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Prenatal Programming of Postnatal Plasticity for Externalizing Behavior: Testing an Integrated Developmental Model of Genetic and Temperamental Sensitivity to the Environment

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Abstract

Although both gene-environment and temperament-environment interactions contribute to the development of youth externalizing problems, it is unclear how these factors jointly affect environmental sensitivity over time. In a seven-year longitudinal study of 232 children (aged 5–10) with and without ADHD, we employed moderated mediation to test a developmentally-sensitive mechanistic model of genetic and temperamental sensitivity to prenatal and postnatal environmental factors. Birth weight, a global measure of the prenatal environment, moderated predictions of child negative emotionality from a composite of dopaminergic polymorphisms (i.e., DRD4 and DAT1), such that birth weight inversely predicted negative emotionality only for children with genetic plasticity. Negative emotionality, in turn, predicted externalizing behavior 4–5 years later, beyond genetic and postnatal parenting effects. Finally, birth weight moderated the indirect effect of dopaminergic genotypes on externalizing problems through negative emotionality, partially supporting a prenatal programming model. We discuss theoretical and empirical implications for models of environmental sensitivity.

Keywords

Human; Externalizing behavior; Prenatal programming; Gene-environment interaction; Temperament; Developmental plasticity

Individual differences in sensitivity to the environment are a central theme of developmental psychopathology. Several complementary theories proposed that biologically-based factors, such as genetic variation, early temperament, and physiology critically affect reactivity or plasticity to the social environment (Boyce, 2016; Pluess, 2015). The diathesis-stress framework contends that risk factors, including specific genotypes or emotionally reactive temperament traits, increase vulnerability to environmental adversity. More recently, vantage sensitivity posits that similar biologically-based characteristics may also heighten reactivity to *positive* environments (Pluess & Belsky, 2013). Finally, guided by evolutionary reasoning and studies including a full range of positive and negative environments, differential susceptibility or biological sensitivity to context theory suggests that children with plasticity factors may be more vulnerable to early environmental stress and, at the same time, benefit

the *most* from environmental enrichment (e.g., parental support; Bakermans-Kranenburg & van IJzendoorn, 2011; Belsky, Bakermans-Kranenburg, & IJzendoorn, 2007; Ellis, Boyce, Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2011)

Although environmental sensitivity models have been applied to many health and socioemotional outcomes, there has been particular interest in its role in the development of youth externalizing behavior problems (EB), such as aggression/violence and rule-breaking behaviors (Bakermans-Kranenburg, Van IJzendoorn, A, Mesman, & Juffer, 2008; Bradley & Corwyn, 2008; Caspi et al., 2002; Jaffee et al., 2005; Kochanska & Kim, 2013). Early EB, particularly when accompanied by attention-deficit/hyperactivity disorder (ADHD), robustly predicts academic, interpersonal, familial, and mental health problems (Armstrong, Lycett, Hiscock, Care, & Sciberras, 2014; Biederman et al., 2008; Faraone et al., 1993; Kuhne, Schachar, & Tannock, 1997). Thus, identifying factors that increase children's risk for EB in the context of adversity, but *also* amplify benefits from supportive environments, including intervention-induced enrichment, will critically innovate prevention programs for children most at risk for EB.

Genetic and Temperamental Sensitivity

Diverse biological systems, including genetic variation, neurotransmitter production, brain circuitry, physiological reactivity, and early temperament have been implicated as conferring environmental sensitivity (Boyce, 2016; Ellis et al., 2011). Among these, early temperament and genetic variation have been most consistently implicated in differential susceptibility for EB. Although numerous candidate genes (e.g., 5-HTTLPR, DRD4, BDNF; Belsky et al., 2015) have been implicated in differential susceptibility, studies of candidate gene-environment interactions (GxE) have been relatively inconsistent (Dick et al., 2015; Duncan & Keller, 2011), which reflects multiple influences including small effect sizes and phenotypic measurement error (of both environmental influences as well as outcomes). Furthermore, proposed candidate “plasticity” genes likely operate together to cumulatively influence different neurotransmitter systems (e.g., dopaminergic vs. serotonergic) that vary in their theorized mechanisms leading to psychopathology. Indeed, many GxE studies have not been based on theorized mechanisms, thus impeding the ability to elucidate *how* GxE influences emotional and behavioral processes that subsequently predict complex behavioral outcomes such as EB (Moffitt, Caspi, & Rutter, 2006).

In particular, candidate genes that influence dopaminergic efficiency and transmission have been linked to EB due to their role in reward sensitivity and attention processes (Janssens et al., 2015). A meta-analysis of GxE studies testing dopamine-related genes [i.e., dopamine receptor D4 (DRD4), dopamine receptor D2 (DRD2), and dopamine transporter (DAT1)] found that children with genotypes associated with lower dopaminergic efficiency (e.g., 7-repeat (7R) allele of DRD4, 10-repeat (10R) allele of DAT1) showed more behavioral and emotional problems in adverse environments than comparison children without these “plasticity alleles,” but they also showed better outcomes when exposed to positive environments (Bakermans-Kranenburg & van IJzendoorn, 2011). For example, consistent with differential susceptibility, DRD4 moderated a parenting intervention on toddler EB with stronger effects for 7R carriers (Bakermans-Kranenburg et al., 2008). Similarly, the

association between maternal unresponsiveness and childhood EB was moderated by child DAT1 genotype, with children's uninhibited temperament mediating these effects (Davies, Cicchetti, & Hentges, 2015). These dopaminergic plasticity genes *cumulatively* affected motivational sensitivity to environmental stimuli by affecting neural structures mediating reward, motivation, and learning (Feder, Nestler, & Charney, 2009; Wise, 2004). Thus, carriers of multiple plasticity alleles (e.g., 7R of DRD4 *and* two copies of the 10R of DAT1) may be particularly sensitive to positive and negative family environments (Belsky & Pluess, 2013a).

