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The Roles of HPA Axis Activity and Attentional Bias in the Development of Anxiety Symptoms in Low-Income Mexican-Origin Children

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THE ROLES OF HPA AXIS ACTIVITY AND ATTENTIONAL BIAS IN THE DEVELOPMENT OF ANXIETY SYMPTOMS IN LOW-INCOME MEXICAN-ORIGIN CHILDREN

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CHAPTER ONE

INTRODUCTION

Children of low-income Mexican-origin immigrants experience multiple chronic stressors related to poverty and immigration (Cervantes, Fisher, Cordova, & Napper, 2012; Cervantes, Padilla, Napper, & Goldbach, 2013). This accumulation of chronic stress is likely related to significant mental health disparities for children of low-income Mexican-origin immigrants, as they display higher rates of anxiety than other groups (Beidel & Alfano, 2011; Varela, Sanchez-Sosa, Biggs, & Luis, 2008). Although accumulated poverty-related stress has been identified as a factor leading to anxiety in low-income children (Beesdo, Knappe, & Pine, 2009; Conger & Donnellan, 2007; Wadsworth, Raviv, Santiago, & Etter, 2011), the present study builds on this literature by examining culturally relevant immigration-related stress alongside poverty-related stress for low-income Mexican-origin children. A likely mediator of this process is hypothalamic–pituitary–adrenal axis (HPA axis) activity, causing a buildup of cortisol in the body. There is a large amount of evidence indicating that chronic HPA axis activity is a mechanism through which accumulated poverty-related stress causes mental illness (Essex et al., 2011; Gunnar & Quevedo, 2007; McEwen & Tucker, 2011), but this mediator has not been examined in relation to immigration-related stress in low-income Mexican-origin children.

Although HPA axis activity has been established as a biomarker of chronic stress (Cunningham, 2015; Gow, Thomson, Rieder, Van Uum, & Koren, 2010; Russell, Koren, Rieder, & Van Uum, 2012; Vanaelst, De Vriendt, Huybrechts, Rinaldi, & De Henauw, 2012; Wippert, Honold, Wang, & Kirschbaum, 2014), which can lead to problems with anxiety (Cunningham, 2015; Essex et al., 2011; Gunnar & Quevedo, 2007; McEwen & Tucker, 2011), not all low-income Mexican-origin children develop anxiety, so there may be a moderating process implicated in anxiety development. Children who develop anxiety disorders tend to be vigilant for threats in their environment, attending to threatening stimuli significantly more than other types of stimuli as they process information (Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007; Muris & Field, 2008; Wolters et al., 2012). Neurocognitive processes such as attentional bias to threat have been shown to determine the trajectory of children's anxious behavior later in life and could be good candidates for moderators of the pathway from HPA axis activity to anxiety (Shechner et al., 2012). Attentional bias to threat is a key component of the development and maintenance of anxiety (Bar-Haim et al., 2007; Pérez-Edgar, Taber-Thomas, Auday, & Morales, 2014; Wolters et al., 2012), yet it has not been examined as a potential moderator distinguishing the low-income Mexican-origin children who develop anxiety from those who do not in a context of elevated HPA axis activity.

The present research focuses on both HPA axis activity (as measured through hair cortisol concentrations; HCCs) and attentional bias to threat in order to explain why some Mexican-origin children display anxiety symptoms in the context of accumulated stress and some do not. Specifically, the present study uses culturally relevant measures of poverty-related and immigration-related stress and examines the association between chronic HPA axis activity and anxiety among a population with which these processes have not been studied. Further, this research examines attentional bias as a moderator of the association between chronic HPA axis activity and anxiety symptoms. Determining whether this neurocognitive process moderates the relation between HPA axis activity and anxiety will help to clarify the causes of mental health disparities in this population and aid in identifying children for early intervention. The present study addresses these gaps with a longitudinal research design (three timepoints each spaced six months apart) in a community sample of children (ages 6-10) of low-income Mexican-origin immigrants.

A Theoretical Foundation for Studying Mental Health in Children

The present study is built on a foundational understanding that neurobiological markers of mental health problems can be used to more accurately understand the development of these problems in the context of chronic stress. The strict categorization of mental disorders developed by clinical consensus does not always align with empirical observations from clinical neuroscience (Insel et al., 2010). Thus, these diagnostic frameworks do not fully capture the underlying neural circuitry of mental illness. To address this problem, the National Institute of Mental Health (NIMH) introduced the neuroscience-based Research Domain Criteria (RDoC) project to provide a new framework for the study of pathophysiology (Cuthbert & Insel, 2013; Insel et al., 2010). Through this new research focus on neural circuits, genes, physiology, and observable behavior, it is hoped that a classification system will emerge that will accurately reflect the nature of mental illness as being dysfunction based in neurobiological systems.

The RDoC matrix is comprised of dimensional constructs (e.g., sustained threat) and the units of analysis used to study these constructs (e.g., physiology and behavior; Cuthbert & Insel, 2013). This matrix is an evidence-based guide for the best neurobiological methodology used to conduct research on dimensional constructs and is intended to be used alongside an awareness of developmental factors and interactions with the environment. The present research focuses on the construct of *sustained threat* in order to explain the development of anxiety in low-income Mexican-origin children. Sustained threat is defined as the aversive result of being chronically exposed to stimuli and experiences that would be adaptive to escape, and the psychophysiological changes caused by sustained threat persist even in the absence of threat (NIMH, 2011). Dysregulation of the HPA axis and attentional bias to threat have been established as responses to sustained threat (NIMH, 2011). Thus, the present study utilizes these physiological and behavioral indicators of exposure to sustained threat to examine the presence of anxiety symptoms in children who are at-risk for anxiety problems. It is particularly important to study sustained threat in low-income Mexican-origin children due to their high levels of immigration-related stress (Cervantes et al., 2012; Cervantes et al., 2013), which may lead to elevated rates of anxiety compared to children of other ethnicities (Beidel & Alfano, 2011; Varela et al., 2008).

Anxiety in Mexican-Origin Children

Anxiety refers to a future-oriented response to threatening stimuli that an organism aims to avoid (Beesdo et al., 2009). Anxiety is thought to be adaptive in many circumstances in which it would be protective to avoid a harmful stimulus. In fact, along the course of normal development, children experience a number of anxieties and fears, most of which are not problematic (Muris & Field, 2008). However, anxiety becomes maladaptive when it interferes with functioning (Beesdo et al., 2009). Mexican and Mexican American children have been found to experience higher rates of anxiety than Caucasian children via self- and parent-report (Beidel & Alfano, 2011; Varela et al., 2008). It is crucial that researchers examine possible culturally relevant causal factors to explain this disparity in children's mental health.

Anxiety is the most common mental health disorder in childhood, with lifetime prevalence rates of any anxiety disorder at 15% to 20% for children and adolescents (Beesdo et al., 2009; Field & Lester, 2010). Notably, prevalence rates over shorter periods of time (e.g., 1-year or 6-month rates) are not considerably lower than lifetime estimates (Beesdo et al., 2009). In addition to the substantial prevalence of anxiety disorders, further support for devoting serious attention to anxiety lies in longitudinal data. Most past research indicates that, with the exception of specific phobias, anxiety disorders are chronic conditions, in that about 40% to 50% of children exhibit homotypic continuity across follow-up periods ranging from six months to six years (Beidel & Alfano, 2011). In addition, 25% to 30% of children exhibit heterotypic continuity, in that they display a different emotional disorder at follow-up. A final group of children (about 20% to 25%) display no disorder at follow-up. Overall, these data reveal that there is a high level of temporal stability for anxiety (Beesdo et al., 2009). Past research has established that rates of anxiety increase with age, and anxiety is more common among females than males, including in Mexican-origin children (Beesdo et al., 2009; Beidel & Alfano, 2011; Varela et al., 2008). Understanding early factors associated with child anxiety is important, because anxiety symptoms during childhood often persist through adolescence and into adulthood (Field & Lester, 2010). Longitudinal studies assessing multiple possible vulnerability and risk factors are needed to identify predictors for the onset of anxiety in children (Beesdo et al., 2009). This type of information is important for improving early recognition as well as prevention and treatment of anxiety in low-income Mexican-origin children.

There is considerable overlap among anxiety disorders, with significant association between virtually all specific anxiety disorders (Beesdo et al., 2009; Beidel & Alfano, 2011).

Across diagnostic categories, anxiety causes significant impairment in multiple domains, including mental health, physical health, academic functioning, peer relationships, and family functioning, and that these impairments continue throughout adolescence and early adulthood (Beidel & Alfano, 2011; Field & Lester, 2010). Research has shown that children and adolescents who do not meet the *DSM* thresholds for anxiety disorders reveal a similar range of adverse outcomes (Beesdo et al., 2009). Given the high rate of anxiety disorder co-occurrence and the significant functional impairments correlated with sub-clinical anxiety, the present study focused on anxiety symptoms independent of *DSM* disorder categories. Through this examination of anxiety symptoms in low-income Mexican-origin children, it may be possible to identify early risk factors to serve as targets for intervention.

Impacts of Chronic Stress on Low-Income Mexican-Origin Children

Of the foreign-born individuals living in the United States today, Mexican-origin immigrants comprise almost one third (29%; Motel & Patten, 2013). This is a large and quickly growing population who are typically under-researched and underserved, despite being at increased risk for developing mental illness (Paral, 2009). One evidence-based theory as to why these individuals display higher rates of anxiety and other mental disorders is that Mexican-origin immigrants face numerous stressors across multiple domains, including economic strain, discrimination, acculturative stress, family separation, legal problems, and exposure to violence (Cervantes et al., 2012; Cervantes et al., 2013). Most families do not face one or two of these immigration-related stressors but multiple stressors simultaneously and over long periods of time. In fact, research has shown that Mexican American children living in disadvantaged neighborhoods may face twice as many stressors as predominantly Caucasian middle-class children (Suarez-Morales & Lopez, 2009). The accumulation of such chronic stress is likely related to significant mental health disparities for children of low-income Mexican-origin immigrants (Cervantes et al., 2013; Varela et al., 2008).

Disproportionately high rates of Mexican-origin immigrants live below the federal poverty line, indicating that they have insufficient income to pay for their basic needs (Paral, 2009). Accumulated poverty-related stress has been identified as a likely mechanism causing anxiety in low-income children (Beesdo et al., 2009; Conger & Donnellan, 2007; Wadsworth et al., 2011), and the present study builds on this literature by examining culturally relevant stress alongside poverty-related stress for low-income Mexican-origin children. Thus, for the purposes of this study, "immigration-related stress" is conceptualized as containing both poverty-related stress (e.g., parents having insufficient income to support the family) and culturally relevant stress (e.g., fears related to deportation, experiences of discrimination, and cultural conflicts between parents and children).

