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Associations between Infant Behaviors during the Face-To-Face Still-Face Paradigm and Oppositional Defiant and Callous-Unemotional Behaviors in Early Childhood

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Abstract Deficits in social orienting (i.e., gazing toward caregivers) during dyadic interactions and reactivity to stressful stimuli have been identified as behavioral correlates of oppositional defiant disorder (ODD) and callous-unemotional (CU) behaviors in older children. The goal of the current study was to investigate infants' mother-directed gaze and reactivity during the face-to-face and still-face episodes of the face-to-face stillface paradigm performed at 6 months in the prediction of ODD and CU behaviors in early childhood. Using data from the Durham Child Health and Development study (n = 206), hierarchical regression analyses revealed that infants' negative reactivity during the still-face episode and mother-directed gaze during the face-to-face episode predicted fewer ODD behaviors in early childhood. Examination of interaction effects suggested that mother-directed gaze attenuated the negative relation between reactivity and ODD and CU behaviors in early childhood. The current study is one of the first to extend downward the investigation of ODD and CU behaviors into infancy.

Keywords Oppositional defiant · Callous-unemotional · Infancy · Reactivity · Parenting

Both developmental and clinical fields of research have invested substantial resources in understanding the course, causes, and consequences of aggression and antisocial behavior. Much of this research in childhood and adolescence has focused on early conduct problems (CP) and callous-unemotional (CU) behaviors (Frick et al. 2014), largely because children exhibiting elevated CP and CU behaviors are at greater risk for later antisocial behavior and psychopathy (Lynam et al. 2007; Rowe et al. 2010). There is growing interest in identifying early behavioral correlates of CP and CU behaviors because of evidence suggesting that externalizing behavior problems may develop into a psychologically

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meaningful construct before age 12 months (Lorber et al. 2014; Van Zeijl et al. 2006) and childhood CP, a consistent predictor of adolescent and adult antisocial behavior, may start as early as age 3 (Shaw and Gross 2008). The current study uses longitudinal data to investigate the direct and interactive associations between infant behaviors during the face-to-face still-face paradigm (FFSFP; Tronick et al. 1978) and later oppositional defiant disorder (ODD) behaviors, a correlate of child CP developmentally appropriate for use in toddlerhood (Burke et al. 2014), and CU behaviors. Whereas ODD behaviors are typically characterized by defiant, disobedient, and uncooperative behaviors and are often accompanied by anger and irritability (Frick et al. 1993; Stringaris and Goodman 2009), CU behaviors describe non-normative emotional, affective, and cognitive deficits (e.g., lack of guilt, empathy, and fear), as well as an over-focus on reward and insensitivity to punishment (Frick and White 2008). Although there is slight variation in factor structure across samples and some debate about the relevancy of specific assessment items (e.g., Hyde et al. 2013), the measurement of CU behaviors has been refined at a younger ages, and there is a general consensus that CU behaviors are distinct from ODD (Frick et al. 2014) and are distinguishable in the first 3 years of life (Waller et al. 2015a; Waller et al. 2015b; Willoughby et al. 2014, 2011). Our ability to measure CU behaviors early allows for the downward extension of a growing body of research examining the behavioral correlates of ODD and CU behaviors in middle childhood (Frick et al. 2014) to infancy and toddlerhood (Willoughby et al. 2013; see Waller et al. 2015b for review). The current study is informed by work examining the early correlates of ODD and CU behaviors but also draws from research on childhood CP and conduct disorder when appropriate.

The caregiver–infant relationship plays an important role in the emergence of adaptive behavioral and emotional functioning and is supported by effective signaling and engagement on the part of the infant, which ideally serves to elicit a contingent, synchronous, and sensitive response on the part of the caregiver (Bowlby 1969; Sroufe 1996). These early interactions with caregivers contribute to the emergence and maturation of behavioral and neurocognitive systems that underlie later adjustment or maladjustment (Cicchetti and Dawson 2002), and have important significance given their influence on the child's behavioral development (Sroufe et al. 1999). Although the literature examining early contextual and temperamental correlates of later CU behaviors is growing (Bedford et al. 2014; Waller et al. 2015a; Waller et al. 2015a), little research has investigated the associations between infants' behaviors during caregiver–infant interactions and later ODD and CU behaviors. Research findings with samples of older children have demonstrated associations between behaviors which may undermine the caregiver–child relationship and externalizing problems and CU behaviors. Such behaviors include deficits in orienting towards caregivers (i.e., less eye contact and mutual orienting) as well as hyporeactivity in response to arousing stimuli (Dadds et al. 2011; Frick et al. 2003; Frick and White 2008; Loney et al. 2006; Willoughby et al. 2011). The current study investigated the extent to which infant behaviors, specifically mother-directed gaze and negative reactivity, during different contexts of mother–infant interactions predict later ODD and CU behaviors in a prospective longitudinal study of infants and their families.

Parent–Child Interactions

There is a rather large research literature examining associations between early familial experiences and the development and persistence of externalizing behavior problems (Deater-Deckard and Dodge 1997; Loeber and Hay 1997), and a growing literature on early experiences and the development of CU behaviors (see Waller et al. 2013 for review). Both harsh (Barker et al. 2011; Willoughby et al. 2014) and insensitive (Pardini et al. 2007; Wagner et al. 2015a) parenting qualities are associated with the development of externalizing and CU behaviors. For example, Willoughby and colleagues found that harsh-intrusive parenting behaviors in infancy predicted later oppositional defiant and CU behaviors (Willoughby et al. 2013). Additionally, research shows that parental hostility in the 2nd and 3rd years of life are predictive of later CP (Campbell et al. 1996) and rejecting parenting at ages 1.5 and 2 years is predictive of persistently high levels of CP from ages 2 to 8 years (Shaw et al. 2003). Additionally, recent work by Waller, Hyde, and colleagues shows that parental harshness during the preschool period was related to increases in CU behaviors from ages 2 to 4 (Waller et al. 2012), that parental warmth was reciprocally related to CU behaviors from ages 2 to 3 (Waller et al. 2014a), and that lower levels of observed positive reinforcement were associated with CU behaviors at 27 months in an adoption design where parents were not genetically related to their children (Waller et al. 2015a).