In a parallel body of work, the association between early parenting and EB was moderated by early temperament traits in a differential susceptibility pattern (Kochanska & Kim, 2013). Temperament is a relatively stable, biologically-based construct that influences children's self-regulation and reactivity to their environments. Meta-analytic evidence suggests that compared to children with "easy" temperaments, children with more reactive temperaments (e.g., high negative emotionality) were more vulnerable to negative parenting, but they also benefited more from positive parenting behaviors (Slagt, Dubas, Deković, & van Aken, 2016). Although most studies have focused on infant temperament and toddler outcomes (Leerkes, Nayena Blankson, & O'Brien, 2009; Pluess & Belsky, 2009), these effects may extend to school-age and early adolescence (Gallitto, 2015; Nikitopoulos et al., 2014; Pluess & Belsky, 2010).

Integrating Models of Sensitivity

Despite its expanding evidence base, most environmental sensitivity studies have focused narrowly on one level of measurement (e.g., genotype) rather than integrating multiple plasticity factors *across* levels of analysis (e.g., genotype *and* temperament). This limitation has prevented understanding of *how* these different plasticity factors, together, contribute to the patterns of environmental sensitivity over time (Boyce, 2016; Weeland, Overbeek, Castro, & Matthys, 2015). Given consistent findings across genotype and temperament, these constructs may reflect a single underlying plasticity factor that affects how individuals perceive, experience, approach, and react to the environment. That is, perhaps the "sensitive" individuals separately identified by each trait (e.g., individuals with DRD4 7R allele or high in negative emotionality) are the *same* individuals across multiple markers of susceptibility. Temperamental sensitivity markers such as negative emotionality may mediate phenotypic markers of underlying genetic plasticity. This is supported by evidence that carriers of plasticity alleles for DRD4 and DAT1 exhibit higher negative emotionality or emotional reactivity (De Luca et al., 2003; Holmboe, Nemoda, Fearon, Sasvari-Szekely, & Johnson, 2011; Ivorra et al., 2011; Oniszczenko & Dragan, 2005). In contrast, in a study of toddlers and school-aged children, Belsky & Pluess (2013b) included both DRD4 x Caregiving and Temperament x Caregiving interactions and found that each interaction *uniquely* predicted EB. These results suggest that negative emotionality is not simply an endophenotype of genetic plasticity, but instead may uniquely interact with the environment beyond genetic variation.

Importantly, beyond genetic effects, early environmental factors also influence the development of negative emotionality (Huizink, 2012; Shiner et al., 2012), highlighting that

developmental plasticity *itself* may be influenced by environmental factors. Proposing an archeology of the mechanisms of differential susceptibility, Boyce (2016) suggested that early gene $\times$ environment interactions (GxE) shape biological sensitivity to the environment (e.g., negative emotionality temperament) as a means of calibrating an organism to maximize its adaptation to the environment. In turn, temperament may then interact with later environmental factors, through Temperament $\times$ Environment interactions (TxE), to affect EB. Thus, different plasticity factors across multiple levels and periods of development provide a certain level of flexibility in adaptive development, enabling the environment to play a continuous role in calibrating plasticity sequentially. In this way, early GxE may affect the most adaptive level of susceptibility to the future environment, and this calibration or “programming” of the organism may be particularly active when adaptive systems are still organizing, such as in utero (Boyce, 2007).

Prenatal Programming of Postnatal Plasticity

From an evolutionary biology perspective, the prenatal period represents a critical stage where programming occurs, calibrating the fetus to the expected postnatal environment to maximize adaptation (Glover, O'Connor, & O'Donnell, 2010). To illustrate, prenatal maternal stress may forecast a stressful and inconsistent postnatal environment, for which elevated infant negative emotionality (e.g., vigilance and reactions to threat) may advantage fitness. In this maladaptive postnatal environmental context, negative emotionality then contributes to heightened risk for the development of EB, which may be *adaptive* for survival and reproduction in an unstable setting (Glover, 2011; Pluess & Belsky, 2011; Talge, Neal, & Glover, 2007). This “prenatal programming of postnatal plasticity” (Pluess & Belsky, 2011) is supported by the link between prenatal stress and infant negative emotionality (Blair, Glynn, Sandman, & Davis, 2011; Glover, 2011). For example, elevated maternal cortisol during pregnancy predicted elevated infant negative emotion at 7 weeks postpartum (de Weerth, van Hees, & Buitelaar, 2003). Similarly, lower birth weight, a correlate of prenatal stress (Rice et al., 2010), predicted higher temperamental negative emotionality in 5-year old children (Pesonen et al., 2006). Although “programming” may traditionally reflect the formation of an unchangeable outcome, the idea that the prenatal environment can calibrate the fetus to exhibit early traits (e.g., negative emotionality) associated with *heightened postnatal plasticity* suggests that the prenatal environment may play a role in programming children's sensitivity to their social environments.

