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A Multilevel Prediction of Physiological Response to Challenge: Interactions among Child Maltreatment, Neighborhood Crime, eNOS and GABRA6

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Abstract

Physiological response to stress has been linked to variety of healthy and pathological conditions. The current study conducted a multilevel examination of interactions among environmental toxins – i.e., neighborhood crime and child maltreatment – and specific genetic polymorphisms of eNOS and GABRA6. A total of 186 children were recruited at age 4. At this time, the presence or absence of child maltreatment was determined, as was the amount of crime that occurred in their neighborhood during the previous year. At age 9, the children were brought to the lab where their physiological response to a cognitive challenge – i.e., change in the amplitude of RSA – was assessed and DNA samples were collected for subsequent genotyping. Results confirmed that complex G x G, E x E, and G x E interactions were associated with different patterns of RSA reactivity. The implications for future research and evidence-based intervention are discussed.

A multilevel approach to examining the consequences of environmental adversity is consistent with an ecological-transactional analysis of development (Belsky, 1980; Bronfenbrenner, 1977; Cicchetti & Lynch, 1995; Cicchetti & Valentino, 2006; Sameroff & Chandler, 1975). The complex ways in which ecological systems interact with each other and with the individual necessitates careful multilevel investigation to identify factors that shape the individual's response to adversity.

Stress associated with adversity has been implicated as a contributing factor to a variety of somatic health problems and psychiatric disorders, particularly mood disorders (McEwen, 2003). By examining individual differences in the responses of autonomic systems, it may be possible to identify groups of people disposed to stress-related pathology. Cardiovascular parameters like heart rate and blood pressure often have been examined as indicators of the physiological stress response and as risk factors for somatic disease and psychopathology

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(Chida & Hamer, 2008). Recently, a more specific index of autonomic cardiac regulation has received attention for its association with stress-resilience and emotion regulation processes (Porges, 2001). The variable of interest is a phenomenon known as respiratory sinus arrhythmia (RSA) and it can be indirectly assessed by measuring the high frequency component of heart rate variability.

RSA is a naturally occurring rhythm in heart rate that may be understood as the synchronization between heart rate and spontaneous breathing (DeGeus & Van Doornen, 1996). Typically, heart rate tends to accelerate during inspiration and decelerate during expiration. RSA is an indicator of parasympathetic activity, and the amplitude of RSA can be quantified to provide a sensitive index of the impact of the myelinated vagus nerve on the heart (Porges, 1995). High levels of RSA indicate a relatively high amount of respiration-linked variability in heart rate which may promote more efficient pulmonary oxygen perfusion under resting conditions (Yasuma & Hayano, 2004), which in turn may facilitate social engagement by the oxygen-dependent central nervous system (Porges, 2007).

In response to non-resting and challenging, or potentially threatening, conditions, RSA may decrease reflecting a shift toward sympathetic nervous system dominance and acceleration of heart rate in order to mobilize metabolic resources. The resulting increase in heart rate facilitates a rapid increase in arousal and engagement in the environment and promotes an increase in metabolic output and behavioral activity to meet the demands of the environment (Porges, 2001; Propper & Moore, 2006; Skowron, Loken, Gatzke-Kopp, et al., 2011). Lowering of RSA during a challenging situation has been related to a number of positive outcomes in infants and older children (Huffman, Bryan, Del Carmen, Pederson, Doussard-Roosevelt, J., & Porges, 1998; Porges et al., 1996; Eisenberg et al., 1996). These findings suggest that regulation of RSA may facilitate behavioral and attentional control linked to different arousal states (Posner & Rothbart, 2000).

By assessing RSA during various resting and challenge conditions, it is possible to measure the dynamic regulation of RSA through an active “vagal brake”. The myelinated vagus nerve functions as a brake in which rapid inhibition and disinhibition of vagal tone to the heart can quickly mobilize or calm an individual (Porges, Doussard-Roosevelt, Portales, & Greenspan, 1996). The myelinated vagus actively inhibits the sympathetic nervous system’s influences on the heart and dampens hypothalamic pituitary adrenal (HPA) axis activity (see Porges, 2001). Functionally, the vagal brake enables the individual to rapidly engage and disengage with objects and people in the environment. As a result, the amplitude of RSA is not constant. The vagal bake is released or lessened to support the metabolic requirements for mobilization in response to challenge. Conversely, the vagal brake is applied or increased to facilitate social engagement and calm, restful states. As a result, RSA varies as a function of the behavioral and psychological demands of different environmental conditions, and the amplitude of RSA provides an assessment of the state of the vagal brake (Porges, 2007).

Porges (2004) proposes that a neural process of “neuroception” allows the individual continuously to monitor and evaluate risk in the environment and modulate vagal output. In safe environments, RSA is regulated to facilitate complex social engagement strategies.

However, when danger or threat is perceived, mylenated vagal control of the heart gives way to the sympathetic nervous system and limbic structures that control fight, flight, and freezing behaviors (Porges, 2007). When the threat passes and the environment is perceived to be safe, parasympathetic control of the heart resumes and RSA increases again.

Effective use of the vagal brake system has been associated with better control over arousal and therefore an increased ability to maintain homeostasis in the face of changing environmental demands (Propper & Moore, 2006). Porges (2001, 2007) suggests that the dynamic control of physiological response has important implications for regulating emotions associated with heightened and prolonged arousal (typically experienced as anger, fear, or agitation), and thus allows the individual to engage in appropriate social interaction. Because of the association of RSA to general regulatory ability, this system is of interest in understanding the relationship between early adversity and the ability to regulate arousal, which likely has implications for risk for maladaptive and pathological outcomes as children develop (Skowron et al., 2011). In fact, the modulation of RSA appears to be a general correlate of psychopathology across both internalizing and externalizing phenomena (Beauchaine, 2001).

Baseline levels of RSA are thought to represent the extent of an individual's regulatory capacity (Porges, 1998, 2001). Low baseline RSA is a common correlate of psychopathology, and seems to reflect reduced flexibility in responding to challenge (Beauchaine, Gatzke-Kopp, & Mead, 2007). In particular, studies that have compared resting RSA levels between patient populations and healthy control groups indicate that significantly lower levels of resting RSA have been reported among depressed (Kemp, Quintana, Gray, Felmingham, Brown, & Gatt, 2010) and anxious patients (Thayer, Friedman & Borkovec, 1996).

However, approaches that focus on RSA at a single point in time fail to capture individual differences in responding to environmental demands, which may be important for predicting adaptation under stress. RSA is a dynamic and stress-responsive phenomenon, increasing or decreasing in order to effectively manage metabolic resources according to environmental demands (Ottaviani, Shapiro, Davydov & Goldstein, 2008). RSA reactivity, the change in RSA observed when transitioning from a resting state to an active state, has been examined across various laboratory tasks. The suppression or reduction of RSA in response to challenge is generally considered to reflect an adaptive response by increasing task engagement without compromising control (e.g., El-Sheikh, Kouros, Erath, Cummings, Keller, Staton, & Moore, 2009).

Multiple studies support the link between aberrant RSA reactivity patterns and risk for both externalizing (El-Sheikh, Hinnant & Erath, 2011) and internalizing problems (Boyce, Quas, Alkon, Smider, Essex, & Kuppfer, 2001; Greaves-Lord, Tulen, Dietrich, et al., 2010). In particular, research indicates a link between excessive vagal suppression and psychopathology (Calkins, Graziano, & Keane, 2007), especially in cases involving early exposure to adversity (e.g., Obradović, Bush, Stamperdahl, Adler, & Boyce, 2010). Conversely, RSA augmentation (i.e., increased parasympathetic activation) in response to challenge has been observed in some settings, and it can be functional when individuals are

required to suppress arousal in the service of social engagement (Butler, Wilhelm, & Gross, 2006). However, this less typical response also has been linked to problematic behavior, especially among children who have experienced early adversity in the form of child neglect or domestic violence (Katz, 2007; Skowron et al., 2006).

While less frequently examined, there is also evidence that RSA recovery, the change observed when returning from an active state to a resting state, may be important for distinguishing those at risk for behavioral health problems. Specifically, deficient RSA recovery has been associated with internalizing problems among children (Boyce et al., 2001) and social anxiety (Movius & Allen, 2005), cognitive rumination (Key, Campbell, Bacon & Gerin, 2008), and negative affective states (Waugh, Panage, Mendes & Gotlib, 2010) among adolescents and young adults. Together, these results suggest that aberrant RSA response patterns during both reactivity and recovery phases may be associated with risk for psychopathology.

Genetic Influence on Heart Rate Variability

Several genetic polymorphisms have been associated with visceral regulation related to heart rate and respiration.

Endothelial nitric oxide synthase gene (eNOS)

eNOS is an important mediator of cardiovascular homeostasis through its role in the production of nitric oxide (NO). Nitric oxide has well-recognized vascular effects, and it significantly influences autonomic nervous system activity (Culotta & Koshland, 1992). Nitric oxide is synthesized by several nitric oxide synthase (NOS) enzymes, including eNOS. The endothelium, or inner lining, of blood vessels uses nitric oxide to signal the surrounding smooth muscle to relax, thus resulting in vasodilation and increasing blood flow (Huang, Huang, Mashimo, Bloch, Moskowitz, Bevan, & Fishman, 1995). Nitric oxide is highly reactive and diffuses freely across membranes, making it an ideal signaling molecule. In humans, it has been shown to augment vagal control of the heart (Binkley, Nunziatta, Liu-Stratton, & Cooke, 2005).

The human gene encoding eNOS is localized on chromosome 7q36. A polymorphism of the promoter region of the eNOS gene is associated with a reduction of eNOS activity. Research indicates that variations in this gene may contribute to autonomic imbalance (Binkley et al., 2005). A polymorphism of the gene has been associated with spontaneous coronary artery spasm, potentially because of decreased eNOS expression and nitric oxide production. A reduction in nitric oxide synthesis has been associated with the T to C polymorphism of the eNOS promoter, and patients who are homozygous for the C allele show autonomic imbalance compared to patients who are heterozygous or homozygous for the T allele (Binkley et al, 2005). In particular, individuals homozygous for the “CC” polymorphism of the eNOS promoter have lower amplitude RSA (Brinkley et al., 2005).

Human research has shown a significant increase in measures of sympathetically influenced low-frequency heart rate variability in patients homozygous for the T to C (i.e., “CC”) transition polymorphism. Conversely, there is a significant reduction in the ratio of

parasympathetically governed high-frequency heart rate variability to total heart rate variability in patients homozygous for the polymorphism. As a result, there is a shift toward increased sympathetic activity in patients homozygous for the polymorphism as reflected by the ratio of low-frequency heart rate variability to high-frequency heart rate variability (Binkley et al., 2005).

Similar research has highlighted the role of other versions of eNOS genes. For example, polymorphisms of the eNOS G894T gene have been linked to difficulties regulating heart rate and blood pressure. The presence of the T allele of this gene has been implicated in pathological conditions, including greater risk for hypertension (Shankarishan, Borah, Ahmed, & Mahanta, 2014). Conversely, homozygosity of the G allele is associated with lower diastolic blood pressure during a physical challenge (Nunes, Barroso, Pereira, Krieger, & Mansur, 2014). It appears that the presence of the T allele alters the structure of eNOS and affects its activity by decreasing production of nitric oxide which ultimately leads to higher blood pressure (Shankarishan et al., 2014).

A somewhat surprising finding in at least one study was a significant gene-environment interaction between eNOS and behavioral risk factors like chewing tobacco and consuming alcohol. Specifically the “GG” genotype was associated with increased risk for hypertension among individuals who used tobacco or consumed alcohol (Shankarishan et al., 2014). The molecular effect of the “GG” polymorphism on eNOS enzyme function in this case is still not clear, although several mechanisms have been proposed including stimulation of the sympathetic nervous system and inhibition of nitric oxide (Randin, Vollenweider, Tappy, Jéquier, Nicod, & Scherrer, 1995; Yamada, Noborisaka, Ishizaki, Tsuritani, Honda, & Yamada, 2004).