In addition to the influence of harsh and insensitive parenting influences on the child, work with older children exhibiting concurrent behavioral problems and CU behaviors make less eye contact with caregivers during emotionally charged discussions (Dadds et al. 2012) and during normal free play experiences (Dadds et al. 2011). In a sample of 92 males (mean age = 8.9 years), youth high on both conduct problems and CU behaviors showed a consistent lack of eye contact towards their parents during a free play task and an emotion talk scenario (Dadds et al. 2011). Similarly, using a mixed-sex sample of youth, Dadds et al. (2012) found similar phenotypic behavior in caregiver-directed eye contact for youth with ODD and high CU behaviors during an

emotionally-charged discussion (mean age = 5.9 years). A number of studies support the associations between behavior problems and CU behaviors and a failure to attend to core emotional features (i.e., the eye region) when interacting with attachment figures (Dadds et al. 2011, 2012) and when freely viewing emotional faces (Dadds et al. 2008). Importantly, recent work with infants demonstrated that reduced face preference at 5 weeks is predictive of higher CU behaviors at 2.5 years, suggesting that these associations may be present in very early life (Bedford et al. 2014).

This growing body of research may have implications for the development of ODD and CU behaviors given that the early parent–infant relationship plays a vital role in the development of the children's emotional and behavioral regulation. The influences and organization of this relationship are often discussed in terms of synchronization between the caregiver and the infant. Mutual gaze, or joint visual attention (Butterworth 1991), between the caregiver and the infant plays a key role in the emergence of autonomous regulation and the development of healthy attachment relationships (Waters et al. 1979; Sroufe 1996). Infants' propensity to contribute to this early dyadic relationship by directing gaze towards the mother may be fundamental to understanding the development of ODD and CU behaviors, especially given that these behaviors are associated with insecure attachments to caregivers (Pasalich et al. 2012). Further, research suggests that mutually responsive orienting between caregivers and their children, deficits in which may be particularly pronounced for youth high on CU behaviors, has implications for children's conscience development (e.g., Kochanska 1997) and the development of moral emotion and empathy (Kochanska et al. 2005). Since poor empathic functioning is an integral dimension of CU behaviors, and deficits in caregiver-directed gaze have been associated with ODD and CU behaviors in older children, the role of gaze to the caregiver during early interactions may be one of several infant predictors of subsequent ODD and CU behaviors. The first goal of the current study was to investigate the extent to which infants' mother-directed gaze during a typical mother-child interaction predicts later ODD and CU behaviors above and beyond the influences of sensitive parenting and harsh-intrusive parenting behaviors.

Infant Negative Reactivity

Studies of affective correlates of CU behaviors consistently identify negative correlations between behavioral reactivity and CU symptomatology in older children (c.f., Blair et al. 2005; Newman and Lorenz 2003; Patterson and Newman 1993); similar to how hyporeactivity to stress-inducing stimuli has been linked with adult antisocial behavior and psychopathy (Hare et al. 1991; Lykken 1995). For example, lower baseline levels and blunted reactivity of other physiological systems including heart rate, skin conductance, and parasympathetic nervous system functioning have also been shown to be associated with externalizing problems and CU behaviors (Raine 2002; Dietrich et al. 2007) suggesting that children and adolescents high on CU behaviors may exhibit distinct physiological and behavioral phenotypes characterized by underreactivity. Importantly, research on developmental precursors of adult antisocial behavior and psychopathy linking hyporeactivity to CU behaviors has largely focused on later childhood (Frick et al. 2003) and adolescence (Loney et al. 2006), very few have extended this model downward into the infancy and toddlerhood years (see Mills-Koonce et al. 2015; Waller et al. 2015a; Wagner et al. 2015b; Willoughby et al. 2011).

Although difficult and reactive temperamental qualities are often linked with eventual behavior problems (e.g., Bradley and Corwyn 2008; Vitaro et al. 2006), there is evidence that early fearlessness or under-reactivity are associated with later externalizing (Colder et al. 2002; Gilliom and Shaw 2004; Raine et al. 1998) and CU behaviors (Frick and White 2008; Frick et al. 2014), and that these links may partially depend on parents' responses to the infants' behaviors (Park et al. 1997; Waller et al. 2014a). Raine and colleagues demonstrated links between fearlessness at age 3 years and later aggressive behaviors (Raine et al. 1998), and low levels of fearfulness coded during an auditory fear task at age 2 are predictive of high levels of externalizing behaviors from ages 2 to 6 years (Gilliom and Shaw 2004). Additionally, fearless or uninhibited temperamental profiles are associated with a lack of crying in laboratory situations in which reactivity would be expected (Kagan 1994), and Waller and colleagues (Waller et al. 2014a) provide evidence from observational research suggesting that the emergence of affective deficits associated with CU behaviors may undermine contingent and reciprocal mother-child responses in infancy and toddlerhood (Waller et al. 2014a).

Developmentalists have stressed the importance of infants' behavioral signaling (e.g. negative affect, orienting) as a means of communicating emotional needs to the parent which, when responded to appropriately, support adaptive social development (Kochanska 1997; Maccoby 1992; Tronick 1989). The Mutual Regulation Model (MRM; Tronick 1989) describes early mother–infant dyadic interactions as being jointly regulated toward reciprocity through a system of infant-directed behavioral and affective feedback. When the behaviors of each member of the dyad are reciprocated, these socio-emotional processes help to generate adaptive mutual states of regulation that support healthy development (Tronick et al. 1998). Because infants' capacities for emotional regulation depend partly on an ability to engage appropriately with caregivers (Waters and Sroufe 1983), it is reasonable to suggest that disruptions in this system, possibly fueled

by infant hyporeactivity during stressful caregiver–infant interactions when reactivity would be expected, may undermine healthy social development and be particularly informative for the etiology of ODD and CU behaviors. As such, another goal of the current study was to investigate the extent to which behavioral hyporeactivity during a stressful mother–infant interaction predicts later ODD and CU behaviors, respectively, above and beyond the influences of sensitive parenting and harsh-intrusive parenting behaviors.

Measuring Social Orienting to the Parent and Negative Reactivity in Infancy

Tronick et al. (1978) developed the Face-to-Face Still-Face Paradigm (FFSFP; Tronick et al. 1978) to demonstrate (1) the importance of mother–infant connectedness and mutual regulation in infancy and (2) what happens when social connectedness and mutual regulation are disrupted (Tronick et al. 1978, 1998). The FFSFP consists of three episodes during which the parent first is asked to engage in typical face-to-face play, then to stop responding to the infant and maintain a neutral facial expression [still-face episode] and, finally, to resume playing with the infant [reunion episode] (see Adamson and Frick 2003 for a review of the FFSFP in infancy research). As such, the FFSFP is well-suited for assessing infants' social orientation towards the caregiver and reactivity to aversive interactions (Weinberg and Tronick 1996), and individual differences in infant behaviors during the FFSFP have been associated with both relational development (Cohn et al. 1991; Ekas et al. 2013; Mesman et al. 2009) and later behavior problems in children (Ekas et al. 2013; Moore et al. 2001).

