ABSTRACT

AN INVESTIGATION OF MATERNAL BIOLOGICAL INDICES OF ANXIETY PRONENESS AS PREDICTORS OF TODDLERS’ DYSREGULATED FEAR THROUGH MATERNAL PROTECTIVE PARENTING BEHAVIORS

by Randi A. Phelps

To help determine who is at heightened risk for anxiety development, research has investigated behaviorally and biologically-based temperament predictors of subsequent disorder. The current study focuses on toddler dysregulated fear (DF) as the outcome variable of interest. Although DF may be the strongest predictor of anxiety development, it is unclear how DF develops. Maternal biological indices of reactivity (i.e., cortisol) have been associated with intergenerational transmission of anxiety risk, but have not been investigated in association with DF. Neural markers of regulation (i.e., delta-beta coupling: “coupling”) were also investigated to better understand how intergenerational transmission might be occurring. The current study investigated cortisol reactivity and coupling as predictors of toddlers’ DF, as mediated by maternal protective parenting. Study aims tested whether protective parenting mediated the relations between maternal reactivity/regulation and child age 3 DF above and beyond age 2 DF. Results suggested that maternal cortisol reactivity and coupling do not significantly predict change in toddler DF through maternal protective parenting behaviors. Furthermore, cortisol reactivity and delta-beta coupling did not additively predict toddlers’ DF through maternal protective parenting behaviors. Future directions include investigating toddler DF’s influence on maternal protective parenting behaviors in the context of indices of regulation and reactivity.
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Introduction

Anxiety disorders are among the most common mental health disorders affecting both children (Beesdo, Knappe, & Pine, 2009) and adults (Kessler, Chiu, Demler, & Walters, 2005) in the United States (Franz et al., 2013; Pine, Cohen, Gurley, Brook, & Ma, 1998). It is estimated that 1-13% of children (Beesdo, Knappe, & Pine, 2009) and 10.6-16.6% of adults (Somers, Goldner, Waraich, & Hsu, 2006) suffer from a clinical anxiety disorder. Due to the large lifetime prevalence rates of anxiety disorders in the population, research has increasingly focused on longitudinal, developmental data, beginning in early toddlerhood, to investigate which individuals are at greatest risk for the development of anxiety disorders. The developmental psychopathology perspective posits that the development of psychopathology occurs across the lifespan and is influenced by a variety of factors including heritability, biology, and environmental contexts (Sroufe & Rutter, 1984; Rutter & Sroufe, 2000). Furthermore, this perspective suggests that individuals have predispositions to developing psychopathology, but it may be environmental triggers that turn on such characteristics (Sroufe & Rutter, 1984; Rutter & Sroufe, 2000). Working under this framework allows for the investigation of individual differences in risk for the development of anxiety, and informs early intervention and prevention techniques.

Temperament refers to individual differences, at the trait level, in behavioral reactivity and regulation, and is often measured in toddlerhood (Goldsmith et al., 1987). Although definitions of temperament vary, there are certain points of consensus across researchers, including the ideas that temperament is partly biologically based and heritable, focuses on individual differences rather than general behavioral characteristics, and, importantly, is dynamic, meaning that there are most likely multiple underpinnings that influence the development of temperament (e.g., environment) (Goldsmith et al., 1987). The current study operated under a definition that places emphasis on the assumption that temperament is moderately stable, biologically based, and focused on individual differences in reactivity and regulation that influence one’s predispositions to psychopathology. Importantly, the current study also operated under the assumption that although temperament originates from biologically based characteristics, the behavioral manifestations of temperament may also be shaped by the environment (Goldsmith et al., 1987).
One dimension of temperament, fearful temperament (i.e., behavioral inhibition), is of particular interest to the current study because it is indicative of increased risk for being diagnosed with an anxiety disorder (Clauss & Blackford, 2012). Behavioral inhibition is recognized as the display of extreme fearfulness (i.e., hesitance, avoidance, withdrawal, distress) in novel situations (Kagan, Reznick, Clarke, Snidman, & Garcia-Coll, 1984). Previous research has discussed whether behavioral inhibition is a temperamental trait or an earlier form of anxiety (Perez-Edgar & Guyer, 2014). Although research remains inconclusive, a review of the literature provides evidence suggesting that behavioral inhibition is a trait-level propensity for anxiety, but does not guarantee anxiety development, further suggesting that behavioral inhibition is not prodromal anxiety (Perez-Edgar & Guyer, 2014). Although this construct has contributed to our ability to predict risk for anxiety, there is still a fair amount of variability in stability in fearfulness and association with anxiety risk, such that not all children with behavioral inhibition will go on to develop an anxiety disorder. Thus, recent research has aimed to identify which individuals displaying behavioral inhibition are at even higher risk for the development of an anxiety disorder (Kagan et al., 1984; Buss, 2011; Buss et al., 2013; Buss & Kiel, 2013).

Buss and colleagues suggest that it is not the amount of fear toddlers display that is indicative of anxiety development, but instead proposed that the consideration of the context in which fear is displayed is vital when predicting who is at heightened risk for anxiety development (Buss, 2011; Buss et al., 2013). More specifically, toddlers at heightened risk are those displaying dysregulated fear, or high-fear in low-threat contexts, or contexts that do not elicit fearful reactions across most children, even those who may display fear in higher-threat contexts (Buss, 2011; Buss et al, 2013). As opposed to high-threat contexts, which usually involve stimuli that are unpredictable, more objectively threatening, and may intrude into the child’s space, low-threat contexts involve stimuli that, although novel, may be more typical of toys or games experienced in daily situations, allowing the child more coping mechanisms (e.g., the ability to withdraw or having toys with which to distract him or herself) during these situations. In high-threat contexts, it may be developmentally appropriate for toddlers to have difficulty regulating their fear independently, but difficulty coping in low-threat contexts is more indicative of extreme difficulties. Biologically, this may be because these children have an amygdala with an even lower threshold for activation than other behaviorally inhibited children, putting their systems in a more vigilant and physiologically aroused state (Kagan, 1994). Due to
the lack of complete stability in behavioral inhibition, it is theorized that behavioral inhibition consists of a relatively heterogeneous group of heightened fear responses; however, dysregulated fear is posited to be a more homogenous and stable display of fear across the group. Importantly, however, although dysregulated fear is possibly the most stable temperamental measure of fearfulness related to anxiety development, it is likely that this measure may also be influenced by the environment. More specifically, other measures of temperament (e.g., behavioral inhibition) related to anxiety development do not perfectly correlate over time and are shown to be influenced by the environment. Although dysregulated fear would putatively target the most stable measure of temperament as a risk factor for anxiety, given the known nature of temperamental measures, it is likely that dysregulated will also fluctuate over time. It is expected that over time dysregulated fear will demonstrate moderate-strong stability with room for environmental influence, but it is unclear what may influence the development of dysregulated fear over time.

Importantly, intergenerational transmission of anxiety risk has been well established, and this may occur through a variety of means. For example, genetics may explain some of the association between parents’ anxiety and their children’s anxiety risk (Sung, Lee, Song, & Kim, 2011; Kendler, Heath, Martin, & Eaves, 1986; Hettema, Neale, & Kendler, 2001; Eley, Rijsdijk, Perrin, O’Connor, & Bolton, 2008; Hallett, Ronald, Rijsdijk, & Eley, 2009; Hettema, Prescott, Myers, Neale, & Kendler, 2005). Genetic influences on anxiety have been shown to account for approximately one-third of intergenerational transmission (Eley, 1999). This suggests that heritability of anxiety proneness may play an important role in intergenerational transmission. More specifically, maternal biomarkers for stress reactivity and anxiety proneness have been found to be positively linked to toddler behavioral inhibition, suggesting that there is a link between maternal biomarkers of anxiety and their toddlers’ behavioral display of anxiety proneness (Buss, Davidson, Kalin, & Goldsmith, 2004).