Nitric oxide can also be produced by eNOS within the central nervous system. However, it remains to be determined whether and how NO release from eNOS can interact directly with neuronal signaling. Animal models show that neocortical long-term potentiation and neurotransmitter release are disrupted in mice lacking the endothelial nitric oxide gene (Haul, Godecke, Schrader, Haas, & Luhmann, 1999; Kano, Shimizu-Sasamata, Huang, Moskowitz, & Lo, 1998; Wilson, Godecke, Brown, Schrader, & Haas, 1999), but the exact cause of these deficiencies is not known.

Interestingly, tests of male mice lacking the endothelial nitric oxide gene (eNOS −/−) revealed an almost complete elimination of some types of aggressive behavior in response to a controlled threat (Demas, Kriegsfeld, Blackshaw, Huang, Gammie, Nelson, & Snyder, 1999). Even in the rare circumstances when these mice did display aggression, its latency was 25 to 30 times longer than eNOS-typical mice. Once again, it is not clearly understood why eNOS −/− mice exhibit low levels of aggression. However, the lack of aggressive (“fight”) behavior in response to threat suggests that eNOS may play a role in modulating arousal and behavioral/physiological response to stress. The research findings indicate that eNOS is involved in long-term potentiation in multiple brain structures within rodents – since eliminating it results in impairment of neocortical and hippocampal long-term potentiation in mice (Haul et al., 1999; Wilson et al., 1999) – may indicate that eNOS somehow plays a role in mouse neuroception.

GABA(a)alpha6 receptor subunit gene (GABRA6)

The glucocorticoid component of the stress response has been the subject of scientific investigation because of the wide ranging pathological consequences that result from excess glucocorticoid exposure, including mood and anxiety disorders, and cognitive impairment (Uhrat et al., 2004). Exposure to stress activates the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic adrenomedullary system, which are regulated by neuronal pathways, including the inhibitory Gamma-aminobutyric acid (GABA) system.

GABA is the chief inhibitory neurotransmitter in the mammalian brain where it acts at GABA-A receptors. It plays a central role in reducing neuronal excitability throughout the central nervous system. In humans, GABA is also directly responsible for the regulation of muscle tone (Watanabe, Maemura, Kanbara, Tamayama, & Hayasaki, 2002).

Research has shown that variations in the GABRA6 gene are associated with risk for pathology, including anxiety (Arias, Aguilera, Moya et al., 2012), drug and alcohol dependence (Han, Bolo, Daniels, Lyoo, Min, Kim, & Renshaw, 2008) and schizophrenia (Duncan, Webster, Rothmond, Bahn, Elashoff, & Shannon Weickert, 2009). More specifically, the T1521C single nucleotide polymorphism in GABRA6 has been shown to attenuate both the HPA-axis and autonomic reactivity during the Trier Social Stress Test (Uhart et al., 2004). Cortisol, diastolic blood pressure, and mean blood pressure responses to this social stressor were significantly greater in subjects homozygous or heterozygous for the T allele of GABRA6 compared to subjects homozygous for the C allele. Along these same lines, the T allele of the T1512C polymorphism has been linked with dimensions of anxiety, including greater harm avoidance, than C allele carriers (Arias et al., 2012). Finally, subjects homozygous for the C allele score significantly lower on extraversion compared to subjects homozygous or heterozygous for the T allele (Uhart et al., 2004). Taken together, these results are indicative of gene-mediated regulation of the stress response. In particular, the T1521C polymorphism in the GABRA6 gene appears to be associated with attenuation in hormonal and blood pressure responses to psychological stress.

Environmental Influences on Stress Response

Through the process of neuroception, the individual is continuously monitoring the environment for evidence of safety versus threat. It is not surprising, then, that a number of studies have demonstrated links between chronic exposure to threat and subsequent alterations in physiological response.

Current conceptualizations about allostatic load (McEwen & Wingfield, 2003) suggest that chronic stress – such as child maltreatment, domestic violence, and growing up in violent neighborhoods – may adversely affect multiple, interrelated stress-mediating systems. A number of studies demonstrate such links across a variety of physiological responses. First, several studies report evidence of physiological *hypo-arousal* associated with chronic exposure to threat and challenge. Exposure to neighborhood violence has been associated with lower baseline heart rates in school-age children at rest (Krenichyn, Saegert, & Evans, 2001), and in lower heart rates in adolescents in response to watching depictions of media violence (Cooley-Quille, Boyd, Frantz, & Walsh, 2001). Moreover, harsh parenting may

exacerbate some of these physiological reactions. Children exposes to high levels of neighborhood violence who also had experienced harsh parenting showed significantly lower systolic and diastolic blood pressure than children whose parenting had not been as harsh (Krenichyn et al., 2001).

In contrast, there also is evidence of *hyper-arousal* in response to chronic exposure to threat. For example, African American children who either witnessed or were victimized by violence were less likely to show a normal drop in blood pressure at night (Wilson, Kliewer, Teasley, Plybon, & Sica, 2002) compared to non-exposed children. In addition, Heim, Newport, Heit, and colleagues (2000) found increased heart rate responses to stress in adults with a history of childhood abuse, whereas children exposed to high levels of family stress show increased PEP reactivity on laboratory challenges (Ellis, Essex, & Boyce, 2005). College-aged participants who had been exposed to neighborhood violence show significantly higher levels of arousal – as measured by galvanic skin conductance and blood pressure volume – in response to viewing movie clips depicting violence (Frost & Stauffer, 1987).

While there is broad evidence for the impact of chronic stress on a wide array of physiological systems, there also is more specific evidence linking experiences of threat and adversity to alterations in RSA. At least one study reports increased vagal reactivity among individuals exposed to long-term adversity. Dale, Carroll, Galen, Hayes, Webb and Porges (2009) reported that self-reported history of abuse was associated greater RSA reactivity and an inability to rapidly re-engage vagal regulation following a physical challenge. This pattern is consistent with research that shows greater RSA reactivity among infants with insecure avoidant attachments compared to those with secure attachment (Hill-Soderlund, Mills-Koonce, Propper, et al., 2008). Taken together, the findings are consistent with clinical impressions that abused individuals may have a lower threshold for a physiological response that supports fight or flight behaviors in response to stress, and may have difficulty shifting from mobilization strategies to states of calm (Dale et al., 2009).

On the other hand, several studies show a different association between experiences of adversity and RSA response. For example, there is evidence that victims of child maltreatment manifest lower absolute levels of RSA in both resting and challenge conditions (Miskovic, Schmidit, Georgiades, Boyle, & MacMillan, 2009; Skowron et al., 2011). In addition, several studies report that children exposed to early chronic adversity in the caregiving environment – including physical and sexual abuse, hostile and controlling caregiving, parental distress and substance use, and poverty – are significantly more likely to display lower levels of vagal withdrawal and less RSA reactivity in response to challenge (Calkins, Smith, Gill, & Johnson 1998; Conradt, Degarmo, Fisher, et al., 2014; Dale et al, 2009). Lower levels of RSA at rest and reduced RSA reactivity during challenge have been predictive of poor outcomes in children due to an underregulation of sympathetic nervous system activity (Beauchaine et al., 2007). There even is evidence that higher levels of RSA may offer protection against the effects of maltreatment on problem behavior (Gordis, Feres, Olezeski, Rabkin, & Trickett, 2010). However, research suggests that early experiences of chronic threat and challenge can alter the growth trajectory of RSA regulation (Conradt et al., 2014), resulting in some children displaying less growth in RSA regulation over time

(Rigterink, Katz, & Hessler, 2010). Regardless of whether children experience early adversity, children with decelerations in RSA reactivity display increases in behavioral dysregulation as they mature (Conradt et al., 2014). Even children who display a typical drop in RSA in response to challenge may be at increased risk for poor outcomes if this system is not coordinated with other physiological systems. For example, Shenk and colleagues (2010) report that victims of childhood sexual abuse were more likely than others to show asymmetry in RSA and cortisol responses to challenge. In these cases, the individual displayed appropriate RSA reactivity, but it was matched with a blunted cortisol response. These poorly coordinated, asymmetrical physiological responses predicted higher levels of depression and antisocial behavior in young adulthood (Shenk, Noll, Putnam, & Trickett, 2010).

Finally, some children exposed to early adversity exhibit a very different pattern of vagal response. For example, rather than lowering RSA in response to challenge, some children display increased levels of RSA during challenge conditions. This up-regulation reflecting a pattern of vagal augmentation has been seen in children exposed to domestic violence (Katz, 2007) and children raised in foster care following experiences of abuse (Oosterman, de Schipper, Fisher, Dozier, & Schuengel, 2010). For some children, chronic exposure to fearful conditions may interfere with sympathetically mediated mobilization strategies associated with fight or flight behaviors. The result may be a disruption in more organized responses to challenge. Along these lines, vagal augmentation in threat-exposed children has been linked to disordered attachment and conduct disordered behavior (Katz, 2007; Oosterman et al., 2010).

Gene-Environment (G x E) Interactions and Differential Response to Adversity

Clearly, not all children respond to conditions of chronic adversity in the same way. Research on the relationship between RSA and childhood adversity reveals a number of different, and seemingly contradictory, physiological responses. The concept of differential susceptibility provides a useful framework for understanding the plasticity observed in RSA responses across different studies (Belsky & Pluess, 2009). Undoubtedly, individual differences in genetic make-up and biological predisposition interact with uniquely specific experiences to shape the developmental response of each person. As a result, research on gene-environment (G x E) interactions has become increasingly common and useful.

Already, there have been numerous important studies examining the interactions between specific environmental pathogens and particular candidate genes (cf. Caspi, Hariri, Holmes, Uher, & Moffitt, 2010; Karg, Burmeister, Shedden, & Sen, 2011; Mehta & Binder, 2012). As useful as these studies have been, there is a need to examine more complex G x E models (Mehta & Binder, 2012). For example, examining G x G x E as well as G x E x E effects is likely to increase our understanding of important phenomena in a manner consistent with a multilevel analysis as prescribed by an ecological-transactional model.

Hypotheses

Based on the research reviewed above, the current study was guided by the following hypotheses in examining determinants of physiological regulation.

1. We expected that polymorphisms of the eNOS G894T and GABRA6 genes, as well as exposure to child maltreatment and neighborhood crime, would predict RSA variability in children.
2. We expected that polymorphisms in eNOS and GABRA6 would moderate the effects of child maltreatment and neighborhood crime on RSA variability in children.
3. We expected to identify complex interactions among genes and environmental pathogens – i.e., (a) G x G, (b) E x E, (c) G x G x E, and (d) G x E x E – to further clarify the multilevel effects of environmental adversity on RSA variability.

Method

Participants

Participants in this investigation included 186 high-risk urban children and their caregivers from upstate New York. All children were living with their biological mothers at the time of enrollment. The sample was ethnically diverse, with African American, Latino, Caucasian, and biracial families represented (88.7% of the sample was non-Caucasian; see Table 1). Children were recruited as preschoolers at the age of four years (*M* age = 4.2, *SD* = 0.17) and were assessed again at age nine (*M* age = 9.3, *SD* = 0.18).

Recruitment of maltreated children (N = 93) focused on children who had been identified through the Department of Human Services (DHS) as having documented histories of physical neglect. A DHS staff member who was assisting with the project identified eligible children and made the initial contact with families to ascertain interest in participation and to obtain permission to share their contact information with the project team.

To obtain a demographically matched comparison group, nonmaltreating families (N=93) were selected randomly from the County recipients of Temporary Assistance for Needy Families (TANF). Because the majority of maltreating families referred to Child Protective Services are socioeconomically disadv-antaged and receiving public assistance (95.7% in the current study; Gaudin, 1999; Sedlak, Mettenburg, Basena, Petta, McPherson, Greene, & Li, 2010), utilization of TANF lists provided access to a demographically similar population. In the initial contact, consents were obtained to verify nonmaltreatment status by reviewing DHS central registry data. Medical records also were reviewed to confirm nonmaltreatment status. Maltreated and nonmaltreated children were demographically similar on a number of characteristics, including gender, ethnicity, number of adults in the home, and family history of public assistance (see Table 1).