Beyond these main effects, some individuals may be particularly sensitive to prenatal effects due to their genetic makeup (Pluess & Belsky, 2011). That is, negative emotionality as a sensitivity factor may reflect independent and interactive effects of genotype *and* prenatal environment. Although genetic influences on the association between prenatal environment and later development are not well characterized, genetic moderation of these prenatal programming effects may occur. Grizenko et al. (2012) reported that DRD4 moderated the association between prenatal stress and child ADHD; moderate to high prenatal stress (retrospectively reported by mothers) predicted more ADHD symptoms, but only for children homozygous for the 7R allele. Similarly, DRD4 genotype moderated the association of prenatal stress and EB in school age and adolescence, such that 7R carriers were more likely to be diagnosed with EB such as conduct disorder or oppositional defiant disorder

when their mothers reported higher levels of stress during pregnancy, whereas prenatal stress was unrelated to EB outcomes for non-carriers (Zohsel et al., 2014). Thus, there is emerging evidence to support a mechanistic model of differential susceptibility in which genes interact with prenatal environment to predict temperamental measures of plasticity, which then interact with the postnatal environment to predict complex behavioral outcomes such as EB.

Present Study

Despite its important theoretical implications and the growing number of GxE and TxE studies supporting environmental sensitivity, much less is known about its underlying developmental mechanisms. Testing a developmentally-sensitive and integrated model of genetic and temperamental sensitivity may critically elucidate *how* developmental plasticity of EB forms and changes over time. However, no empirical studies of EB have integrated these multiple levels of analysis by explicitly modeling these interactive associations across prenatal and postnatal development in a single study. The present study employed a longitudinal sample of children to integrate GxE and TxE hypotheses in prediction of EB. Based on emerging evidence of prenatal programming GxE effects (Pluess & Belsky, 2011), our aims were two-fold: (1) to model the independent and interactive effects of prenatal stress and dopaminergic genes on the formation of early temperament traits linked to plasticity (i.e., negative emotionality), and (2) to explore how negative emotionality interacts with postnatal environment (i.e., positive and negative parenting behavior) to predict EB in pre-/early adolescence. See Figure 1 for a conceptual model. Thus, using a developmentally-sensitive moderated mediation model, we aimed to elucidate how plasticity is *shaped* by modeling the mechanisms underlying EB development. If supported, results would suggest that developmental plasticity itself is a function of both nature and nurture and can, thus, be shaped by modifiable developmental experiences (e.g., prenatal stress) to prospectively influence EB outcomes in childhood and adolescence.

Methods

Participants

Participants were 232 children (aged 5–10 at baseline) and their parents (88% mothers) from a prospective longitudinal case-control study of children with and without ADHD. The means, standard deviations, and bivariate correlations among all demographic and study variables appear in Table 1. Participants were racial-ethnically diverse (52.5% White Non-Hispanic, 10.8% Hispanic, 8.5% Black, 3.6% Asian, 24.7% Mixed or Other) and mostly male (68%). Families were recruited from a large metropolitan city in the Western United States through presentations to self-help groups, advertisements mailed to local elementary schools, and referrals from pediatric offices and mental health clinics. All children were required to have an IQ of at least 70, live with one biological parent at least half time, and be fluent in English. Participants were excluded if they had a current/previous autism spectrum, seizure, or neurological disorder that prevented full participation in the study. As part of the larger case-control study, ADHD diagnostic status was ascertained at baseline from the Diagnostic Interview Schedule for Children, 4th edition (DISC-IV-P; Shaffer, Fisher, Lucas, Dulcan, & Schwab-Stone, 2000), a fully structured parent interview keyed to DSM-IV

criteria (e.g., age of onset, symptom persistence, cross-situational). Non-ADHD comparison youth were negative for ADHD according to the DISC but were allowed to meet diagnostic criteria for other mental disorders (e.g., anxiety, ODD).

Procedure

At baseline (timepoint 1 or T1; ages 5–10), study eligibility for interested families was determined through a telephone screening based on the inclusion and exclusion criteria listed above. Eligible families were mailed rating scales and invited to complete a laboratory-based assessment. After obtaining parental consent and child assent, parents completed multi-method measures of child psychopathology and temperament; in a separate room, children were assessed with measures beyond the scope of the present study including cognitive, academic, and social-emotional functioning. Finally, parents and children were videotaped during a parent-child interaction task. Children and parents provided saliva samples for genotyping. The child's diagnostic status was initially masked to all interviewers, although this was difficult to maintain following the completion of the DISC-IV. Approximately 94% of children were assessed in our laboratory without psychotropic medications. If a child was normally medicated (17%), we asked that parents provide ratings based on the child's unmedicated behavior.

Families were invited back to the laboratory for a follow-up assessment approximately four to five years after baseline (time 2; T2) when children were aged 9–15 years of age. Procedures for the follow-up visit were highly parallel to those in the baseline assessment. Approximately 83% of the initial sample ($n = 183$) participated in the 4–5-year follow-up assessment; participants who dropped-out did not differ significantly from participants included the present study on any baseline demographic (i.e., child age, sex, race-ethnicity), clinical (i.e., child ADHD symptoms), or genetic and temperament variables. We employed Full Information Maximum Likelihood procedures (FIML; described below) so that analyses were conducted on the full sample of 232 youth. The Institutional Review Board approved all study procedures.