**Maternal Biology and Toddler Temperamental Risk for Anxiety**

One physiological measure of stress reactivity that may indicate anxiety proneness is cortisol reactivity. Research suggests that cortisol, a stress hormone that is the glucocorticoid end-product of the hypothalamic-pituitary-adrenocortical (HPA) axis, is released in response to emotional stressors in the environment (Gunnar & Quevedo, 2007). Cortisol is released in efforts to reinforce and sustain other regulatory systems as well as assist in the release of other stress
hormones when threat is perceived. Heightened release of cortisol compared to a baseline measure (i.e., cortisol reactivity) has been positively linked to anxiety risk in adults (e.g., Takahashi et al., 2005) and children (e.g., Gunnar & Quevedo, 2007; Gunnar, Talge, & Herrera, 2009). While previous literature has not investigated maternal cortisol reactivity in relation to toddler anxiety risk in an intergenerational transmission framework, prenatal and postnatal cortisol exposure through amniotic fluid and breast milk positively related to negative reactivity in infant temperament (Davis, Glynn, Hobel, Chicz-Demet & Sandman, 2007; Glynn et al., 2007). Given findings of positive relations between cortisol reactivity and anxiety in children (e.g., Gunnar & Quevedo, 2007; Gunnar, Talge, & Herrera, 2009) and adults (e.g., Takahashi et al., 2005), it is reasonable to investigate an intergenerational transmission model in with maternal cortisol reactivity predicts toddlers’ temperament. Two barriers exist to understanding how maternal cortisol relates to child anxiety risk. First, the association between maternal cortisol reactivity and toddlers’ dysregulated fear has not been investigated. Secondly, maternal biomarkers of stress reactivity have been shown to be difficult to parse apart from other reactive and regulatory systems in the body. As part of the neuroendocrine system, cortisol production and reactivity is influenced by neural commands associated by regulation. Therefore, it is important to understand the unique versus additive effects of biomarkers of reactivity versus neural markers of regulation when studying trait-like propensities for anxiety. Thus, as dysregulated fear provides a better predictor for risk of anxiety development, it is vital to investigate the association between maternal cortisol reactivity as well as neural systems associated with regulation and toddlers’ dysregulated fear.

A promising neural marker that is related to anxiety-proneness is delta-beta coupling, a proxy for neural regulation derived from data collected via electroencephalograph (EEG). Neural regulatory efforts have been investigated during baseline tasks in both children (Miskovic et al., 2011a) and adults (Miskovic et al., 2011b) through links in oscillations between fast (i.e., beta) and slow (i.e., delta) wave frequency bands, derived from EEG data. Power in the delta frequency band is thought to reflect activity in the subcortical region of the brain, which is linked to emotional processes (Knyazev, 2007; Uhlhaas & Singer, 2006), whereas power in the beta frequency band is thought to reflect activity in the cortical region of the brain, which is associated with cognitive processing (Ray & Cole, 1985). It is theorized that, the more the delta and beta power bands are oscillating together, the more that cortical regions of the brain are
putting effort towards regulating subcortical systems (Engel, Fries, & Singer, 2001; Robinson, 1999). Delta-beta coupling, or the correlation between delta and beta frequency bands, is interpreted as cortical-subcortical crosstalk, or efforts of cognitively-oriented, cortical systems to regulate emotional reactivity in subcortical systems in real time. Thus, delta-beta coupling is described as a proxy for neural regulation.

Importantly, high delta-beta coupling is a promising marker for anxiety proneness. More specifically, delta-beta coupling is positively associated with increased risk for the development of anxiety disorders at multiple time points during the lifespan (i.e., toddlerhood and adulthood). In toddlerhood, an association between delta-beta coupling and dysregulated fear suggests that toddlers displaying dysregulated fear may have a tendency to overregulate their emotions in low-threat contexts at the neural level, or are in chronic anticipation of threat (Phelps, Brooker, & Buss, 2016). To provide further evidence of a proxy for neural regulation, Miskovic et al. (2011b) measured coupling in a clinical sample of adults with social anxiety disorder before and after 12 weekly standardized sessions of group cognitive behavioral therapy. Results showed a significant decrease in delta-beta coupling in socially anxious participants post treatment that were comparable to delta-beta coupling levels in a subclinical control group (Miskovic et al., 2011b; Miskovic et al., 2011a). These results suggest that after participants learned to appropriately modulate their emotions according to context, a similar pattern was seen at the neural level. This provides further evidence that delta-beta coupling is a proxy for neural regulation, such that as anxiety symptoms decrease to subclinical levels, coupling decreases to become more normative. Importantly, children of parents with anxiety disorders (i.e., social anxiety) have displayed heightened delta-beta coupling, suggesting that children of parents with anxiety disorders are at heightened risk for the development of anxiety as seen on the neural level (Miskovic et al., 2011a).

The association between delta-beta coupling and anxiety risk in both adults and children, and the association between maternal anxiety disorder and child neural markers of anxiety suggest that there may be an intergenerational transmission of neural markers of anxiety. It is reasonable, then, to investigate whether mothers with anxiety proneness at the neural level, shown through delta-beta coupling, may be transmitting anxiety risk to their children, which is visible through toddlers’ dysregulated fear. However, maternal delta-beta coupling has not been investigated in relation to toddlers’ behavioral outcomes.
Importantly, as mentioned previously, it is difficult to attribute anxiety proneness to a sole biological system. It is likely that multiple biological systems are influencing anxiety risk. Furthermore, it is difficult to determine the degree to which each of these systems is acting independently. Research suggests that neural circuitry is associated with fear responses that modulate neuroendocrine responses, and that perceived stress then activates the HPA axis, which then results in the release of cortisol (Van der Kolk, 1994). This process, however, is bidirectional, and the release of cortisol, in turn, reinforces and sustains neural responses. The scant research investigating the association between cortisol reactivity and delta-beta coupling has resulted in inconsistent findings. van Peer and colleagues (2008) found that the administration of cortisol in a small sample of male adults resulted in increased delta-beta coupling. These results support theory suggesting that neural and neuroendocrine processes influence each other and may result in an additive effect. Importantly, results suggest that cortisol and delta-beta coupling are positively linked and that cortisol may influence the magnitude of delta-beta coupling. During early developmental periods, however, preliminary findings from Phelps, Howe, Brooker, and Buss (2015) found no relation between cortisol reactivity and delta-beta coupling at baseline in toddlerhood, whereas Brooker, Phelps, Davidson, and Goldsmith (2015) found that delta-beta coupling was associated with cortisol reactivity in non-positive contexts in infants. It is possible that these two systems are not reliably regulating together during early developmental stages as regulatory systems have not yet matured. Moreover, it is unclear whether cortisol reactivity and delta-beta coupling, specifically, are working together as regulatory constructs or if these constructs contribute unique variance in the transmission of anxiety risk. Thus, the current study will investigate maternal cortisol reactivity and delta-beta coupling both separately as well as together to understand unique versus shared relations to toddlers’ dysregulated fear.

Although heritability seems to be playing a role in intergenerational transmission of anxiety risk, environmental context has also received support in the investigation of anxiety transmission. It is suggested that children can learn maladaptive emotion regulation skills by modeling a parent with an anxiety disorder (Askew & Field, 2008). It has also been suggested that mothers with anxiety disorders may use maladaptive parenting techniques, such as overprotection, more than mothers without anxiety, increasing the child’s risk for anxiety development (Creswell, Apetroaia, Murray, & Cooper, 2013; Creswell, Cooper, & Murray,
This environment may influence children’s regulatory abilities not only at the behavioral level, but also at the biological level. For example, as a parent works to soothe a crying child, the child learns to behaviorally regulate their emotions, but, importantly, their biological systems are learning to regulate appropriately as well. Whether or not this pattern of behavioral intervention is ultimately adaptive may depend upon the environmental context. Thus, although maternal biological indices of reactivity and regulation may directly influence toddlers’ behavioral outcomes, it seems even more likely, given previous research, that maternal biology may indirectly affect toddlers’ behavioral outcomes through the caregiving environment mothers create for their children. Maternal biology and protective parenting may thus be involved in the development of dysregulated fear and are subsequently reviewed to support the model proposed by the current study.

Parenting Behavior as a Mechanism of Association between Maternal Biology and Toddler Dysregulated Fear

Protective parenting behaviors (also, overprotection) are characterized by the broad restriction of the child’s autonomy (Mills & Rubin, 1998) through excessive warmth, restriction of independence, and encouragement of avoidance (Parker, Tupling & Brown, 1979; Kiel & Buss, 2011). Protective parenting can be seen behaviorally in examples such as shielding a child from a stimulus when protection is not warranted or appropriate, high levels of physical affection that distract the child from independent coping, and the encouragement of avoidant coping when faced with a stressful situation (Kiel & Buss, 2011; Barrett & Fleming, 2011).

Protective parenting is one of the most robustly studied parenting behaviors in relation to child anxiety and temperamental risk for anxiety. Protective parenting behaviors have been associated with the development of anxiety disorders in children (Rapee, 1997) and have been shown to predict child internalizing difficulties, behavioral inhibition, anxiety risk, and anxiety development (Bayer, Sanson, & Hemphill, 2006; Kagan, 1994; Siqueland, Kendall, & Steinberg, 1996). Protective parenting behaviors have only begun to be investigated in relation to dysregulated fear. Kiel & Buss (2014) found that protective parenting, as solicited by the child, concurrently related to toddlers’ dysregulated fear, however, the directionality of this relation was not tested.

Although protective parenting behaviors, specifically, have not been investigated in relation to cortisol reactivity, relations between maternal biology and other maladaptive
parenting behaviors provide evidence to suggest that there may be a positive relation between cortisol reactivity and protective parenting behaviors. Importantly, high maternal cortisol reactivity is associated with maladaptive parenting behaviors (i.e., intrusive parenting) in mothers of children with inhibited temperament (Kiel & Buss, 2013; Martorell & Bugental, 2006). Increases in cortisol reactivity in adults have also been positively linked to avoidance behaviors (Roelofs, Elzing, & Rotteveel, 2005).