Procedure

Parents of all maltreated and nonmaltreated children provided informed consent for their child's participation, as well as consent for examination of any Department of Human Services (DHS) records pertaining to the family. Participating families completed initial assessments of children's functioning and the family environment, including demographic information on socioeconomic status, children's race, and ethnicity. Family addresses were obtained at age four to link with neighborhood crime statistics. Families were re-contacted when the children turned nine, and follow-up visits assessed children's physiological response during periods of rest and challenge. Parents signed consent for their children's participation – including the obtaining of DNA samples from the children, as described below. In addition to parental consent, children gave assent for their participation at age nine.

Measures

Child Neglect and Abuse Histories—Permission to review DHS records was obtained from all participating families. Histories of maltreatment were coded using the structured nosological system developed by Barnett, Manly, & Cicchetti (1993). This classification system captures detailed information from CPS records by using a structured approach to obtain systematic data from the narrative and investigation determinations contained in DHS records (Manly, 2005). This system provides independent confirmation of neglect and other forms of maltreatment through detailed coding of records. It captures information on the occurrence of multiple subtypes of maltreatment, including extensive information on severity, chronicity, perpetrator of maltreatment, and the developmental period during which it occurred. Neglect is coded when there is evidence that a caregiver has failed to exercise a minimum degree of care in meeting the child's needs or failed to take adequate precautions to ensure the child's safety. Neglect can range from frequently missed meals, unsanitary living conditions, and failure to provide adequate supervision to severe malnutrition, gross inattention to medical needs, or failure to supervise the child that resulted in life-threatening situations. Adequate reliability for coding of maltreatment subtypes and severity has been demonstrated, with intraclass correlations ranging from .81 to 1.0 across subtypes (Manly, 2005).

Before the age of 4, 93 children had been maltreated and 93 children had no documented history of maltreatment as confirmed by a review of the records. Among the group of maltreated children, 47 children had documented cases of physical neglect (in the absence of other forms of maltreatment), including reports of Lack of Supervision, Failure to Provide, and Moral/Legal Maltreatment (Barnett, et al., 1993). The remaining 46 maltreated children had experienced other forms of abuse prior to age 4 in addition to physical neglect; 28.3% had experienced physical abuse, 2.2% had experienced sexual abuse, and 80.4% had experience documented emotional maltreatment.

Neighborhood Crime—Violence occurring in neighborhoods was assessed through the use of crime reports within census tracts. Government census tracts provide units of analysis for which numerous types of data are readily available. We focused on data pertaining to violent crime within census tracts. Children's addresses at age 4 were linked to annual

Police Department crime statistics for the census tract in which they lived at that time. The number of violent crime incidents (including homicide, rape, aggravated assault, and robbery) for that current year was recorded for each geographic area. Across census tracts, incidents of violent crime ranged from 0 to 4 homicides (mean = 0.71, SD = 0.88), 0 to 6 rapes (mean = 1.56, SD = 1.38), 0 to 41 aggravated assaults (mean = 13.6, SD = 8.77), and 2 to 66 robberies (mean = 14.08, SD = 10.53). These data gave us an approximation of neighborhood conditions that provided a backdrop for children's exposure to violence. Although, census tracts do not necessarily represent the neighborhood as it would be defined by the residents themselves (Coulton, Korbin, Su, & Chow, 1995), data based on census tracts have been used successfully in a number of studies examining the functioning of high-risk samples (Aber, Jones, Brown, Chaudry, & Samples, 1998; Korbin, Coulton, Chard, Platt-Houston, & Su, 1998). A principle components factor analysis was used to create a weighted composite of neighborhood crime based on analyzing the homicide, rape, aggravated assault, and robbery data. A median split of the data was used to identify children who at age 4 were living in neighborhoods characterized by high versus low levels of crime.

RSA—When the children were 9 years old, they were brought to the lab for a follow-up assessment. During this visit, heart period was recorded continuously during the conditions described below via three electrodes with an ECG100C Electrocardiogram Amplifier (BIOPAC Systems, Goleta, CA) using Acknowledge data acquisition software to facilitate R-wave detection. The ground electrode was placed above the collarbone on the right side, and the two active electrodes were placed on the left side (ribs) at heart level and on the right lower abdomen.

Data were collected across three conditions. During the initial baseline, children sat quietly and for five minutes and watched a peaceful clip from a children's video (*The Snowman*). The video segment contained music and animation, but no spoken language. Following the completion of the video, children were administered three subscales from the WISC-IV (i.e., Digit Span, Coding, and Block Design). These tasks represented a cognitive challenge for the children. Finally, children watched a different 4-minute clip from the same *Snowman* video. This final condition represented a second baseline. As a result, we were able to measure heart period variability in an initial resting state, during a subsequent challenge condition, and finally during a return to rest.

Throughout each condition, the ECG monitor was able to measure the R-R interval (heart period) to the nearest millisecond. The heart period data was stored for off-line editing and analysis. Data files for each subject initially were input into CardioEdit software (Brain-Body Center, University of Illinois at Chicago, 2007) to visually display the sequential heart period data, and the data were then manually edited by a trained and reliable research assistant to remove R-wave detection artifacts (Porges & Byrne, 1992). RSA was quantified according to the technique established by Porges (Porges, 1985; Porges & Bohrer, 1990). Using CardioBatch software (Brain-Body Center, University of Illinois at Chicago, 2007), this method detrends the data and accurately quantifies the variance in heart period within the estimated frequency band associated with spontaneous breathing. Finally, the natural logarithm of the variance is calculated as the measure of RSA (Riniolo & Porges, 1997).

This method enables the calculation of RSA during relatively short time periods. The amplitude of RSA was calculated during sequential 30 second epochs. For each of the three conditions, the initial 30 second epoch was truncated. For the two baseline conditions, the subsequent 3.5 minutes of data were used for analysis. For the cognitive challenge, the subsequent 10 minutes of data were used. The average of the 30-second epoch values within each condition (i.e., baselines and cognitive challenge) was used in the data analysis.

DNA Collection, Extraction, and Genotyping—Using an established protocol, trained research assistants obtained DNA samples from participants during their age-9 follow-up visit by collecting buccal cells with the Epicentre Catch-All Collection Swabs. Subsequently, DNA from the participants was submitted to the BioMedical Genomics Center at the University of Minnesota for quantity and quality testing and subsequent SNP genotyping. Using the conventional method, human genomic DNA was extracted with the Epicentre BuccalAmp DNA Extraction Kit (Epicentre, Cat. No. BQ0901SSC) and amplified using the Repli-g kit (Qiagen, Catalog No. 150043) per the kit instructions. Amplified samples were then diluted to a working concentration and genotyped for SNPs rs1799983 – *Endothelial nitric oxide synthase gene (eNOS)* – and rs3219151 – *GABA(a)alpha6 receptor subunit gene (GABRA6)* – using pre-degisned TaqMan assays from Applied Biosystems, Inc. (Catalog C___3219460_20, C__11263956_10 respectively). Individual allele determinations were made using these assays with TaqMan Genotyping Master Mix (Applied Biosystems, Catalog 4371357) and amplification on an ABI 9700 thermal cycler then analyzing the endpoint fluorescence using a Tecan M200 using JMP 10.0 (SAS, Inc.).

Genotype distributions for both SNPs included in the analyses are presented in Table 2. All genetic polymorphisms were in Hardy-Weinberg equilibrium.

Ancestral Proportion Determinations—DNA samples were subjected to SNP genotyping of the Burchard et al. panel of 106 SNPs (Lai et al., 2009; Yaeger et al., 2008), known to be informative for ancestry from Africa, Europe, and Native America. The SNPs were genotyped using the iPLEX platform from Sequenom Bioscience, Inc which uses the Sequenom MassArray. The SNP genotyping results were then recoded and uploaded into STRUCTURE v2.3.4 which uses algorithms developed by Pritchard et al. (Falush Stephens, & Pritchard, 2003, 2007; Hubisz, Falush, Stephens, & Pritchard, 2009). The data were uploaded into the software and set to analyze with an Admixture model of ancestry and initialization of the simulation on the GALA cohort. The simulation was set to run with a Burn-in of 10,000, MCMC Reps of 1,000 and assuming 3 populations within the group. The results of the simulations were subsequently identified as the percent association to each ancestry group – African, Native American, and European – based on the known ancestry of the GALA cohort. It was possible, therefore, to generate three separate ancestry variables reflecting continuous proportion scores of the ancestrally important markers (AIMs) for each participant.

Results

Plan of Analysis

We used repeated measures analysis of covariance (ANCOVA) to examine the influence of maltreatment status, neighborhood crime, polymorphisms for each of the two candidate genes, and their 2-way (G x E, G x G, E x E) and 3-way interactions (G x G x E, and G x E x E) on individual differences in the amplitude of RSA across measurement conditions. Bonferroni-corrected contrasts were used in follow-up analyses to identify significant group differences and simple effects within any significant interaction effects.

Gender and ancestry were included as covariates in the ANCOVA model given the relevance of these potentially confounding variables in interpreting the results. Following the recommendations of Keller (2014), rather than simply control for these variables as covariates, we initially evaluated a model that included both gender and ancestry main effects and their interactions with maltreatment status, neighborhood crime, and genotypes. Before testing this model, we used the continuous AIMs scores as independent variables in a k-means cluster analysis in order to identify distinct ancestral groups, rather than rely on self-reported race to examine the effects of ancestry/ethnicity. Three groups were identified. The first group (n = 130) was highly homogeneous for the African ancestral markers (M_{Af} = .92, SD=.10). The second group (n = 32) was strongly homogeneous for the Euopean ancestral markers (M_{Eu} = .81, SD=.20). The third group (n = 24) was most strongly associated with the Native American ancestral markers (M_{NA} = .70, SD=.20). None of the interactions with gender or ancestry group were significant. Moreover, the other main effects and interaction effects involving the study's independent variable were all consistent with those same effects when the interactions with gender and ancestry group were excluded. Since we did not have specific hypotheses about interactions with gender or ancestry, for the sake of parsimony we included gender and individual AIMs scores as covariates in the model. Those results are presented below.

Evidence for Gene-Environment Correlations

We first examined whether there were differences between the maltreated and nonmaltreated groups on the frequency distributions of the eNOS and GABRA6 genotypes. Chisquare analyses revealed that for eNOS, genetic variation was unrelated to maltreatment status (see Table 3). Conversely, the distributions of CC versus CT or TT variants of GABRA6 did differ according to maltreatment status, χ^2 (2) = 6.11, p=.047. Homozygosity for the C allele of GABRA6 tended to be more common among maltreated children in this sample, suggesting possible evidence of a gene–environment correlation. Genetic variation on eNOS and GABRA6 was not associated with exposure to Low vs. High levels of neighborhood crime (see Table 4).

Change in RSA and G x E Effects

After controlling for the covariates, repeated measures ANCOVA with a Greenhouse-Geisser correction indicated that there was not a significant within-subject main effect of experimental condition on RSA, F (1.87, 299.59) = 1.05, p = .35. This suggests that RSA did not change in response to a cognitive challenge in a consistent way across all levels of

the between-subject independent variables. Moreover, none of the between subject variables themselves demonstrated significant overall effects on RSA: eNOS, F (1, 160) = 0.06, p = .81; GABRA6, F (1, 160) = 2.32, p = .13; maltreatment status, F (2, 160) = 0.41, p = .66; and neighborhood crime, F (1, 160) = 1.68, p = .20.

However, more than these main effects on RSA the primary concern for the current study was whether genes, environments, and their coactions influenced RSA response to, and recovery from, challenge. Individual genotypes by themselves did not interact with experimental condition to influence changes in RSA: Condition x eNOS, F (1.87, 299.59) = 2.16, p = .12; Condition x GABRA6, F (1.87, 299.59) = 0.65, p = .52. However, a significant Condition x G (eNOS) x G (GABRA6) interaction was associated with change in RSA, F (1.87, 299.59) = 3.69, p = .029.

