to assess the validity of psychophysiological markers of EC and FP, bivariate correlations were conducted between the ERN (as measured by the Go/No-Go and flanker tasks), startle magnitude, and laboratory-assessed and maternal-reported EC and FP (see Table 6).

Behavioral and psychophysiological assessments of EC and FP. Results suggested that laboratory-assessed FP across all tasks designed to elicit fear were unrelated to laboratoryassessed EC (r = 0.07, p = 0.70). This finding is consistent with recent literature suggesting that these two constructs are orthogonal dimensions (Durbin, Mendelsohn, & Wilson, 2013). While not statistically significant, results indicated a medium-to-large effect in the association between the ERN measured by the Go/No-Go and ERN measured by the flanker task (r = 0.54, p = 0.11), and the association between the ERN measured by the Go/No-Go task and startle magnitude (r =0.42, p = 0.11). The ERN as measured by both the Go/No-Go and flanker tasks was not associated with laboratory-assessed EC, whereas a positive association between the ERN and laboratory-assessed FP trended toward significance in the Go/No-Go task (r = 0.41, p = 0.07) and similarly for the flanker task (r = 0.33, p = 0.14). In order to further examine the relationship between the ERN and laboratory-assessed FP in the flanker task, its association was explored separately for boys and girls. While results indicated there was a strong positive relationship between the ERN and laboratory-assessed FP in girls, (r = 0.79, p < 0.01), this relationship did not hold in boys (r = -0.08, p = 0.81). This suggested that a smaller or more positive ERN was associated with higher ratings of laboratory-assessed FP in girls, but not boys. Results also indicated that larger startle magnitude was associated with higher ratings of laboratory-assessed FP (r = 0.53, p = 0.03), but not associated with high ratings of laboratory-assessed EC (r = 0.01, p = 0.96).

**Maternal-reported EC and FP.** Analyses focused on maternal-reported CBQ scales tapping EC (Low Intensity Pleasure, Inhibitory Control, Attentional Shifting, Attentional Focus, and Impulsivity) and FP (Fear) dimensions. Laboratory-assessed FP was unrelated to maternalreported EC and FP. In contrast, higher ratings of coded behavior indicative of EC in lab tasks was associated with higher scores on the Attentional Focus (r = 0.45, p < 0.01) scale and unrelated to Low Intensity Pleasure, Inhibitory Control, Attentional Shifting, and Impulsivity scales (see Table 6). Maternal-reported EC and FP were unrelated to the ERN measured by both Go/No-Go and flanker tasks. In contrast, there was a moderate association between higher scores on the Attentional Shifting scale and larger startle magnitude (r = 0.45, p = 0.06).

Maternal-reported internalizing problems. To address the second aim of study to further assess the validity of psychophysiological markers of EC and FP, bivariate correlations were also conducted with frank internalizing symptoms as reported by the child's mother. Maternal-reported internalizing symptoms were unrelated to laboratory-assessed EC and FP. With regard to psychophysiological markers of EC and FP, higher maternal-reported internalizing symptoms that were indicative of anxiety problems consistent with those described in the DSM-IV were associated with a larger or more negative ERN as measured by the Go/NoGo task, r = -0.41, p = 0.05 and flanker task, r = -0.41, p = .05. Maternal-reported internalizing problems were unrelated to startle magnitude.

#### DISCUSSION

The present study examined the relationship between behavioral and psychophysiological assessments of EC and FP dimensions of temperament, and their associations to maternal-reported internalizing problems in children. This is the first known study to not only compare psychophysiological correlates of EC and FP to laboratory-assessed and maternal-reported temperament, but also compare these associations across different psychophysiological tasks. Consistent with our first hypothesis, psychophysiological correlates commonly implicated in risk for internalizing psychopathology in adults were associated with laboratory assessments of FP. A smaller ERN measured from both Go/No-Go and flanker tasks, and a larger startle response to negative-valenced video clips were associated with higher ratings of behaviors indicative of FP in tasks designed to elicit fear. While these findings were in the expected direction and consistent with literature examining these associations in young children, the small effect in association between the ERN and laboratory-assessed EC suggested that either the ERN is not a valid psychophysiological correlate of EC or that its relationship to the EC is more complicated than what was captured in the present study.