Beyond the main effects of observed infant reactivity and mother-directed gaze, the current study poses the question of whether the qualities of the mother–child interaction during the FFSFP may interactively predict later CU behaviors. This question is motivated by recent research highlighting the importance of mother-directed gaze as a correlate of CU behaviors in children (i.e., Dadds et al. 2011) and work suggesting that variability in social orienting in the first weeks of life may be associated with later CU behaviors (Bedford et al. 2014). The current study considered the extent to which mother-directed gaze during the face-to-face episode buffers any relation between infant reactivity and later and CU behaviors. Such a prediction is consistent with work by Ekas et al. (2013) linking infant mother-directed gaze during the FFSFP to later attachment security (see also Cohn et al. 1991), and evidence suggesting that early secure relationships with caregivers may buffer against pathways to antisocial conduct (Kochanska et al. 2009). Given evidence that reactivity and social orienting may represent both independent and interactive pathways to CU behaviors, empirically testing both possibilities may offer new insight into the heterotypic continuity of elevated CU behaviors over time.

Current Study

Data from a prospective longitudinal study were used to examine the relation between child behavior in the FFSFP at 6 months and later ODD and CU behaviors in early childhood. Given the importance of caregiver-directed visual attention for the emergence of autonomous regulation and the development of healthy attachment relationships (Waters et al. 1979; Sroufe 1996), it was hypothesized that infants' mother-directed gaze during the face-to-face episode would be negatively associated with both ODD and CU behaviors. Second, it was hypothesized that infants' negative reactivity during the still-face episode would be negatively correlated with later ODD and later CU behaviors. Third, it was hypothesized that infants' mother-directed gaze during the face-to-face episode would attenuate the associations between infants' reactivity during the still-face episode and later CU behaviors. Analyses presented in the current study controlled for demographic variables (i.e., child's gender, race, temperament, and family income), as well as mother's behavior during the FFSFP in an attempt to isolate the relation between infants' behavioral and affective responses to the FFSFP and later ODD and CU behaviors. Additionally, observed measures of sensitive parenting and harsh-intrusive parenting during a separate free play task were included in each analytic model to control for the broader relationship quality between child and mother.

The current study is informed by work from research groups which have derived and implemented "homegrown" measures of CU behaviors drawn from commonly assessed behavioral questionnaires (e.g., Song et al. 2015; Waller et al. 2015c; Waller et al. 2015a; Willoughby et al. 2011; Willoughby et al. 2014). Despite slight differences in factor structure across "homegrown" measurement approaches resulting in different labels (e.g., deceitful-callous vs. callous-unemotional; Hyde et al. 2013; Waller et al. 2012, Waller et al. 2014a, b), there is now considerable evidence to suggest that CU behaviors can be validly and reliably assessed in early childhood using common behavioral measurement approaches such as the Achenbach System of Empirically Based Assessment, Preschool Forms (ASEBA; Achenbach and Rescorla 2000).

Methods

Participants

The current study used participants from the Durham Child Health and Development Study (DCHDS), a prospective

longitudinal study of 206 full-term infants and their families who were recruited when their children were 3 months old. The sample was 57 % African American and 43 % European American, and approximately 53 % of families were low income (below 200 % of the poverty level). Of the 206 children recruited into the DCHDS, 185 (89.8 %) had data for ODD and CU behaviors at one of the three time points of interest (24, 30, or 36 months). There was no evidence that children with ($N = 185$) and without ($N = 21$) outcome data varied as a function of sex (51 % male vs. 57 % male; $p = 0.58$), race (56 % African American vs. 62 % African American; $p = 0.62$), or total family income at 6 months ($p = 0.79$). Furthermore, there was no evidence that missingness was related to sensitive parenting at 6 months ($p = 0.12$), harsh-intrusive parenting at 6 months ($p = 0.26$), or mother (infant directed gaze during face-to-face, $p = 0.27$) and infant (mother directed gaze during the face-to-face, $p = 0.38$; reactivity during still-face, $p = 0.53$) behaviors during the FFSFP.

Procedure

The current study used observational and questionnaire data that were collected at visits completed when the children were 6, 24, 30, and 36 months old. Information on children's sex and race was collected upon entry into the study. All ratings and observations occurred in a laboratory setting, except for the observation of parent–child interactions during free play (coded for maternal sensitivity and harsh-intrusiveness), which were conducted at the participants' homes. At each visit, infants and their mothers participated in a number of joint and individual activities and mothers completed a standardized interview and demographic questionnaires. Transportation was provided to families who required assistance getting to and from the laboratory. Families were compensated $50 for their participation at each time point.

The infants' were observed in the FFSFP (Adamson and Frick 2003; Tronick et al. 1978) during the 6 month lab visit to assess infants' behaviors, specifically mother-directed gaze and negative reactivity. Mothers placed infants in an infant chair on a table and situated themselves in a chair that was placed directly in front of the infants' chair. Mothers were given a set of standardized instructions for each episode of the FFSFP (i.e., face-to-face, still-face, reunion). As the behaviors in which we are interested, mother-directed gaze and negative reactivity, are demonstrated most often during the face-to-face and still-face episodes (Ekas et al. 2013; Mesman et al. 2009), the reunion phase of the FFSFP is of less interest to the current study and was not included in subsequent analyses. During the face-to-face episode, mothers were instructed to play with their babies as they normally would for 2 min. Then, mothers were told to turn away from their infant for 15 s, and then to turn back toward their infants for the still-face episode. Mothers were to look at their infant for 2 min without providing any verbal or facial response to the infant (i.e., maintaining a still face). The FFSFP was stopped if the infant was unable to be soothed at any point during the procedure. The episodes were video recorded using a split-screen procedure to ensure that the behaviors of both mothers and infants could be observed during the entire interaction.

Measures

Coding Infants' and Mothers' Behavior during FFSFP As previously described by Moore et al. (2009), infants' and mothers' affect and gaze during the FFSFP episodes were coded by trained coders. In separate viewings of the videotapes, different research assistants coded infants' and mothers' facial affect and direction of gaze in 1-s intervals. Affect was coded as positive, neutral, or negative, and gaze was coded as toward or away from the partner. Coders were initially trained to reliability using pre-existing video recorded FFSFP interactions. Inter-observer agreement was determined by randomly selecting 15 % of the interactions to be coded by a second coder. The coders were considered to be in agreement if they coded the same behavior within one second of each other. Reliability was calculated using kappa to correct for chance agreement. Overall, coders reliably identified mother affect (K = 0.83), infant affect (K = 0.89), infants' direction of gaze (K = 0.90), and mothers' direction of gaze (K = 0.85). Mother affect is not used in the current analyses because observed measures of maternal parenting and mothers' direction of gaze were included as covariates. Affect and gaze scores used in the current analyses were computed as proportions of the total valid interaction time.