As seen in Figure 1, there are a several simple effects of experimental condition on RSA for different combinations of the eNOS and GABRA6 genotypes. Specifically, for the GABRA6 CT or GT genotypes, there is significant change in RSA in response to challenge, as well as significant recovery in RSA following the challenge, regardless of the eNOS genotype (see Figure 1b). However, among children who were homozygous for the C allele of GABRA6, polymorphisms of eNOS were associated with differences in vagal regulation of the heart (see Figure 1a). Only homozygosity of the G allele of eNOS was associated with significant changes in RSA among the GABRA6 CC genotype group. The presence of the T allele of eNOS in the GABRA6 CC genotype was associated with no significant modulation of RSA in response to challenge.

There also was a simple effect of GABRA6 on RSA for one particular combination of experimental condition and eNOS genotype. For children who had either the GT or TT eNOS genotype, RSA measured during the challenge condition was significantly higher for the GABRA6 CC genotype than it was for the CT or TT genotypes, F (1, 160) = 3.97, p = .048 (see Figures 1a and 1b). Homozygosity for C allele of GABRA6 seems to have prevented a drop in RSA in response to challenge for those whose eNOS genotype includes the T allele, but not for those who are homozygous for the G allele of eNOS. The simple effects of eNOS on RSA within each combination of experimental condition and GABRA6 genotype were all nonsignficant.

There were trends in the data suggesting that individual indices of environmental adversity affected vagal regulation of the heart in response to challenge, but the interactions between experimental condition and both maltreatment status [F (3.75, 299.59) = 1.87, p = .12] and neighborhood crime [F (1.87, 299.59) = 2.53, p = .08] did not exert a statistically significant effect on RSA. However, a significant Condition x E (maltreatment) x E (neighborhood crime) interaction did predict change in RSA, F (3.75, 299.59) = 3.49, p = .01. As shown in Figure 2, there are a number of simple effects of experimental condition on RSA for different combinations of maltreatment status and neighborhood crime. Specifically, when children lived in low crime neighborhoods at age 4 they showed more consistent ability to modulate RSA in response to challenge at age 9 regardless of their maltreatment status (see Figure 2a). However, when children lived in high crime neighborhoods, only nonmaltreated children showed significant modulation of RSA across conditions (see Figure 2b). The

simple effects of neighborhood crime on RSA within each combination of experimental condition and maltreatment status, and the simple effects of maltreatment status on RSA within each combination of experimental condition and neighborhood crime were all nonsignficant.

Finally, there was evidence that genes modified the effects of this E x E interaction with experimental condition. There was a significant Condition x G (GABRA6) x E (maltreatment) x E (neighborhood crime) interaction that predicted changes in RSA, F (3.75, 299.59) = 4.29, p = .003. Tests of the simple effects of experimental condition on RSA revealed that the interaction between child maltreatment and neighborhood crime predicted different patterns of change in vagal regulation depending on the child's GABRA6 genotype (see Figure 3).

Specifically, within the GABRA6 CC genotype only nonmaltreated children demonstrated statistically significant modulation of RSA in response to challenge (see Figures 3a and 3b). Neither of the maltreated groups of children with the CC genotype displayed significant changes in RSA across conditions. It is noteworthy that nonmaltreated children with the CC genotype who had lived in high crime neighborhoods at age 4 demonstrated the most robust regulation of RSA, displaying both a significant drop in RSA in response to challenge, followed by a significant increase in RSA after the challenge had passed (see Figure 3b). A different pattern emerged among children with the T allele of GABRA6 (see Figures 3c and 3d). Once again, nonmaltreated children displayed significant modulation of RSA across conditions. However, even children who had been neglected with additional maltreatment were able to modulate RSA if they had the T allele of GABRA6. This was especially true for these maltreated children if they had lived in low crime neighborhoods at age 4, in which case they displayed both a significant decrease in RSA followed by a significant increase (see Figure 3c). Although the data suggested trends in the modulation of RSA among CT or TT genotype children who had only been neglected, changes in their RSA scores across conditions were not significantly different.

Tests of the simple effects of neighborhood crime on RSA revealed a significant effect for one particular group. Children with the T allele of GABRA6 who had been neglected without other forms of maltreatment displayed significantly higher RSA across all three experimental conditions – Baseline 1, F (1, 160) = 4.68, p = .032; Challenge, F (1, 160) = 4.91, p = .028; Baseline 2, F (1, 160) = 4.32, p = .039 – if they had lived in high crime neighborhoods at age 4 rather than low crime neighborhoods (see Figures 3c and 3d). The effects of neighborhood crime for all other combinations of maltreatment status and GABRA6 genotype were nonsignificant. Likewise, the simple effects of maltreatment status on RSA for all combinations of neighborhood crime and GABRA6 genotype were nonsignificant. Finally, tests of the simple effects of the GABRA6 genotype on RSA revealed significant effects for some groups of children. In particular, neglected children who had lived in low crime neighborhoods at age 4 displayed significantly higher RSA across all three experimental conditions – Baseline 1, F (1, 160) = 4.83, p = .029; Challenge, F (1, 160) = 3.97, p = .048; Baseline 2, F (1, 160) = 4.96, p = .027 – if they were homozygous for the C allele of GABRA6 rather than heterozygous for the T allele (see Figures 3a and 3c). In addition, nonmaltreated children who had lived in high crime

neighborhoods at age 4 showed significantly higher levels of RSA during the final baseline condition if they were homozygous for the C allele of GABRA6 rather than heterozygous for the T allele, F (1, 160) = 6.49, p = .012 (see Figures 3b and 3d).

None of the other repeated measures interaction effects that were tested were statistically significant: Condition x eNOS x Maltreatment Status, F (3.75, 299.59) = 0.44, p = .78; Condition x eNOS x Neighborhood Crime, F (1.87, 299.59) = 1.31, p = .27; Condition x GABRA6 x Maltreatment Status, F (3.75, 299.59) = 1.59, p = .18; Condition x GABRA6 x Neighborhood Crime, F (1.87, 299.59) = 0.35, p = .69; Condition x eNOS x GABRA6 x Maltreatment Status, F (3.75, 299.59) = 1.49, p = .21; Condition x eNOS x GABRA6 x Neighborhood Crime, F (1.87, 299.59) = 0.33, p = .70; Condition x eNOS x Maltreatment Status x Neighborhood Crime, F (3.75, 299.59) = 0.35, p = .83.

Discussion

Data from the current study provide evidence of complex multilevel interactions among objective neighborhood conditions, caregiving adequacy in the home, and children's genotype in predicting their physiological response to challenge. Of particular note for this multilevel analysis is the finding that neighborhood crime at age 4 played a consistent role in children's vagal response five years later. Crime rates were measured objectively via police reports within particular geographic regions. However, the children in this study were not necessarily victims of these crimes, and as four year olds they may have barely been aware of most of these crimes. Nonetheless, neighborhood crime – through its interaction with caregiving and children's genes – contributed to processes that shaped how individual children characteristically regulated their heart rate in response to challenge. The fact that such a distal ecological system could influence such a proximal biological system highlights the importance of multilevel analyses. In this case, it points out that in order to understand the impact of child maltreatment on children's development, it is critical to simultaneously examine the back-drop in which it occurs (e.g., neighborhood crime) and the endogenous characteristics of the individual to whom it is happening (e.g., genetic polymorphisms).

The importance of vagal regulation of the heart for adaptive functioning and its role in risk for psychopathology has been articulated by a number of researchers (Beauchaine, 2001; Beauchaine et al., 2007; Porges, 2001, 2007). High levels of RSA and its reliable suppression or reduction are positive indices of social and emotional regulation (Porges, 2007). In particular, withdrawal of the vagal break in response to challenge shifts neurological regulation of the heart to the sympathetic nervous system in order to provide the cardiac and metabolic output necessary for behavioral mobilization. When the challenge has passed, vagal pathways restore parasympathetic control of the heart to promote calm states and allow for social engagement. In the current study, this typical pattern of change in RSA was not consistently observed in our multi-risk sample.

In contrast, low levels of RSA and its unreliable modulation appear to increase risk for difficulties in social and emotional regulation, and in some cases may contribute to psychopathology (Porges, 2007). It seems likely that there may be an optimal level of vagal control of the heart that both promotes calm states while simultaneously facilitating a

preparedness to respond to perceived challenges (Beauchaine, 2001). Deficient levels of RSA may interfere with social engagement in non-challenge conditions and – because RSA is already low – may limit the deployment of additional resources to constructively engage the environment in response to challenge.

However, since RSA is a dynamic system it is important to consider how RSA changes from its baseline state. For example, excessive vagal withdrawal in response to challenge has been linked to a variety of behavior problem profiles (Calkins et al., 2007). Beauchaine et al. (2007) has argued that when the vagal system is compromised response strategies may shift to sympathetic nervous system mediated fight or flight response that activate strong approach or avoidance emotions. In contrast to excessive vagal withdrawal, some people show evidence of vagal augmentation in response to challenge. It has been argued that this increase in RSA may occur in the service of focused attention (Jacobson, Gottman, Shortt, & Wu, 1995). For example, children who have been exposed to domestic violence, or who otherwise may be at risk for conduct problems, may be particularly sensitized to violence and may have become hypervigilant to signs of interpersonal threat. Several studies have reported evidence of vagal augmentation among such children (Calkins et al., 2007; Katz, 2007; Kwong, Bartholomew, Henderson, & Trinke, 2003). If vagal augmentation is associated with hypervigilance and focused attention, it may suggest these children are paying especially close attention to violence and aggression in their environment, which may increase their encoding of aggressive behavior. As a result, a pattern of vagal augmentation may mediate the relationship between exposure to violence and the subsequent enactment of aggression and hostility toward others (Katz, 2007).

Findings from the current study provide evidence of factors from multiple levels that may contribute to alterations in RSA and vagal regulation of the heart among maltreated children. To begin, there was evidence of a G x G interaction – regardless of children's maltreatment history – that predicted differences in RSA response across conditions. Among children with the T allele of GABRA6, similar patterns of significant RSA reactivity and recovery were observed regardless of the child's eNOS genotype. However, among children who were homozygous for the C allele of GABRA6, different polymorphisms of eNOS were associated with different patterns of vagal response. Children who were homozygous for the G allele of eNOS displayed a typical reduction in RSA during the cognitive challenge, followed by a re-engagement of parasympathetic control of the heart after the challenge had ended, as evidenced by an increase in RSA to its previous level. Children with the T allele of eNOS displayed a different pattern. Although their initial level of RSA was similar to the G allele children, these children did not show a drop in RSA in response to the challenge, and their RSA remained unchanged after the challenge had passed. This pattern of unmodulated RSA (and possible vagal augmentation) may have been in service of increasing focused attention during the challenge. However, the molecular effects of the two polymorphisms associated with this atypical vagal response require further examination and explanation. The T allele of eNOS has been associated with decreased production of nitric oxide, which would impact vasodilation and blood flow (Huang et al., 1995). In addition, GABA is the chief inhibitory neurotransmitter in humans, and homozygosity for the C allele of the gene regulating this GABA-A receptor has been associated with less, or attenuated, physiological reactivity (Uhart et al., 2004). Taken together, it may be possible to develop a model

indicating how these two genetic polymorphisms contribute to a reduction and alteration in RSA reactivity.

There also was evidence of an E (neighborhood crime) x E (child maltreatment) interaction associated with different patterns of RSA change across conditions. For example, RSA reactivity among maltreated and nonmaltreated children from low crime neighborhoods was highly similar. However, maltreatment experiences were associated with different patterns of RSA reactivity among children whose families lived in neighborhoods characterized by high levels of crime when the children were four years old. It appears that the environmental toxin of child maltreatment may interfere with successful physiological regulation and modulation of RSA among children who are faced with the additional adversity of neighborhood crime.

It is important to note that this E x E interaction was qualified by its interaction with GABRA6. Among children who were homozygous for the C allele of GABRA6, only nonmaltreated children demonstrated the ability to modulate RSA in response to challenge. Evidence of vagal regulation was especially apparent among nonmaltreated children who had lived in high crime neighborhoods at age 4, indicating that these children are able to deploy mobilization resources in response to challenge and then return to a state of homeostatic calm once the challenge has passed. On the other hand, children who experienced child neglect – either in isolation or in combination with other forms of maltreatment – did not show changes in RSA across experimental conditions if they had the CC GABRA6 genotype, whether they came from high or low crime neighborhoods.