As reviewed previously, research suggests that the ERN reflects cognitive control functions supported by the ACC. In other words, larger or more negative ERNs would be expected to be associated with higher levels of executive functioning skills such as EC. However, this hypothesis has largely been tested in the adult literature. Researchers assessing the relationship between the ERN and EC in younger populations have observed contrasting associations between these two variables. For example, one study investigating the difference in error monitoring in children 9- to 11-years- old with Attention Deficit-Hyperactivity Disorder (ADHD) found smaller or more positive ERNs in children with ADHD as compared to children

without ADHD, possibly suggesting that children with ADHD had impairment in cognitive control functions (Liotti, Pliszka, Perez, Kothmann, & Woldorff, 2005). In contrast, Bugio-Murphy and colleagues (2007) examined the same relationship among different ADHD subtypes and found that children between the ages of 7 and 13 with ADHD-Combined symptoms had larger or more negative ERNs compared to Non-ADHD subjects, suggesting that children with ADHD-Combined symptoms may have been more vigilant to their mistakes.

There are several possible explanations to the discrepancies seen in the developmental literature, particularly with studies investigating psychophysiological measures such as the ERN. First, researchers have adopted a range of methods for assessing the ERN, and evidence suggests that the differences between these tasks such as the stimuli may influence the children's behavioral performance (McDermott, Perez-Edgar, & Fox, 2007). These different paradigms used across research groups make it challenging to compare results, and these differences may play a role in the discrepancies seen between the location, latency, and amplitude of the ERN. Secondly, more recent studies have produced evidence that the ERN and startle response can be elicited in younger samples than previously believed. While development itself could help account for discrepancies such as those reviewed previously, it is difficult to disentangle this explanation from the inherent problem of nonequivalence of measures in developmental research. In other words, since many of the psychophysiological tasks used with young children have been adopted from the adult literature and adapted to be more age-appropriate, it is possible that these tasks tap different constructs than they do in studies of adults.

In addition to these basic methodological concerns, EC as a construct may be more complicated than previously thought, making it unclear whether the ERN is a psychophysiological marker of EC or a marker of a facet of EC such as attentional control or

response inhibition. It is important to recognize this distinction particularly because specific cognitive processes of EC, attentional and inhibitory control processes, might differentially contribute to adaptive or maladaptive regulation of trait FP (e.g., Muris, Meesters, & Blijlevens, 2007; White et al., 2011). The relationship between the ERN and other constructs such as FP may be dependent on what facet of EC the ERN may actually be tapping. Results revealed a moderate effect in the association between a more positive ERN as measured by the Go/No-Go task and larger startle response, whereas a smaller effect was found with the ERN as measured by the flanker task. The variation in these associations may suggest that the observed ERNs from these tasks may reflect different dimensions of EC. So while both tasks involve response-inhibition, the design of each task suggests that the Go/No-Go task could be more specific to inhibitory control processes while the flanker task may be more sensitive to attentional control processes. However, both measures of the ERN share similar associations with external correlates such as maternal-reported internalizing problems, suggesting that both tasks may also be tapping a shared underlying construct.