Similarly to cortisol reactivity, although parenting behaviors have not been investigated in relation to delta-beta coupling, evidence has shown that delta-beta coupling fluctuates in concert with anxiety symptoms and behavioral displays of anxiety; thus, it is reasonable to assert the idea that high delta-beta coupling is associated with behavioral displays of anxiety, which in mothers may be manifested as maladaptive parenting behaviors. Indeed, maternal anxiety has been linked to maladaptive parenting such as overprotectiveness (e.g., Schneider et al., 2009; Parking & Lipscombe, 1981). Therefore, the field will also benefit from the investigation of maternal protective parenting behaviors as a mechanism through which maternal delta-beta coupling may relate to toddlers’ dysregulated fear.

**Current Study Aims**

The extant literature has provided evidence for relations among maternal biology, parenting behavior, and toddlers’ risk for developing anxiety problems, but several gaps remain in understanding the developmental consequences of these associations. First, although delta-beta coupling has been linked to anxiety, it is unclear whether maternal delta-beta coupling has implications for parenting behavior and toddlers’ anxiety risk, similarly to other aspects of psychobiology. Second, whether maternal biology and parenting behaviors relate to children’s dysregulated fear, a more recently advanced measure of child risk for anxiety, in the same manner as more traditional measures of anxiety risk (e.g., behavioral inhibition), remains unknown. Finally, although bivariate relations among maternal biology, parenting behavior, and child anxiety risk have been shown, whether maternal biology relates to anxiety risk indirectly through parenting behavior has received scant attention. Understanding these gaps would allow for more complete models of child anxiety risk, augmenting both theory and practice surrounding anxiety.

Thus, Aim 1 (see Figure 1) of the current study investigated a mediation model in which maternal cortisol reactivity was a predictor of toddlers’ dysregulated fear through a mechanism
of maternal protective parenting behaviors. I hypothesized that maternal cortisol reactivity would relate to maternal protective parenting behaviors, and that those behaviors would, in turn, predict increased dysregulated fear across one year. Further, I predicted that the indirect effect of the relation between maternal cortisol reactivity and toddlers’ dysregulated fear through maternal protective parenting would be significant.

Aim 2 (see Figure 2) of the current study investigated the relation between maternal delta-beta coupling and toddlers’ dysregulated fear, as mediated by maternal protective parenting behaviors. I hypothesized that maternal delta-beta coupling would relate to maternal protective parenting behaviors which would, in turn, predict an increase in toddlers’ dysregulated fear. I also predicted that the indirect effect of the relation between maternal delta-beta coupling and toddlers’ dysregulated fear through maternal protective parenting behaviors would be significant.

Aim 3 (see Figure 3) of the current study investigated the additive effect of maternal biological indices of reactivity and regulation (i.e., maternal cortisol reactivity and maternal delta-beta coupling) as predictors of toddlers’ dysregulated fear through the mechanism of maternal protective parenting behaviors in a larger mediation model. I hypothesized that each predictor would account for unique variance in protective parenting, therefore providing an additive effect in the indirect effect.

Method

Participants

Toddlers and their mothers (n = 62) were recruited from public birth announcements, from the Women, Infants, and Children Program, and at local events and offices (e.g., farmer’s markets, child care centers, pediatrician’s offices) in a Midwestern county of Ohio. This sample was broadly recruited, but there was a slight emphasis on the inclusion of participants with rural and/or low socioeconomic background. Families were predominantly European American (93.6%). Gross household income ranged from less than $15,000 to greater than $100,000 with an average annual income between $51,000-60,000 and 19.4% of participants reporting a gross annual income less than $20,000. Dyads participated in a larger investigation of temperament and parenting. In this study, mother-child dyads participated in laboratory assessments of temperament, emotion regulation, and family relationships at child age 1, 2, and 3 years. Additionally at child age 2, mothers’ electrophysiology was assessed via EEG at a separate visit. The current study focuses on dyads who have participated in the child age 2 visit, maternal EEG
visit, and child age 3 visit. The subset of participants from the larger study were chosen based on usable delta-beta coupling data because a within subjects delta-beta coupling measure has not been used in previous literature. One-hundred and three mothers participated in the EEG portion, however, only 62 participants (60.2%) provided usable EEG data. Therefore, 100% of participants ($n = 62$) in the current study provided delta-beta coupling data and dysregulated fear at age 2. Fifty-one (82.25%) of participants provided cortisol samples and 58 (93.54%) of participants provided usable video data for coding dysregulated fear at age 3. Consistent with previous cortisol literature, 1 participant in her third trimester of pregnancy was removed from analyses. Importantly, this participant was removed from all analyses as it is unclear how pregnancy may influence EEG measures (final $n = 61$, female toddlers = 17).

**Procedure**

**Age 2 laboratory visit.** Dyads who participated in the child age 1 visit were invited to participate in a child age 2 laboratory visit. Recruitment was left open for the age 2 visit, which allowed for new participants to join the study during this visit. Once the age 2 visit had been scheduled, mothers were mailed a questionnaire packet that contained a consent form. Mothers were asked to bring the completed questionnaire packet and consent form to the laboratory visit. Upon arrival to the laboratory, packets and consent forms were collected. The primary experimenter then informed the mother that her child would be participating in a variety of tasks designed to elicit a range of reactions to novel situations, in which she would always be present. Mothers were given a detailed explanation of each episode, including an indication of the episodes in which she would be asked to limit her interaction with her child. The visit was video recorded by cameras visible to the dyad, controlled from a separate room. During the visit, saliva was collected from both mothers and toddlers to later be assayed for cortisol. After completion of the visit, the primary experimenter provided mothers with a debriefing form and answered any questions she had. Mothers were compensated $50 (+$5 if the dyad lived outside of city limits) for their time. Children were given a small, stuffed teddy bear as a token of our appreciation.

Although dyads were asked to participate in various psychophysiological procedures and a variety of episodes modeled after the Toddler Version of the Laboratory Assessment Battery (Lab-TAB; Buss & Goldsmith, 2000) and other studies in the literature (Buss, 2011) during this visit, the current study will focus on maternal cortisol measures collected during the visit and
puppet show and clown episodes considered to be low in threat to children (Buss, 2011; Buss et al., 2013).

**Cortisol collection.** Mothers provided saliva samples at three time points during the visit (20 minutes after acclimating to the lab, midway through the visit, and approximately 20 minutes after the last episode) through passive drool. Mothers were instructed to allow saliva to collect in the mouth. If mothers indicated that they had a dry mouth, they were instructed to think of their favorite food to increase saliva production. Mothers then allowed the saliva to pass into the collection tube until a sufficient amount of saliva was provided. Time of day was recorded for each sample as well as the length of time it took for mothers to provide samples (Pruessner et al., 2003). Cortisol samples were stored at -80°C until they were shipped on dry ice to Biochemisches Labor (Trier, Germany), where they were be stored at -20°C. According to the laboratory’s stated practices, saliva was be centrifuged for 10 minutes at 2000 g and assayed using competitive solid phase time-resolved fluorescence immunoassay with flouromeric end point detection (DELFIA). Testing is sensitive for values between 0.30-100 nmol/l. Average intra- (6.11%) and inter- (7.1-9.0%) assay coefficients of variation were acceptable.

**Behavioral episodes.** Dysregulated fear and maternal protective behaviors were assessed from both a 3 minute puppet show and a 5 minute clown episode, which have been validated as low-threat episodes for toddlers (Buss, 2011). During the puppet show episode, the room was set up so that the puppet show stage was on the opposite side of the room from a chair where mothers could sit. Prior to the puppet show episode, mothers were instructed to interact naturally with their children. During the puppet show, performed by a female research assistant, a lion puppet and an elephant puppet played two games: catch (1 minute in length) and fishing (1 minute in length). Finally, the puppets offered the child a sticker as a prize before saying goodbye (1 minute in length). As a debriefing period after the puppet show, the female research assistant revealed herself to the dyad and asked the child if s/he would like to play with the puppets.

During the clown episode (Buss, 2011), mothers were also instructed to interact naturally with their children. The room was empty except for a chair in which the mother was instructed to sit. The dyad sat in the room for approximately 10 seconds before a female research assistant dressed as a clown entered the room and introduced herself as “Floppy the Clown.” The female research assistant wore a clown suit with a wig and red nose. Floppy the Clown played a series
of games with the child: blowing bubbles (1 minute in length), playing with 2 large beach balls (1 minute in length), and playing with musical instruments (1 minute in length). The clown worked to engage the child in each game. For example, the clown encouraged involvement in the bubble game by making statements such as, “Bubbles are so fun! Would you like to try blowing bubbles?” Between playing with the beach balls and musical instruments, Floppy the Clown stated that it was getting hot in the room and removed the wig and nose. This portion of the episode served as a debriefing period for the child. After playing with the toys, Floppy the Clown asked the child to help clean up the toys. Once the toys were put away, the clown said goodbye.