ODD and CU Behaviors Measures of ODD and CU behaviors were derived from the Achenbach System of Empirically Based Assessment, Preschool Forms (ASEBA; Achenbach and Rescorla 2000) which was completed by primary caregivers at the 24-, 30-, and 36-month visits. This standardized assessment indexes children's behavioral and emotional problems using caregivers' ratings of their child's behavior currently or within the last 2 months (Achenbach and Rescorla 2000). The ASEBA includes a scoring profile drawn from DSM-referenced scales for ODD comprised of six items including ("defiant", "disobedient", "angry moods", "stubborn", "temper tantrum", and "uncooperative"). Further, drawing from the sample used in the current study, Willoughby et al. (2011) demonstrated that five items drawn from the ASEBA ("no guilt after misbehave", "punish does not change behavior", "unresponsive to affection", "shows little affection", and "too little fear") could be used to measure individual differences in CU behaviors at these early ages. The approach to measuring CU behaviors used in the current study has been validated in multiple longitudinal samples (e.g.,

Waller et al. 2015a, b, c; Waller et al. 2015a; Willoughby et al. 2014, 2013).

Measures of ODD behaviors at 24, 30, and 36 months were significantly correlated between 0.48 to 0.62. Similarly, measures of CU behaviors at 24, 30, and 36 months were significantly correlated between 0.41 to 0.51. Unconditional latent curve models (LCM; Meredith and Tisak 1990) were estimated to examine the possibility of individual variability in ODD and CU behaviors the three assessment periods. Results indicated there is not significant individual variability in the rates of change of ODD or CU behaviors over the three time points used in the study ($\eta = 0.369$, $p = 0.21$ and $\eta = 0.162$, $p = 0.22$, respectively). Further, there was a non-significant covariance between the intercept and the linear slope components of growth for both ODD and CU behaviors ($\phi = 0.75$, $p = 0.83$ and $\phi = -0.23$, $p = 0.87$, respectively) indicating that mean levels of behavior at 24 months are not related to variability in the rate of change. Given the strong intra-correlation between time points for ODD and for CU behaviors, respectively, and the results from LCM analyses, separate unweighted mean scores from the ASEBA at 24, 30, and 36 months were used to represent the two outcomes of interest. The ODD behavior measure items demonstrated adequate internal consistency at 24 months ($\alpha = 0.74$), 30 months ($\alpha = 0.77$), and 36 months ($\alpha = 0.83$). Although internal consistencies for the CU behavior measure items were modest at 24 months ($\alpha = 0.53$), 30 months ($\alpha = 0.60$), and 36 months ($\alpha = 0.65$), they were comparable to those reported by other studies using the ASEBA at these ages (e.g., Song et al. 2015; Waller et al. 2012, Waller et al. 2014a) and with other measures of CU behaviors in older samples of children and adolescents (e.g., Hipwell et al. 2007). Centered scores for ODD and CU behaviors in early childhood were used to support interpretability of regression coefficients and interaction plots.

Observed Parent–Infant Interactions Mothers and their infant were observed during a free play task as part of the home visit completed when the infants were 6 months of age. A set of standard toys were arranged on a blanket and the mothers were asked to play with their infants as they normally would on a typical day. The mother–child free play task was structured to last 10 min. All interactions were videotaped and later viewed by trained and reliable coders who rated the interactions using 5-point subscales to measure parental sensitivity, intrusiveness, detachment, stimulation of development, positive regard, negative regard, and animation (measures adapted from the NICHD ECCRN 1999). Previous factor analysis supported the creation of two composite measures of maternal parenting at 6 months. The first composite was harsh and intrusive parenting, what we refer to as harsh parenting, and included measures of intrusiveness and negative regard. The second composite was sensitive parenting and included measures of sensitivity, detachment (reverse scored), stimulation of development, positive regard, and animation. Each coding team consisted of four to five coders and included one or two master coders. Each coder was trained to be reliable with the master coder(s). Reliability was calculated using the intraclass correlation for the independent ratings made for the overlapping coding assignments. Reliability across subscales and composites was high (intraclass correlations >0.80 for all subscales).

Additional Covariates Child's sex and race were collected at the time of recruitment. Family income was collected at the 6 month home visit. Infant's distress to limitations was included as a measure of temperament and was collected at the 6 month home visit using the Infant Behavior Questionnaire – Revised (IBQ-R; Rothbart 1981). Sex, race, income, and temperament were included in the first covariate model and in all subsequent OLS regression models.

Analytic Strategy

The primary analytic approach involved estimating a series of ordinary least squares multiple regression models in which ODD and CU behaviors were separately regressed on infant mother-directed gaze during the face-to-face and infant negative reactivity during the still-face episodes of the FFSFP. Child gender, race, temperament, and total family income at 6 months were included as covariates in all OLS regression models. Measures of observed sensitive and harsh parenting at 6 months and mothers' infant-directed gaze during the face-to-face episode of the FFSFP were also included as covariates. In order to test the hypothesis that the relation between infant reactivity and later ODD and CU behaviors is moderated by mother-directed gaze, the final model tested the interaction between infant gaze during the face-to-face episode and infant negative reactivity during the still-face in the prediction of ODD and CU behaviors in early childhood. Each set of OLS regression models was completed for ODD and CU behaviors, separately. Significant interactions were probed using the online utility and computational tools for probing two-way interaction effects in multiple linear regressions (Preacher et al. 2006).

Results

Descriptive Analyses

Table 1 presents the bivariate correlations, means, and standard deviations for the model covariates and variables of interest. Observed measures of sensitive and harsh parenting were significantly correlated with race and income. ODD and CU behaviors were both positively correlated with sex indicating higher scores for boys. CU behaviors in early

Table 1 Zero-order bivariate correlations between model outcomes

	1	2	3	4	5	6	7	8	9	10	11
1. Sex (0 = female)	–										
2.Race (0 = white)	−0.08	–									
3. Household Income (6 m)	0.08	−0.21**	–								
4. Distress to Limitations (6 m)	−0.03	0.23**	−0.23**	–							
5. Sensitive parenting (6 m)	0.05	−0.30**	0.32**	−0.24**	–						
6. Harsh parenting (6 m)	−0.05	0.31**	−0.32**	0.09	−0.37**	–					
7. Mother's Child-Directed Gaze (FF)	0.23**	0.01	0.06	−0.3	0.13	0.05	–				
8. Childs' Mother-Directed Gaze (FF)	−0.05	0.21**	−0.19*	0.07	−0.02	0.17*	0.06	–			
9. Child's Negative Affect (SF)	0.11	0.02	−0.18*	0.08	−0.05	−0.01	−0.06	0.09	–		
10. ODD (24, 20, 36 month mean)	0.17*	−0.09	−0.00	0.12	−0.09	0.13	0.06	−0.16*	−0.17*	–	
11. CU (24, 20, 36 month mean)	0.16*	−0.04	−0.13	0.09	−0.19*	0.21**	0.12	−0.02	−0.10	0.69**	–
Number	206	206	170	179	175	175	151	165	159	184	184
Mean	0.51	0.57	49,405	3.55	3.2	2.5	0.90	0.40	0.19	2.8	1.2
Standard Deviation	0.50	0.49	40,670	0.87	0.80	0.91	0.08	0.22	0.27	1.9	1.1

$p \leq 0.05$*, $p \leq 0.01$**; *FF* face-to-face episode, *SF* still-face episode

childhood were associated with more harsh and less positive parenting at 6 months. ODD behaviors in early childhood were associated with less infants' mother-directed gaze during the face-to-face episode and less negative reactivity during the still-face episode. ODD and CU behaviors in early childhood were strongly positively correlated with each other, which is consistent with previous work at these ages (e.g., Waller et al. 2014b, 2015c; Willoughby et al. 2011; Willoughby et al. 2013).