Consistent with our second hypothesis, laboratory-assessed FP was not related to maternal-reports of child EC or FP. In contrast, higher ratings of coded behavior indicative of EC in lab tasks were associated with higher scores on maternal-reported scales related to attentional focus. One possible explanation for this finding is that behaviors or emotions representative of FP occur at a lower base rate whereas behaviors indicative of EC may be more readily observed and easily reported by mothers. Results also indicated that maternal-reported EC and FP were unrelated to the ERN measured by both Go/No-Go and flanker tasks. This finding is unsurprising given that the ERN is a measure of a biobehavioral process, and this microlevel analysis may not be captured by maternal report. In contrast, there was a moderate effect in the association

between higher maternal-reported attentional shifting and a larger startle response. This finding suggests that a child's ability to effectively shift attention from one task to another was associated with a larger startle response. This association was not found with maternal-reported inhibitory control, providing further support that different dimensions of EC may differentially contribute to the manifestation of FP.

Report of how these biobehavioral processes might manifest, such as maternal-reported internalizing symptoms, was moderately associated with the ERN as measured by both the Go/No-Go and flanker task. The moderate effect in the relationship between a more negative ERN and higher maternal-reported internalizing symptoms indicative of anxiety problems as described by the DSM-IV is inconsistent with recent studies investigating the relationship between the ERN and anxiety in young children (Meyer et al., 2012). One possible explanation for this unexpected result is that the association between the ERN and anxiety may differ depending on risk and disorder status. More specifically, a recent study found that maternal history of anxiety disorders, a risk factor for the development of anxiety disorders, was related to a smaller ERN in 6 year-old children (Torpey et al., 2013). However, a diagnostic assessment for clinical anxiety disorders in this sample of children revealed that anxious children were characterized by a larger ERN (Meyer et al., 2013). Extending these findings to the present study, the association between a larger ERN and maternal-reported internalizing problems may be independent from the opposing influence of risk factors such as increased FP in childhood on the ERN. Maternal-reported internalizing problems were unrelated to the startle response. There are several possible explanations for this unexpected result. First, results indicated that the startle paradigm used in the present study did not elicit a FPS, and therefore the largest startle magnitude was used as a baseline startle measure. This finding suggests that either the newly

designed task was ineffective at eliciting FPS (i.e., video clips did not elicit expected affective states) or provides evidence that the fear-defense circuit responsible for producing FPS is not fully developed in young children. Given that current literature has only investigated this question in older children (Quevedo et al., 2010), future studies will examine observed startle responses to this novel paradigm in adult samples to first determine whether this finding is a function of methodological design.

While it was not a primary aim of the present study, analyses exploring gender differences revealed important implications for future studies. This is the first known study to examine the association between the ERN and anxiety separately by gender in young children. Results indicated that in girls, there was a large effect in the relationship between a smaller or more positive ERN and higher ratings of behaviors indicative of FP in laboratory assessments of temperament, whereas in boys, this relationship was not found. These findings suggest that there is a potential moderating factor of gender on the relationship between the ERN and FP, and on the association between the ERN and related anxiety problems in preschool-age children. Only one study has examined this relationship in adults (Moran, Taylor, & Moser, 2012). Moran and colleagues (2012) examined the moderating effect of sex on the relationship between the ERN and anxiety problems in an undergraduate college sample. The association between enhanced ERN and higher levels of worry was specific to females. This relationship is consistent with the findings from the present study when considering the observed shift in the association between the ERN and anxiety problems in the developmental literature. The present results highlight the importance of collecting longitudinal data across transitional periods such as adolescence to better understand the shift in association between the ERN and internalizing problems, in addition to further examining how these gender differences may influence developmental

trajectories associated with FP.

The present results should be considered in light of several limitations. First, the different parameters of the flanker task (combining both unmodified and modified versions of the task) confound the findings, given that it is challenging to attribute findings to the nature of the task rather than the effect of the construct itself. Second, there are a number of child-level factors including engagement with the task and effective induction of an affective state that were not explored in the present study that may play a role in the child's performance or observed response. Despite these limitations, the present study utilized a novel approach in investigating EC and FP across behavioral, psychophysiological, and maternal-report methods that provided support for integrating assessments of temperament across multiple methods to better understand biobehavioral processes implicated in risk for developing internalizing disorders.