Both episodes were video-recorded for later scoring of maternal protective parenting behaviors as well as toddler dysregulated fear. Coders received 15-20 hours of training from a master coder and were required to achieve a minimum inter-rater reliability (intraclass correlation coefficient, or κ) of .80 before coding independently. A master coder double-scored approximately 20% of cases, and discrepancies in scores and coding questions were discussed at regular meetings to prevent coder drift (distress in puppet show: κ = 1.00; shyness in puppet show: κ = 0.82; distress in clown: κ = 0.90; shyness in clown: κ = 0.95; age 3: distress in puppet show: κ = 1.00; shyness in puppet show: κ = 0.94; distress in clown: κ = 0.97; shyness in clown: κ = 1.00).

**Maternal EEG visit.** Approximately 2-4 weeks after the child age 2 visit, mothers were asked to come back to participate in an hour-long EEG visit. During this visit, mothers were fitted with a 256-channel Hydrocel Geodesic Sensor Net (Electrical Geodesics, Inc.). EEG data was recorded during an 8 minute baseline. During the baseline period, mothers were instructed to sit as quietly and still as possible, alternating 1 minute of eyes open with 1 minute eyes closed for 8 minutes. EEG activity was also recorded while mothers viewed video clips of their child in various episodes from the age 2 visit, although this was not included in the current study. After completion of the EEG visit, the primary experimenter provided mothers with a debriefing form and answered any questions she had. Mothers were compensated with for this visit in the same way as previously discussed.

Cleaning and analysis of EEG data was completed offline using MatLab to derive a within-subjects measure of delta-beta coupling. According to the American Board of Registration of Electroencephalographic and Evoke Potential Technologists (ABRET), the
electrodes that were used in deriving delta-beta coupling (F3, F4, C3, C4, P3, P4) have acceptable equivalence with the 10/10 electrode positions (Luu & Ferree, 2005).

**Age 3 laboratory visit.** Mother-child dyads who participated in child age 2 visits were invited to participate in an age 3 laboratory visit. Recruitment was open to new participants during the age 3 visits, however, current analyses will only focus on children who have longitudinal data. Similar to age 2 practices, mothers were mailed a questionnaire packet that contained a consent form. Mothers were asked to complete the packet prior to the visit and bring it with them to the laboratory. Again, at the beginning of the visit, the primary experimenter informed the mother that her child would be participating in a variety of tasks designed to elicit a variety of reactions to novel situations, and mothers were given a detailed description of the entire visit. The visit was video recorded in the same manner as the age 2 visit. Upon completion of the visit, again a debriefing form was provided for mothers and the primary experimenter answered any questions she had. Mothers were compensated for this visit in the same way as previously discussed. Children were given a small, stuffed teddy bear as a token of our appreciation.

Although mother-child dyads were asked to engage in a number of tasks during this laboratory visit, the current analyses will focus on the puppet show and clown episodes, which followed the exact same procedures as at the age 2 visit. Scoring of observational behaviors relevant to dysregulated fear also proceeded similarly.

**Measures**

**Cortisol Reactivity.** Cortisol value outliers were winsorized such that extreme values (> 3 SD above the mean) were replaced with the highest value within 3 SD of the mean. In order to correct for skewness and kurtosis, cortisol values were log transformed, consistent with previous practices (Gunnar, Morison, Chisholm, & Schuder, 2001). Effects of taking any medications (i.e., allergy medications, anti-depressants, multivitamin, analgesics, narcotics, antibiotics, thyroid medication, heartburn medication, ADHD medication, beta-blockers, and epilepsy) versus not taking medication on cortisol samples were investigated and were not significant (r = -0.12, p = .50), therefore, medication was not included in further analyses. Previous work has suggested that birth control use may influence cortisol production; however, birth control use was unrelated to cortisol reactivity in the current sample (p = .71) and was not considered further. Effects of sample time on cortisol samples were also examined. Sample time was not
correlated with pre-visit samples ($r = 0.15, p = .29$), mid-visit samples ($r = 0.15, p = .29$), or post-visit samples ($r = 0.14, p = .32$), therefore sample time was not included in subsequent analyses. Area under the curve with respect to increase was computed to determine cortisol reactivity for each mother (Pruessner et al., 2003). Area under the curve was calculated using a formula in which, essentially, the baseline measurement is subtracted from an average of the three samples collected across the visit to provide a dimensional measure of average change from baseline as reactivity.

**Dysregulated fear.** Dysregulated fear was scored from the puppet show and clown episodes during the age 2 and age 3 visits. Dysregulated fear was computed from scores capturing children’s displays of distress and shyness, inhibition, and withdrawal during each of the episodes. Distress was considered to occur when the child displayed negative facial affect (e.g., sadness, fear) and/or negative vocalizations (e.g., crying, whimpering) and was scored on a scale of 1-5 for the entire episode as follows: 1 = No distress shown, or a very fleeting display, 2 = One or two displays of low intensity distress, 3 = Many long displays of low intensity distress, 4 = A few intense displays of distress, or consistent display of low intensity distress, 5 = Display of distress that lasts the whole episode, is very intense or the episode is stopped because of the child’s distress. Shyness/inhibition was considered to occur when the child displayed behaviors such as avoidance of or withdrawal from the stimulus. Shyness/inhibition was scored on the 1-5 scale as follows: 1 = No shyness/inhibition shown, or a very fleeting display, 2 = Child withdrawals from stimulus once and/or is fidgety or reduced activity, 3 = Very tense, may be fidgety, frozen, asks to leave, or asks stimulus to leave, or avoidant for most of the episode, 5 = Extremely shy, freezes, never leaves mother’s lap, avoidant or resistant of stimulus throughout the entire episode. Distress and shyness/inhibition were each scored in the puppet show and clown episodes. Distress and shyness within the clown episode were correlated at both ages 2 ($r = .37, p = .00$) and 3 ($r = .65, p = .00$). Distress and shyness within the puppet show episode were not correlated at age 2 ($r = .22, p = .09$), but were significantly correlated at age 3 ($r = .26, p = .05$). While these latter correlations are relatively low to justify a full composite, the current study’s small sample size may be driving this effect. The current study created a composite of distress and shyness during clown and puppet show that is consistent with previous work. After scores were averaged within episodes, these composites were significantly correlated across episodes (age 2: $r = .35, p = .007$; age 3: $r = .41, p = .002$). Thus, these scores were averaged
within each time point to yield the final scores of age 2 dysregulated fear and age 3 dysregulated fear.

**Observed maternal protective behaviors.** Maternal protective behaviors were scored during the puppet show and clown episodes during the age 2 visit. Protective behaviors were considered to have occurred when mothers shielded their children from a stimulus or engaged in avoidant behavior (e.g., moving the child away from the clown) as well as when mothers engaged in comforting behaviors. A 0-3 scale was used for both comforting and protective behaviors each 10-second epoch of each episode. Protective behaviors were scored as follows: 0 = no behavior shown, 1 = slight behavior (e.g., leaning child away from stimulus), 2 = moderate behavior (e.g., physically moving the child away from the stimulus), 3 = intense or prolonged behavior (turning child completely away from stimulus). Comforting behaviors were scored as follows: 0 = no comforting behavior shown, 1 = mother touches child, or says something comforting, 2 = mother actively soothes child (e.g., rubs arm), 3 = mother hugs or embraces child generally with both arms. In addition, scores were qualified by whether they were “solicited” (i.e., in response to the child’s bid for comfort or support) or “unsolicited” (i.e., spontaneous, not in response to the child). Given previous research showing that solicited behaviors were related to children’s anxiety development while unsolicited behaviors were not (Kiel & Buss, 2010), only solicited behaviors were used. Solicited comforting and protective behavior scores were each averaged across epochs within each episode. Solicited comforting and protective behaviors within the puppet show episode were not significantly correlated (r = 0.07, p = .58). Solicited comforting and protective behaviors within the clown episode were significantly correlated (r = 0.38, p = 0.00). While these correlations are not consistent with creating a composite, the current study’s small sample size may be driving this effect. In order to remain consistent with the literature, a composite was made. Scores of comforting and protection were averaged within each episode. These composites were not correlated across the episodes (r = 0.12, p = .37). They were then averaged across episodes. These final scores were used for the protectiveness variable.

**Maternal delta-beta coupling.** Netstation was used to collect and clean EEG data. EEG data were filtered using a highpass filter of 0.50 Hz and a lowpass filter of 48 Hz during collection. All channels were online referenced to Cz during data acquisition. Data from each participant was run through an Independent Components Analysis (ICA) in EEGLab to extract
eye blink and eye movement artifacts. ICA returns maximally independent sources of electrical activity in the neural recordings. An algorithm then plots each component and the researcher identifies which components have a pattern consistent with eye blink or ocular movement artifacts. These components are then deleted, and the voltage data are reconstructed for analysis. Artifacts were identified using independent component analysis and common artifacts were removed according to typical topography and component characteristics (Makeig & Onton, 2012).