Measures

Dopaminergic gene index—During the baseline evaluation, DNA was extracted from saliva samples using DNA Genotek Oragene Self-Collection Kits (DNA Genotek, Inc., Ottawa, CA). The present study created a dopaminergic gene index based on the dopamine-related polymorphisms meta-analytically implicated in differential susceptibility (Bakermans-Kranenburg & van Ijzendoorn, 2011; Belsky & Beaver, 2011). First, the 48-base pair (bp) variable number tandem repeat (VNTR) polymorphism located on chromosome band 11p15.5 of the DRD4 gene was genotyped using standard primers. The allele frequencies for the most common genotypes (4/4, 4/7, 2/4, 3/4, 7/7) of DRD4 were in Hardy-Weinberg equilibrium, $\chi^2(4) = .97, p = .21$. Following previous strategies (Bakermans-Kranenburg & van Ijzendoorn, 2011), participants with at least one 7R allele (36.2%) were compared to non-carriers (63.8%). Next, the 40-bp VNTR polymorphism in the 3' untranslated region of the DAT1 gene was genotyped, which produced the two most common polymorphisms (9R and 10R) in the following distribution: 10/10 (56.5%), 9/10 (36.4%), and 9/9 (7.0%). These frequencies were in Hardy-Weinberg equilibrium, $\chi^2(1) =$

0.25, $p = .62$. Of note, we initially planned to include DRD2 in addition to DRD4 and DAT 1 to parallel the three dopamine genes identified in a meta-analysis of dopamine genes linked to plasticity (Bakermans-Kranenburg & van Ijzendoorn, 2011); however, we encountered genotyping errors for DRD2, thus rendering those data unusable for this study. Thus, we created an index of dopaminergic genotypes that represented the number of plasticity genotypes for each participant where participants with at least one 7R allele of DRD4 were assigned one plasticity “point,” and participants with the 10/10 genotype of DAT1 were assigned one point. Dopaminergic gene index scores ranged from 0–2, with higher scores representing more genetic plasticity.

Negative emotionality—At baseline, child negative emotionality was assessed using the Child and Adolescent Dispositions Scale (CADS), a parent interview of that assesses temperament or dispositional traits specifically linked to psychopathology (e.g., conduct disorder) (Lahey et al., 2008). Because the CADS was explicitly designed for studies of psychopathology, it excludes synonyms and antonyms of psychiatric symptoms in its measurement of temperament dimensions to increase specificity in predictions of psychopathology. Parents rated 50 items based on how well the item described their child on a 4-point Likert scale, ranging from 1 (*not at all*) to 4 (*very much/very often*). Previous psychometric studies identified three factors: negative emotionality, prosociality, and daring, which evidenced excellent internal consistency, high re-test reliability, and predictive and concurrent validity in multiple samples (Lahey et al., 2008; Trentacosta, Hyde, Shaw, & Cheong, 2009). The present study used the total score from the negative emotionality factor (11 items, Cronbach’s $\alpha = .82$), which includes items such as “does [your child] react intensely when he/she gets upset?” and “does [your child] get upset easily?”

Birth weight—We followed Pluess & Belsky (2011) and treated birth weight as reported by the primary parent as a proxy for global prenatal stress. Birth weight reported through maternal recall is highly correlated with medical record data into offspring adulthood (e.g., intraclass correlation [ICC] = .99 in Yawn, Suman, & Jacobsen, 1998; also see Buka, Goldstein, Spartos, & Tsuang, 2004; Jaspers, de Meer, Verhulst, Ormel, & Reijneveld, 2010; O’Sullivan, Pearce, & Parker, 2000; Rice et al., 2007). Given its sensitivity to multiple forms of prenatal stress, including prenatal exposure to trauma and violence (Hill, Pallitto, McCleary-Sills, & Garcia-Moreno, 2016), chronic stress and poverty (Bolten et al., 2010; Kayode et al., 2014; Strutz et al., 2014), cigarette smoking (Bailey, McCook, Hodge, & McGrady, 2011), and pregnancy-related stress and anxiety (Bussieres et al., 2015), we interpreted lower birth weight as suggesting more prenatal stress. Children in the present study represented a full range of birth weights (from 2.6–9.8 lbs).

Parenting behavior—Observed measures of positive and negative parenting behavior were coded from a parent-child interaction task administered at baseline using the Dyadic Parent Child Interaction Coding System (DPICS; Eyberg, Nelson, Duke, & Boggs, 2005). The DPICS is a well-validated system of rating parent-child interaction in children with disruptive behavior disorders. Discrete parent and child behaviors were coded continuously, and then composite categories of parenting were created (Chronis-Tuscano et al., 2008; Eyberg et al., 2001; Li & Lee, 2013). Negativity was coded when parents made hostile or

critical comments to their child (e.g., “You’re so irritating sometimes,” “You’re doing that wrong”), negative commands (e.g., “Stop doing that!”), or sarcastic and condescending remarks (e.g., “You think you’re so clever, don’t you?”). Praise was coded when parents made positive appraisals of their children’s behavior, attributes, or products that their children created (e.g., “You’re a good builder,” “That’s a really pretty picture of a dog you drew”). All parent-child interactions were digitally recorded. Research assistants completed intensive training on DPICS coding procedures until at least 70% agreement was attained. The present study used the negativity (ICC = .75) and praise composites (ICC = .88) as measures of negative parenting and positive parenting, respectively. Please see Li and Lee (2013) for further details about the DPICS coding system.