Hierarchical OLS Regression Models

We estimated four OLS regressions to test the associations between infants' mother-directed gaze during the face-to-face episode and infants' negative reactivity during the still-face episode and ODD and CU behaviors in early childhood. The first set of models separately regressed the centered means of ODD and CU behaviors from 24, 30, and 36 months on the mother-directed gaze and negative reactivity controlling for sex, race, income and temperament, sensitive parenting, harsh parenting, and mothers' child-directed gaze during face-to-face. The second set of models also separately regressed ODD and CU behaviors from 24, 30, and 36 months on the mother-directed gaze and negative reactivity controlling for sex, race, income and temperament, sensitive parenting, harsh parenting, and mothers' child-directed gaze during face-to-face, but included an interaction term to test the hypothesis that infants' mother-directed gaze during the face-to-face moderates the hypothesized association between negative reactivity during the still-face and ODD and CU behaviors in early childhood. A final step involved exploratory follow-up analyses which involved rerunning the four OLS regressions controlling for the shared variance of ODD or CU behaviors. Unstandardized coefficients, standardized coefficients, confidence intervals, and R^2 statistics for each of the OLS regression models can be found in Table 2.

ODD Behaviors Infant behaviors during the face-to-face and still-face episodes of the FFSFP, measures of harsh and sensitive parenting, and other covariates explained a significant amount of variance for Model 1 of ODD behaviors, $R^2 = 0.20$, $F(9, 108) = 3.05$, $p < 0.01$. Harsh parenting at 6 months was positively associated with ODD behavior scores in early childhood, $B = 0.47$, $\beta = 0.19$, $p \leq 0.05$. Further, infants' negative reactivity, $B = -1.8$, $\beta = -0.22$, $p < 0.05$, during the still-face and mother-directed gaze, $B = -1.82$, $\beta = -0.19$, $p < 0.05$, during the face-to-face episode were negatively associated with ODD behaviors in early childhood. Exploratory follow-up analyses indicated that infants' negative reactivity, $B = -1.1$, $\beta = -0.14$, $p < 0.05$, during the still-face remained significant and infants' mother-directed gaze, $B = -1.0$, $\beta = -0.12$, $p = 0.07$, during the face-to-face episode approached significance in the prediction of ODD behaviors even after controlling for concurrent CU behaviors.

For model 2, the interaction between infants' mother-directed gaze during the face-to-face episode and infants' negative affect during the still-face episode significantly predicted ODD behaviors, $B = 5.85$, $\beta = 0.20$, $p < 0.05$. This interaction was probed at one standard deviation above and below the mean for infants' mother-directed gaze during the face-to-face episode (Fig. 1). The association between reduced reactivity to the still-face episode and later ODD behaviors was significant for children at (mean simple slope = −2.36 [SE = 0.76], $t = -3.09$, $p < 0.01$) or below (−1 SD simple slope = −3.64 [SE = 1.10], $t = -3.34$, $p < 0.01$) the mean for mother-directed gaze during the face-to-face episode. This association was not significant for children with high levels

Table 2 Hierarchical regression models predicting mean ODD and CU behaviors from 24 to 36 months

	ODD Behaviors				CU Behaviors			
	Model 1		Model 2		Model 1		Model 2	
Parameter	β (B)	95 % CI	β (B)	95 % CI	β (B)	95 % CI	β (B)	95 % CI
Sex (0 = female)	0.21 (0.89)*	0.14 to 1.64	0.20 (0.81)*	0.08 to 1.55	0.12 (0.28)	−0.17 to 0.74	0.10 (0.24)	−0.21 to 0.68
Race (0 = white)	−0.12 (−0.49)	−1.29 to 0.29	−0.10 (−0.42)	−1.21 to 0.36	−0.12 (−0.29)	−0.77 to 0.19	−0.10 (−0.25)	−0.72 to 0.22
Household Income (6 m)	0.04 (0.001)	−0.01 to 0.02	0.05 (0.001)	−0.01 to 0.03	−0.11 (−0.001)	−0.02 to 0.02	−0.11 (−0.001)	−0.00 to 0.02
Distress to Limitations (6 m)	0.14 (0.34)	−0.09 to 0.78	0.13 (0.32)	−0.11 to 0.75	−0.02 (−0.03)	−0.29 to 0.24	−0.03 (−0.05)	−0.31 to 0.21
Sensitive parenting (6 m)	−0.02 (−0.07)	−0.61 to 0.46	−0.06 (−0.16)	−0.71 to 0.37	−0.15 (−0.23)	−0.55 to 0.10	−0.19 (−0.29)^	−0.61 to 0.03
Harsh parenting (6 m)	0.19 (0.47)*	0.00 to 0.94	0.18 (0.44)^	−0.02 to 0.90	0.25 (0.34)*	0.06 to 0.62	0.23 (0.32)*	0.04 to 0.60
Mother's Child-Directed Gaze (FF)	0.03 (0.76)	−3.57 to 5.10	0.03 (0.75)	−3.51 to 5.02	0.10 (1.35)	−1.26 to 3.97	0.10 (1.35)	−1.21 to 3.92
Child's Mother-Directed Gaze (FF)	−0.19 (−1.82)*	−3.51 to −0.14	−0.21 (−1.94)*	−3.61 to −0.28	−0.11 (−0.63)	−1.65 to 0.39	−0.13 (−0.71)	−1.71 to 0.29
Child's Negative Affect (SF)	−0.22 (−1.86)*	−3.32 to −0.39	−0.29 (−2.35)*	−3.87 to −0.85	−0.13 (−0.61)	−1.49 to 0.27	−0.19 (−0.93)*	−1.84 to −0.15
Negative Reactivity (SF) *Gaze (FF)	–	–	0.20 (5.85)*	0.52 to 11.18	–	–	0.21 (3.72)*	0.50 to 6.93
Total R^2 (Adjusted R^2)	0.20* (0.14)*		0.24* (0.17)*		0.16* (0.09)*		0.20* (0.13)*	