Cleaned data were submitted to a Fast-Fourier Transform using a Hamming window with a 50% segment overlap. Power spectral densities were computed on each channel in each cluster (the frontal [F3 cluster: electrodes 23, 29, 35, 36, 30, 41, 40; F4 cluster: electrodes 4, 5, 206, 214, 215, 223, 224], central [C3 cluster: 52, 51, 59, 60, 65, 58, 66; C4 cluster: 195, 196, 183, 182, 164, 155, 184], and parietal [P3 cluster: 88, 78, 87, 99, 98, 86, 77; P4 cluster: 162, 163, 154, 153, 142, 141, 152] electrode sites) for each epoch separately using the Welch’s moving window procedure as programmed in the Matlab function pwelch. Power (mV^2) was derived in the delta (0.5-4 Hz) and beta (18-30 Hz) frequency bands for each 15-second epoch for the frontal, central, and parietal clusters. These cutoffs for delta and beta frequency bands and the specified electrode sites were selected based on previous delta-beta coupling investigations with adults (Miskovic et al., 2011a; Miskovic et al., 2011b). A laplacian correction for remove global volume conductance, or noise consistent across all electrodes, was applied to get a more localized representative of neural activity in each electrode cluster. However, it was unclear whether this transformation corrected values accurately. Additionally, after the laplacian correction, correlations within participants (frontal: range = 0.76-0.99; central: range = 0.73-1.00; central: range = 0.72-0.99) did not seem to significantly differ from within participant correlations without the correction (frontal: range = 0.74-0.99; central: range = 0.69-1.00; parietal: range = 0.69-0.99). Therefore, delta-beta coupling was investigated without using the laplacian correction. Raw delta and beta values were positively skewed and transformed using the natural logarithm which produced normally distributed variables. A correlation among the 28 delta and beta power values within an individual for each of the frontal (F3, F4), central (C3, C4), and parietal (P3, P4) electrode sites were used as continuous variables.
Results

Preliminary Analyses

Missing Data. The Little’s Missing Completely at Random (MCAR) analysis of patterns of missing data was used to examine missing data. A non-significant MCAR test ($\chi^2[26] = 25.75, p = .48$) suggested that the data were consistent with the pattern of missing completely at random. Missingness was also investigated using t-tests to determine any significant differences on other variables between participants who were and were not missing values; however, no significant t-tests emerged.

All missing data were imputed. Multiple imputation using biological variables is not widely practiced. However, multiple imputation does not change the shape or characteristics of the data, but instead uses the existing data to create estimates for values that maintain the properties of the data (e.g., Rubin, 1996; Yuan, 2010). Importantly, multiple imputation is considered best practice for missing data (Graham, 2009). Data are lost using more traditional methods of dealing with missing data (e.g., listwise deletion), but multiple imputation maximizes the utility of the existing data. This practice is particularly useful for longitudinal data (Jelićić, Phelps & Lerner, 2009) and has been consistently used with questionnaire and behavioral data. Given the difficult nature of collection of biological measures, multiple imputation is useful in creating estimates for values whilst maintaining descriptive properties of the dataset.

Descriptive statistics were computed (Table 1), and variables were examined for outliers and skew and kurtosis as indicators of non-normality. Maternal protective parenting behavior was corrected for skewness and kurtosis by adding 1 and taking the square root of the variable. Bivariate correlations were examined among primary variables, and correlations between variables acting as dependent variables and sociodemographic variables were examined for identification of potential covariates (Table 1). Toddler race, maternal race, maternal education, and marital status were not significantly correlated with any primary variables and were not considered further. Total family income was significantly associated with toddler dysregulated fear at age 3 ($r = 0.29, p = 0.04$). Income (0 = $15,000 or less, 1 = $16,000-$20,000, 2 = $21,000-$30,000, 3 = $31,000-$40,000, 4 = $41,000-$50,000, 5 = $51,000-$60,000, 6 = $61,000-$70,000, 7 = $71,000-$80,000, 8 = $81,000-$90,000, 9 = $91,000-$100,000, 10 = $100,000 or more) was included as a covariate in all subsequent analyses using age 3 dysregulated fear as the dependent variable. It was expected that age 2 and age 3 dysregulated fear would be significantly
and moderately to strongly correlated. Our sample suggested that age 2 and 3 dysregulated fear were significantly, positively correlated \((r = 0.59, p = .00)\). This moderate to strong correlation suggests stability; however, the imperfect correlation also suggests that there is a proportion of variance unaccounted for between the two variables.

**Primary Analyses**

Mediation analyses were used to investigate all aims. Aim 1 investigated the indirect relation between maternal cortisol reactivity and toddler dysregulated fear through maternal protective parenting behaviors. Multiple regression was used to investigate each individual path, and the PROCESS macro was used to investigate the indirect effect. Age 2 dysregulated fear was included as a covariate in analyses for which dysregulated fear at age 3 was the dependent variable to assess for change in dysregulated fear over time. Raw, non-imputed biological variables with missing data (i.e., cortisol reactivity) were also investigated to ensure imputation provided similar results. Multiple regressions revealed similar results. Therefore, the imputed data results will be discussed.

**Aim 1: Cortisol analyses.** The first aim was to investigate the relation between maternal cortisol reactivity and toddler dysregulated fear through maternal protective parenting behaviors. Multiple regression revealed a nonsignificant relation between maternal cortisol reactivity and maternal protective parenting, or the A path \((b = 0.00, SE = 0.00, \beta = 0.17, t = 1.34, p = .18)\) (Table 2). The relation between maternal protective parenting and dysregulated fear at age 3 above and beyond cortisol reactivity, or the B path, was not significant \((b = 0.39, SE = 0.48, \beta = 0.10, t = 0.80, p = .43)\) (Table 2). The total effect between maternal cortisol reactivity and toddler dysregulated fear at age 3, or the C path, was not significant \((b = -0.00, SE = 0.01, \beta = -0.05, t = -0.50, p = .62)\) (Table 2). The direct relation between maternal cortisol reactivity and toddler dysregulated fear at age 3 controlling for covariates and maternal protective parenting was not significant \((b = -0.00, SE = 0.01, \beta = -0.07, t = -0.65, p = .52)\) (Table 2). Contrary to my hypothesis, maternal cortisol reactivity did not relate to age 3 dysregulated fear through maternal protective parenting above and beyond age 2 dysregulated fear.

Due to current move towards testing the indirect effect without reliance on the significance of individual paths (Hayes, 2013), the PROCESS macro in SPSS was used to investigate the indirect effect of maternal protective parenting and toddler dysregulated fear at age 3. The indirect relation between maternal cortisol reactivity and toddler dysregulated fear...
through maternal protective parenting was not significant \((ab = 0.00, SE = 0.00, 95\% CI[-0.00, 0.01])\). This result suggests that maternal cortisol reactivity does not relate to toddler dysregulated fear at age 3 through maternal protective parenting.

**Aim 2: Delta-beta coupling analyses.** The second aim was to investigate the relation between maternal delta-beta coupling and toddler dysregulated fear through maternal protective parenting behaviors. Delta-beta coupling at frontal, central, and parietal electrode sites were each investigated as predictors in separate models. At all, multiple regression revealed a nonsignificant relation between maternal delta-beta coupling and maternal protective parenting, or the A path (frontal electrodes sites: \(b = -0.10, SE = 0.33, \beta = -0.04, t = -0.29, p = .77\); central electrode sites: \(b = -0.32, SE = 0.32, \beta = -0.13, t = -0.99, p = .33\); parietal electrode sites: \(b = -0.06, SE = 0.38, \beta = -0.02, t = -0.15, p = .88\)) (Table 3). At frontal, central, and parietal electrode sites, the relation between maternal protective parenting and dysregulated fear at age 3 above and beyond delta-beta coupling, or the B path, was also not significant (frontal electrode sites: \(b = 0.32, SE = 0.47, \beta = 0.09, t = 0.68, p = .50\); central electrode sites: \(b = 0.33, SE = 0.48, \beta = 0.09, t = 0.69, p = .49\); parietal electrode sites: \(b = 0.36, SE = 0.47, \beta = 0.10, t = 0.76, p = .45\)) (Table 3). The total relation between maternal delta-beta coupling and toddler dysregulated fear at age 3, or the C path, was not significant at any electrode site (frontal electrode sites: \(b = 0.18, SE = 0.98, \beta = 0.02, t = 0.18, p = .86\); central electrode sites: \(b = 0.04, SE = 0.95, \beta = 0.00, t = 0.04, p = .97\); parietal electrode sites: \(b = 1.15, SE = 1.10, \beta = 0.11, t = 1.04, p = .30\)) (Table 3). The direct relation between maternal delta-beta coupling and toddler dysregulated fear at age 3 after controlling for covariates and dysregulated fear at age 2 was also nonsignificant (frontal electrode sites: \(b = 0.67, SE = 0.98, \beta = 0.02, t = 0.17, p = .87\); central electrode sites: \(b = 0.14, SE = 0.97, \beta = 0.02, t = 0.14, p = .89\); parietal electrode sites: \(b = 1.21, SE = 1.11, \beta = 0.11, t = 1.09, p = .28\)) (Table 3). Maternal delta-beta coupling did not relate to age 3 dysregulated fear through maternal protective parenting above and beyond age 2 dysregulated fear.