EB—Children’s EB at the follow-up evaluation was assessed using the Child Behavior Checklist (CBCL; Achenbach & Rescorla, 2001), a widely-used 113-item parent rating scale that yields multiple scales of internalizing and externalizing problems. Parents rated each behavior based on the preceding 6 months as 0 (*Not True*), 1 (*Somewhat or Sometimes True*), or 2 (*Very True or Often True*). Normative data are available for boys and girls ranging from 6–18, and the scales have demonstrated convergent validity with other common measures of behavioral and emotional functioning (Bender et al., 2008). The scales also discriminated between disordered and non-disordered children (Brasil & Bordin, 2010). The present study used total scores from the Externalizing Behavior broadband scale, which includes aggressive behavior (e.g., “cruelty, bullying, or meanness to others,” “gets in many fights”) and rule-breaking behaviors (e.g., “breaks rules at home, school, or elsewhere,” “steals at home”).

Data analysis

Prenatal programming of postnatal sensitivity to the environment was explored using two complementary moderated mediation models (also called conditional indirect effects models; Hayes, 2015) to examine sensitivity to negative and positive parenting (see Figure 1 for the conceptual model). Each model simultaneously estimated (1) coefficients for the direct effects between dopaminergic genes, negative emotionality, and EB, (2) the indirect effect of dopaminergic genes on EB through negative emotionality, and (3) separate Indices of Moderated mediation by birth weight and parenting behavior (two separate models for positive and negative parenting). That is, we tested whether the indirect effect of dopaminergic genes on EB through negative emotionality was *moderated* by prenatal and/or postnatal environment, as the Indices of Moderated mediation reflect the individual effects of the moderators (i.e., birth weight, parenting behavior) on the overall indirect effect from dopaminergic genes to EB through negative emotionality (Hayes, 2015). Significant moderated mediation was indicated when the Index of Moderated Mediation for birth weight or parenting behavior was significantly different from zero. Each model described here also estimated conditional indirect effects across different levels of birth weight (–1SD, grand mean, +1SD) or parenting behaviors (–1SD, grand mean, +1SD; Hayes, 2015) to aide interpretability of observed moderated mediation. All models controlled for baseline child ADHD symptoms to account for the case-control design of the sample. Given that age ranged from 5–10 years at baseline and the sample was majority (68%) male, we additionally controlled for child age and sex as covariates in the models to improve

specificity of effects. Finally, although DRD4 was randomly distributed by race-ethnicity groups in the current sample ($\chi^2(4) = 5.20, p = .27$), DAT1 genotype was not ($\chi^2(4) = 11.05, p = .03$). Thus, we controlled for race-ethnicity in the models to reduce risk for population stratification due to racial-ethnic differences in allelic frequencies (Hutchison, Stallings, McGeary, & Bryan, 2004).

Both moderated mediation models were conducted in Mplus 6.12 (Muthén & Muthén, 1998–2011). Parameter estimates and 95% bias-corrected (BC) confidence intervals (CIs) for point estimates of the indirect effects, Indices of Moderated Mediation, and conditional indirect effects were calculated based on 5,000 bootstrap resamples (statistical significance is assumed when the interval excludes zero; Hayes, 2015). Bootstrapping is a powerful nonparametric re-sampling procedure that enables simultaneous evaluation of mediators and moderators with adjustment for potential covariates (Hayes, 2013; Preacher & Hayes, 2008). In addition to these critical advantages, bootstrapping-based mediation is also statistically more powerful than traditional approaches (i.e., Sobel test; Zhao, Lynch, & Chen, 2010). For all moderated mediation analyses, variables were centered and FIML estimation with robust standard errors was used to address missing data and to increase model power. Modern missing data approaches such as FIML are significantly advantaged over common methods (e.g., listwise deletion, mean imputation) given that they produce less biased parameter and standard error estimates and decrease Type 1 error (Collins, Schafer, & Kam, 2001; Little & Rubin, 1987).

Results

Preliminary analyses

Descriptive statistics and bivariate correlations among all demographic and study variables appear in Table 1. As expected, negative emotionality was significantly and positively correlated with EB 4–5 years later, whereas dopaminergic plasticity was not directly correlated with negative emotionality nor EB. Bivariate correlations between the environmental moderator and outcome variables were mixed and supported the plausibility of interactive effects. Specifically, the correlation between birth weight and negative emotionality was not significant. Negative parenting was significantly correlated with EB 4–5 years later, whereas positive parenting was not. Finally, dopaminergic plasticity was unrelated to the environmental variables (i.e., birth weight, positive parenting, negative parenting; all p 's $> .05$), reducing concerns about evocative gene-environment correlations.