^$p \leq 0.10$, *$p \leq 0.05$; cont. predictors centered; *FF* face-to-face, *SF* still-face

of mother-directed gaze during the face-to-face episode (+1 SD simple slope = −1.07 [SE = 0.81], $t = 1.31$, $p = 0.19$). The lower bound of the region of significance (RoS) was 0.15 indicating that the interactive association between reduced reactivity to the still face episode and mother-directed gaze during the face-to-face episode in the prediction of ODD behaviors was significant for infants who exhibited levels of mother-directed gaze at or below the sample mean of zero (mean-centered). The upper bound of the region of significance (upper bound RoS = 3.79) was outside of the observed data. These findings indicate that the risk for later ODD behaviors as a function of not displaying negative reactivity to the mother's still-face is attenuated by above average levels of mother-directed gaze in infancy. Exploratory follow-up analyses indicated that the interaction between infants' mother-directed gaze during the face-to-face episode and expressed negative affect during the still-face episode no longer predicted ODD behaviors after controlling for concurrent CU behaviors.

CU Behaviors Infant behaviors during the face-to-face and still-face episodes of the FFSFP, measures of harsh and sensitive parenting, and other covariates explained a significant amount of variance for Model 1of CU behaviors in early childhood, $R^2 = 0.16$, $F(9, 107) = 2.24$, $p < 0.05$. Harsh parenting at 6 months was positively associated with CU behaviors in early childhood, $B = 0.34$, $\beta = 0.25$, $p < 0.05$. The main effects of infants' negative reactivity during the still-face and mother-directed gaze during the face-to-face episode were not significant in the prediction of CU behaviors. Exploratory follow-up analyses indicated that the findings were unchanged after controlling for concurrent ODD.

For Model 2, the interaction between infants' mother-directed gaze during the face-to-face episode and infants' expressed negative affect during the still-face episode significantly predicted CU behaviors, $B = 3.72$, $\beta = 0.21$, $p < 0.05$. This interaction was probed at 1 standard deviation above and below the mean for infants' mother-directed gaze during the face-to-face episode (Fig. 2). The association between reduced reactivity to the still-face episode and later CU behaviors was significant for children at (mean simple slope = −0.93 [SE = 0.46], $t = -2.02$, $p < 0.05$) or below (−1 SD simple slope = −1.74 [SE = 0.66], $t = -2.64$, $p < 0.01$) the mean for mother-directed gaze during the face-to-face episode. This association was not significant for children with high levels of mother-directed gaze during the face-to-face episode (+1 SD simple slope = −0.12 [SE = 0.49], $t = -0.22$, $p = 0.82$). The lower bound of the region of significance was 0.006 indicating that the interactive association between reduced reactivity to the still face episode and mother-directed gaze during the face-to-face episode in the prediction of CU behaviors was significant for infants who exhibited levels of mother-directed gaze at or below the sample mean of zero (mean-centered). The

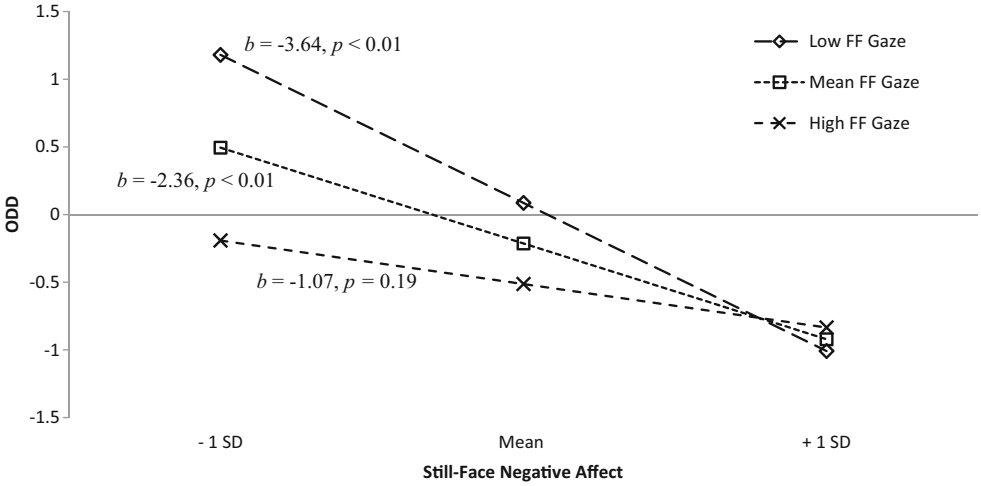

Fig. 1 The relation between negative reactivity during the still-face episode and ODD behaviors in early childhood is plotted as a function of mother-directed gaze during the face-to-face episode. The slope representing the relation between reactivity and ODD behaviors is not significantly different from zero for participants high on face-to-face gaze

upper bound of the region of significance (upper bound RoS = 1.46) was outside of the observed data. These findings indicate that the risk for later CU behaviors as a function of not displaying negative reactivity to the mother's still-face is attenuated by high levels of mother-directed gaze in infancy. Exploratory follow-up analyses indicated that the interaction between infants' mother-directed gaze during the face-to-face episode and expressed negative affect during the still-face episode no longer predicted CU behaviors after controlling for concurrent ODD behaviors.

Discussion

Empirical investigations of the etiology of behavior problems in early childhood have been the focus of substantial developmental and clinical research, given the evidence for their relative stability (Hawes and Dadds 2007), their associations with disruptions in other domains of functioning like social competence (Campbell 2002), and their predictive relation with other, more serious antisocial behaviors later in development (Lynam et al. 2007; Rowe et al. 2010). Consistent with existing research on the topic, the initial regression models indicated that mothers' harsh parenting at 6 months is associated with children's later ODD and CU behaviors (Waller et al. 2012; Willoughby et al. 2011). This association was stronger for CU behaviors than it was for ODD behaviors. Next, consistent with study hypotheses, hierarchical regression analyses revealed that infants' mother-directed gaze during the face-to-face episode predicted fewer ODD behavior scores in early childhood. Additionally, the current analyses suggest that infants' negative reactivity during the still-face episode predicts fewer ODD behaviors in early childhood. Finally, examination of interaction effects showed that mother-directed gaze during the face-to-face episode moderates the relation between negative reactivity during the still-face episode and ODD and CU behaviors in early childhood.