The PROCESS macro in SPSS was used to investigate the indirect effect between maternal delta-beta coupling and toddler dysregulated fear at age 3 through maternal protective parenting. The indirect relation between transformed maternal delta-beta coupling and toddler dysregulated fear through maternal protective parenting was not significant at frontal \((ab = -0.03, SE = 0.18, 95\% CI[-0.70,0.19])\), central \((ab = -0.10, SE = 0.28, 95\% CI[-1.17, 0.15])\), or parietal \((ab = -0.02, SE = 0.24, 95\% CI[-0.89, 0.28])\) electrode sites. Results suggest that maternal delta-
beta coupling does not relate to toddler dysregulated fear at age 3 through maternal protective parenting behaviors.

**Aim 3: Cortisol and delta-beta coupling analyses.** The final models investigated the additive effect of maternal cortisol reactivity and maternal delta-beta coupling in this larger mediation model adding both predictors into the same model. Multiple regression revealed a nonsignificant relation between maternal cortisol reactivity and maternal protective parenting after covarying frontal ($b = 0.00$, $SE = 0.00$, $\beta = 0.18$, $t = 1.36$, $p = .18$), central ($b = 0.00$, $SE = 0.00$, $\beta = 0.17$, $t = 1.28$, $p = .21$), or parietal ($b = 0.00$, $SE = 0.00$, $\beta = 0.17$, $t = 1.32$, $p = .19$) delta-beta coupling (Table 4). The relation between maternal protective parenting and dysregulated fear at age 3 above and beyond maternal cortisol reactivity and delta-beta coupling, or the B path, was also not significant (frontal: $b = 0.38$, $SE = 0.49$, $\beta = 0.10$, $t = 0.79$, $p = .43$; central: $b = 0.39$, $SE = 0.49$, $\beta = 0.11$, $t = 0.80$, $p = .43$; parietal: $b = 0.41$, $SE = 0.48$, $\beta = 0.11$, $t = 0.86$, $p = .39$) (Table 4). The total relation between maternal cortisol reactivity and toddler dysregulated fear at age 3, or the C path, after controlling for frontal ($b = -0.00$, $SE = 0.01$, $\beta = -0.05$, $t = -0.51$, $p = .62$), central ($b = -0.00$, $SE = 0.01$, $\beta = -0.05$, $t = -0.49$, $p = .63$), or parietal ($b = -0.00$, $SE = 0.01$, $\beta = -0.04$, $t = -0.41$, $p = .68$) delta-beta coupling was not significant (Table 4). The direct relation between maternal cortisol reactivity and toddler dysregulated fear at age 3 after controlling for dysregulated fear at age 2 and frontal ($b = -0.00$, $SE = 0.01$, $\beta = -0.07$, $t = -0.65$, $p = .52$), central ($b = -0.00$, $SE = 0.01$, $\beta = -0.07$, $t = -0.64$, $p = .53$), and parietal ($b = -0.00$, $SE = 0.01$, $\beta = -0.06$, $t = -0.57$, $p = .57$) delta-beta coupling was not significant (Table 4).

Maternal cortisol reactivity did not account for significant variance in predicting dysregulated fear at age 3 above and beyond maternal delta-beta coupling at any electrode site or dysregulated fear at age 2.

Finally, multiple regression revealed a nonsignificant relation between maternal delta-beta coupling and maternal protective parenting after accounting for maternal cortisol reactivity at frontal ($b = -0.13$, $SE = 0.33$, $\beta = -0.05$, $t = 0.39$, $p = .70$), central ($b = -0.29$, $SE = 0.32$, $\beta = -0.12$, $t = -0.92$, $p = .37$), or parietal ($b = -0.02$, $SE = 0.37$, $\beta = -0.01$, $t = -0.04$, $p = .97$) delta-beta coupling (Table 4). The relation between maternal protective parenting and dysregulated fear at age 3 above and beyond maternal cortisol reactivity and delta-beta coupling, or the B path, was also not significant (frontal: $b = 0.38$, $SE = 0.49$, $\beta = 0.10$, $t = 0.79$, $p = .43$; central: $b = 0.39$, $SE = 0.49$, $\beta = 0.11$, $t = 0.80$, $p = .43$; parietal: $b = 0.41$, $SE = 0.48$, $\beta = 0.11$, $t = 0.86$, $p = .39$)
The total relation between maternal delta-beta coupling and toddler dysregulated fear at age 3, or the C path, after controlling for maternal cortisol reactivity was not significant at any electrode site (frontal: $b = 0.21, SE = 0.99, \beta = 0.02, t = 0.22, p = .83$; central: $b = 0.01, SE = 0.96, \beta = 0.00, t = 0.01, p = .99$; parietal: $b = 1.11, SE = 1.11, \beta = 0.10, t = 1.00, p = .32$) (Table 4). The direct relation between maternal delta-beta coupling and toddler dysregulated fear at age 3 after controlling for dysregulated fear at age 2 and cortisol reactivity was not significant for frontal ($b = 0.21, SE = 0.99, \beta = 0.02, t = 0.21, p = .84$), central ($b = 0.11, SE = 0.97, \beta = 0.01, t = 0.12, p = .91$), or parietal ($b = 1.16, SE = 1.12, \beta = 0.11, t = 1.04, p = .30$) electrode sites (Table 4). Maternal delta-beta coupling did not account for significant variance in predicting dysregulated fear at age 3 above and beyond maternal cortisol reactivity or dysregulated fear at age 2.

Because the PROCESS macro in SPSS cannot accommodate two predictors in a mediation model, two separate mediation models were assessed where each model contained one main predictor while the other predictor was included as a covariate. This allowed for the assessment of unique contribution above and beyond the other variable for each predictor. For protective parenting, no significant indirect effects emerged when investigating maternal cortisol reactivity as the main predictor in this larger mediation model with frontal ($ab = 0.00, SE = 0.00, 95\% CI[-0.00, 0.01]$), central ($ab = 0.00, SE = 0.00, 95\% CI[-0.00, 0.01]$), or parietal ($ab = 0.00, SE = 0.00, 95\% CI[-0.00, 0.01]$) delta-beta coupling serving as a covariate. No significant indirect effects emerged when investigating maternal delta-beta coupling as the main predictor and cortisol reactivity as a covariate for frontal ($ab = -0.04, SE = 0.19, 95\% CI[-0.66, 0.21]$), central ($ab = -0.12, SE = 0.29, 95\% CI[-1.23, 0.14]$), or parietal ($ab = -0.02, SE = 0.24, 95\% CI[-0.82, 0.30]$) electrode sites. Results suggest that cortisol reactivity or delta-beta coupling do not account for unique variance above and beyond the covaried variable when predicting change in dysregulated fear through maternal protective parenting behavior.

**Discussion**

The influence of maternal psychophysiology on parenting behaviors has been previously supported (Kiel & Buss, 2013; Martorell & Bugental, 2006; Roelofs et al., 2005). Further, the influence of parenting behaviors on temperament and child risk for anxiety development (e.g., behavioral inhibition, dysregulated fear) has also been supported (Bayer, Sanson, & Hemphill, 2006; Kagan, 1994; Siqueland, Kendall, & Steinberg, 1996; Kiel & Buss, 2014). The current
study sought to expand the extent literature by investigating maternal psychophysiological markers of anxiety risk as predictors of change in toddler dysregulated through protective parenting behaviors.

Contrary to hypotheses, no significant results emerged. First, results suggested that maternal cortisol reactivity does not predict change in toddler dysregulated fear over the course of a year through maternal protective parenting behaviors. Second, results suggested that maternal delta-beta coupling does not predict change in toddler dysregulated fear through maternal protective parenting behaviors. Finally, maternal cortisol reactivity and delta-beta coupling do not account for unique variance in the prediction of change in toddler dysregulated fear through protective parenting to provide an additive effect in the indirect effect. Interestingly, these results are in contrast with much of the existing literature investigating maternal biological indices of reactivity and regulation in relation to anxiety and parenting behaviors. Previous research suggested influences of cortisol in prenatal and postnatal periods on child temperament (Davis et al., 2007; Glynn et al., 2007), and cortisol reactivity has been linked to anxiety in children (e.g., Gunnar & Quevedo, 2007; Gunnar et al., 2009) and adults (e.g., Takahashi et al., 2005). Therefore, it was hypothesized that maternal cortisol reactivity would predict change in measures of temperament (i.e., dysregulated fear) through parenting behaviors. This relation was also expected given theory suggesting that cortisol reactivity and anxiety measures are positively correlated. It could be the case that toddler dysregulated fear and maternal cortisol reactivity are unrelated; however, it is important to note that the sample size of the current study was small and results may change with a larger sample. Previous work has suggested that maternal cortisol reactivity interacted with toddler inhibited temperament to positively relate to maladaptive parenting behaviors associated with anxiety development (i.e., intrusive parenting, harsh parenting) (Kiel & Buss, 2013; Martorell & Bugental, 2006). Moreover, the authors did not find a direct association between maternal cortisol reactivity and maladaptive parenting behaviors which is consistent with the current findings. Given these findings and the current results, maternal biological indices of reactivity may not directly predict toddler outcomes, but instead maternal biology may interact with temperament to predict the parenting environment.