It is possible that this lack of RSA reactivity may represent a type of adaptation among maltreated children that is facilitated by the CC GABRA6 genotype. Children whose parents neglect and/or maltreat them may have learned that it is important to always be vigilant since they may not be able to count on their parents for help in time of need. Maintaining stable levels of vagal control may facilitate processes of neuroception that focus attention on signs of threat, and it is possible that homozygosity for the C allele of GABRA6 facilitates or potentiates attenuated vagal reactivity for these children. However, this particular adaptation to the environment may interfere with these children being able to appropriately deploy mobilization resources to engage the environment in response to threat, and may leave maltreated children who face additional environmental adversity – such as those living in violent neighborhoods – especially unprepared to cope with new challenges. Moreover, the amplitude of the initial RSA set-point may be important to consider, especially when vagal reactivity is suppressed. A low RSA set-point for maltreated children with the CC GABRA6 genotype may leave them in a less well-regulated, more sympathetically controlled physiological state with a compromised ability to deploy further resources for mobilization in response to challenge (Beauchaine, 2001). Taken together, these findings suggest that homozygosity for the C allele of GABRA6 may contribute to risk for psychopathology among neglected and multiply maltreated children due to its association with the attenuation of appropriate change in RSA in response to, and following a challenge (Beauchaine et al., 2007; Porges, 2007).

In contrast, both nonmaltreated and maltreated children with the T allele of GABRA6 were able to modulate their RSA levels in response to challenge, although the trends in RSA for children who only experienced neglect were not statistically significant. The RSA levels of the neglected children in this study were less a function of experimental condition, and more a function of neighborhood crime and GABRA6 genotype. Among neglected children from low crime neighborhoods, those with the CC GABRA6 genotype displayed significantly higher levels of RSA across all conditions than those neglected children with the T allele of GABRA6. In addition, neglected children with the T allele of GABRA6 showed significantly higher RSA across all conditions if they had lived in a high crime neighborhood at age 4, rather than a low crime neighborhood. The vagal regulation of neglected children relative to that of those who have been multiply maltreated warrants further investigation.

It is also worth noting that there was evidence in the current study of a possible gene-environment correlation between GABRA6 and child maltreatment. Homozygosity for the C allele of GABRA6 was significantly related to maltreatment status, such that multiply maltreated children were mostly likely to be homozygous for the C allele and nonmaltreated children were least likely. It is not clear how to interpret this correlation or specify its possible impact. It is possible that the correlation is passive in nature. Perhaps GABRA6 in the parents contributes to the caregiving environment they create, and these parents then contribute their genetic material to their children. Or it could be the case that the association between GABRA6 and child maltreatment reflects an evocative correlation in which the children's phenotype (based on their genotype) elicits a particular caregiving response. In either case, it is important to further investigate this possible gene-environment correlation so that we can properly interpret the G x E interactions reported in the current study (Eaves, Foley, & Silberg, 2003; Mehta & Binder, 2012). In this regard, it is interesting to note that there is evidence that physically abusive and neglecting mothers have been found to exhibit significantly lower amplitude RSA (at baseline and in response to challenge) than nonmaltreating mothers (Skowron et al., 2011). Although these investigators did not genotype their participants, the current study provided evidence that homozygosity of the C allele for GABRA6 was associated with attenuated RSA reactivity in maltreated children. It is possible that lower and attenuated RSA responses among maltreating mothers (Skowron et al., 2011) may also be associated with polymorphisms of GABRA6, in which case the gene-environment correlation observed in the current study may be passive in nature. These issues warrant careful examination and replication in the future.

It should be noted that there has been some debate in the literature about the appropriate measurement of RSA (Denver, Reed, & Porges, 2007; Lewis, Furman, McCool, & Porges, 2012). In particular, since RSA reflects coordination between cardiac activity and respiration, the assumptions is that the frequency of respiration needs to be monitored – in addition to variability in heart period – in order accurately measure the amplitude of RSA. The measure of RSA used in the current study did not incorporate a direct assessment of respiration. Rather, RSA was calculated following the procedure described by Porges (Porges, 1985; Porges & Bohrer, 1990) based solely on data derived from heart period variability. A review of studies that compared the amplitude of RSA based on measures that included respiratory parameters and those that did not concluded that all the measures of

RSA were highly inter-correlated. However, RSA quantification using the Porges-Bohrer method actually may have the strongest psychometric properties (Lewis et al., 2012).

As indicated above, it will be critically important in the future to understand more fully the complex and interacting molecular effects of particular genetic polymorphisms on the vagal regulation of maltreated children. Understanding these effects may help us to identify children who are most at risk for the atypical physiological responses to stress that may contribute to disregulated behavior and emotion. Moreover, findings from the current study confirm the importance of including multiple levels of analysis in studies of the effects of maltreatment (and other toxic environments). In fact, these findings suggest that more complex G x E interactions should routinely be examined, including G x G x E and G x E x E interactions. It will also be important for future studies to carefully examine potential gene-environment correlations and devise longitudinal strategies for effectively understanding the nature of these correlations in order to interpret apparent G x E interactions accurately. Along these lines, it may be wise for future studies of child maltreatment to genotype both parents and children when possible. However, such requests for genetic material will need to handled with heightened sensitivity to any concerns that multi-risk families may have.

It was also noteworthy that neighborhood crime at age 4 was consistently related to vagal regulation five years later, despite the fact that children may have been minimally – or indirectly – aware of this characteristic of their neighborhoods. Further examination of the processes through which this more distal ecological factor exerted influence on subsequent physiological response is warranted. Perhaps our measure of neighborhood crime acted as a proxy measure for more broad environmental stress that influenced the development of children's stress regulation systems. Data from the current study indicate that neighborhood crime interacted with child maltreatment in predicting RSA, and thus provided a back-drop in which maltreatment occurred for some children. But how do these characteristics of communities interact with the capabilities and vulnerabilities of parents in ways that shape their caregiving and the subsequent development of their children? More fine-grained longitudinal studies are required to model these complex multilevel processes. For example, one thing that was not measured in the current study – and thus not controlled – was children's age-4 RSA reactivity. As a result, it is not possible to say with certainty that these early experiences of maltreatment and exposure to neighborhood crime actually changed children's pattern of RSA reactivity. Subsequent attempts to replicate the current findings should specifically examine change in RSA reactivity over time in order to specify the potential causal effects of adverse environments – interacting with genes – on physiological regulation.

Finally, findings from the current study have implications for intervention. Although improvements in parenting quality and declines in the recidivism of child maltreatment have been achieved through randomized controlled interventions (e.g., Chaffin, Funderburk, Bard, Valle, & Gurwitch, 2011), it remains true that child maltreatment is particularly resistant to intervention (Skowron & Reinneman, 2005). Mechanisms of change associated with effective interventions revolve around increasing parents' positive social engagement with their children (Hakman, Chaffin, Funderburk, & Silovsky, 2009). However, positive

social engagement with their children is exactly the issue that is problematic for maltreating parents, and it may be the case that atypical and maladaptive patterns of vagal regulation (Skowron et al., 2011) interfere with some parents' ability to effectively activate and regulate their social engagement systems with their children (Porges, 2007). Findings from the current study indicate that parents' extreme difficulties with appropriate social engagement with children – seen in the form of child maltreatment – may influence their children's vagal regulation, with cascading effects that impact children's behavioral and emotional regulation and risk for psychopathology. Moreover, the current study makes it clear that the impact of child maltreatment on children's physiological response is modified by additional factors at multiple levels of the ecology, including the neighborhood and the child's own genes.

As the transactions among these factors across multiple systems continue to be examined and understood, it suggests that interventions should be designed and implemented at multiple levels. For example, beyond reducing levels of crime within neighborhoods, helping families develop effective strategies to buffer themselves from the effects of crime may result in cascading effects on caregiving and on children's stress regulation. Since the data continue to indicate that people are differentially susceptible to particular environmental adversity, identifying people – both parents and children – who are genotypically vulnerable to particular adversity may be an important step in designing individualized interventions. The goal of these approaches would be to promote healthier responses to stress and challenge that allow people to more effectively modulate arousal levels. The result would be more effective mobilization of cardiac and metabolic resources during times of challenge, and more efficient return to homeostasis and parasympathetic regulation during times of calm. Along these lines, the measurement of RSA reactivity might be an effective tool to help monitor an individual's progress through the course of intervention and identify areas of continued need. There even is some suggestive evidence that techniques such as the use of RSA biofeedback and progressive muscle relaxation may be effective in helping people moderate heart rate variability and reduce related symptoms of pathology (Lehrer, Smetankin, & Potapova, 2000; Zucker, Samuelson, Muench, Greenberg, & Gevirtz, 2009). RSA is understood to be a dynamic system, and thus should be modifiable. If these techniques prove to be effective, it may help individuals who are vulnerable to disregulated stress responses – due to genetics, environmental adversity, or both – to more effectively modulate themselves in ways that allow them to meet the particular environmental challenges they encounter.

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References

Aber JL, Jones SM, Brown JA, Chaudry N, Samples F. Resolving conflict creatively: Evaluating the developmental effects of a school-based violence prevention program in neighborhood and classroom context. Development and Psychopathology. 1998; 10:187–213. [PubMed: 9635221]

Arias B, Aguilera M, Moya J, Sáiz PA, Villa H, Ibáñez MI, García-Portillo MP, Bobes J, Ortet G, Fañanás L. The role of genetic variability in the SLC6A4, BDNF and GABRA6 genes in anxiety-related traits. Acta Psychiatrica Scandinavica. 2012; 125(3):194–202. [PubMed: 21902679]

Barnett, D.; Manly, JT.; Cicchetti, D. Defining child maltreatment: The interface between policy and research. In: Cicchetti, D.; Toth, S., editors. Child abuse, child development, and social policy. Norwood, NJ: Ablex; 1993. p. 7-73.

Beauchaine T. Vagal tone, development, and Gray's motivational theory: Toward an integrated model of autonomic nervous system functioning in psychopathology. Development and Psychopathology. 2001; 13:183–214. [PubMed: 11393643]

Beauchaine T, Gatzke-Kopp L, Mead HK. Polyvagal theory and developmental psychopathology: Emotion dysregulation and conduct problems from preschool to adolescence. Biological Psychology. 2007; 74:174–184. [PubMed: 17045726]

Belsky J. Child maltreatment: An ecological integration. American Psychologist. 1980; 35:320–335. [PubMed: 7386966]

Belsky J, Pluess M. Beyond diathesis stress: Differential susceptibility to environmental influences. Psychological Bulletin. 2009; 135:885–908. [PubMed: 19883141]

Binkley PF, Nuziatta E, Liu-Stratton Y, Cooke G. A polymorphism of the endothelial nitric oxide synthase promoter is associated with an increase in autonomic imbalance in patients with congestive heart failure. American Heart Journal. 2005; 149:342–348. [PubMed: 15846275]

Boyce WT, Quas J, Alkon A, Smider NA, Essex MJ, Kuppfer DJ. Autonomic reactivity and psychopathology in middle childhood. The British Journal of Psychiatry. 2001; 179(2):144–150. [PubMed: 11483476]

Bronfenbrenner U. Toward an experimental ecology of human development. American Psychologist. 1977; 32:513–531.

Butler EA, Wilhelm FH, Gross JJ. Respiratory sinus arrhythmia, emotion, and emotion regulation during social interaction. Psychophysiology. 2006; 43:612–622. [PubMed: 17076818]

Calkins SD, Graziano P, Keane SP. Cardiac vagal regulation differentiates among children at risk for behavior problems. Biological Psychology. 2007; 74:144–153. [PubMed: 17055141]

Calkins SD, Smith CL, Gill K, Johnson MC. Maternal interactive style across contexts: relations to emotional, behavioral and physiological regulation during toddlerhood. Social Development. 1998; 7:350–369.