Prenatal Programming of Sensitivity to Negative Parenting

Conditional direct effects—The first model examined moderation of the direct and indirect effects of dopaminergic genes on EB through negative emotionality by birth weight and negative parenting behaviors, controlling for child age, sex, race-ethnicity, and ADHD. Path coefficients for this full model are presented in Figure 2. First, neither dopaminergic genes nor birth weight had a significant direct effect on negative emotionality (Figure 2). Instead, they significantly interacted to predict negative emotionality (Figure 3). To examine *how* dopaminergic plasticity influenced the association of birth weight (representing prenatal environment) and later temperamental sensitivity, post hoc regression models separately

tested the association between birth weight and negative emotionality for children across 0, 1, and 2 dopaminergic plasticity genotypes. For children with high dopaminergic plasticity (i.e., with 7R allele of DRD4 and the 10/10 genotype of DAT1), birth weight inversely predicted negative emotionality ($\beta = -.08$, $SE = .04$, $p = .03$) such that children with high birth weights were less negatively emotional (i.e., theoretically “less sensitive” temperament) whereas low birth weight children had high negative emotionality. In contrast, birth weight did not influence negative emotionality for children with moderate or low dopaminergic plasticity ($\beta = .01$, $SE = .03$, $p = .80$ and $\beta = .04$, $SE = .04$, $p = .23$, respectively).

In addition to prenatal programming effects, this model also tested the direct effects of negative emotionality, negative parenting, and their interaction on later EB. Controlling for age, sex, race-ethnicity, ADHD symptoms, and dopaminergic genes, negative emotionality had a significant positive direct effect on EB 4–5 years later, and negative parenting had a marginally significant positive direct effect on EB (Figure 2). Furthermore, negative parenting marginally moderated the prospective association between negative emotionality and EB (Figure 2).

Conditional indirect effects—As expected, neither the total effect of dopaminergic genes on EB nor its indirect effect through negative emotionality were significant, supporting the need to examine potential moderating effects by environmental factors. The moderating effects of birth weight and negative parenting on the *indirect* effect from dopaminergic genes to EB through negative emotionality are presented in Table 2. The Index of Moderated Mediation for birth weight was significantly different from zero, suggesting that birth weight moderated the indirect effect of dopaminergic genes on EB through negative emotionality (Table 2). Specifically, for children with high birth weights (i.e., +1 SD representing a stable/enriching prenatal environment), high genetic plasticity predicted *low* negative emotionality, which in turn predicted lower levels of EB 4–5 years later. In contrast, this indirect effect was not significant for children with low (i.e., –1 SD) or average birth weights. The Index of Moderated Mediation for negative parenting was not significantly different from zero (Table 2). Thus, negative parenting did not moderate the indirect effect of dopaminergic genes on EB through negative emotionality (Table 2).

Prenatal Programming of Sensitivity to Positive Parenting

Conditional direct effects—Next, to explore how prenatal programming effects may influence sensitivity to *positive* postnatal parenting behaviors, we examined moderation of the direct and indirect effects of dopaminergic genes on EB through negative emotionality by birth weight and positive parenting behaviors, controlling for age, sex, race-ethnicity, and ADHD symptoms. Path coefficients for this full model are presented in Figure 3. Similar to results from the negative parenting model, neither birth weight nor dopaminergic genes directly predicted negative emotionality, although their interaction did so marginally. Negative emotionality, in turn, had a significant direct effect on EB, but positive parenting behaviors and the interaction between positive parenting and negative emotionality were not significant (Figure 3).

Conditional indirect effects—In the positive parenting model, birth weight marginally moderated the indirect effect of dopaminergic genes on EB through negative emotionality in a pattern consistent with results in the negative parenting model (Table 2). However, the Index of Moderated Mediation for positive parenting behavior was non-significant (Table 2).

Discussion

In a multi-method longitudinal study of youth that spanned across multiple periods of development (i.e., prenatal and postnatal environment), we tested an integrated model of genetic and temperamental sensitivity to the environment to explore prenatal and postnatal mechanisms underlying EB development. Whereas previous studies of environmental sensitivity typically focused on a single plasticity marker (e.g., single genotype), we integrated these biologically-based levels into a single moderated mediation model to explore how plasticity is formed. First, birth weight moderated the association between dopaminergic genes and negative emotionality in childhood, supporting genetic moderation of prenatal programming effects on temperament traits linked to plasticity (Pluess & Belsky, 2011). Childhood negative emotionality, in turn, positively predicted EB 4–5 years later in pre/early adolescence. The association between negative emotionality and EB was marginally moderated by negative parenting, but not by positive parenting. Thus, we observed partial support for postnatal plasticity, with these marginal effects following a diathesis-stress pattern of environmental sensitivity. Finally, birth weight moderated the indirect effect of dopaminergic genes on EB through negative emotionality, such that the mediating pathway was only significant for children with high birth weights (representing stable, nurturing prenatal environments).

These preliminary findings suggest that individual differences in childhood negative emotionality were sensitive to genetic x prenatal environment interactions. Specifically, birth weight inversely predicted negative emotionality, but only for children with greater genetic plasticity (i.e., with both the 7R allele of DRD4 and the 10/10 genotype of DAT1). These results suggest that temperamental traits linked to plasticity (such as negative emotionality) are not direct phenotypic markers of underlying genetic plasticity. Rather, the prenatal environment likely plays a key adaptive role in guiding the fetus to exhibit either a highly *reactive* postnatal temperament (i.e., high negative emotionality) when the prenatal environment forecasts a chaotic postnatal environment, or a less sensitive and “easy” temperament when exposed to a stable, nurturing prenatal environment (Boyce, 2016; Pluess & Belsky, 2011). Thus, these results implicate genetic moderation of prenatal programming effects on negative emotionality. Considering the moderating role of the prenatal environment helps shed light on why dopaminergic plasticity predicted *higher* negative emotionality in some studies (De Luca et al., 2003; Holmboe et al., 2011; Ivorra et al., 2011; Oniszczenko & Dragan, 2005), but predicted *lower* negative emotionality in other studies (Auerbach et al., 1999; Auerbach, Faroy, Ebstein, Kahana, & Levine, 2001; Papageorgiou & Ronald, 2013).