The still-face episode of the FFSFP (Tronick et al. 1978), during which mothers are instructed to become verbally and emotionally unresponsive, typically elicits negative reactivity from the infant. Our findings indicate that, in the context of this relational stressor, reduced reactivity relative to other infants at 6 months was associated with higher levels of ODD behaviors in early childhood, controlling for observed

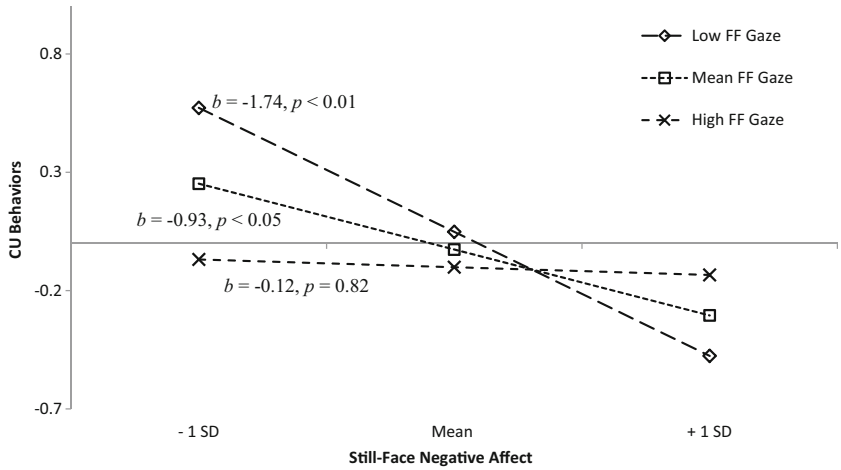

Fig. 2 The relation between negative reactivity during the still-face episode and CU in early childhood is plotted as a function of mother-directed gaze during the face-to-face episode. The simple slopes for low face-to-face gaze and mean face-to-face gaze are significantly different from zero. The slope for high face-to-face gaze is not significantly different from zero. Mother-directed gaze during the face-to-face episode seems to attenuate the negative association between reactivity and CU

parenting behaviors outside of the FFSFP and mother behavior during the FFSFP. Although oppositional and reactive behaviors (i.e., ODD) are often associated with elevated reactivity and negative or difficult temperamental characteristics (Scarpa et al. 2010; Vitaro et al. 2006), infant reactivity in the context of threat or discomfort represents normative signals to the caregiver which are meant to prompt responsiveness, proximity, and promote the infant's capacity for arousal modulation (Sroufe 1996). As such, an infant's emotional and behavioral development is partially influenced by the extent to which the infant signals for support in the face of disruptions of synchronous caregiver–infant interactions. Appropriate signaling on the part of the infant provides a foundation for the development of regulatory capacities and healthy relationships (Tronick 1989).

The current findings are consistent with research that demonstrates associations between infant behaviors during the FFSFP and later externalizing problems using a community sample (Moore et al. 2001) and with clinical research on older children showing that behavioral problems are associated with underarousal to presumed stressful or aversive stimuli (Frick and Morris 2004; Stadler et al. 2011). These findings also align with those of Barker et al. (2011), in which they found that maternal-rated fearless temperament at age 2 predicts conduct problems in early adolescence, controlling for other familial and contextual risks (Barker et al. 2011). The results of the current study do not preclude the possibility of links between reactive and difficult temperamental profiles but rather, suggest that a lack of infant reactivity, or signaling, in response to a dyadic experience where reactivity would be expected may signify risk for later ODD behaviors.

Findings also indicate that less mother-directed gaze during the face-to-face episode of the FFSFP at 6 months is associated with more ODD behaviors in later childhood, but we did not find this main effect for CU behaviors. Additionally, and contrary to prediction, we did not find a significant main effect of negative reactivity during the still-face episode in the prediction of later CU behaviors. However, moderation analyses indicated that high levels of mother-directed gaze diminished the observed relation between reduced reactivity to mothers' unresponsiveness during the still-face episode and later ODD and CU behaviors. This is partially consistent with extant findings that older children and adolescents high on CU behaviors exhibit deficits in the extent to which they make eye contact with caregivers during dyadic interactions (see Frick et al. 2014 for review) and that these deficits contribute to maladaptive social functioning (Dadds et al. 2011).

A possible explanation of the current findings comes from classic attachment theory (Bowlby 1969), and identifies the importance of infants' affective displays and visual orientation as primary mechanisms of communication with caregivers. When viewed through this lens, infants' mother-directed gaze during the face-to-face episode of the FFSFP may promote affiliation and continued reciprocity which, when responded to appropriately, influence sensitive parenting and the formation of secure attachment relationships (Ainsworth et al. 1978; Bowlby 1969). Interestingly, children and adolescents exhibiting high levels of behavior problems and CU behaviors commonly display low levels of eye contact with attachment figures (Dadds et al. 2014, 2011, 2012) and are more likely to form insecure attachments than youth with normative levels of behavior problems and CU behaviors (Fearon et al. 2010; Pasalich et al. 2012). Work by Kochanska and colleagues has shown that early attachment security may enhance the positive outcomes associated with adaptive parenting (Kochanska et al. 2004) and buffer against maladaptive trajectories of antisocial behaviors (Kochanska et al. 2009). Thus, the current findings could suggest that high levels of infants' mother-directed gaze indicate the foundations of a secure attachment relationship which may buffer the child from affective and emotional risks for ODD and CU behaviors.

Another possible explanation for the current findings stems from the research literatures on the neurobiological and psychophysiological correlates of CU behaviors, arousal states, and social orientation. The notion of heterotypic continuity, as it applies to potential etiological processes associated with ODD and CU behaviors in early life, would suggest that common underlying mechanisms influence multiple phenotypic behaviors and that dynamic relations between these underlying mechanisms may result in one influencing the developmental progression of another. Consistent with the concept of heterotypic continuity, damage to the amygdala, a neural system implicated in emotional dysfunction and fearlessness in children and adults with CU behaviors (Blair 2008), has also been shown to be associated with reduced eye contact (Spezio et al. 2007). Furthermore, there is research to suggest that older samples exhibiting CU behaviors show low levels of attention to affective cues (Newman et al. 2010) and that these attentional deficits, especially when referring to orienting and attending to the eye region of others, may contribute to dysfunctional recognition and interpretation of emotionally salient stimuli (Richell et al. 2003). Our findings might suggest that very early deficits in relational attention (as demonstrated by orienting towards another during a dyadic interaction) and under-reactivity to arousing stimuli may be independent precursors of later ODD and CU behaviors, and their co-occurrence may multiplicatively affect the likelihood of developing elevated ODD and CU behaviors. More specifically, given the associations between attentional processes and successful interpretation of emotionally salient stimuli, an early ability to socially attend to one's caregiver in infancy may attenuate the risk of developing ODD and CU behaviors, regardless of affective reactivity to a mild relational stressor.