Caspi A, Hariri AR, Holmes A, Uher R, Moffitt TE. Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. The American Journal of Psychiatry. 2010; 167(5):509–527. [PubMed: 20231323]

Chaffin M, Funderburk B, Bard D, Valle LA, Gurwitch R. A combined motivation and parent– child interaction therapy package reduces child welfare recidivism in a randomized dismantling field trial. Journal of Consulting and Clinical Psychology. 2011; 79:84–95. [PubMed: 21171738]

Chida Y, Hamer M. Chronic psychosocial factors and acute physiological responses to laboratory-induced stress in healthy populations: A quantitative review of 30 years of investigations. Psychological Bulletin. 2008; 134(6):829–885. [PubMed: 18954159]

Cicchetti, D.; Lynch, M. Failures in the expectable environment and their impact on individual development: The case of child maltreatment. In: Cicchetti, D.; Cohen, DJ., editors. Developmental Psychopathology, Volume 2: Risk, Disorder, and Adaptation. New York, NY: John Wiley & Sons, Inc; 1995. p. 32-71.

Cicchetti, D.; Valentino, K. An ecological-transactional perspective on child maltreatment: Failure of the average expectable environment and its influence on child development. In: Cicchetti, D.; Cohen, D., editors. Development and Psychopathology: Risk, disorder, and adaptation. 2. Vol. 3. New York, NY: John Wiley; 2006. p. 129-201.

Conradt E, Degarmo D, Fisher P, Abar B, Lester BM, Lagasse LL, Shankaran S, Bada H, Bauer CR, Whitaker TM, Hammong JA. The contributions of early adverse experiences and trajectories of respiratory sinus arrhythmia on the development of neurobehavioral disinhibition among children with prenatal substance exposure. Development and Psychopathology. 2014; 26:901–916. [PubMed: 24909973]

Cooley-Quille M, Boyd RC, Frantz F, Walsh J. Emotional and behavioral impact of exposure to community violence in inner-city adolescents. Journal of Community Psychology. 2001; 30:199–206.

Coulton C, Korbin J, Su M, Chow J. Community level factors and child maltreatment rates. Child Development. 1995; 66(5):1262–1276. [PubMed: 7555215]

Culotta E, Koshland DE. NO news is good news. Science. 1992; 258:1862–1864. [PubMed: 1361684]

Dale LP, Carroll LE, Galen G, Hayes JA, Webb KW, Porges SW. Abuse history is related to autonomic regulation to mild exercise and psychological wellbeing. Applied Psychophysiology Biofeedback. 2009; 34:299–308. [PubMed: 19707870]

De Geus, EJC.; Van Doornen, LJP. Ambulatory assessment of parasympathetic/sympathetic balance by impedance cardiography. In: Fahrenberg, J.; Myrtek, M., editors. Ambulatory assessment: Computer assisted psychological and psychophysiological methods in monitoring and field studies. Berlin: Hogrefe & Huber; 1996. p. 141-164.

Demas GE, Kriegsfeld LJ, Blackshaw S, Huang PL, Gammie SC, Nelson RJ, Snyder SH. Elimination of aggressive behavior in male mice lacking endothelial nitric oxide synthase. The Journal of Neuroscience. 1999; 19:RC30, 1–5. [PubMed: 10493775]

Denver JW, Reed SF, Porges SW. Methodological issues in the quantification of respiratory sinus arrhythmia. Biological Psychology. 2007; 74:286–294. [PubMed: 17067734]

Duncan CE, Webster MJ, Rothmond DA, Bahn S, Elashoff M, Shannon Weickert C. Prefrontal GABA(A) receptor alpha-subunit expression in normal postnatal human development and schizophrenia. Journal of Psychiatric Research. 2010; 44(10):673–81. [PubMed: 20100621]

Eaves L, Foley D, Silberg J. Has the “Equal Environments” assumption been tested in twin studies? Twin Research. 2003; 6:486–489. [PubMed: 14965458]

Eisenberg N, Fabes RA, Karbon M, Murphy BC, Wosinski M, Polazzi L, et al. The relations of children’s dispositional prosocial behavior to emotionality, regulation, and social functioning. Child Development. 1996; 67:974–992. [PubMed: 8706539]

Ellis BJ, Essex MJ, Boyce WT. Biological sensitivity to context: II. Empirical explorations of an evolutionary–developmental theory. Development and Psychopathology. 2005; 17:303–328. [PubMed: 16761547]

El-Sheikh M, Hinnant JB, Erath S. Developmental trajectories of delinquency symptoms in childhood: The role of marital conflict and autonomic nervous system activity. Journal of Abnormal Psychology. 2011; 120(1):16–32. [PubMed: 20919788]

El-Sheikh M, Kouros CD, Erath S, Cummings EM, Keller P, Staton L, Moore G. Marital conflict and children’s externalizing behavior: Interactions between parasympathetic and sympathetic nervous system activity. Monographs of the Society for Research in Child Development. 2009; 74:1–69.

Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. Genetics. 2003; 164:1567–1587. [PubMed: 12930761]

Falush D, Stephens M, Pritchard JK. Inference of population structure using multilocus genotype data: Dominant and null alleles. Molecular Ecology Notes. 2007; 7:574–578. [PubMed: 18784791]

Frost R, Stauffer J. The effects of social class, gender, and personality on physiological responses to filmed violence. Journal of Communication. 1987; 37:29–45.

Gaudin, JM. Child neglect: Short-term and long-term consequences. In: Dubowitz, H., editor. Neglected children: Research, practice, and policy. Thousand Oaks, CA: Sage; 1999. p. 89-108.

Gordis EB, Feres N, Olezeski CL, Rabkin AN, Trickett PK. Skin conductance reactivity and respiratory sinus arrhythmia among maltreated and comparison youth: Relations with aggressive behavior. Journal of Pediatric Psychology. 2010; 35:547–558. [PubMed: 19995866]

Greaves-Lord K, Tulen J, Dietrich A, Sondeijker F, van Roon A, Oldehinkel A, Ormel J, et al. Reduced autonomic flexibility as a predictor for future anxiety in girls from the general population: The TRAILS study. Psychiatry Research. 2010; 179(2):187–193. [PubMed: 20483486]

Hakman M, Chaffin M, Funderburk B, Silovsky JF. Change trajectories for parent-child interaction sequences during parent-child interaction therapy for child physical abuse. Child Abuse & Neglect. 2009; 33:461–470. [PubMed: 19581001]

Han DH, Bolo N, Daniels MA, Lyoo IK, Min KJ, Kim CH, Renshaw PF. Craving for alcohol and food during treatment for alcohol dependence: Modulation by T allele of 1519T>C GABAAalpha6. Alcoholism, Clinical and Experimental Research. 2008; 32(9):1593–1599.

Haul S, Godecke A, Schrader J, Haas HL, Luhmann HJ. Impairment of neocortical long-term potentiation in mice deficient of endothelial nitric oxide synthase. Journal of Neurophysiology. 1999; 81:494–497. [PubMed: 10036253]

Heim C, Newport DJ, Heit S, Graham YP, Wilcox M, Bonsall R, et al. Pituitary–adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. Journal of the American Medical Association. 2000; 284:592–597. [PubMed: 10918705]

Hill-Soderlund AL, Mills-Koonce WR, Propper C, Calkins SD, Granger DA, Moore GA, et al. Parasympathetic and sympathetic responses to the strange situation in infants and mothers from avoidant and securely attached dyads. Developmental Psychobiology. 2008; 50:361–376. [PubMed: 18393278]

Huang PL, Huang Z, Mashimo H, Bloch KD, Moskowitz MA, Bevan JA, Fishman MC. Hypertension in mice lacking the gene for endothelial nitric oxide synthase. Nature. 1995; 377(6546):239–242. [PubMed: 7545787]

Hubisz MJ, Falush D, Stephens M, Pritchard JK. Inferring weak population structure with assistance of sample group information. Molecular Ecology Resources. 2009; 5:1322–1332. [PubMed: 21564903]

Huffman LC, Bryan Y, Del Carmen R, Pederson F, Doussard-Roosevelt J, Porges SW. Infant temperament and cardiac vagal tone: assessments at twelve weeks of age. Child Development. 1998; 69:624–635. [PubMed: 9680676]

Jacobson NS, Gottman JM, Shortt JW, Wu J. The distinction between Type 1 and Type 2 batterers – further considerations: Reply to Ornduff et al. (1995), Margolin et al. (1995), and Walker (1995). Journal of Family Psychology. 1995; 9:272–279.

Kano T, Shimizu-Sasamata M, Huang PL, Moskowitz MA, Lo EH. Effects of nitric oxide synthase gene knockout on neurotransmitter release in vivo. Neuroscience. 1998; 86:695–699. [PubMed: 9692709]

Karg K, Burmeister M, Shedden K, Sen S. The serotonin transporter promoter variant (5-HTTLPR), stress, and depression meta-analysis revisited: evidence of genetic moderation. Archives of General Psychiatry. 2011; 68(5):444–54. [PubMed: 21199959]

Katz LF. Domestic violence and vagal reactivity to peer provocation. Biological Psychology. 2007; 74:154–164. [PubMed: 17118516]

Keller MC. Gene x Environment interaction studies have not properly controlled for potential confounders: The problem and the (simple) solution. Biological Psychiatry. 2014; 75:18–24. [PubMed: 24135711]

Kemp AH, Quintana DS, Gray MA, Felmingham KL, Brown K, Gatt JM. Impact of depression and antidepressant treatment on heart rate variability: A review and meta-analysis. Biological Psychiatry. 2010; 67(11):1067–1074. [PubMed: 20138254]

Key BL, Campbell TS, Bacon SL, Gerin W. The influence of trait and state rumination on cardiovascular recovery from a negative emotional stressor. Journal of Behavioral Medicine. 2008; 31(3):237–248. [PubMed: 18350377]

Korbin JE, Coulton CJ, Chard S, Platt-Houston C, Su M. Impoverishment and child maltreatment in African American and European American neighborhoods. Development and Psychopathology. 1998; 10:215–233. [PubMed: 9635222]

Krenichyn K, Saegert S, Evans GW. Parents as moderators of psychological and physiological correlates of innercity children's exposure to violence. Applied Developmental Psychology. 2001; 22:581–602.

Kwong M, Bartholomew K, Henderson A, Trinke S. The intergenerational transmission of relationship violence. Journal of Family Psychology. 2003; 17:288–301. [PubMed: 14562454]

Lai CQ, Tucker KL, Choudhry S, Parnell LD, Mattei J, Garcia-Bailo B, et al. Population admixture associated with disease prevalence in the Boston Puerto Rican health study. Human Genetics. 2009; 125:199–205. [PubMed: 19107526]

Lehrer P, Smetankin A, Potapova T. Respiratory sinus arrhythmia biofeedback therapy for asthma: A report of 20 unmedicated pediatric cases using the Smetankin Method. Applied Psychophysiology and Biofeedback. 2000; 24:193–200. [PubMed: 10999237]

Lewis GF, Furman SA, McCool MF, Porges SW. Statistical strategies to quantify respiratory sinus arrhythmia: Are commonly used metrics equivalent? Biological Psychology. 2012; 89:349–364. [PubMed: 22138367]

Manly JT. Invited commentary: Advances in research definitions of child maltreatment. Child Abuse and Neglect: The International Journal. 2005; 29(5):413–619.