Studies on cultural stress find that in addition to general stressors, Mexican-origin children face culturally relevant stressors that are related to significant mental health concerns. These culturally relevant stressors include the anti-immigrant attitudes portrayed by others, family separation related to immigration status, and stress related to gaps in acculturation between youth and their peers and/or parents (Cervantes, Cardoso, & Goldbach, 2015) and likely contribute to the disproportionate mental health difficulties faced by Mexican-origin children. For example, experiencing stressors such as discrimination, immigration stress, bicultural stress, peer pressure to conform to one's ethnic group, intergenerational acculturation-gap conflict, and family stress has been tied to poorer educational outcomes (Lui, 2015), poorer interpersonal functioning (Piña-Watson, Dornhecker, & Salinas, 2015), worse externalizing problems such as rule-breaking and aggression (Cano et al., 2015), worse depressive symptoms (Cano et al., 2015; Cervantes et al., 2015; Piña-Watson et al., 2015), more suicidal ideation (Cervantes, Goldbach, Varela, & Santisteban, 2014; Piña-Watson et al., 2015), and more self-harm (Cervantes et al., 2014). In addition, culturally relevant stress has been implicated in the experiences of physiological, concentration, and worry-related symptoms of anxiety for Latino children (Suarez-Morales & Lopez, 2009).

Past research has shown that Mexican and Mexican American children experience more symptoms of anxiety than Caucasian children (Beidel & Alfano, 2011; Varela et al., 2008). Further, pathological anxiety is widely conceptualized as being exacerbated by stressful life events (Beesdo et al., 2009; Beidel & Alfano, 2011). Thus, the present study examines whether the multiple chronic immigration-related stressors experienced by children of Mexican-origin immigrants are related to the presence of anxiety in this population. Given that much of the research on culturally relevant stress has focused on adolescents, the present study focuses on the experiences of Mexican-origin children ages 6-10 in order to identify early risk factors for the development of anxiety.

Studying Chronic Stress and Anxiety in Middle Childhood

When examining the effect of accumulated stress on the development of anxiety, the optimal developmental period to examine is middle childhood (Beesdo et al., 2009). Anxiety is the most common mental health disorder in childhood, so identifying etiological factors implicated in anxiety development can have a large impact on public health (Beesdo et al., 2009). Scholars have called for longitudinal research examining which developmental and contextual processes contribute to the emergence of anxiety problems, and this is even more crucial when examining children with disproportionately high rates of anxiety, such as Mexican-origin children (Varela et al., 2008). As younger children (ages 7-12) report experiencing more anxiety symptoms across more types of anxiety than older children (ages 13-16), the process of anxiety development must be examined during middle childhood, as this is the period when anxiety disorders commonly onset (Beidel & Alfano, 2011; Varela et al., 2008).

Normative changes that occur during middle childhood heighten the risk for anxiety development. For example, cognitive development occurring during this time affects the emergence of anxiety. Around age six or seven, children begin to understand the concept of talking to oneself, which is implicated in the negative cognitions that are a key component of anxiety (Beidel & Alfano, 2011). Due to the cognitive immaturity of very young children, anxiety may not be as salient or complex before the age of six. In addition, with increasing cognitive maturity, children develop the ability to consider the future, which is central to anxiety, as anxiety is conceptualized as fear related to the future (Beesdo et al., 2009; Beidel & Alfano, 2011). Further, as children begin to contemplate concepts that are more abstract and nuanced, they develop the ability to think about concerns related to social evaluation, the wellbeing of others besides themselves, and other topics that younger children often do not readily conceptualize (Beidel & Alfano, 2011). Finally, when children attend school beginning around age six, this stimulates the emergence of school-related fears, including anxiety related to separation from caregivers, social interactions with peers and unfamiliar adults, and academic performance (Beidel & Alfano, 2011). Thus, risk factors for anxiety development increase during middle childhood, making this developmental period the optimal time to examine factors associated with anxiety symptomatology.

For the same reasons, the negative impact of culturally relevant stress may be stronger during middle childhood. During this time, children develop new skills across cognitive and social domains, enabling them to notice stressful events more readily, and increasing the likelihood that they will worry about stressful events after they occur. Given that anxiety is experienced more often by Mexican-origin children than by Caucasian children, research that attempts to explain this health disparity must include children of this age range (Beidel & Alfano, 2011; Varela et al., 2008). As Mexican-origin children confront culturally relevant stress (e.g., immigration-related stress) in addition to stressors faced by youth in other ethnic groups (e.g., peer-related stress, academic stress), it is particularly important to examine anxiety development during middle childhood in Mexican-origin children (Cervantes et al., 2015; Varela et al., 2008). The additional stress faced by Mexican-origin children may help to explain their disproportionately high rates of anxiety, and longitudinal research including school-aged children has the capacity to confirm or refine this hypothesis.

HPA Axis Activity and Anxiety in Mexican-Origin Children

Chronic HPA axis activity may be a key mechanism through which low-income Mexican-origin children exposed to high immigration-related stress develop anxiety symptoms. The HPA axis is a physiological system designed to activate in response to stress and aid in the management of such experiences (McEwen & Tucker, 2011). Through a cascade of events occurring during the stress response, activation of the HPA axis leads to elevations in glucocorticoid hormones, including cortisol (Tarullo, & Gunnar, 2006). During a typical HPA axis response, when the stressor terminates and high circulating glucocorticoids are no longer needed, glucocorticoids occupy receptors in the hippocampus, leading to the inhibition of activity of the HPA axis (Tottenham & Sheridan, 2010). Although the HPA axis can adaptively manage acute stressors, when this regulatory system is repeatedly and chronically activated, dysregulation of the HPA axis occurs.

As the HPA axis is repeatedly activated, chronic occupation of glucocorticoid receptors impairs the negative feedback process, resulting in extended HPA axis activation following stressful events (Tottenham & Sheridan, 2010). This prolonged HPA axis activation in response to stress results in an elevation in cortisol secretion (Essex et al., 2011; Saxbe, 2008). Because the HPA axis continues to develop throughout childhood, excessive exposure to cortisol during these formative years may alter the sensitivity of the HPA axis, thereby resulting in dysregulation of the HPA axis (Essex et al., 2011). Long-term cortisol accumulation is one effect of such dysregulation, conveying a host of physical and psychological risks (Essex et al., 2011; Gunnar & Quevedo, 2007; McEwen & Tucker, 2011). Given the harmful impact of persistent activation of the HPA axis, the present study examines the effect of chronic HPA axis activity (i.e., accumulated cortisol in hair over a period of three to six months) rather than immediate cortisol reactivity or diurnal cortisol rhythms. *Allostatic load* is a related construct that consists of the cumulative impacts of stress across multiple physiological pathways (McEwen & Tucker, 2011). Elevated allostatic load prevents individuals from habituating to stressors, leaving them with persistent elevations in cortisol and psychological problems like anxiety (Essex et al., 2011). As the present study focuses on only one physiological measure of stress (cortisol accumulation), the construct employed in this study is *chronic HPA axis activity*.

The most widely used samples for measuring cortisol are blood and saliva, though these samples yield estimates of HPA axis activity that fluctuate according to circadian variation, and even the collection of multiple samples over time does not produce a long-term index of HPA activity and its response to chronic stress (Meyer & Novak, 2012). Thus, measuring cortisol in hair has begun to fill that gap in the stress literature. As cortisol is incorporated in hair as it grows, hair samples are being used to measure HPA activity over prolonged periods of time retrospectively (Wippert et al., 2014). Hair cortisol concentrations (HCCs) are both valid and reliable as a measure of chronic HPA axis activity (Wippert et al., 2014). Although HCC is a relatively new measure, it has been established as a biomarker of chronic stress (Russell et al., 2012), for both adults and children (Vanaelst et al., 2012). Biomarkers have been defined as objective indicators of biological processes that can be measured accurately and reliably (Strimbu & Tavel, 2010). Hair cortisol analysis is a non-invasive technique that can uniquely examine the physiological effects of temporally distant stressors, making it an exciting and promising biobehavioral research tool (Gow et al., 2010; Stalder & Kirschbaum, 2012; Russell et al., 2012). Thus, the present study utilizes HCCs to measure chronic HPA axis activity in low-income Mexican-origin children.

An impressive body of research has implicated chronic HPA axis activity as a mechanism through which high levels of chronic stress lead to mental health problems (Essex et al., 2011; Gunnar & Quevedo, 2007; McEwen & Tucker, 2011). Chronic HPA axis activity has been tied to threats to both mental and physical health, making long-term cortisol secretion "a vital parameter" in biobehavioral research (Chrousos & Kino, 2007; Cunningham, 2015; Essex et al., 2011; Stalder & Kirschbaum, 2012). Ongoing buildup of cortisol can also increase the severity of conditions such as anxiety (Cunningham, 2015; Essex et al., 2011), and elevated levels of cortisol have been implicated in the development of anxiety symptoms (Qin et al., 2014). Chronic HPA axis activity has been shown to cause individuals to fail to habituate to stressors, leaving them with persistent elevations in cortisol and resulting in psychological and medical problems (Essex et al., 2011; McEwen & Tucker, 2011).

Despite mounting evidence for HPA axis activity as a key mechanism explaining the development of anxiety in the context of chronic stress, there are some inconsistencies in the literature. Although the majority of stress research has demonstrated hypercortisolism in the context of chronic stress, some studies have also found hypocortisolism in stressed populations. As a result, current theories view cortisol deviations in both directions as indicators of HPA axis dysregulation and as potentially harmful (Miller, Chen, & Zhou, 2007). Miller and colleagues (2007) conducted a meta-analysis of studies examining the effects of chronic stress on HPA axis functioning. This research revealed that exposure to chronic stress is associated with a significantly higher daily volume of cortisol output ($d = 0.31$). However, HPA axis function depended on the nature of the stress and the individual. One of the most robust findings of the meta-analysis was that the amount of time since the onset of stress was negatively associated with HPA activity (Miller et al., 2007). This supports past research in which studies focusing on recent and ongoing stressors have typically documented increases in cortisol output, whereas studies focusing on distant traumas have often found the opposite. Further, two recent reviews of studies on hair cortisol have found significantly increased HCCs associated with a wide range of chronic stress contexts (e.g., major life events, unemployment, chronic pain), early life adversity, stress-related psychiatric conditions, and medical conditions indicating chronic activation of the

HPA axis or high stress levels (Staufenbiel, Penninx, Spijker, Elzinga, & van Rossum, 2013; Wosu, Valdimarsdóttir, Shields, Williams, & Williams, 2013). Effect sizes for the impact of exposure to recent and/or ongoing stressors on hair cortisol levels were medium to large, indicating an impressive increase in HCC resulting from chronic stress. These findings provide strong support for the ability of hair cortisol research to distinguish individuals with increased cortisol production from individuals with normal HPA axis activity (Staufenbiel et al., 2013).

Because the aggregate of stress research examining both short-term and long-term cortisol secretion in response to chronic stress demonstrates an increase in cortisol, the present study focuses on hypercortisolism as an indicator of chronic, ongoing immigration-related stress for Mexican-origin children. Given the dearth of longitudinal research on chronic stress and HPA axis activity, leaders in this area have called for future research using prospective longitudinal designs to substantiate past meta-analytic findings (Miller et al., 2007; Wosu et al., 2013). The present study has the opportunity to contribute to the field's understanding of chronic stress and its consequences as well as extend this understanding to a new population. The process of chronic stress causing elevated HPA axis activity that precipitates the development of anxiety symptoms has not been established in low-income Mexican-origin children, especially not using culturally relevant measures of chronic stress, including stressors related to immigration. The present study examined cortisol accumulation over three months as a mediator of the association between immigration-related stress and anxiety symptoms within low-income Mexican-origin children.