Next, this study partially supported temperament-based postnatal plasticity, such that negative emotionality predicted significantly higher EB 4–5 years later, and this effect was marginally heightened if the postnatal parenting environment was *negative*; negative

emotionality did not affect sensitivity to positive parenting behaviors. Furthermore, given that the interaction between negative emotionality and negative parenting was marginal, the more prominent postnatal effect was the *direct* effect of negative emotionality on EB 4–5 years later, beyond dopamine genotypes, parenting behaviors, ADHD symptoms, and demographics (e.g., age, sex, race-ethnicity). The prospective prediction of EB from negative emotionality is consistent with evidence that negative emotionality is an etiological marker for EB (Singh & Waldman, 2010). Thus, understanding the early developmental processes that predict lower negative emotionality may be a critical way to target early prevention of later EB.

Finally, we evaluated a moderated mediation model to examine how prenatal and postnatal environmental factors influence the *mediating* pathway from dopaminergic genes to negative emotionality to EB. Birth weight moderated the indirect effect of dopaminergic genes on EB through negative emotionality, supporting a genetically-moderated prenatal programming model of EB. Surprisingly, calculation of conditional indirect effects revealed that the mediating pathway from dopaminergic genes to EB through negative emotionality was only significant for children with *high* birth weights (representing stable, nurturing prenatal environment). When exposed to a positive prenatal environment, children with high genetic plasticity exhibited less negative emotionality, which directly predicted lower EB in pre/early adolescence, beyond the postnatal environment. In other words, compared to children with moderate or low genetic plasticity, children with high genetic plasticity *benefited more* from a stable, nurturing prenatal environment, which in turn predicted less negative emotionality and lower EB. However, low birth weight did not predict negative emotionality and EB for children with high genetic plasticity. This pattern of prenatal plasticity is consistent with a vantage sensitivity framework (Pluess & Belsky, 2013), in which genetic markers heightened sensitivity to *positive* prenatal environments, but not negative prenatal environments.

It is important to interpret these preliminary results in the context of this sample characteristics and relevant methods. The sample included primarily middle to middle-upper SES families, which may have limited the amount of severe prenatal stress experienced. We did observe a full range of birth weight (ranging from 2.6 to 9.8 lbs), but low birth weight likely reflects multiple factors beyond prenatal stress (e.g., genetic effects; Lunde, Melve, Gjessing, Skjærven, & Irgens, 2007). Thus, future studies are needed to replicate this model in high-risk samples, such as infants exposed to prenatal maltreatment, chronic poverty, or community violence. Second, due to medical record data being unavailable, birth weight was assessed via maternal recall, which is highly correlated with medical record data (e.g., Yawn et al., 1998) but less accurate. Furthermore, although birth weight meaningfully approximates a global measure of the prenatal environment, future studies that examine separable measured aspects of the prenatal environment are needed to clarify which specific prenatal experiences have the largest influence on later postnatal plasticity. These results should also be interpreted in the context of the relatively modest sample size, which may have been underpowered to detect all moderated mediation effects and/or to sufficiently probe significant interactions. Given that several marginal effects were observed in the current model, larger sample sizes are warranted to clarify the nature of these effects. Finally, it is important to acknowledge the challenges inherent to GxE research, including

replication failure and false positives (Duncan & Keller, 2011). Given that this study did not employ a built-in replication sample, our preliminary results should be interpreted in the context of well-powered follow-up studies that can further characterize genetic moderation of prenatal programming effects. Furthermore, although the present study represents an exploratory test of a theoretical model and focused on one neurotransmitter system in the role of prenatal programming of postnatal plasticity, it is likely that other unmeasured candidate genes that influence dopamine efficiency as well as other neurotransmitter systems (e.g., serotonin, GABA) influence these processes as well. We encourage future studies to integrate this theoretical framework in tests of environmental sensitivity while attending to the specific neurotransmitter systems likely underlying GxE, a critical step to begin uncovering the *mechanisms* underlying children's sensitivity to their environments.

The rapidly growing number of GxE and TxE studies in the past decade make clear that biologically-based processes of environmental sensitivity critically underlie EB development. However, environmental sensitivity itself is likely a dynamic process, fluctuating based on developmental period and influenced by multiple levels of biology (Boyce, 2016; Ellis et al., 2011). Because most environmental studies have narrowly focused on one biological level or developmental period, the causal mechanisms underlying EB development remain elusive. Studies integrating these multiple levels of analysis are timely and needed to understand not only *who* is most sensitive to the environment, but *how* this environmental sensitivity is shaped across time (Boyce, 2016; Weeland et al., 2015). If replicated, our exploratory findings have clinically meaningful implications for the timing and targets of intervention and prevention programs for EB. We found that for children with plasticity variations of dopaminergic genes (i.e., the 7R allele of DRD4 and 10/10 genotype of DAT1), a stable and positive prenatal environment led to significantly lower childhood negative emotionality, which in turn predicted lower EB 4–5 years later. In contrast, postnatal negative parenting behavior had only a marginal independent and interactive effect on EB above and beyond the direct effects of negative emotionality, and positive parenting did not prospectively predict EB. These preliminary findings speak to a preventative rather than a reactive approach to reducing child and adolescent EB. Reducing EB may require interventions directly targeted at temperamental factors underlying EB, such as negative emotionality, that may need to start as early as the prenatal environment for at-risk mothers. Ultimately, this exploratory study emphasizes that to understand the development of complex behavioral outcomes such as EB, we must consider individual and environmental factors across multiple levels. Future replication studies testing integrated models of genetic and temperamental sensitivity that also consider different sources of environmental stress and support across developmental periods (e.g., maternal depression during pregnancy vs. infancy vs. childhood) are needed to clarify how plasticity is shaped over time.