McEwen BS. Mood disorders and allostatic load. Biological Psychiatry. 2003; 54(3):200–207. [PubMed: 12893096]

McEwen BS, Wingfield JC. The concept of allostasis in biology and biomedicine. Hormones and Behavior. 2003; 43:2–15. [PubMed: 12614627]

Mehta D, Binder EB. Gene x environment vulnerability factors for PTSD: The HPA-axis. Neuropharmacology. 2012; 62:654–662. [PubMed: 21439305]

Miskovic V, Schmidt LA, Georgiades K, Boyle M, MacMillan HL. Stability of resting frontal electroencephalogram (EEG) asymmetry and cardiac vagal tone in adolescent females exposed to child maltreatment. Developmental Psychobiology. 2009; 51:474–487. [PubMed: 19629997]

Movius HL, Allen JJB. Cardiac vagal tone, defensiveness, and motivational style. Biological Psychology. 2005; 68(2):147–162. [PubMed: 15450694]

Nunes RAB, Barroso LP, Pereira AdaC, Krieger JE, Mansur AJ. Gender-related associations of genetic polymorphisms of α-adrenergic receptors, endothelial nitric oxide synthase and bradykinin B2 receptor with treadmill exercise test responses. Open Heart. 2014; 1(1):e000132. [PubMed: 25544888]

Obradović J, Bush NR, Stamperdahl J, Adler NE, Boyce WT. Biological sensitivity to context: The interactive effects of stress reactivity and family adversity on socioemotional behavior and school readiness. Child Development. 2010; 81:270–289. [PubMed: 20331667]

Oosterman M, de Schipper JC, Fisher P, Dozier M, Schuengel C. Autonomic reactivity in relation to attachment and early adversity among foster children. Development and Psychopathology. 2010; 22:109–118. [PubMed: 20102650]

Ottaviani C, Shapiro D, Davydov DM, Goldstein IB. Autonomic stress response modes and ambulatory heart rate level and variability. Journal of Psychophysiology. 2008; 22(1):28–40.

Porges, SW. Method and Apparatus for Evaluating Rhythmic Oscillations in Aperiodic Physiological Response Systems. Patent Number. 4,510,944. 1985 Apr 16. 1985

Porges SW. Orienting in a defensive world: mammalian modifications of our evolutionary heritage: a polyvagal theory. Psychophysiology. 1995; 32:301–318. [PubMed: 7652107]

Porges SW. Love: An emergent property of the mammalian autonomic nervous system. Psychoneuroendocrinology. 1998; 23:837–861. [PubMed: 9924740]

Porges SW. The polyvagal theory: Phylogenetic substrates of a social nervous system. International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology. 2001; 42(2):123–146. [PubMed: 11587772]

Porges SW. The polyvagal perspective. Biological Psychology. 2007; 74:116–143. [PubMed: 17049418]

Porges, SW.; Bohrer, RE. Analyses of periodic processes in psychophysiological research. In: Cacioppo, JT.; Tassinary, LG., editors. Principles of Psychophysiology: Physical, Social, and Inferential Elements. New York: Cambridge University Press; 1990. p. 708-753.

Porges SW, Byrne EA. Research methods for measurement of heart rate and respiration. Biological Psychology. 1992; 34:93–130. [PubMed: 1467397]

Porges SW, Doussard-Roosevelt JA, Portales AL, Greenspan SI. Infant regulation of the vagal “brake” predicts child behavior problems: a psychobiological model of social behavior. Developmental Psychobiology. 1996; 29:697–712. [PubMed: 8958482]

Posner MI, Rothbart MK. Developing mechanisms of self-regulation. Development and Psychopathology. 2000; 12:427–441. [PubMed: 11014746]

Propper C, Moore GA. The influence of parenting on infant emotionality: A multi-level psychobiological perspective. Developmental Review. 2006; 26:427–460.

Randin D, Vollenweider P, Tappy L, Jéquier E, Nicod P, Scherrer U. Suppression of alcohol-induced hypertension by dexamethasone. The New England Journal of Medicine. 1995; 332:1733–1737. [PubMed: 7760888]

Rigterink T, Katz LF, Hessler DM. Domestic violence and longitudinal associations with children's physiological regulation abilities. Journal of Interpersonal Violence. 2010; 25:1669–1683. [PubMed: 20587477]

Riniolo TC, Porges SW. Inferential and descriptive influences on measures of respiratory sinus arrhythmia: sampling rate, r-wave trigger accuracy, and variance estimates. Psychophysiology. 1997; 34:613–621. [PubMed: 9299916]

Samerfoff, A.; Chandler, M. Reproductive risk and the continuum of caretaking casualty. In: Horowitz, F., editor. Review of child development research. Vol. 4. Chicago: University of Chicago Press; 1975. p. 187-244.

Sedlak, AJ.; Mettenburg, J.; Basena, M.; Petta, I.; McPherson, K.; Greene, A.; Li, S. Fourth national incidence study of child abuse and neglect (NIS–4): Report to congress. Washington, DC: U.S. Department of Health and Human Services, Administration for Children and Families; 2010.

Shankarishan P, Borah PK, Ahmed G, Mahanta J. Endothelial nitric oxide synthase gene polymorphisms and the risk of hypertension in an Indian population. BioMed Research International. 2014; 2014:Article ID 793040, 11.

Shenk CE, Noll JG, Putnam FW, Trickett PK. A prospective examination of the role of childhoos sexual abuse and physiological asymmetry in the development of psychopathology. Child Abuse & Neglect. 2010; 34:752–761. [PubMed: 20850183]

Skowron EA, Loken E, Gatzke-Kopp LM, Cipriano-Essel EA, Woehrle PL, Van Epps JJ, Gowda A, Ammerman RT. Mapping cardiac physiology and parenting processes in maltreating mother-child dyads. Journal of Family Psychology. 2011; 25:663–674. [PubMed: 21842991]

Skowron EA, Reinneman DHS. Psychological interventions for child maltreatment: A meta-analysis. Psychotherapy: Theory, Research, Practice, and Training. 2005; 42:52–71.

Thayer JF, Friedman BH, Borkovec TD. Autonomic characteristics of generalized anxiety disorder and worry. Biological Psychiatry. 1996; 39(4):255–266. [PubMed: 8645772]

Uhart M, McCaul ME, Oswald LM, Choi L, Wand GS. GABRA6 gene polymorphism and an attenuated stress response. Molecular Psychiatry. 2004; 9:998–1006. [PubMed: 15197399]

Watanabe M, Maemura K, Kanbara K, Tamayama T, Hayasaki H. GABA and GABA receptors in the central nervous system and other organs. International Review of Cytology. 2002; 213:1–47. [PubMed: 11837891]

Waugh CE, Panage S, Mendes WB, Gotlib IH. Cardiovascular and affective recovery from anticipatory threat. Biological Psychology. 2010; 84:169–175. [PubMed: 20096747]

Wilson D, Kliewer W, Teasley N, Plybon L, Sica D. Violence exposure, catecholamine excretion, and blood pressure non-dipping status in African-American male versus female adolescents. Psychosomatic Medicine. 2002; 64:906–915. [PubMed: 12461196]

Wilson RI, Godecke A, Brown RE, Schrader J, Haas HL. Mice deficient in endothelial nitric oxide synthase exhibit a selective deficit in hippocampal long-term potentiation. Neuroscience. 1999; 90(4):1157–1165. [PubMed: 10338286]

Yaeger R, Alvial-Bront A, Abdul K, Nolan PC, Grann VR, Birchette MG, et al. Comparing genetic ancestry and self-described race in African Americans born in the United States and in Africa. Cancer Epidemiology, Biomarkers & Prevention. 2008; 17:1329–1338.

Yamada Y, Noborisaka Y, Ishizaki M, Tsuritani I, Honda R, Yamada S. Alcohol consumption, homeostasis model assessment indices and blood pressure in middle-aged healthy men. Journal of Human Hypertension. 2004; 18(5):343–350. [PubMed: 14739909]

Yasuma F, Hayano JI. Respiratory sinus arrhythmia: Why does the heartbeat synchronize with respiratory rhythm? Chest. 2004; 125(2):683–690. [PubMed: 14769752]

Zucker TL, Samuelson KW, Muench F, Greenberg MA, Gevirtz RN. The effects of Respiratory Sinus Arrhythmia biofeedback on heart rate variability and posttraumatic stress disorder symptoms: A pilot study. Applied Psychophysiology and Biofeedback. 2009; 34:135–143. [PubMed: 19396540]

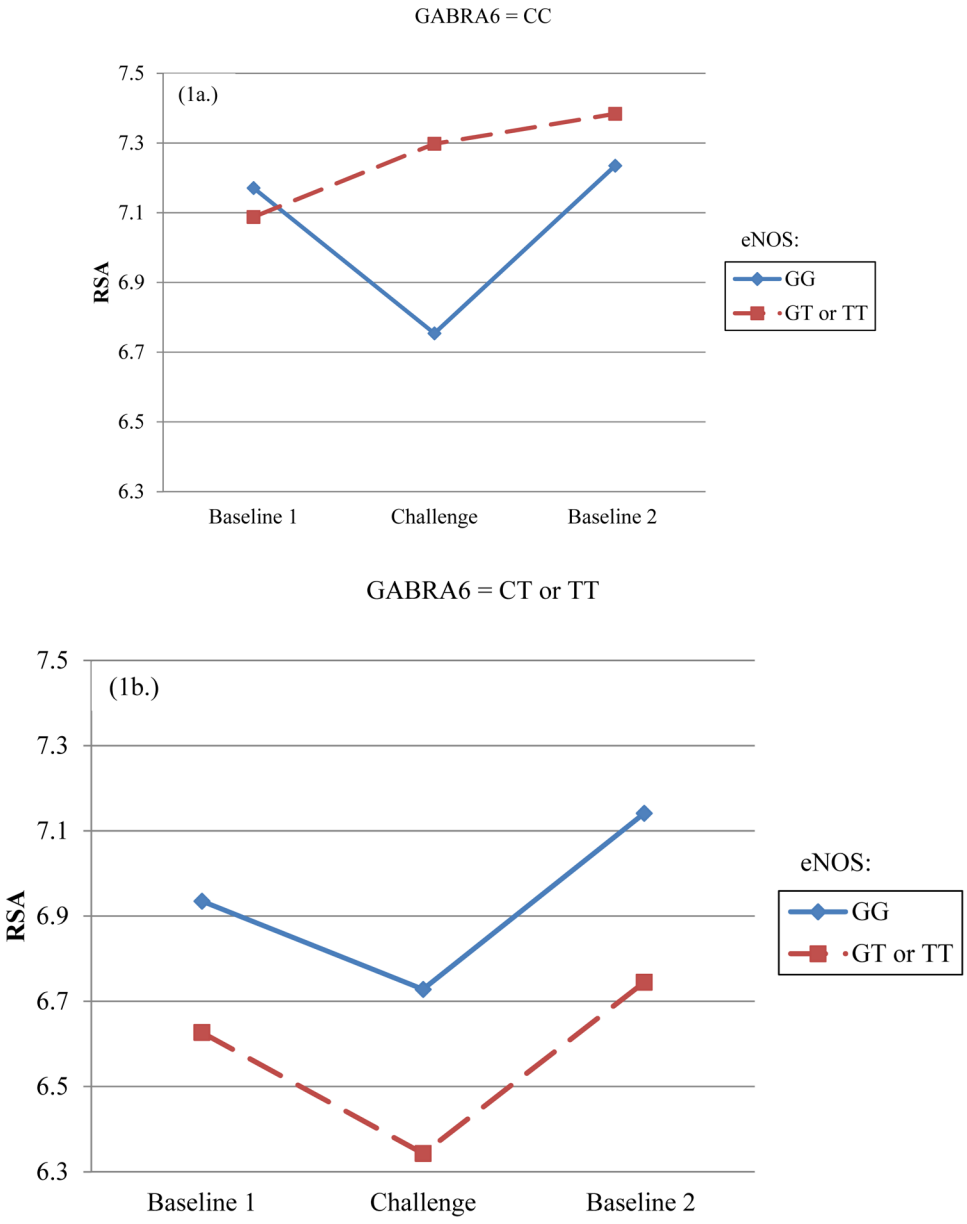

Figure 1.

Change in RSA as a function of experimental condition, eNOS and GABRA6: (1a.) Condition x eNOS within the GABRA6 CC genotype group.

Note: Group contrasts.

eNOS = GG:	Challenge < Baseline 1 ***
	Baseline 2 > Challenge ***
eNOS = GT or TT:	ns

* $p < .05$, ** $p < .01$, *** $p < .001$

Change in RSA as a function of experimental condition, eNOS and GABRA6: (1b.) Condition x eNOS within the GABRA6 CT or TT genotype group.