Attentional Bias to Threat in Child Anxiety

Although chronic HPA axis activity may be a key mechanism in the development of anxiety, not all individuals with elevated cortisol accumulation develop mental illness (Essex et al., 2011; Gunnar & Quevedo, 2007; McEwen & Tucker, 2011). Thus, studies evaluating possible moderators of this process are needed. Neurocognitive processes such as attentional bias to threat have been shown to determine the trajectory of children's anxious behavior later in life and could be good candidates for moderators of this process (Shechner et al., 2012). Selectively attending to threatening stimuli is typically an adaptive response that enables individuals to evade dangerous and distressing situations (Wolters et al., 2012). However, exaggerated attentional bias to threat has been widely theorized to be a causal and maintaining factor in anxiety disorders (Bar-Haim et al., 2007; Shechner et al., 2012). Thus, attentional bias to threat may be the neurocognitive process that distinguishes the children who display anxiety symptomatology from those who do not.

Since the 1980s, several prominent theorists have emphasized the role of neurocognitive processes in anxiety (Muris & Field, 2008). For example, Kendall's (1985) cognitive theory of anxiety posits that anxious children have overactive schemas involving themes of vulnerability and danger. Thus, when presented with novel or ambiguous information, these schemas focus information-processing resources on threat-relevant information and manifest themselves in cognitive distortions. Further, Crick and Dodge's (1994) well-known model of information processing has been applied to child anxiety, emphasizing a focus on threat throughout all stages of information processing (Daleiden & Vasey, 1997). For example, during encoding, anxious children tend to attend to threatening stimuli (i.e., attention bias). Further, during information interpretation, anxious children tend to attach threatening meanings to ambiguous stimuli (i.e., interpretation bias), and finally, anxious children display enhanced memory for information about danger (i.e., memory bias). This biased information processing chronically increases anxiety, which in turn may further strengthen the maladaptive vulnerability and danger schemas (Muris & Field, 2008). These theoretical models of cognitive biases in anxiety have been supported by strong evidence that anxious children selectively attend to threatening stimuli in their environment (i.e., attentional bias) and form overly threatening interpretations of ambiguous stimuli (i.e., interpretation bias; Field & Lester, 2010). In fact, in both animal models and human brain-imaging research, attentional bias to threat has been observed across diagnostic categories of anxiety disorders, suggesting that this neurocognitive bias is a shared process across the spectrum of anxiety (Bar-Haim et al., 2007; Shechner et al., 2012).

Several arguments support the premise that attentional bias to threat is a better candidate for a moderator of anxiety development than interpretation bias or memory bias. First, reviews of research on anxiety-related information-processing biases have yielded strong evidence for the conclusion that anxious children display attentional bias to threat during encoding and interpretation bias during the more conceptual stages of information processing; however, few studies have demonstrated memory bias in youth with anxiety problems (Muris & Field, 2008). Thus, biases in attention and interpretation are stronger indicators of pathological anxiety than memory bias. Second, experts in this research area suggest that attentional bias to threat may be causally implicated in the development of pathological anxiety, because attention acts a “gate-keeper,” determining which environmental stimuli are processed, and therefore, what children learn about the world (Pérez-Edgar et al., 2014). Consequently, attentional bias early in the processing of information may significantly influence later interpretive bias. Research has shown that experimentally manipulating attention bias to threat affects the manner in which ambiguous information is interpreted (White, Suway, Pine, Bar-Haim, & Fox, 2011). This finding suggests that anxiety-related abnormalities in the initial stages of information processing may lead to a cascade of subsequent processing biases. Indeed, developmental research has established that attention orienting determines the trajectory of anxious behavior later in life (Shechner et al., 2012). Studies examining behaviorally inhibited temperament and attentional bias to threat have found that of the children with anxious temperaments early on, it is only those who also display attentional bias to threat who go on to develop anxiety symptomatology (Pérez-Edgar, Bar-Haim, et al., 2010; Pérez-Edgar, McDermott, et al., 2010; Pérez-Edgar et al., 2011). Attentional bias to threat is a strong potential moderator determining which children develop anxiety symptoms in the context of chronic stress. Thus, the present study focuses on attentional bias to threat as the factor that determines which low-income Mexican-origin children display anxiety symptoms in the context of chronic stress and dysregulated HPA axis activity.

The most direct way to measure attentional bias to threat is the dot-probe task (Shechner et al., 2012). In this experimental paradigm, two stimuli (i.e., two pictures) are briefly presented on the left and right sides of a computer screen; one stimulus is threatening, and the other is neutral. Following the disappearance of the stimuli, a small dot (i.e., the probe) appears in a location previously occupied by one of the stimuli. The child is instructed to identify the probe location via a key press (i.e., pressing one key for the left side of the screen and another key for the right side of the screen). It is hypothesized that the time required to detect the dot probe indicates the extent to which visual attention was directed to the stimulus in that location. Thus, faster latencies to detect a probe following threatening stimuli relative to neutral stimuli indicate an attentional bias toward threat, whereas the opposite pattern reflects a tendency to direct attention away from threat (Muris & Field, 2008). Research utilizing a dot-probe task has demonstrated that anxious children, relative to controls, are faster to react to a probe if it is preceded by a threatening stimulus than a neutral stimulus. Similar findings have been established in populations of children who display non-clinical levels of anxiety (Muris & Field, 2008); thus, attentional bias to threat appears to be present in both highly anxious and anxiety-disordered youth, just like in anxious adults (see Bar-Haim, Lamy, Pergamin, Bakermans-Kranenburg, & van IJzendoorn, 2007, for a meta-analytic review). Researchers in the area of child anxiety have called for further examination of information processing biases such as attentional bias to threat in order to understand whether these processes pose a risk for subsequent anxiety disorder onset (Beesdo et al., 2009).

Research on attentional bias has rarely, if ever, focused on low-income children of Mexican-origin immigrants (Wolters et al., 2012), who display disproportionate rates of anxiety compared to children of other ethnicities (Varela et al., 2008). If attentional bias to threat moderates the association between accumulated stress and anxiety symptoms, this would indicate that despite experiencing chronic stress, children may be protected from developing pathological anxiety if they do not excessively attend to threats in their environment. Further, if attentional bias to threat is the factor that determines which children display anxiety symptoms, tests of this neurocognitive process may aid in the early identification of children in need of intervention (Bar-Haim, 2010; Wolters et al., 2012). Specifically, if attentional bias to threat moderates the link between chronic stress and anxiety symptoms, this would suggest that the low-income

Mexican-origin children with early attentional bias to threat are those who would benefit most from early intervention efforts. Attention bias modification treatment has been shown through meta-analysis to have a moderate effect, d = .61, on training individuals to orient away from threat and thereby reduce anxiety (Hakamata et al., 2010). However, there is a need for further evaluation of attentional bias to threat in low-income Mexican-origin children, especially in the context of the elevated chronic stress faced by these children (Bar-Haim, 2010; Shechner et al., 2012).

An Integrated Model of Anxiety Development

A contemporary priority of the National Institutes of Health (NIH) is to “promote research to understand and to improve minority health,” which includes “advancing scientific understanding of the causes of health disparities” (NIMHD, 2018). It is important to understand the disproportionately high rates of anxiety problems in Mexican American children, because in doing so, we may begin to identify and intervene in processes that precede the onset of anxiety in these children (Beesdo et al., 2009). It is clear that low-income Mexican-origin children are exposed to an accumulation of chronic stressors, perhaps twice as many stressors as predominantly Caucasian middle-class children (Cervantes et al., 2012; Cervantes et al., 2013; Suarez-Morales & Lopez, 2009). This accumulated stress stimulates increased HPA axis activity, likely resulting in negative mental health outcomes (Essex et al., 2011; Gunnar & Quevedo, 2007; McEwen & Tucker, 2011). Although this process has been observed in other contexts, this model of anxiety development has not been tested among Mexican-origin children. Despite the promise of this mediational model in gleaning new understanding of the high rates of anxiety problems in Mexican American children, this model requires refinement, as not all low-income

Mexican-origin children develop anxiety problems. The link between accumulated stress and increased HPA axis activity is likely universal, as HCCs have been established as a biomarker of chronic stress for both adults and children (Russell et al., 2012; Vanaelst et al., 2012). However, there may be a moderating process implicated in anxiety development following repeated HPA axis activation. Given the strong research literature tying attentional bias to threat to the onset and maintenance of anxiety, and given the conceptualization of attention as a “gate-keeper,” determining which environmental stimuli are processed, this construct may explain why some low-income Mexican-origin children display anxiety after experiencing chronic stress and some do not (Bar-Haim et al., 2007; Pérez-Edgar et al., 2014; Shechner et al., 2012). This research was designed to test this integrated model of anxiety development in low-income Mexican-origin children.

The Current Study

Past research has highlighted the detrimental effect of exposure to stress on mental health, and the link between accumulated stress and mental health problems is intensified for ethnic minority youth (McLeod & Owens, 2004). This is partially a result of the greater number of stressors facing ethnic minority children, particularly those who are low-income (Beesdo et al., 2009; Conger & Donnellan, 2007; Wadsworth et al., 2011). In addition, the stronger impact of stress on mental health for ethnic minority children is caused by the additive effect of discrimination. Discrimination is conceptualized as a persistent type of stress that accompanies other major stressors and magnifies their detrimental effect on mental health (Edwards & Romero, 2008; Kessler, Mickelson, & Williams, 1999). For example, a child who is a member of a non-minority group may experience the negative emotions associated with academic stress, but for a child who is a member of a minority group, this same academic stress may be accompanied by the additional stressor of discrimination, given the interplay between race/ethnicity and stress in the United States (Kessler et al., 1999; Torres, Santiago, Walts, & Richards, 2018). The current sociopolitical context in the U.S. magnifies this effect, as immigration-related policies and associated social attitudes have created a harsh environment for Mexican-origin children (Torres et al., 2018). This is why it is important to document the negative impact of culturally relevant stress (e.g., immigration-related stress)—it is likely that this type of stress causes more harm than other major types of stress. By demonstrating the detrimental effect of accumulated stress on mental health for low-income Mexican-origin children, we may begin to understand the reasons for the disproportionately high rates of anxiety in this group, creating an opportunity for change.

Despite emerging evidence of the risk conveyed by constructs examined in this research, no previous study has focused on both HPA axis activity and attentional bias to threat in order to explain why some low-income Mexican-origin children display anxiety in the context of accumulated stress and some do not. The current study included a community sample of children (ages 6-10) of low-income Mexican-origin immigrants and a longitudinal research design (three timepoints each spaced six months apart). This study used culturally relevant measures of poverty-related and immigration-related stress and examined chronic HPA axis activity as a mechanism in the development of anxiety among a population where these processes have not been previously studied. Further, this research examined attentional bias to threat as a moderator of the association between chronic HPA axis activity and anxiety symptoms. The first goal of this research is to determine whether elevated HPA axis activity mediates the relation between culturally relevant stress and later anxiety symptoms in this population. The second goal is to determine whether attentional bias to threatening stimuli moderates the relation between cortisol accumulation and later anxiety symptoms in this population. The third and final goal is to test an integrative longitudinal model of anxiety development in the context of chronic immigration-related stress. This integrated model combines the first two goals of this project, examining whether elevated HPA axis activity mediates the relation between culturally relevant stress and later anxiety symptoms, with the effect of HPA axis activity on later anxiety symptoms being conditional on attentional bias to threatening stimuli.