APPENDICES

# APPENDIX A

# TABLES

Table 1										
Multi-method Assessment of Temperament Dimensions										
	Temperament Di	mensions	Internalizing problems							
	Fear-Proneness	Effortful Control								
Behavioral	Lab tasks: Coded	Lab tasks: Global behavioral								
Assessment	expressions of	ratings of activity level,								
	fear	compliance, attentional								
		control, and behavioral								
		control								
Psychophysiological	FPS	ERN								
Assessment										
Parent-reported	CBQ: Fear scale	CBQ: Low Intensity	CBCL: Anxious/							
Measure		Pleasure, Inhibitory Control,	depression scale,							
	Attentional Focus,	Withdrawn/depression								
		Attentional Shifting, and	scale, DSM-oriented							
		Impulsivity scales	anxiety and mood scales							

Behavioral Performance on No-Go Error and Go Correct trials in the Go/No-Go Task.

	Mean	SD	Range
Error No-Go trials	30.77	10.45	13 - 54
Correct Go trials	226.00	15.21	180 - 240
Percent error on No-Go trials	38.46%	0.13	16.00 - 68.00%
Percent error on Go trials	5.83%	0.06	0.00 - 25.00%
Reaction time error	439.86	66.42	303.15 - 599.52
Reaction time correct	557.05	71.94	434.33 - 672.46

Table 3				
Behavioral Performance on Error a	nd Correct	Trials in	the Flanker	Task.

	Mean	SD	Range
Error trials	30.18	18.62	8 - 80
Correct trials	125.14	42.70	46 - 185
Total Accuracy	80.20%	0.10	63.00 - 96.00%
Reaction time error	550.26	173.36	320.56 - 1156.17
Reaction time correct	648.77	121.71	468.00 - 993.00

		mpnesaes (h + )	,		er 2, ana r 2.
Components	Fz	FCz	Cz	CPz	Pz
Go/No-Go ERN	-1.65 (4.56)	-2.17 (4.67)	-1.28 (4.06)	-0.48 (4.12)	-0.73 (4.08)
Go/No-Go CRN	3.31 (2.41)	4.15 (2.51)	4.10 (2.78)	0.90 (2.83)	1.66 (2.94)
Go/No-Go $\Delta$ ERN	4.96 (5.51)	6.32 (5.34)	5.38 (5.31)	1.37 (4.94)	2.39 (4.02)
Flanker ERN	-1.29 (4.56)	-1.34 (4.50)	-1.18 (4.35)	-0.10 (4.10)	-0.19 (4.18)
Flanker CRN	0.68 (2.55)	0.68 (2.87)	1.46 (3.36)	1.00 (3.50)	3.73 (3.60)
Flanker $\Delta$ ERN	1.97 (5.01)	2.02 (4.67)	2.64 (4.72)	1.10 (3.78)	0.92 (3.64)

Mean (SD) ERN and CRN Voltage Amplitudes (µV) at Midline Sites Fz, FCz, Cz, CPz, and Pz.

*Note*. ERN = Error-related Negativity. CRN = Correct-response Negativity, the voltage amplitude on correct trials identified in the same time window as the ERN.  $\Delta$  ERN = Difference between the CRN and ERN.

Mean (SD) Startle Responses and Negative-Valenced	onse Magnitud Video Clips.	es ( $\mu V$ ) to Neut	tral-, Positive-,
	2.6	<b>GD</b>	D

	Mean	SD	Range
Neutral Peak	0.85	0.37	0.28 - 1.56
Positive Peak	0.74	0.30	0.15 - 1.40
Negative Peak	0.89	0.35	0.49 - 1.65