Note: Group contrasts.

eNOS = GG:	Challenge < Baseline 1 **
	Baseline 2 > Challenge ***
	Baseline 2 > Baseline 1 **
eNOS = GT or TT:	Challenge < Baseline 1 **
	Baseline 2 > Challenge **

* $p < .05$, ** $p < .01$, *** $p < .001$

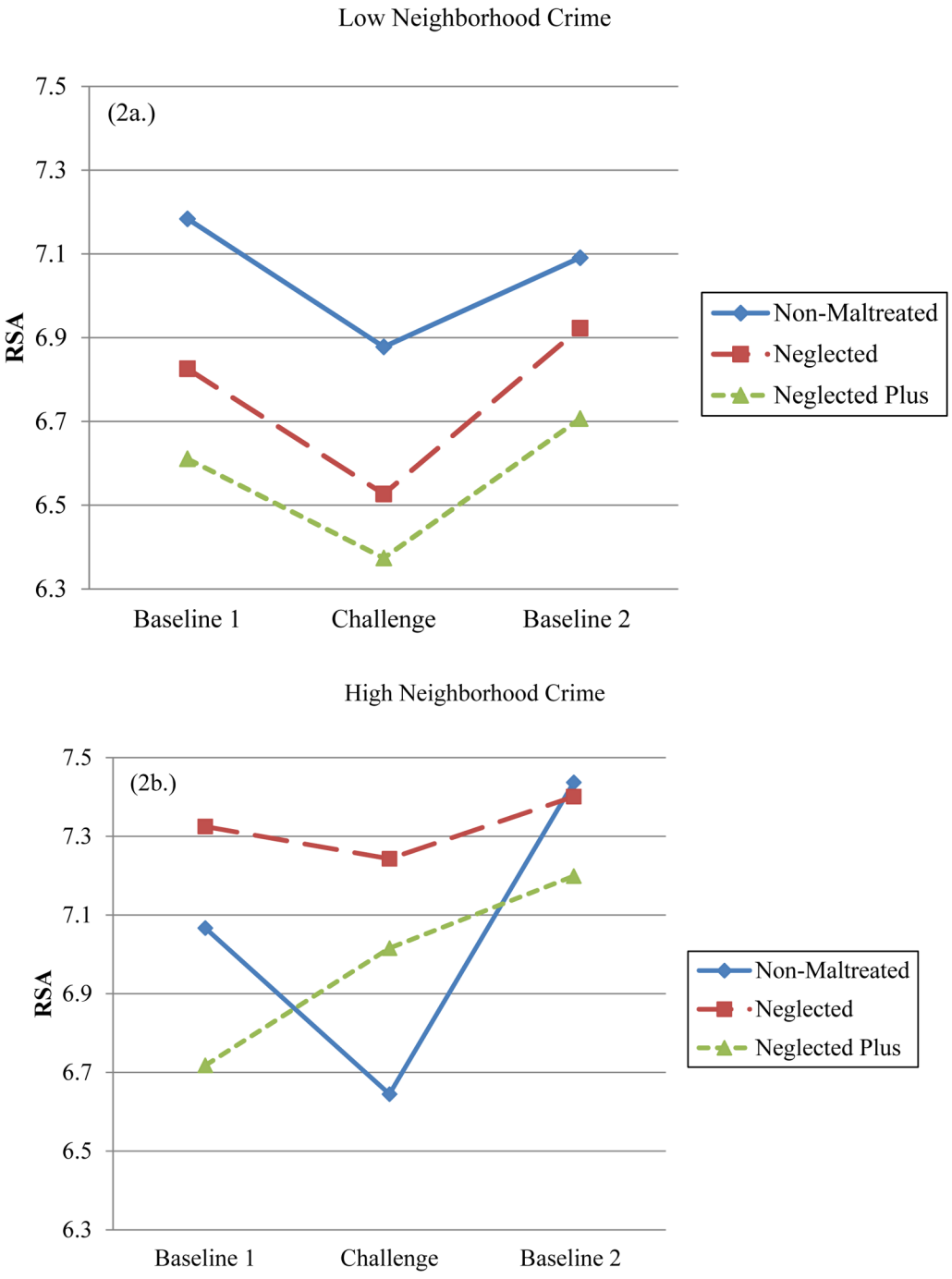

Figure 2.

Change in RSA as a function of experimental condition, child maltreatment and neighborhood crime: (2a.) Condition x Maltreatment Status within the Low Neighborhood Crime group.

Note: Group contrasts.

Non-Maltreated:	Challenge < Baseline 1 ***
Neglected:	Challenge < Baseline 1 *

	Baseline 2 > Challenge *
Neglected Plus:	Baseline 2 > Challenge *

* $p < .05$, ** $p < .01$, *** $p < .001$

Change in RSA as a function of experimental condition, child maltreatment and neighborhood crime: (2b.) Condition x Maltreatment Status within the High Neighborhood Crime group.

Note: Group contrasts.

Non-Maltreated:	Challenge < Baseline 1 **
	Baseline 2 > Challenge ***
	Baseline 2 > Baseline 1 *
Neglected:	ns
Neglected Plus:	ns

* $p < .05$, ** $p < .01$, *** $p < .001$

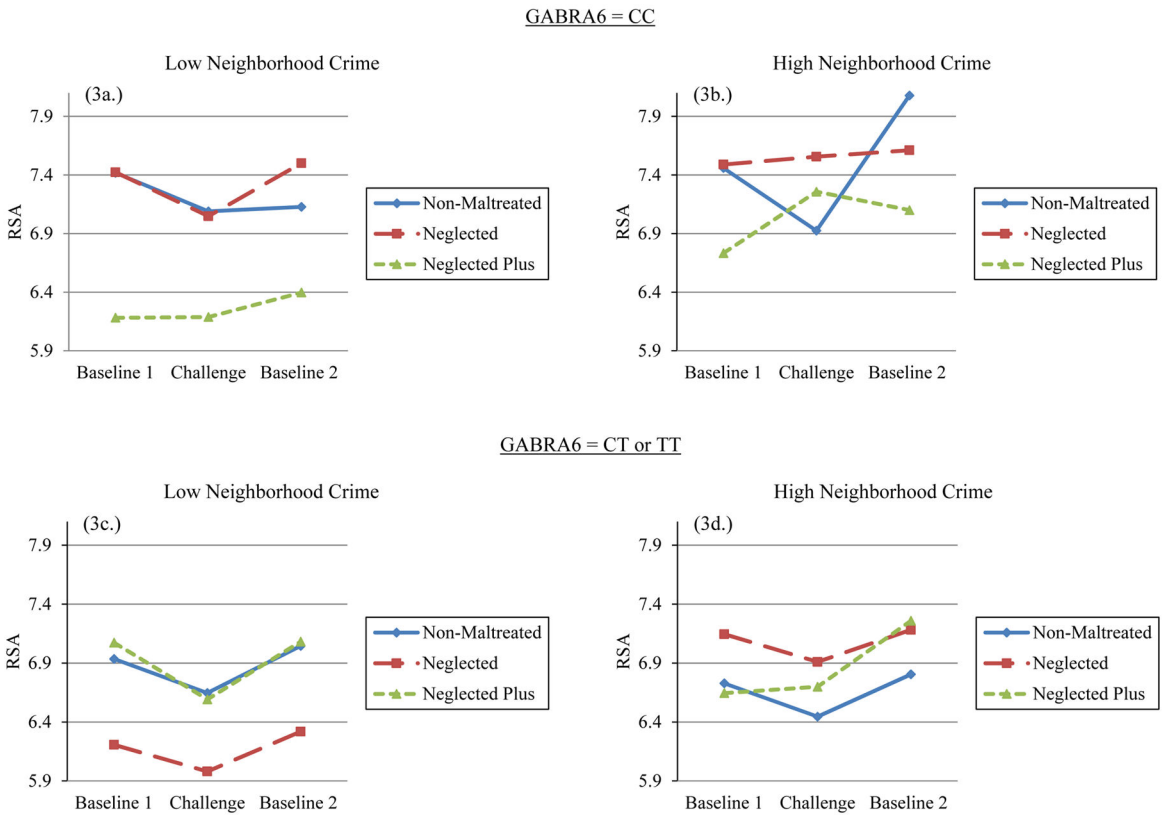

Figure 3.

Change in RSA as a function of experimental condition, child maltreatment, neighborhood crime, and GABRA6 genotype: (3a.) Condition x Maltreatment Status within the Low Neighborhood Crime and GABRA6 CC genotype group; (3b.) Condition x Maltreatment Status within the High Neighborhood Crime and GABRA6 CC genotype group.

Note: Group contrasts.

Non-Maltreated:	Challenge < Baseline 1 *
Neglected:	ns
Neglected Plus:	ns

* $p < .05$, ** $p < .01$, *** $p < .001$

Note: Group contrasts.

Non-Maltreated:	Challenge < Baseline 1 *
	Baseline 2 > Challenge ***
	Baseline 2 > Baseline 1 *
Neglected:	ns
Neglected Plus:	ns

* $p < .05$, ** $p < .01$, *** $p < .001$

Change in RSA as a function of experimental condition, child maltreatment, neighborhood crime, and GABRA6 genotype: (3c.) Condition x Maltreatment Status within the Low Neighborhood Crime and GABRA6 CT or TT genotype group; (3d.) Condition x

Maltreatment Status within the High Neighborhood Crime and GABRA6 CT or TT genotype group.

Note: Group contrasts.

Non-Maltreated:	Challenge < Baseline 1 ***
	Baseline 2 > Challenge ***
Neglected:	ns
Neglected Plus:	Challenge < Baseline 1 ***
	Baseline 2 > Challenge **

* $p < .05$, ** $p < .01$, *** $p < .001$

Note: Group contrasts.

Non-Maltreated:	Challenge < Baseline 1 *
	Baseline 2 > Challenge *
Neglected:	ns
Neglected Plus:	Baseline 2 > Challenge *
	Baseline 2 > Baseline 1 **

* $p < .05$, ** $p < .01$, *** $p < .001$

Table 1

Comparison of demographic characteristics

Characteristic	Nonmaltreated	Neglected	Neglected Plus Other Maltreatment	Significance
Child Gender (% female)	54.8%	51.1%	52.2%	χ^2 (2) = 0.21, *p = 0.902*
Child Ethnicity				χ^2 (6) = 4.91, *p = 0.556*
% African American	58.1%	63.8%	65.2%	
% Caucasian	11.8%	6.4%	15.2%	
% Latino	12.9%	8.5%	4.3%	
% Other	17.2%	21.3%	15.2%	
Number of Adults in Home	1.7	1.5	1.5	F (2,183) = 2.44, *p = 0.09*
History of Public Assistance (% Yes)	91.4%	91.5%	100%	χ^2 (2) = 4.22, *p = 0.122*

Table 2

Genotype frequencies and Hardy-Weinberg equilibrium for eNOS and GABRA6

Gene	Major Allele Homozygote *N*	Heterozygote *N*	Minor Allele Homozygote *N*	HWE χ^2	*p*
eNOS	GG	GT	TT	0.12	.73
	129	51	6		
GABRA6	CC	CT	TT	0.17	.68
	54	95	37		

Table 3

Comparison of genotype distributions based on maltreatment status (*N* in parentheses)

Polymorphism	**Genotype/Percentage**		χ^2	***p***
eNOS	GG	GT/TT		
Nonmaltreated	67.7 (63)	32.3 (30)	0.60	.741
Neglected	68.1 (32)	31.9 (15)		
Neglect plus other maltreatment	73.9 (34)	26.1 (12)		
GABRA6	CC	CT/TT		
Nonmaltreated	21.5 (20)	78.5 (73)	6.11	.047
Neglected	31.9 (15)	68.1 (32)		
Neglect plus other maltreatment	41.3 (19)	58.7 (27)		

Table 4

Comparison of genotype distributions based on exposure to neighborhood crime (*N* in parentheses)

Polymorphism	**Genotype/Percentage**		χ^2	*p*
eNOS	GG	GT/TT		
High Neighborhood Crime	76.2 (64)	23.8 (20)	3.37	.066
Low Neighborhood Crime	63.7 (65)	36.3 (37)		
GABRA6	CC	CT/TT		
High Neighborhood Crime	31.0 (26)	69.0 (58)	0.27	.601
Low Neighborhood Crime	27.5 (28)	72.5 (74)		