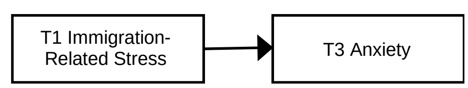
Specific Aims and Hypotheses

Specific Aim #1

The first aim of this study is to determine whether high immigration-related stress at the first timepoint (T1) relates to elevated HPA axis activity six months later (Pre-T3), which relates to anxiety symptomatology six months after that (T3).

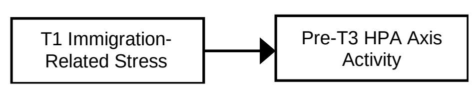
Hypothesis 1a. Children's high levels of immigration-related stress at T1 will be related to their anxiety symptoms at T3 (see Figure 1).

Figure 1. Model of hypothesis 1a



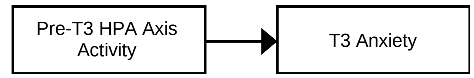
Hypothesis 1b. Children's high levels of immigration-related stress at T1 will be related to their Pre-T3 HPA axis activity (measured by cortisol concentrations in hair samples collected at T3; see Figure 2).

Figure 2. Model of hypothesis 1b



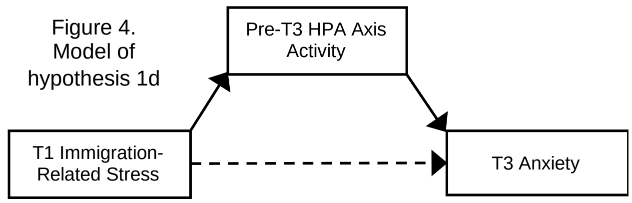
Hypothesis 1c. Children's Pre-T3 HPA axis activity (measured by cortisol concentrations in hair samples collected at T3) will be related to their anxiety symptoms at T3 (see Figure 3).

Figure 3. Model of hypothesis 1c



Hypothesis 1d. The association between children's T1 immigration-related stress and T3 anxiety symptoms will be statistically accounted for by elevations in Pre-T3 HPA axis activity (see Figure 4).

Figure 4. Model of hypothesis 1d

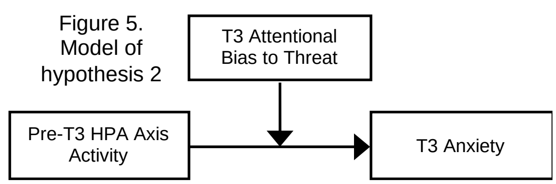


Specific Aim #2

The second aim of this study is to determine whether attentional bias to threatening faces at T3 (as measured by a dot-probe task) moderates the association between Pre-T3 HPA axis activity and T3 anxiety symptomatology.

Hypothesis 2. For children who demonstrate attentional bias to threatening faces (relative to neutral faces) in a dot-probe task at T3, elevated Pre-T3 HPA axis activity will be related to high anxiety symptoms at T3, whereas for children who do not demonstrate attentional bias to threatening faces at T3, anxiety symptoms at T3 will be lower (regardless of their levels of Pre-T3 HPA axis activity; see Figure 5).

Figure 5. Model of hypothesis 2



Specific Aim #3

The third and final aim of this study is to evaluate a longitudinal model of the development of anxiety symptoms that integrates attentional bias to threat within a context of chronic immigration-related stress and HPA axis activity. This model combines Hypothesis 1d and Hypothesis 2, examining whether children's T1 immigration-related stress is tied to Pre-T3 HPA axis activity, which is tied to T3 anxiety—with Pre-T3 HPA axis activity being more strongly associated with T3 anxiety for children with high levels of T3 attentional bias to threat.

Hypothesis 3. The association between children's T1 immigration-related stress and T3 anxiety symptoms will be statistically accounted for by elevations in Pre-T3 HPA axis activity. Additionally, the association between Pre-T3 HPA axis activity and T3 anxiety symptoms will differ depending on T3 attentional bias to threat. For children who demonstrate T3 attentional bias to threat, elevated Pre-T3 HPA axis activity will be related to high anxiety symptoms at T3, whereas for children who do not demonstrate T3 attentional bias to threat, anxiety symptoms at T3 will be lower (regardless of Pre-T3 HPA axis activity; see Figure 6).

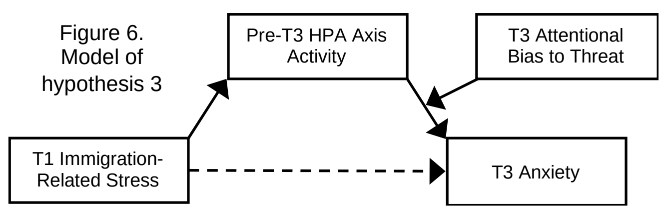


Figure 6. Model of hypothesis 3

CHAPTER TWO

METHOD

The present study expanded on a longitudinal study conducted by Catherine DeCarlo Santiago, Ph.D. and funded by the Foundation for Child Development. In the larger study, home visits were conducted for each participating family once every six months over three timepoints (T1, T2, and T3). This dissertation study added the collection of hair samples that were assayed for cortisol, a computerized dot-probe task assessing attentional bias to threat, and a measure of anxiety symptoms in order to accomplish the Specific Aims of this research. Data collection for this study (across T1, T2, and T3) was completed from June 2013 to September 2015.

Participants

Participants were recruited from the community. Eligibility criteria included: at least one Mexican-origin immigrant parent, at least one child between the ages of 6-10, and family income at or below 150% of the federal poverty line. Although efforts were made to recruit secondary caregivers from each family, if the family satisfied all eligibility criteria and no secondary caregiver was available, the family was still permitted to participate. Out of 162 interested families, 58 did not meet eligibility criteria, were no longer interested, or were not able to be contacted or scheduled, yielding a final sample of 104 low-income Mexican-origin families.

Retention rates for this study were strong. At Time 1, 104 children, 104 primary caregivers, and 72 secondary caregivers participated. At Time 2, 97 children, 99 primary caregivers, and 67 secondary caregivers participated. At Time 3, 97 children, 97 primary caregivers, and 61 secondary caregivers participated.

At Time 1, 61% of children were female (M_{age} = 8.39, *SD* = 1.33), and 97% identified as Latino (2% identified as African American & Latino, and 1% identified as Caucasian & Latino). Most children (97%) were born in the U.S., and 3% of children were born in Mexico. Among caregivers at Time 1, 97% of primary caregivers were female (M_{age} = 37.13, *SD* = 5.61), and 82% of secondary caregivers were male (M_{age} = 43.14, *SD* = 10.03). The majority of primary caregivers (98%) were mothers, and the majority of secondary caregivers (79%) were fathers, although participating caregivers also included grandparents, aunt/uncles, an older sibling, and a stepfather. The majority of mothers (99%) and fathers (98%) identified as Latino, 1% of mothers identified as Caucasian, and 2% of fathers identified as African American. Most mothers (91%) and fathers (87%) were born outside of the U.S. All immigrant parents were born in Mexico, except for one mother born in Guatemala, one mother born in Uruguay, and three fathers born in Guatemala (given the inclusion criteria for this study, families with one parent born in Guatemala or Uruguay had another parent born in Mexico). The majority of primary caregivers (62%) and secondary caregivers (80%) were married. Families reported an average monthly income of $1,806.53 (*SD* = $928.05) at Time 1, with this income supporting an average of 3.98 people (*SD* = 1.50). Regarding the educational attainment of primary caregivers: 32% did not finish high school; 26% received a high school diploma or a GED; 35% attended some college and/or obtained a training certificate, associate's degree, or college degree; 2% obtained an advanced degree; and 5% were currently attending school. Regarding educational attainment of secondary caregivers: 51% did not finish high school; 24% received a high school diploma or a

GED; 20% attended some college and/or obtained a training certificate, associate's degree, or college degree; 4% obtained an advanced degree; and 1% were currently attending school.

Procedures

For the parent study, recruitment was completed via community partnerships, including human service organizations, local parochial schools, and community centers, and relevant family-oriented community resources, including libraries, churches, and doctors' offices using flyers, letters, and in-person presentations. Interested families were screened in person or by phone to ensure eligibility. If families met eligibility criteria, a home visit appointment was scheduled. Home visits were conducted at all timepoints to eliminate the burden placed on research participants related to transportation and childcare. Questionnaires were administered in an interview format to eliminate possible barriers related to literacy. All procedures were conducted in the language preferred by participants (Spanish or English) by bilingual members of the research team. Parents were informed that their family's participation would entail three visits spaced six months apart. Back-up contact information for a close friend or relative was collected to aid with participant retention. Time 1 and Time 2 home visits consisted of the larger study's parent and child questionnaires and family observational tasks. In addition to these procedures, Time 3 home visits included the present study's novel procedures (described in detail below): the collection of children's hair samples, the administration of the dot-probe task, and the completion of a self-report measure of child anxiety symptoms. Families received a $100 gift card for their participation at each visit. Also, families who participated in this research's novel procedures at Time 3 received an additional $20 gift card.

Hair Sample

At the Time 3 home visit, children were asked to provide a hair sample to be assayed for cortisol. When taking the hair sample, children were given a game to play on a laptop to limit distraction and to focus their attention downward. A nondetectible amount of hair was cut from the vertex posterior as close to the scalp as possible using thinning shears. Hair samples for girls were carefully secured in aluminum foil and labeled with the scalp end, and hair samples for boys with short hair were collected in a secure container. The most proximal 3 cm hair segments to the scalp were used to measure the past three months' cortisol accumulation (Russell et al., 2012). Thus, hair cortisol data is conceptualized as "Pre-T3." All samples were assayed by the Behavioral Immunology and Endocrinology Laboratory at the University of Colorado Denver Anschutz Medical Campus in collaboration with Mark Laudenslager, Ph.D., as described below.