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(a)

(b)

Figure 1.

Conceptual diagrams of moderated mediation models exploring prenatal programming of postnatal sensitivity to (1a) negative parenting and (1b) positive parenting

Figure 2.

Path coefficients from the model estimating moderation by birth weight and negative parenting behavior of the indirect effect of dopaminergic genes on EB through negative emotionality, controlling for child age, sex, race-ethnicity, and ADHD. *Note.* Numbers shown reflect unstandardized beta coefficients; $^+p < .10$ $^*p < .05$ $^{**}p < .01$

Figure 3.
Genetic moderation of prenatal effects (measured by birth weight) on child negative emotionality

Figure 4.

Path coefficients from the model estimating moderation by birth weight and positive parenting behavior of the indirect effect of dopaminergic genes on EB through negative emotionality, controlling for child baseline age, sex, race-ethnicity, and ADHD symptoms.

Note. Numbers shown reflect unstandardized beta coefficients; $^{+}p < .10$ $^{*}p < .05$ $^{**}p < .01$

Table 1

Descriptive statistics and correlations among demographic and study variables

Variable	M (SD) or %	1	2	3	4	5	6	7	8	9	10
1. Age in years (T1)	7.42 (1.14)	--									
2. Sex (% girls) ^a	31.9%	-.04	--								
3. Race-ethnicity (% Caucasian) ^b	52.5%	-.01	.02	--							
4. ADHD symptoms (T1)	7.96 (5.52)	-.11	.18**	-.08	--						
5. Dopaminergic plasticity score	0.91 (0.67)	-.05	-.04	-.06	-.03	--					
6. Negative emotionality (T1)	25.15 (5.64)	<.01	.04	.09	.54**	-.07	--				
7. Birth weight in ounces	117.92 (19.11)	-.07	.18*	<.01	-.04	-.01	-.01	--			
8. Parental Negativity (T1)	9.22 (9.00)	-.09	.04	-.15	.18*	-.03	.08	-.08	--		
9. Parental Praise (T1)	12.49 (10.92)	-.20**	.09	.10	.10	-.02	-.04	.01	-.01	--	
10. Externalizing Problems (T2)	7.48 (7.38)	.06	.01	-.06	.40**	-.08	.46**	<.01	.20*	-.11	--

Note. T1 = timepoint 1 (age 5–10 years old); T2 = timepoint 2 (age 7–14 years old).

^a 1 = boys, 0 = girls.^b 1 = Caucasian, 0 = non-Caucasian.* $p < .05$ ** $p < .01$

Table 2

Indirect effects of dopaminergic genes on EB through negative emotionality conditioned on birth weight and parenting behavior

	<i>Point-Est.</i>	<i>SE</i>	<i>95% BCa Bootstrap CI</i>	
			<i>Lower</i>	<i>Upper</i>
Negative Parenting Model				
Total effect	-0.588	0.830	-2.279	0.980
Indirect effect	-0.214	0.259	-0.814	0.221
Index of Moderated Mediation by Birth Weight	-0.029	0.016	-0.070	-0.005
Conditional indirect: Low birth weight	0.341	0.340	-0.266	1.102
Conditional indirect: Moderate birth weight	-0.214	0.259	-0.814	0.221
Conditional indirect: High birth weight	-0.769	0.447	-1.973	-0.104
Index of Moderated Mediation by Negative Parenting	-0.016	0.023	-0.081	0.014
Conditional indirect: Low negative parenting	-0.066	0.163	-0.647	0.097
Conditional indirect: Moderate negative parenting	-0.214	0.259	-0.814	0.221
Conditional indirect: High negative parenting	-0.361	0.436	-1.404	0.359
Positive Parenting Model				
Total effect	-0.702	0.818	-2.251	0.975
Indirect effect	-0.169	0.243	-0.722	0.235
Index of Moderated Mediation by Birth Weight	-0.024	0.015	-0.062	< 0.001
Conditional indirect: Low birth weight	0.287	0.322	-0.297	0.987
Conditional indirect: Moderate birth weight	-0.169	0.243	-0.722	0.235
Conditional indirect: High birth weight	-0.626	0.425	-1.720	0.019
Index of Moderated Mediation by Positive Parenting	0.001	0.010	-0.014	0.027
Conditional indirect: Low positive parenting	-0.183	0.275	-0.977	0.178
Conditional indirect: Moderate positive parenting	-0.169	0.243	-0.722	0.235
Conditional indirect: High positive parenting	-0.156	0.257	-0.828	0.223

Note. Models controlled for child age, sex, race-ethnicity, and ADHD as covariates. Boldface indicates significant conditional indirect effect and/or significant index of moderated mediation; Point est. = point estimate of the indirect effect; BCa Bootstrap CI = bias corrected and accelerated confidence intervals