Previous research has positively linked delta-beta coupling to anxiety outcomes in both children and adults (Miskovic et al., 2011a; Miskovic et al., 2011b; Phelps et al., 2016). However, in the current study, no significant relation was found. It is important to note that the
current measure of delta-beta coupling is a within-subjects measure, whereas previous studies have used a between-subjects measure. Given that delta-beta coupling is theorized to be a measure of regulation, and in order to understand individual differences in neurobiological markers of regulation, a within-subjects measure of delta-beta coupling is necessary. However, a within-subjects measure of delta-beta coupling has not been developed or tested up to this point, and this is the first study known to have utilized a within-subjects measure of delta-beta coupling. Therefore, it is difficult to determine whether the current results are consistent with how the field would expect this measure to relate to other variables. While the current within-subjects variable of delta-beta coupling had less range in correlation than between-subjects measures, it is expected that delta and beta are more highly correlated within individuals than between individuals. Future directions include validating a within-subjects measure of coupling by comparing how a between-subjects measure and the newly developed within-subjects measure relate to concomitants of regulation.

The non-significant relation between maternal protective parenting behaviors and toddler dysregulated fear at age 3 after controlling for toddler dysregulated fear at age 2 was inconsistent from what was expected. Although this had not been previously studied, the existent literature suggests concurrent relations between protective parenting and dysregulated fear. Importantly, multiple regression analyses revealed that protective parenting significantly related to dysregulated fear at age 2 and at age 3, but only when dysregulated fear at age 2 was not included as a covariate. This suggests that protective parenting does not predict dysregulated fear above and beyond the previous year’s measure; however, the concurrent significance may shed light on another way these variables may relate to each other. The current results are consistent with the existing literature investigating the relation between protective parenting and dysregulated fear. Kiel & Buss (2014) found a concurrent relation between protective parenting behaviors and concurrent dysregulated fear. If parenting relates to dysregulated fear at each time point, but not to change in dysregulated fear, perhaps the direction of effect is in the opposite direction. It could be the case that dysregulated fear is driving maternal protective parenting behaviors. This could be related to why no direct relations were found between maternal biology and protective parenting. More specifically, maternal biology may serve a different role than a direct predictor, such as a moderator. This would be consistent with how biology has been found to function in children (Hastings et al., 2008). It could be the case that child dysregulated fear
may predict more protective parenting behaviors only in the context of maternal biological predispositions for certain patterns of reactivity and regulation.

The current study has multiple strengths. Behavioral data were collected for both children and mothers. Maternal biological indices of regulation and reactivity were collected as well. A longitudinal design allowed for the investigation of change in temperamental characteristics over time. This is the first study, to our knowledge, to provide an estimate of the stability of dysregulated fear. In the current study’s sample, dysregulated fear appears to have a moderate to strong correlation from age 2 to age 3 however, this correlation also suggested a proportion of variance was unaccounted for by age 2. Moreover, this suggests that the development of dysregulated fear has room to be influenced by environmental factors.

The current study is not without limitations. Biological data were imputed; however, multiple imputation using biological variables is not widely practiced. Importantly, analyses were completed with a dataset in which biological variables were imputed and a dataset for which biological variables were not imputed. Results followed similar patterns and were nonsignificant in both cases. Thus, the multiply imputed dataset was used in order to maximize the utility of the existing data in an already small sample. The current study has a small sample size and was also primarily middle class and European American. The homogeneity of the current sample limits generalizability. Thus, future research would benefit from analyzing these relations in a larger, more diverse sample.

Additionally, future directions include investigating the same variables in a different theoretical framework. The current study assumed parent-directed effects on child anxiety risk, however, it could be the case that child-anxiety risk may drive anxiety-related parenting behaviors. Bidirectional relations between child temperament and parenting behaviors have been evident in the literature for a number of years (Rubin, Nelson, Hastings & Asendorpf, 1999; Sameroff, 2009). Theory suggests not only that children are influenced by parenting behaviors, but also that mothers are influenced by their toddlers’ temperaments. Although a bidirectional relation exists, it may be the case that when mothers have children with dysregulated fear and biological markers of anxiety, they may be more likely to engage in maladaptive parenting behaviors that are linked to increased toddler anxiety risk. It is also important to consider that maternal biological indices of reactivity and regulation may not serve as direct predictors of parenting behavior, but rather determine when the environment influences parenting behaviors.
More specifically, we will investigate whether maternal parenting behavior is influenced by toddler dysregulated fear as moderated by psychophysiological predispositions to anxiety.

Overall, this study contributes to the existing literature by providing insight into how measures of maternal cortisol reactivity and delta-beta coupling may relate to the development of toddler dysregulated fear through parenting behaviors. More specifically, results suggested that these measures of maternal biological indices of reactivity and regulation did not predict change in toddler dysregulated fear through maternal protective parenting behaviors. Further, the current study provides an estimate of the stability of dysregulated fear suggesting that it may be a more stable measure of fearfulness than behavioral inhibition, yet has variance unaccounted for suggesting other factors may influence the further development of dysregulated fear. Future directions should consider investigating a transactional model of temperament development where maternal biology acts a moderator to understand the role of maternal biology in the complex relationship between temperament and parenting behaviors.
References


### Table 1

**Descriptive statistics and bivariate correlations.**

<table>
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<th>Variable</th>
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<td>-0.09</td>
<td>0.13</td>
<td>0.29</td>
<td>0.02</td>
</tr>
<tr>
<td>2. Maternal Cortisol Reactivity</td>
<td>-19.08</td>
<td>13.76</td>
<td>-</td>
<td>0.07</td>
<td>-0.06</td>
<td>-0.08</td>
<td>0.01</td>
<td>-0.05</td>
<td>0.17</td>
</tr>
<tr>
<td>3. Maternal Delta-Beta Coupling (frontal)</td>
<td>0.90</td>
<td>0.07</td>
<td>-</td>
<td>0.75**</td>
<td>0.69***</td>
<td>-0.10</td>
<td>-0.05</td>
<td>-0.04</td>
<td></td>
</tr>
<tr>
<td>4. Maternal Delta-Beta Coupling (central)</td>
<td>0.90</td>
<td>0.07</td>
<td>-</td>
<td>-</td>
<td>-0.03</td>
<td>-0.05</td>
<td>-0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Maternal Delta-Beta Coupling (parietal)</td>
<td>0.92</td>
<td>0.06</td>
<td>-</td>
<td>0.06</td>
<td>0.12</td>
<td>-0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6. Dysregulated Fear (age 2)</td>
<td>1.86</td>
<td>0.57</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.59***</td>
<td>0.58***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. Dysregulated Fear (age 3)</td>
<td>1.66</td>
<td>0.62</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.39***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8. Maternal Protective Parenting</td>
<td>0.18</td>
<td>0.17</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*p < .05, p < .01, p < .00*
### Table 2
*Aim 1: Maternal Cortisol Mediation Models.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Path A (DV = protective parenting)</th>
<th>Path C (DV = age 3 DF)</th>
<th>Paths C’, and B (DV = age 3 DF)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maternal cortisol reactivity</td>
<td>Income</td>
<td>Family income</td>
</tr>
<tr>
<td></td>
<td>b 0.00</td>
<td>SE 0.00</td>
<td>β 0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note.* Income and age 2 dysregulated fear were included as covariates in paths C, C’, and B. No predictor variables of interest emerged as significant. A path model: $R^2 = 0.03$, $F[1,59] = 1.80, p = .18$; C path model: $R^2 = 0.40$, $F[3,57] = 12.60, p = .00$). C’ and B path model: $R^2 = 0.41$, $F[4,56] = 9.55, p = .00$. *p < 0.05, ***p < .001.
Table 3