Dot-Probe Task

Also at the Time 3 home visit, children were asked to complete a computerized dot-probe task. In this study, the dot-probe task was presented on a laptop computer using E-Prime software (version 2.0 Professional; Psychology Software Tools Inc., 2014). Each trial began with a fixation cross for 500 ms, followed by the presentation of a pair of faces (one angry and one neutral) for 500 ms, followed by a dot appearing in the location of one of the faces. Using the laptop keyboard, children indicated on which side of the screen the dot appeared, and then a new trial commenced. The inter-trial period was varied randomly from 750 to 1,250 ms; this variation has been used in previous child anxiety research to facilitate subject engagement by minimizing the predictability of trial onset (Salum et al., 2013). Although studies employing the dot-probe task have used a range of stimulus presentation durations, the present duration of 500 ms was selected based on research indicating that this is the most reliable assessment of attentional bias to threat in children with anxiety symptoms (Shechner et al., 2012). For facial stimuli, the Karolinska Directed Emotional Faces (KDEF; Lundqvist, Flykt, & Öhman, 1998) were used. The KDEF database includes 490 images of faces displaying emotional expressions from 70 individuals between 20 and 30 years of age (35 men and 35 women). For this study, the 20 most accurate angry face actors (10 male and 10 female) were selected based on ratings reported by Goeleven, De Raedt, Leyman, and Verschuere (2008). Each photo was presented in color. There were three types of face pairs presented: Angry/Neutral (32 trials), Happy/Neutral (32 trials), and Neutral/Neutral (16 trials), and each trial was presented twice, for a total of 160 trials presented over 7-8 minutes. In order to minimize distraction and ensure that the environment in which the dot-probe task was completed was as similar to a structured lab setting as possible, there were a number of steps that were implemented: place the laptop on a table/desk with a chair directly in front of it, turn off all lights and close all window coverings, maximize laptop screen brightness, arrange the child in front of the laptop so that his/her eyes are level with the center of the screen, measure the distance from the child's eyes to the center of the screen, use a wired keyboard on a lap desk in order to standardize the ease of input across children, and administer practice trials with opportunities for children to ask questions. See measure description below for calculation of attentional bias to threat.

Measures

Demographic Information

At the Time 1 home visit, caregivers reported demographic information on the questionnaires they were read by research staff. Child and caregiver ages, gender, generational/immigration status, length of time in the U.S., and language preference/use were assessed.

Immigration-Related Stress

Immigration-related stress. At the Time 1 home visit, caregivers completed the Hispanic Stress Inventory (HSI), a culturally informed measure of psychosocial stress among immigrant and non-immigrant Latinos (Cervantes, Padilla, & Salgado de Snyder, 1991). The HSI comprises 73 items organized into five subscales: Immigration Stress, Family/Culture Stress, Parental Stress, Marital Stress, and Occupational/Economic Stress. In order to capture the accumulated stress that has been examined in past research along with culturally relevant stress, the present research used the following HSI subscales: Immigration Stress, Family/Culture Stress, Parental Stress, and Occupational/Economic Stress. These subscales were examined together, as a composite of immigration-related stress, and separately, to determine whether distinct domains of stress are differentially related to other key variables. Sample items include "I feared the consequences of deportation" (Immigration Stress), "There has been physical violence among members of my family" (Family/Cultural Stress), "My children have been influenced by bad friends" (Parent Stress), and "My income has not been sufficient to support my family or myself" (Occupational/Economic Stress). For each item, parents indicated whether their family had experienced that particular stressor during the past three months. They then rated the perceived stressfulness of each endorsed item on a five-point scale (1 = not at all, 2 = somewhat, 3 = moderately, 4 = very, 5 = extremely). Previous research with the HSI has achieved high levels of internal consistency (alphas ranging from .77 to .91) and adequate test-retest reliability (.61 to .86). In the present sample, internal consistency was adequate (composite alpha was .88, and subscale alphas were .65 to .80).

For the present study, it was determined that if stress totals from the two participating caregivers correlated at or above .80 for all participants, a composite of both caregivers' scores would be used. It was found that caregivers' reports of immigration-related stress did not correlate above this threshold. Thus, it was decided that primary caregiver data would be used, except in families with a primary caregiver who was not an immigrant; in these families, secondary caregiver data would be used in order to accurately assess immigration-related stress in the family.

Child-specific immigration-related stress. At the Time 1 home visit, caregivers also reported on immigration-related stress that was experienced specifically by the participating child. Caregivers answered "yes" or "no" to a series of nine items, indicating which of these stressors had been a problem for their child during the past six months. These nine items describing child-specific stress were drawn from the Hispanic Stress Inventory - Adolescent Version (HSI-A), a culturally informed measure of psychosocial stress that was designed for use with Latino adolescents (Cervantes, Fisher, Córdova, & Napper, 2012). The HSI-A consists of 71 items organized into eight subscales: Family Economic Stress, Culture and Educational Stress, Acculturation-Gap Stress, Immigration Stress, Discrimination Stress, Family Immigration Stress, Community and Gang-Related Stress, and Family and Drug-Related Stress. In order to capture accumulated stress across these domains and adapt this measure so that it was briefer and relevant to school-aged children, nine items were chosen across seven of these eight subscales (excluding Family and Drug-Related Stress). Sample items include "Members of his/her family had problems with immigration papers," "There was not enough money for everyone in his/her family," "S/he experienced racial tensions at school," and "S/he was picked on by other students." Previous research with the HSI-A has achieved high internal consistency (alphas ranging from .92 to .96), and in the present study, internal consistency was .68. Primary caregivers' reports of child-specific stress were used.

Chronic HPA Axis Activity

At the Time 3 home visit, children provided a hair sample to be assayed for cortisol (n = 92 out of 97 families participating at Time 3). Given that HCCs reflect the preceding months' accumulation of cortisol, the samples collected at Time 3 home visits reflect Pre-Time 3 HPA axis activity. All samples were assayed by the Behavioral Immunology and Endocrinology Laboratory at the University of Colorado Denver Anschutz Medical Campus in collaboration with Mark Laudenslager, Ph.D. Some hair samples (n = 8) were problematic, in that the hair was so tangled that the scalp end was undetectable, or too few strands of hair were collected, so they could not be processed. The remaining hair samples (n = 84) were processed according to best practices.

For some long hair samples (n = 20), the most proximal 3 cm hair segment to the scalp was cut and used to measure the past three months of cortisol accumulation (Russell et al., 2012). Given that some long hair samples were of insufficient weight for processing (n = 34), the most proximal 6 cm hair segment to the scalp was cut in order to include those samples in the assay. For samples less than 3 cm in length (n = 30), Dr. Laudenslager's laboratory processed the entire sample. The length of hair samples was evaluated as a potential covariate in all analyses.

Each hair sample was placed in a pre-weighed 2 ml cryovial (Wheaton, Millville, NJ, USA) and washed three times in 100% isopropanol and dried as previously described (D'Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011). After washing, drying, and re-weighing on a high sensitivity electronic balance (Mettler Toledo Model MS105, Greifense, Switzerland), the hair was ground in the same tube using a ball mill (Retsch, Haan, Germany) with a single 3/16 inch (~4.8 mm) stainless steel ball bearing. Specially milled aluminum cassettes were designed to hold three of these cryovials for grinding. The cassettes containing the cryovials were submerged in liquid nitrogen for approximately 3 minutes before grinding to freeze hair samples, rendering them brittle for easier grinding. Samples were ground for 4-5 minutes. The powdered hair (2-36 mg based on weights after washing) was extracted in the same cryovial in 1000µl HPLC grade methanol for 24 hours at room temperature on a side-to-side shaker platform. This self-contained process ensured no loss of hair in the processing by confining initial extraction steps to the same cryovial. Following the methanol extraction, the cryovial was spun for 3 minutes in a centrifuge at 1700 g to pellet the hair, and the supernatant was removed, placed into a microcentrifuge tube, and dried under a stream of nitrogen under a fume hood in a drying rack. The extracts were then reconstituted with assay diluent based on amount of hair and extraction volume. Cortisol levels were determined using a commercial high sensitivity EIA kit (Salimetrics LLC, State College, PA, USA) per manufacturer's protocol as described previously (D'Anna-Hernandez et al., 2011). HCCs are reported in the metric pg/mg (picogram per milligram), indicating the amount of cortisol in picograms per milligram of hair.

To assess the repeatability of results, intra- and inter-assay coefficients of variation (CV) were computed. A pooled control sample of previously ground hair was extracted as above and included on each EIA plate in duplicate for determination of inter-assay coefficients of variation. Inter-assay CV for the control hair pool was 16%, and intra-assay CV was 3%. During hair sample processing (n = 84), some samples were found to be of insufficient weight to yield HCCs (n = 22). Further, some samples yielded nondetectable HCCs (n = 2), resulting in a final sample of assayed samples of n = 60. Within this sample of HCCs, some values fell below the limit of sensitivity for assay (n = 8). This means that these samples' HCCs fell below the detection limit of the assay, but the HCC value could be computed using the standard curve software. These HCCs are generally very low values that are less reliable due to assay error. Thus, all analyses were run using the subsample that excluded values that fell below the limit of sensitivity for assay (n = 52).

Further examination of hair cortisol samples revealed that girls' samples were more problematic because of the difficulty involved in appropriately collecting hair samples and maintaining hair samples' integrity when girls had very long hair, especially when hair was coarse or curly. Research assistants and lab technicians who collected and processed the samples reported that several girls' hair samples were compromised. Given the sample collection method (i.e., using thinning shears), when girls had very long or curly hair, it was difficult to separate the cut strands from those around them, while maintaining alignment across all hair strands in order to detect which were the most proximal 3 cm hair segments to the scalp. Thus, during hair sample processing, when the samples contained very long or curly hair, it was difficult to select the appropriate segments of hair to include in the assay. This caused girls to be overrepresented in the group with hair samples that were impossible to assay.

Attentional Bias to Threat

At the Time 3 home visit, children completed a dot-probe task, which is considered the most direct way to measure attentional bias to threat (Shechner et al., 2012). Dot-probe data were cleaned using contemporary methods used in research on attentional bias in children (Briggs-Gowan et al., 2016; Mian et al., 2015). Trials were excluded if they contained missing responses, incorrect responses, reaction times (RTs) less than 200ms, and RTs greater than 2.5 standard deviations from the individual's mean (average 2.5 *SD* cutoff = 808.38 ms, *SD* = 150.47, min = 390.61, max = 1267.16). In addition, two participants' data were excluded, as their accuracy on the dot-probe task fell below 65% (average accuracy = 95%). Review for outliers (+/−3.5 *SD* from the sample mean) identified no outliers.

Attentional bias was calculated according to standard procedures based on reaction times. Reaction times for trials in which the dot replaces the angry face were subtracted from reaction times for trials in which the dot replaces the neutral face. Thus, positive values indicate attentional bias toward threat, and negative values indicate attentional bias away from threat. This procedure for computing attentional bias has yielded valid assessments among 6-10 year olds in past research with community samples (Salum et al., 2013).

Anxiety Symptoms

Self-report of child anxiety. At the Time 3 home visit, children completed the Revised Children's Manifest Anxiety Scale, Second Edition (RCMAS-2), the most updated version of one of the most widely used questionnaires in research on child anxiety (Reynolds & Richmond, 2008). The RCMAS-2 includes 49 items, which yield a Total Anxiety score that indicates the overall level of anxiety symptoms, as well as scores on three subscales (Physiological Anxiety, Worry, and Social Anxiety) that provide information on the specific nature of the anxiety. Sample items include “Often I feel sick in my stomach” (Physiological Anxiety), “I often worry about something bad happening to me” (Worry), and “I feel someone will tell me I do things the wrong way” (Social Anxiety). For each item, children mark “yes” or “no” to indicate whether or not the statement describes them. A review of the literature on evidence-based assessment of childhood anxiety indicates that the RCMAS is psychometrically strong, with internal consistency estimates greater than .80 and test-retest reliability ranging from .64 to .76 (Silverman & Ollendick, 2005), and reliability estimates for the RCMAS-2 are improved over those for the RCMAS, with a value of .92 for the Total score and values of .75 to .86 for the subscale scores (Reynolds & Richmond, 2008). In the present sample, internal consistency was .93 for the Total Anxiety score and ranged from .75 to .86 for the subscales.