Bivariate Correlations Between Behavioral and Psychophysiological Measures of EC and FP, and Maternal-Reported EC and FP, and Internalizing Problems.														
Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. Lab EC														
2. Lab FP	0.07													
3. Go/No-Go ERN	-0.18	$0.41^{\dagger}$												
4. Flanker ERN	0.24	0.33	0.54											
5. Startle	0.02	$0.51^{*}$	0.42	0.13										
6. CBQ Low Pleas	0.04	0.16	0.10	0.04	0.17									
7. CBQ Inhib Cont	$0.29^{\dagger}$	-0.21	0.08	0.12	0.02	$0.60^{**}$								
8. CBQ Attn Shift	0.19	0.06	0.12	-0.29	$0.45^{\dagger}$	$0.38^{*}$	$0.37^{*}$							
9. CBQ Attn Foc	$0.45^{**}$	-0.22	-0.11	-0.07	-0.18	$0.33^{*}$	$0.54^{**}$	0.12						
10. CBQ Impuls	-0.33†	0.03	-0.09	0.01	-0.29	-0.35*	-0.71**	-0.44**	-0.46**					
11. CBQ Fear	-0.06	0.15	0.09	-0.18	-0.13	0.11	0.06	$0.27^{\dagger}$	-0.16	-0.24				
12. CBCL Anx/Dep	-0.04	0.13	-0.05	0.00	-0.22	-0.15	-0.17	-0.23	-0.15	-0.01	0.21			
13. CBCL With/Dep	0.03	0.13	0.06	-0.22	0.11	0.06	-0.03	-0.01	0.12	-0.32*	0.19	$0.53^{**}$		
14. CBCL DSManx	0.19	-0.17	$-0.41^{*}$	$-0.41^{\dagger}$	-0.33	-0.25	-0.26	-0.13	-0.24	0.11	0.22	$0.55^{**}$	0.16	
15. CBCL DSMmood	-0.02	0.18	0.10	0.01	-0.08	-0.04	-0.10	$-0.29^{\dagger}$	-0.21	0.09	0.01	$0.64^{**}$	$0.57^{**}$	$0.32^{*}$
Mean	0.05	-0.13	-2.17	-1.34	0.89	5.26	4.75	4.15	4.75	4.45	3.84	0.26	0.21	0.49
SD	1.05	0.99	4.67	4.05	0.35	0.74	0.96	0.62	1.65	1.03	0.83	0.25	0.21	0.35

*Note.*  ${}^{\dagger}p \le 0.10$ .  ${}^{*}p \le 0.05$ .  ${}^{**}p \le 0.01$ . EC = Effortful Control. FP = Fear-proneness. ERN = Error-related Negativity. CBQ = Child Behavior Questionnaire. CBQ Low Pleas = Low Pleasure Scale. CBQ Inhib Cont = Inhibitory Control Scale. CBQ Attn Shift = Attentional Shifting Scale. CBQ Attn Foc = Attentional Focus Scale. CBQ Impuls = Impulsivity Scale. CBQ Fear = Fear Scale. CBCL = Child Behavior Checklist. CBCL Anx/Dep = Anxious/Depressed Scale. CBCL With/Dep = Withdrawn/Depressed Scale. CBCL DSManx = DSM-IV Anxiety Problems Scale. CBCL DSMmood = DSM-IV Affective Problems Scale.

APPENDIX B

FIGURES



Figure 1. Sample trials of the Go/No-Go zoo task.



*Figure 2.* Zoo Map slide used before the beginning of the Go/No-Go task.



*Figure 3.* Sample trials of the flanker fish task.



*Figure 4*. Treasure Map slide used before the beginning of the flanker task.



Figure 5. Response-locked error and correct waveforms for the Go/No-Go task at Fz, FCz, and Cz.



*Figure 6.* Scalp topographies depicting voltages in the 0-100 ms window following error and correct trials in the Go/No-Go task.



Figure 7. Response-locked error and correct waveforms for the flanker task at Fz, FCz, and Cz.



*Figure 8.* Scalp topographies depicting voltages in the time window 39 ms preceding error and correct trials and 60 ms following error and correct trials in the flanker task.

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