*Aim 2: Delta-Beta Coupling Mediation Models*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frontal</th>
<th></th>
<th>Central</th>
<th></th>
<th>Parietal</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>b</td>
<td>SE</td>
<td>β</td>
<td>t</td>
<td>b</td>
<td>SE</td>
</tr>
<tr>
<td>Path A (DV = protective parenting)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal delta-beta coupling</td>
<td>-0.10</td>
<td>0.33</td>
<td>-0.04</td>
<td>-0.29</td>
<td>-0.32</td>
<td>0.32</td>
</tr>
<tr>
<td>Path C (DV = age 3 DF)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Income</td>
<td>0.04</td>
<td>0.02</td>
<td>0.21</td>
<td>2.06*</td>
<td>0.40</td>
<td>0.02</td>
</tr>
<tr>
<td>Dysregulated fear (age 2)</td>
<td>0.61</td>
<td>0.11</td>
<td>0.57</td>
<td>5.44***</td>
<td>0.61</td>
<td>0.11</td>
</tr>
<tr>
<td>Maternal delta-beta coupling</td>
<td>0.18</td>
<td>0.98</td>
<td>0.02</td>
<td>0.18</td>
<td>0.04</td>
<td>0.95</td>
</tr>
<tr>
<td>Paths C’, and B (DV = age 3 DF)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family income</td>
<td>0.04</td>
<td>0.02</td>
<td>0.22</td>
<td>2.09*</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Dysregulated fear (age 2)</td>
<td>0.56</td>
<td>0.14</td>
<td>0.52</td>
<td>4.00***</td>
<td>0.55</td>
<td>0.14</td>
</tr>
<tr>
<td>Maternal delta-beta coupling</td>
<td>0.67</td>
<td>0.98</td>
<td>0.02</td>
<td>0.17</td>
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<td>0.97</td>
</tr>
<tr>
<td>Maternal protective parenting</td>
<td>0.32</td>
<td>0.47</td>
<td>0.09</td>
<td>0.68</td>
<td>0.33</td>
<td>0.48</td>
</tr>
</tbody>
</table>

*Note.* Income and age 2 dysregulated fear were included as covariates in paths C, C’, and B. No predictor variables of interest emerged as significant. Model information was as follows for frontal (A path: $R^2 = 0.00$, $F[1,59] = 0.09$, $p = .77$; C path: $R^2 = 0.40$, $F[3,57] = 12.49$, $p = .00$; C’ and B paths: $R^2 = 0.40$, $F[4,56] = 9.39$, $p = .000$), central (A path: $R^2 = 0.02$, $F[1,59] = 0.99$, $p = 0.33$; C path: $R^2 = 0.40$, $F[3,57] = 12.47$, $p = .00$; C’ and B paths: $R^2 = 0.40$, $F[4,56] = 9.39$, $p = .000$), and parietal (A path: $R^2 = 0.00$, $F[1,59] = 0.02$, $p = .88$; C path: $R^2 = 0.41$, $F[3,57] = 13.07$, $p = .00$; C’ and B paths: $R^2 = 0.41$, $F[4,56] = 9.88$, $p = .000$) electrode sites. *$p < 0.05$, ***$p < .001$. 

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Table 4.  
*Aim 3: Cortisol Reactivity and Delta-Beta Coupling Mediation Models.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frontal</th>
<th></th>
<th>Central</th>
<th></th>
<th>Parietal</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$b$</td>
<td>$SE$</td>
<td>$\beta$</td>
<td>$t$</td>
<td>$b$</td>
<td>$SE$</td>
</tr>
<tr>
<td>Path A (DV = protective parenting)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal cortisol reactivity</td>
<td>0.00</td>
<td>0.00</td>
<td>0.18</td>
<td>1.36</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Maternal delta-beta coupling</td>
<td>-0.13</td>
<td>0.33</td>
<td>-0.05</td>
<td>-0.39</td>
<td>-0.29</td>
<td>0.32</td>
</tr>
<tr>
<td>Path C (DV = age 3 DF)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Income</td>
<td>0.04</td>
<td>0.02</td>
<td>0.21</td>
<td>2.05$^*$</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Dysregulated fear (age 2)</td>
<td>0.61</td>
<td>0.11</td>
<td>0.57</td>
<td>5.41$^{***}$</td>
<td>0.61</td>
<td>0.11</td>
</tr>
<tr>
<td>Maternal cortisol reactivity</td>
<td>-0.00</td>
<td>0.01</td>
<td>-0.05</td>
<td>-0.51</td>
<td>-0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Maternal delta-beta coupling</td>
<td>0.21</td>
<td>0.09</td>
<td>0.02</td>
<td>0.22</td>
<td>0.01</td>
<td>0.96</td>
</tr>
<tr>
<td>Paths C’, and B (DV = age 3 DF)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family income</td>
<td>0.04</td>
<td>0.02</td>
<td>0.22</td>
<td>2.09$^*$</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>Dysregulated fear (age 2)</td>
<td>0.55</td>
<td>0.14</td>
<td>0.51</td>
<td>3.89$^{***}$</td>
<td>0.54</td>
<td>0.14</td>
</tr>
<tr>
<td>Maternal cortisol reactivity</td>
<td>-0.00</td>
<td>0.01</td>
<td>-0.07</td>
<td>-0.65</td>
<td>-0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>Maternal delta-beta coupling</td>
<td>0.21</td>
<td>0.99</td>
<td>0.02</td>
<td>0.21</td>
<td>0.11</td>
<td>0.97</td>
</tr>
<tr>
<td>Maternal protective parenting</td>
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<td>0.49</td>
<td>0.10</td>
<td>0.79</td>
<td>0.39</td>
<td>0.49</td>
</tr>
</tbody>
</table>

*Note.* Income and age 2 dysregulated fear were included as covariates in paths C, C’, and B. No predictor variables of interest emerged as significant. Model information was as follows for frontal (A path: $R^2 = 0.03$, $F[2,58] = 0.96$, $p = 0.39$; C path: $R^2 = 0.40$, $F[4,56] = 9.31$, $p = .00$; C’ and B paths: $R^2 = 0.41$, $F[5,55] = 7.52$, $p = .000$), central (A path: $R^2 = 0.04$, $F[2,58] = 1.32$, $p = 0.28$; C path: $R^2 = 0.40$, $F[4,56] = 9.29$, $p = .00$; C’ and B paths: $R^2 = 0.41$, $F[5,55] = 7.51$, $p = .000$), and parietal (A path: $R^2 = 0.03$, $F[2,58] = 0.89$, $p = .42$; C path: $R^2 = 0.41$, $F[4,56] = 9.70$, $p = .00$; C’ and B paths: $R^2 = 0.42$, $F[5,55] = 7.87$, $p = .000$) electrode sites.  
*$p < 0.05$, ***$p < .001$. 

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Figure 1. Maternal protective parenting behavior did not mediate the relation between maternal cortisol reactivity and toddlers' dysregulated fear at age 3 above and beyond toddlers’ dysregulated fear at age 2.
Figure 2. Maternal protective parenting behavior did not mediate the relation between maternal frontal delta-beta coupling and toddlers’ dysregulated fear at age 3 above and beyond toddlers’ dysregulated fear at age 2.
Figure 3. Maternal protective parenting behavior did not mediate the relation between maternal central delta-beta coupling and toddlers’ dysregulated fear at age 3 above and beyond toddlers’ dysregulated fear at age 2.
Figure 4. Maternal protective parenting behavior did not mediate the relation between maternal parietal delta-beta coupling and toddlers’ dysregulated fear at age 3 above and beyond toddlers’ dysregulated fear at age 2.
Maternal protective parenting behavior did not mediate the relation between maternal cortisol reactivity and toddlers’ dysregulated fear at age 3 above and beyond maternal frontal delta-beta coupling and toddlers’ dysregulated fear at age 2.

**Figure 5.** Maternal protective parenting behavior did not mediate the relation between maternal cortisol reactivity and toddlers’ dysregulated fear at age 3 above and beyond maternal frontal delta-beta coupling and toddlers’ dysregulated fear at age 2.
Figure 6. Maternal protective parenting behavior did not mediate the relation between maternal cortisol reactivity and toddlers’ dysregulated fear at age 3 above and beyond maternal central delta-beta coupling and toddlers’ dysregulated fear at age 2.
Maternal protective parenting behavior did not mediate the relation between maternal cortisol reactivity and toddlers’ dysregulated fear at age 3 above and beyond maternal parietal delta-beta coupling and toddlers’ dysregulated fear at age 2.

**Figure 7.** Maternal protective parenting behavior did not mediate the relation between maternal cortisol reactivity and toddlers’ dysregulated fear at age 3 above and beyond maternal parietal delta-beta coupling and toddlers’ dysregulated fear at age 2.
Figure 8. Maternal protective parenting behavior did not mediate the relation between maternal frontal delta-beta coupling and toddlers’ dysregulated fear at age 3 above and beyond maternal cortisol reactivity and toddlers’ dysregulated fear at age 2.
Figure 9. Maternal protective parenting behavior did not mediate the relation between maternal central delta-beta coupling and toddlers’ dysregulated fear at age 3 above and beyond maternal cortisol reactivity and toddlers’ dysregulated fear at age 2.
Figure 10. Maternal protective parenting behavior did not mediate the relation between maternal parietal delta-beta coupling and toddlers’ dysregulated fear at age 3 above and beyond maternal cortisol reactivity and toddlers’ dysregulated fear at age 2.