The cross-group factorial invariance and the construct validity equivalence of the RCMAS have been examined in multiple studies including groups of Caucasian, Hispanic/Latino, Mexican, and Mexican American youth. Based on the findings of these studies, the RCMAS has demonstrated measurement equivalence across relevant ethnic groups (Pina, Little, Knight, & Silverman, 2009; Varela et al., 2008; Varela & Biggs, 2006). This is important, because it indicates that past research showing variations in anxiety (as reported by the RCMAS) between Latino and Caucasian youth likely reflect true group differences in anxiety symptoms. The equivalence of RCMAS-2 scores with RCMAS scores indicates that these findings likely generalize to samples tested with the RCMAS-2 (Reynolds & Richmond, 2008).

Caregiver-report of child anxiety. At the Time 3 home visit, caregivers also reported on each child’s anxiety using the Child Behavior Checklist for Ages 6 to 18, *DSM*-oriented anxiety subscale (CBCL; Achenbach & Rescorla, 2001). This subscale was developed to reflect anxiety problems that span three diagnostic categories: generalized anxiety disorder, separation anxiety disorder, and specific phobia, and it can successfully discriminate between youth with these anxiety disorders vs. other affective disorders, such as major depressive disorder or dysthymic disorder (Ebesutani et al., 2010). Caregivers rated each item on the anxiety subscale by indicating that the item was either 0 = not true, 1 = somewhat or sometimes true, or 2 = very true or often true. The CBCL has consistently demonstrated excellent reliability and validity (Achenbach & Rescorla, 2001).

Power Analyses

Hypothesis 1a, Hypothesis 1b, and Hypothesis 1c

Power analyses were conducted using G*Power 3.1 (Faul, Erdfelder, Buchner, & Lang, 2009; Faul, Erdfelder, Lang, & Buchner, 2007). A power of .80 and an alpha of .05 were assumed for all power calculations. A sample size of 93-97 was available for testing Hypothesis 1a, depending on the variable examined. For a fixed linear multiple regression model with one predictor and one covariate, a sample size of 485 is required to detect small effects ($f^2 = .02$), a sample size of 68 is required to detect medium effects ($f^2 = .15$), and a sample of 31 is required to detect large effects ($f^2 = .35$). Given the sample size available to test this hypothesis (n = 93-97), an effect size between small and medium, specifically, a minimum of $f^2 = .110$, was detectable.

A sample size of 47-50 was available for testing Hypothesis 1b, and a sample size of 49-50 was available for testing Hypothesis 1c (due to problematic hair samples). For a fixed linear multiple regression model with one predictor and two covariates, a sample size of 550 is required to detect small effects ($f^2 = .02$), a sample size of 77 is required to detect medium effects (f^2 =

.15), and a sample of 36 is required to detect large effects (f^2 = .35). Given the sample size available to test these hypotheses (n = 47-50), an effect size between medium and large, specifically, a minimum of f^2 = .255, was detectable.

Hypothesis 1d

Using simulations to empirically compute power estimates of .8, Fritz and MacKinnon (2007) determined the smallest sample sizes needed to detect mediated effects given various parameter values (0.14, 0.39, and 0.59, corresponding to Cohen's (1988) criteria for small (2% of the variance), medium (13% of the variance), and large (26% of the variance) effect sizes). The results for the bias-corrected bootstrap showed it to be consistently the most powerful test of mediation when compared to Baron and Kenny's (1986) causal-steps test, a variation of Baron and Kenny's (1986) causal-steps test known as the joint significance test, the Sobel (1982) first-order test, the percentile bootstrap test, and the bias-corrected bootstrap test. When using bias-corrected bootstrapping to detect a mediated effect, a sample of 71 provided sufficient statistical power, as long as the standardized A → B coefficient and the standardized B → C coefficient were both at least 0.39 in value. If either path had a large effect (at least 0.59 in value), the necessary sample size decreased to 53-54, and if both paths were large effects, a sample of 34 provided sufficient power. When using bias-corrected bootstrapping, these results were similar across all values of the mediational effect (i.e., full mediation and all effect sizes of partial mediation). Thus, the sample size of the present study was sufficient to detect a mediated effect if at least one A → B or B → C path (T1 immigration-related stress → Pre-T3 HPA axis activity or Pre-T3 HPA axis activity → T3 anxiety symptoms) constituted a large effect.

Hypothesis 2

A sample size of 48-49 was available for testing Hypothesis 2. For a fixed linear multiple regression model with five predictors (one predictor, one moderator, one interaction term, and two covariates), a sample size of 647 is required to detect small effects (f^2 = .02), a sample size of 92 is required to detect medium effects (f^2 = .15), and a sample of 43 is required to detect large effects (f^2 = .35). Given the sample size available to test this hypothesis (*n* = 48-49), an effect size between medium and large, specifically, a minimum of f^2 = .305, was detectable.

Hypothesis 3

A sample size of 45-48 was available for testing Hypothesis 3. For a fixed linear multiple regression model with six predictors (one predictor, one moderator, one interaction term, one mediator, and two covariates), a sample size of 688 is required to detect small effects (f^2 = .02), a sample size of 98 is required to detect medium effects (f^2 = .15), and a sample of 46 is required to detect large effects (f^2 = .35). Given the sample size available to test this hypothesis (*n* = 45-48), a large effect size, specifically, f^2 = .356, was detectable.

CHAPTER THREE

RESULTS

Preliminary and Descriptive Analyses

Descriptive statistics for all study variables are presented in Table 1, and correlations are presented in Table 2. Preliminary analyses included plots of data, evaluation of assumptions (i.e., skewness and kurtosis), and tests of possible covariates to better understand the data and their limitations.

Table 1. Descriptive Statistics of Study Variables

Variable	*n*	*M*	*SD*	Range	Variance	Skew	Kurtosis
T1 immigration-related stress							
T1 immigration-related stress composite	103	85.21	27.76	56.00 – 187.00	770.66	1.56	2.59
T1 Occupational /Economic Stress subscale	103	20.74	8.60	13.00 – 54.00	74.04	1.44	1.91
T1 Parental Stress subscale	103	16.76	5.83	12.00 – 41.00	33.95	2.31	5.50
T1 Family/Culture Stress subscale	103	19.48	7.41	13.00 – 44.00	54.94	1.37	1.54
T1 Immigration Stress subscale	103	28.24	11.05	17.00 – 63.00	122.01	1.26	1.09
Child-specific T1 immigration-related stress	98	1.80	1.85	0.00 – 9.00	3.42	1.35	2.05
Pre-T3 HPA axis activity							
Pre-T3 HPA axis activity	50	0.80	0.40	-0.27 – 2.10	0.16	0.63	2.17
T3 attentional bias to threat							
T3 attentional bias to threat	90	-3.66	26.10	-56.15 – 74.77	681.04	-0.08	-0.26
T3 anxiety							
Self-reported T3 overall anxiety	97	46.72	10.97	29.00 – 73.00	120.37	0.47	-0.63
Self-reported T3 physiological anxiety	97	43.94	10.23	29.00 – 68.00	104.64	0.45	-0.38
Self-reported T3 worry-related anxiety	97	49.66	10.33	29.00 – 78.00	106.75	0.45	-0.31
Self-reported T3 social anxiety	97	46.48	10.86	32.00 – 78.00	117.96	0.57	-0.46
Caregiver-reported T3 child anxiety	96	55.10	6.54	50.00 – 75.00	42.75	1.22	0.48

Note. When needed, variables were corrected for extreme skewness, extreme kurtosis, and outliers. This table presents corrected values.

Table 2. Correlations among Study Variables

	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	13.
1. T1 immigration-related stress composite	---												
2. T1 Occupational /Economic Stress subscale	.903***	---											
3. T1 Parental Stress subscale	.744***	.544***	---										
4. T1 Family/Culture Stress subscale	.808***	.664***	.622***	---									
5. T1 Immigration Stress subscale	.875***	.757***	.501***	.515***	---								
6. Child-specific T1 immigration-related stress	.396***	.351***	.285**	.389***	.314**	---							
7. Pre-T3 HPA axis activity	-.250	-.245	.007	-.231	-.231	-.037	---						
8. T3 attentional bias to threat	.055	.034	-.044	.079	.083	.122	-.087	---					
9. Self-reported T3 overall anxiety	.025	-.019	.045	.196	-.076	.200	.193	.085	---				
10. Self-reported T3 physiological anxiety	.080	.045	.085	.194	-.008	.176	.233	.024	.827***	---			
11. Self-reported T3 worry-related anxiety	-.034	-.080	-.013	.140	-.111	.171	.074	.132	.941***	.678***	---		
12. Self-reported T3 social anxiety	.062	.016	.090	.246*	-.068	.245*	.187	.036	.899***	.629***	.794***	---	
13. Caregiver-reported T3 child anxiety	.141	.147	.109	.044	.154	.358***	.229	-.137	.136	.115	.152	.127	---

Note. * $p < .05$, ** $p < .01$, *** $p < .001$, *n*s range from 49 to 103.

Descriptive Information

Immigration-related stress. In the present sample, 97.1% of caregivers reported that their family experienced immigration-related stress. Most families experienced multiple stressors, with a mean number of immigration-related stressors of 14.58 (*SD* = 9.57; this is different from the stressfulness ratings presented in Table 1). There was variability across the sample, with a maximum number of different stressors experienced of 49. When considering caregiver reports of child-specific immigration-related stress, 71.4% of caregivers stated that immigration-related stress impacted their children directly. Caregivers selected from a list of nine possible immigration-related stressors experienced by children, and they endorsed a range of zero to nine child-specific stressors experienced (M = 1.80, SD = 1.85).

Chronic HPA axis activity. The HCCs found in the present sample are within the ranges of HCCs in children found in past research overall; however, the HCCs found in this study are generally lower than those found in previous studies of hair cortisol in middle childhood (e.g., Dettenborn, Tietze, Kirschbaum, & Stalder, 2012; Noppe et al., 2014; Vanaelst et al., 2012). Thus, in the present sample, some children may have normative chronic HPA axis activity, whereas other children may have blunted HPA axis activity.