It is important to note that the extent to which these infant behaviors are behavioral expressions of underlying

neurological functioning *cannot* be inferred from this study. The current findings also *cannot* speak to the nature or direction of the relation between attentional behavior and recognition of emotionally salient stimuli. That being said, the presence of these behaviors in infancy and their relation to ODD and CU behaviors in early childhood should provide support for future studies to more thoroughly investigate such questions.

Strengths and Limitations

To our knowledge, this is one of the first studies to extend downward the investigation of child affective and eye-gaze correlates of ODD and CU behaviors from middle childhood into infancy and toddlerhood. The use of the FFSFP, a paradigm that elicits infants' behavioral responses to normative and disrupted interactions with a caregiver, to measure infant behavior provides an objective assessment of specific affective and social behaviors that serve as the bases of emotional and social processes in infancy (as compared to maternal report). Furthermore, this assessment provides increased specificity with regard to the potential etiological processes of interest for later ODD and CU behaviors that more broad measures of infant temperament might not. Our findings are also strengthened by the longitudinal prospective design of the DCHDS and its demographic diversity, which allows for greater generalizability than is possible with convenience or clinically-based samples.

There are limitations of this study that are important to note. First, the reported findings should be interpreted with the understanding that development is influenced by a host of endogenous and exogenous factors which probabilistically interact over time (Gottlieb 1992). Although the current study included well designed and validated measures of maternal and infant behaviors in multiple contexts, it did not include potential child-level variables (e.g., psychophysiology, genetics, etc.) that have been evidenced to predict externalizing behavior problems and CU behaviors. With specific regard to CU behaviors, there is strong support for the role of genetic heritability (Viding et al. 2005). The current study also extends the work by Willoughby et al. (2013), which found that children's genotype interacted with observed harsh parenting to predict ODD and CU behaviors, by investigating the associations between infant behaviors during the FFSFP and later ODD and CU behaviors while controlling for measures of both sensitive and harsh parenting. However, future studies would benefit from simultaneously considering both family and child biology in a longitudinal framework, given the coaction effects (see Gottlieb 1992) they are likely to exert in the emergence of psychopathology.

Second, similar to correlations reported by other research groups using related measurement approaches at these ages (e.g., Hyde et al. 2013; Waller et al. 2014a, b; Waller et al. 2015a), there was a moderately high correlation between ODD and CU behaviors which contributed to similar findings in both sets of regression models. The similar pattern of predictors at this age may offer insight into the extent to which these constructs, which are clearly distinct at later ages, share a common set of predictors early in life which may contribute to a conceptual or methodological lack of differentiation at these ages. Exploratory follow-up analyses indicated that the associations between infants' negative reactivity and mother-directed gaze and later ODD behaviors remained relatively unchanged even after controlling for concurrent CU behaviors. However, subsequent interactive findings reported in this study were no longer significant when analytically controlling for the shared variance between ODD and CU behaviors, suggesting that, although literature suggests that ODD and CU behaviors are distinct and can be measured in early in life (Willoughby et al. 2011), additional work should be done to further elucidate the developmental processes unique to these behaviors. Further, the non-significant findings in the exploratory follow-up analyses should prompt future research that investigates the extent to which ODD and CU behaviors become more differentiated across time, including continued work on the measurement of CU behaviors in early life (i.e., Hyde et al. 2013; Kimonis et al. 2015). The current study offers an important first step by providing initial insight into the contributions of specific behaviors in infancy to the etiological pathways of ODD and CU behaviors.

Third, the age at which we collected the outcomes of interest restricted the extent which we could include truly diagnostic measures of child behavior. Therefore, we refer throughout to "CU behaviors", rather than "CU traits", although individual differences in these behaviors are predictive of clinically-relevant outcomes. Relatedly, the use of a community sample in this study, rather than a clinical sample, restricted the extent to which graphed estimates of the moderating relation between gaze and negative reactivity (Figs. 1 and 2) were able to predict ODD and CU behaviors at clinically relevant levels. However, it is important to note that the sample used in the current study had 29 participants whose ASEBA ODD T-scores were greater than or equal to 63. A T-score of 63 or higher denotes developmentally inappropriate levels of behavior (90th percentile by norms). Of these 29 participants, about one third scored in the 96th percentile on CU behaviors, a proportion that has been observed in other samples (e.g., Frick and White 2008). Although we were successful in demonstrating a link between infant behaviors and elevated ODD and CU behaviors in early childhood, the use of an older, clinically informed, sample might yield stronger predictive associations or demonstrate links between elevated arousal and reactivity and later behavior problems that have been reported in other studies (Bradley and Corwyn 2008; Vitaro et al. 2006).

Conclusions and Next Steps

This study has several implications for future research in this domain. First, potential etiological models of behavior problems and CU behaviors now include physiological (e.g. Loney et al. 2006; Stadler et al. 2011; Wagner et al. 2015c; Willoughby et al. 2011) and neurological (e.g. Kiehl et al. 2001) systems that influence these responses. Future studies should investigate infants' concurrent biological and behavioral functioning in response to a stressor and the extent to which individual differences in dual-system functioning predict ODD and CU behaviors later in childhood. Second, a remaining empirical question is whether the relation between underarousal to stimuli and the development of ODD and CU behaviors differs as a function of relational (i.e., interacting with a caregiver) versus static (i.e., laboratory fear paradigm) stressors. Mills-Koonce and colleagues (2015) found associations between higher levels of behavioral and biological reactivity to a lab-based fear paradigm at 15 months of age and later conduct problems and CU behaviors, suggesting that the context in which arousal is measured may have implications for our understanding of the associations between arousal and CU behaviors. Third, given the emerging evidence linking ODD and CU behaviors to insecure attachment relationships in older samples (e.g., Fearon et al. 2010; Pasalich et al. 2012), a highly relevant line of research would involve attempts to replicate and extend the current findings by including measures of attachment.

Both maternal and infant influences are theorized to contribute to the development of behavior problems and their relative contributions to ODD and CU behaviors cannot be definitely separated in current study. However, our findings support links between maternal predictors of ODD and CU behaviors, and also show additional unique contributions of specific infant behaviors which are similar to those identified at later ages, even after maternal contributions are controlled. The downward extension of the study of behavioral precursors in infancy to ODD and CU behaviors in childhood and adolescence should be a focus of future research and has potential to aid in the development of early and targeted preventive interventions.

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Compliance with Ethical Standards

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Conflict of Interest Nicholas Wagner declares that he has no conflict of interest. Roger Mills-Koonce declares that he has no conflict of interest. Cathi Propper declares that she has no conflict of interest. Michael Willoughby declares that he has no conflict of interest. Pete Rehder declares that he has no conflict of interest. Ginger Moore declares that she has no conflict of interest. *Martha* Cox declares that she has no conflict of interest.

Ethical Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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