Attentional Bias to Threat. On the dot-probe task, mean accuracy was 95% (SD = 9.1%), with 90.1% of individuals evidencing accuracy of 90% or better. Accuracy did not differ across trial types. Mean reaction time was 497.96ms (SD = 87.55ms), and reaction time did not differ across trial types. When looking at the attentional bias to threat score, the average value was -3.66 (SD = 26.10), and this was not significantly different from zero, t = -1.33, p = .187. Thus, when examined all together, the overall sample did not display attentional bias *toward* threat or attentional bias *away* from threat. However, when the sample was examined based on tertile groupings of the attentional bias score, there were children who displayed attentional bias *toward* threat, no attentional bias, and attentional bias *away* from threat. Across these groups, the attentional bias to threat score differed significantly from zero in the attentional bias *toward* threat group, n = 30, M = 24.03, t = 10.89, $p < .001$, and in the attentional bias *away* from threat group, $n = 30$, $M = -33.93$, $t = -14.94$, $p < .001$, but not in the no attentional bias group, $n = 30$, $M = -1.08$, $t = -0.92$, $p = .364$. When examining the whole sample, children across these attentional bias groups did not differ in their ratings of anxiety. However, when examining children in the subsample with complete hair cortisol data (included in the primary analyses examining chronic HPA axis activity), children had higher total anxiety when they displayed attentional bias (either *toward* threat or *away* from threat, *M*s = 50.53 and 50.17 respectively) than when they displayed no attentional bias, $M = 42.00$, $F(2, 48) = 3.27$, $p = .047$.

Anxiety Symptoms. Children and caregivers reported a range of anxiety symptoms experienced by children in the present sample. Based on self-report, children fell into clinical ranges as follows: 28.9% "less problematic than for most children," 54.6% "no more problematic than for most children," 14.4% "moderately problematic," and 2.1% "extremely problematic." The present sample evidenced lower rates of anxiety compared to the RCMAS-2 full reference sample of 3,086 children (Reynolds & Richmond, 2008). Based on caregiver-report, 86.5% of children fell in the "normal range," 8.3% of children fell in the "borderline clinical range," and 5.2% of children fell in the "clinical range."

Tests of Normality

In the present research, data transformations were conducted in cases of extreme skewness or kurtosis (i.e., skewness > 3 and kurtosis > 10). It is common to encounter some degree of skewness and kurtosis in research employing self-report or caregiver-report measures of stress and mental health symptoms, as most people endorse values at the low end of the scale on these measures. Thus, data transformations were only considered necessary in cases of extreme skewness or kurtosis. Data transformations were unnecessary for all variables except for

HCCs. As is typical in hair cortisol research, HCCs were positively skewed and deviated from normality significantly (*p* < .001, Kolmogorov-Smirnov test). Data handling procedures were based on prior research examining HCCs in children (Groeneveld et al., 2013). First, two children were excluded because of extremely high cortisol values (>3 *SD*s above the mean). Then, after $\log_{10}$ transformation, HCCs did not deviate from normality (*p* = .055).

Tests of Possible Covariates

Several aspects of hair samples (i.e., length, weight, value above/below the limit of sensitivity) were examined in relation to demographic variables in order to determine whether these factors should be included as covariates in primary analyses. In addition, to better understand the challenges in hair cortisol sample collection and assay, comparisons were examined across three groups: complete HCC data (*n* = 52; 50%), HCC data below the limit of sensitivity (*n* = 8; 8%), missing HCC data (*n* = 44; 42%). These analyses examined demographic differences between participants in the three groups. Across these groups, participants did not differ in age. However, girls were disproportionately represented in the HCC data below the limit of sensitivity and missing HCC data groups, $X^2(1) = 11.53$, $p = .003$, Cramer's $V = .33$, likely due to methodological difficulties when collecting and processing hair samples (see Method). Thus, gender was included as a covariate in primary analyses. It was found that variables such as hair sample length and weight were also highly influenced by child gender. Thus, these characteristics of the hair samples were not included as covariates in primary analyses, but gender was.

As discussed in the Method, the type of caregiver reporting on key constructs was examined as another potential covariate to be included in primary analyses. Caregiver type was examined in relation to all caregiver-report variables used in this study's primary analyses (i.e., immigration-related stress, caregiver-reported child anxiety), and it was determined that the caregiver type variable did not need to be included as a covariate in primary analyses.

In addition, demographic variables such as child gender and child age were examined in relation to the primary variables of interest and considered for inclusion in the primary analyses. Most primary variables did not differ based on gender and age. However, the following variables were related to these demographic characteristics: 1) The subscale of immigration-related stress called Occupational/Economic Stress differed by child gender, in that it was higher in boys (M = 22.85) than in girls (M = 19.40), t = 2.02, p = .047. 2) Pre-T3 HPA axis activity differed marginally by child gender and child age. Those with higher HCCs were boys (M = 0.88; girls M = 0.69), t = 1.72, p = .093, and younger children, $r(48)$ = -.27, p = .063. 3) Child anxiety differed by child age, in that those with higher anxiety symptoms were younger. These results were similar across the self-reported child anxiety total score, $r(95)$ = -.30, p = .003, and self-reported child anxiety subscales, $r(95)$ = -.19 to -.30; p = .003 to .062, in addition to the caregiver-reported child anxiety score, $r(94)$ = -.25, p = .014. Given these differences, all analyses including these variables were tested while covarying out the applicable demographic variable(s).

Primary Analyses

Hypothesis 1a

Hierarchical regression analysis was used to determine whether children's T1 immigration-related stress was related to T3 anxiety symptoms, while covarying out child age for all self-reported child anxiety variables and covarying out child gender for Occupational/Economic Stress (see Table 3).

Table 3. Results of T1 Immigration-Related Stress Predicting T3 Anxiety

Variable	***β***	***t***	***p***
Regressions predicting Self-reported T3 overall anxiety			
T1 immigration-related stress composite	-0.01	-0.13	.90
T1 Occupational/Economic Stress subscale	-0.04	-0.42	.68
T1 Parental Stress subscale	0.02	0.19	.85
T1 Family/Culture Stress subscale	0.16	1.59	.11
T1 Immigration Stress subscale	-0.11	-1.14	.26
Child-specific T1 immigration-related stress	0.18	1.86	.07
Regressions predicting Self-reported T3 physiological anxiety			
T1 immigration-related stress composite	0.04	0.45	.66
T1 Occupational/Economic Stress subscale	0.02	0.16	.88
T1 Parental Stress subscale	0.06	0.60	.55
T1 Family/Culture Stress subscale	0.16	1.59	.12
T1 Immigration Stress subscale	-0.04	-0.42	.68
Child-specific T1 immigration-related stress	0.16	1.61	.11
Regressions predicting Self-reported T3 worry-related anxiety			
T1 immigration-related stress composite	-0.06	-0.59	.56
T1 Occupational/Economic Stress subscale	-0.09	-0.89	.37
T1 Parental Stress subscale	-0.03	-0.30	.76
T1 Family/Culture Stress subscale	0.12	1.13	.26
T1 Immigration Stress subscale	-0.14	-1.33	.19
Child-specific T1 immigration-related stress	0.16	1.57	.12
Regressions predicting Self-reported T3 social anxiety			
T1 immigration-related stress composite	0.02	0.24	.81
T1 Occupational/Economic Stress subscale	-0.01	-0.08	.94
T1 Parental Stress subscale	0.06	0.65	.52
T1 Family/Culture Stress subscale	0.21	2.14	**.04**
T1 Immigration Stress subscale	-0.10	-1.06	.29

Child-specific T1 immigration-related stress	0.23	2.34	**.02**
Regressions and correlations predicting Caregiver-reported T3 child anxiety			
T1 immigration-related stress composite	0.11	1.12	.26
T1 Occupational/Economic Stress subscale	0.11	1.11	.27
T1 Parental Stress subscale	0.09	0.89	.38
T1 Family/Culture Stress subscale	0.01	0.10	.92
T1 Immigration Stress subscale	0.13	1.28	.21
Child-specific T1 immigration-related stress	0.35	3.63	**< .001**

Note. Each row reports the results of a separate regression analysis. Bolded *p* values are statistically significant.

Self-report of child anxiety. Contrary to hypothesis, the composite of T1 immigration-related stress was not associated with self-reported T3 overall child anxiety symptoms or any subscale of self-reported T3 child anxiety symptoms. However, consistent with hypothesis, the subscale of T1 Family/Culture Stress was significantly associated with self-reported T3 social anxiety, $\beta = 0.21$, $t = 2.14$, $p = .035$. Additionally, child-specific T1 immigration-related stress was significantly associated with self-reported T3 social anxiety, $\beta = 0.23$, $t = 2.34$, $p = .021$.

Caregiver-report of child anxiety. Contrary to hypothesis, the composite of T1 immigration-related stress and its subscales were not associated with caregiver-reported T3 child anxiety. However, consistent with hypothesis, child-specific T1 immigration-related stress was significantly associated with caregiver-reported T3 child anxiety, $\beta = 0.35$, $t = 3.63$, $p < .001$.

Hypothesis 1b

Hierarchical regression analysis was used to determine whether children's T1 immigration-related stress was related to Pre-T3 HPA axis activity, while covarying out child gender and child age (see Table 4).

Table 4. Results of T1 Immigration-Related Stress Predicting Pre-T3 HPA Axis Activity

Variable	***β***	***t***	***p***
Regressions predicting HCCs			
T1 immigration-related stress composite	-0.32	-2.40	**.02**
T1 Occupational/Economic Stress subscale	-0.30	-2.28	**.03**
T1 Parental Stress subscale	-0.04	-0.28	.78
T1 Family/Culture Stress subscale	-0.30	-2.19	**.03**
T1 Immigration Stress subscale	-0.28	-2.06	**.05**
Child-specific T1 immigration-related stress	-0.03	-0.20	.85

Note. Each row reports the results of a separate regression analysis. Bolded *p* values are statistically significant.

Contrary to hypothesis, the composite of T1 immigration-related stress was significantly and negatively associated with Pre-T3 HPA axis activity, $\beta = -0.32$, $t = -2.40$, $p = .020$. When examining subscales of immigration-related stress, T1 Occupational/Economic Stress was significantly and negatively associated with Pre-T3 HPA axis activity, $\beta = -0.30$, $t = -2.28$, $p = .027$, as was T1 Family/Culture Stress, $\beta = -0.30$, $t = -2.19$, $p = .034$, and T1 Immigration Stress, $\beta = -0.28$, $t = -2.06$, $p = .045$. No significant associations were found between child-specific T1 immigration-related stress and Pre-T3 HPA axis activity.

Hypothesis 1c

Hierarchical regression analysis was used to determine whether children's Pre-T3 HPA axis activity was related to T3 anxiety symptoms, while covarying out child gender and child age (see Table 5).

Table 5. Results of Pre-T3 HPA Axis Activity Predicting T3 Anxiety

Variable	***β***	***t***	***p***
Regression predicting **Self-reported T3 overall anxiety**			
Pre-T3 HCCs	0.20	1.36	.18

Regression predicting Self-reported T3 physiological anxiety			
Pre-T3 HCCs	0.22	1.45	.16
Regression predicting Self-reported T3 worry-related anxiety			
Pre-T3 HCCs	0.10	0.66	.52
Regression predicting Self-reported T3 social anxiety			
Pre-T3 HCCs	0.19	1.29	.20
Regression and correlations predicting Caregiver-reported T3 child anxiety			
Pre-T3 HCCs	0.21	1.34	.19

Note. Each row reports the results of a separate regression analysis. Bolded *p* values are statistically significant.

Contrary to hypothesis, Pre-T3 HPA axis activity was not associated with self-reported T3 child anxiety or caregiver-reported T3 child anxiety.

Hypothesis 1d

To test the mediating function of Pre-T3 HPA axis activity in the longitudinal relation between children's T1 immigration-related stress and T3 anxiety, bootstrapping (Hayes, 2009) was used. Models were run using PROCESS v3.0 (Hayes, 2018) with 10,000 bootstrap samples, resulting in 95% confidence intervals of the indirect effect for each model. Models included child gender and child age as covariates. Models were run with the following variables measuring T1 immigration-related stress: composite of immigration-related stress, subscales of immigration-related stress, and measure of child-specific immigration-related stress. In addition, models were run with the following variables measuring T3 anxiety: child-reported overall anxiety, child-reported subscales of anxiety, and caregiver-reported anxiety. Contrary to hypothesis, none of these models evidenced a significant indirect effect.

Hypothesis 2

To test the moderating function of T3 attentional bias to threat on the relation between Pre-T3 HPA axis activity and T3 anxiety, PROCESS v3.0 (Hayes, 2018) was used. Models included child gender and child age as covariates. Models were run with the following variables measuring T3 anxiety: child-reported overall anxiety, child-reported subscales of anxiety, and caregiver-reported anxiety. Contrary to hypothesis, none of these models evidenced a significant interactive effect.

Hypothesis 3

The final analysis of this study was a longitudinal moderated mediation model (or conditional process model; Hayes, 2018). This model combines Hypothesis 1d and Hypothesis 2, examining whether children's T1 immigration-related stress is tied to Pre-T3 HPA axis activity, which is tied to T3 anxiety—with Pre-T3 HPA axis activity being more strongly associated with T3 anxiety for children with high levels of T3 attentional bias to threat. PROCESS v3.0 (Hayes, 2018) was used to estimate the indirect effect of a single independent variable on an outcome variable through a mediator variable, conditional on a moderator of the path from the mediator to the dependent variable. Models included child gender and child age as covariates. Models were run with the following variables measuring T1 immigration-related stress: composite of immigration-related stress, subscales of immigration-related stress, and measure of child-specific immigration-related stress. In addition, models were run with the following variables measuring T3 anxiety: child-reported overall anxiety, child-reported subscales of anxiety, and caregiver-reported anxiety.

Four of these models evidenced a significant index of moderated mediation. The outcome variable in all of these models was T3 child-reported social anxiety. Significant moderated mediation was found when the predictor variable was 1) the composite of T1 immigration-related stress (moderated mediation index = 0.002; SE = 0.001; CI = 0.0003 – 0.004), 2) the subscale of T1 Occupational/Economic Stress (moderated mediation index = 0.005; SE = 0.003; CI = 0.0002 – 0.011), 3) the subscale of T1 Family/Culture Stress (moderated mediation index = 0.005; SE = 0.003; CI = 0.0005 – 0.012), and 4) the subscale of T1 Immigration Stress (moderated mediation index = 0.004; SE = 0.002; CI = 0.0002 – 0.009). Findings were different from the stated hypothesis. In all of these models, the indirect effect of T1 stress to Pre-T3 HCCs to T3 social anxiety was nonsignificant when there was no T3 attentional bias to threat or when there was a high T3 attentional bias *toward* threat; however, when there was a high T3 attentional bias *away* from threat, the indirect effect was negative. In all of these models, T1 stress was significantly and negatively associated with Pre-T3 HCCs, meaning that greater T1 stress was related to lower Pre-T3 HCCs. Then, the association between Pre-T3 HCCs and T3 social anxiety depended on T3 attentional bias. There was no association between Pre-T3 HCCs and T3 social anxiety when there was no T3 attentional bias to threat or when there was a high T3 attentional bias *toward* threat; however, when there was a high T3 attentional bias *away* from threat, Pre-T3 HCCs were significantly and positively associated with T3 social anxiety, meaning that greater Pre-T3 HCCs were related to greater T3 social anxiety.

Exploratory Analyses

Exploratory analyses were conducted to examine the following: 1) An immigrant caregiver's time living in the U.S. in place of the immigration-related stress variable, 2)

Curvilinear analysis for both hypo- and hypercortisolism relating to anxiety, 3) Hair cortisol as a moderator, instead of a mediator, 4) Analyses with the attentional bias moderator in the stress to hair cortisol pathway.

Time in the U.S.

Analyses were run that mirrored the primary analyses of this study and replaced the T1 immigration-related stress variable with the immigrant parent's time in the U.S. in each analysis. Analyses were run while covarying out the relevant covariates (i.e., child gender and/or age). None of these results were statistically significant.

Curvilinear Effects of HCCs

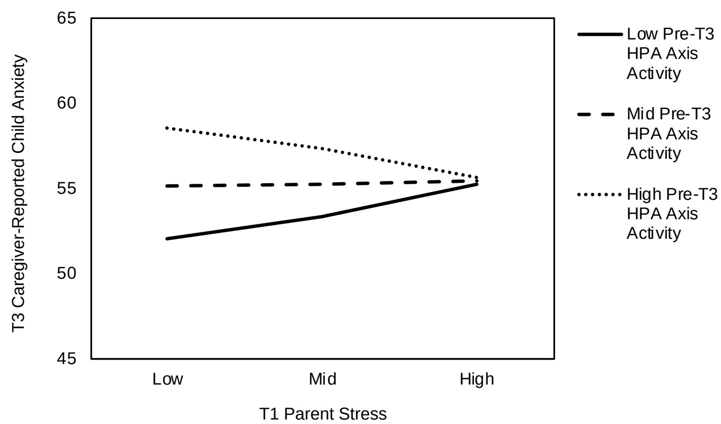
Analyses were run to examine whether the relation of Pre-T3 HPA axis activity to T3 anxiety may be quadratic, in that both hypo- and hypercortisolism might be related to T3 anxiety. Analyses were run while covarying out child gender and child age. No statistically significant curvilinear effects of Pre-T3 HPA axis activity were found.

Pre-T3 HPA Axis Activity as a Moderator

Self-report of child anxiety. No significant interactions were found between T1 immigration-related stress and Pre-T3 HPA axis activity related to T3 self-reported child anxiety symptoms.

Caregiver-report of child anxiety. A significant interaction was found between the subscale of T1 Parent Stress and Pre-T3 HPA axis activity related to T3 caregiver-reported child anxiety. Specifically, for children with very low levels of Pre-T3 HPA axis activity (less than $M - 1SD$), greater T1 Parent Stress was followed by marginally higher caregiver-reported T3 anxiety, simple slope = 0.76 to 1.71, SE = 0.44 to 0.87, t = 1.71 to 1.97, p = .094 to .055. For children with moderate levels of Pre-T3 HPA axis activity, T1 stress was not tied to T3 caregiver-reported anxiety. For children with very high levels of Pre-T3 HPA axis activity (greater than *M* + 1*SD*), greater T1 Parent Stress was followed by marginally lower caregiver-reported T3 anxiety, simple slope = -0.76 to -2.09, *SE* = 0.42 to 1.02, *t* = -1.80 to -2.05, *p* = .079 to .046 (see Figure 7).

Figure 7. T1 stress × HPA axis activity predicting child anxiety



Attentional Bias Moderating the Stress to HPA Axis Activity Pathway

Analyses were run that mirrored the primary analyses of this study and moved the attentional bias moderator variable from the Pre-T3 HPA axis activity to T3 anxiety pathway to the T1 immigration-related stress to Pre-T3 HPA axis activity pathway in each analysis. Models included child gender and child age as covariates. None of these models evidenced a significant interactive effect.

CHAPTER FOUR

DISCUSSION

This study is the first to integrate important constructs from psychophysiology and neurocognition within a context of culturally relevant stress. Immigration-related stress is additive in nature, so it has the potential to cause more harm than other stressors facing children in the U.S. (Kessler et al., 1999; Torres et al., 2018). The present findings confirmed some a priori hypotheses, and other hypotheses were not confirmed, indicating alternative conceptualizations. These interpretations are discussed below.

Specific Aim #1

The first aim of this study was to examine the links between T1 immigration-related stress, Pre-T3 HPA axis activity, and T3 anxiety symptomatology. The first set of findings showed that family/cultural concerns and immigration-related stress experienced by the child were related to children's self-reported social anxiety. This provides evidence for the detrimental effect of immigration policies, anti-immigrant sentiments, and racial/ethnic discrimination on children's mental health. Most research examining the negative impacts of immigration-related stress on children's mental health examines broad categories of outcomes (e.g., Sirin, Ryce, Gupta, & Rogers-Sirin, 2013). Thus, there is not a large body of research indicating that this type of stress causes social anxiety more often than other types of anxiety. However, it seems reasonable that, for children growing up in a sociopolitical context that is overwhelmingly unfriendly to them and their family members, anxiety may manifest in a form that is sensitive to experiences of social threat. The items measuring children's self-reported social anxiety in this study included statements such as "I feel someone will tell me I do things the wrong way" and "A lot of people are against me," which are beliefs that may reasonably result from repeated experiences of discrimination, in addition to being beliefs that may indicate social anxiety (Reynolds & Richmond, 2008). Thus, it may be that this finding also reflects the ongoing discrimination experienced by low-income Mexican-origin children in the U.S. This finding was not robust across other types of anxiety reported by children in the present study, so it may be that children's responses on the measure of social anxiety displayed a reasonable set of beliefs resulting from repeated discrimination. Past research examining immigration-related stress and/or experiences of discrimination in Latino youth has largely focused on measuring outcomes such as depression, self-esteem, and internalizing symptoms as a broad category (Sirin et al., 2013; Zeiders, Umaña-Taylor, & Derlan, 2013). Thus, there is less evidence supporting a direct link between immigration-related stress and social anxiety specifically. Although a large body of research has demonstrated the negative impact of immigration-related stress on children's mental health (Torres et al., 2018), it may be that previous research has failed to focus on the direct impact that immigration-related stress can have by increasing children's sensitivity to experiences of social threat. It appears that immigration-related stress engenders a set of beliefs about the self, others, and the world that are tied to high levels of stress related to post-immigration experiences, cultural differences, and family conflict.

In addition to this link between immigration-related stress and social anxiety, there was also a link between immigration-related stress experienced by the child and caregiver-reported child anxiety, which was measured using the *DSM*-oriented anxiety subscale of the CBCL

(Achenbach & Rescorla, 2001). The items on the *DSM*-oriented anxiety subscale of the CBCL were designed to reflect anxiety that is experienced across generalized anxiety disorder, separation anxiety disorder, and specific phobia (Ebesutani et al., 2010). This finding provides further evidence for the detrimental effect of immigration policies and the resulting experiences of Mexican-origin children on mental health. The harm caused by immigration-related stress has been documented in a large body of research, and it is important to continue to highlight this finding in order to advocate for policy change (Torres et al., 2018). In particular, the association between immigration-related stress and caregiver-reported child anxiety is interesting because this finding suggests a broad impact of culturally relevant stress on child mental health (beyond social anxiety). This result is consistent with the findings of many previous research efforts showing broad impacts on child functioning (Cano et al., 2015; Cervantes et al., 2014; Cervantes et al., 2015; Lui, 2015; Piña-Watson et al., 2015; Suarez-Morales & Lopez, 2009). It is also possible that this finding could be the result of a third variable, such as the caregiver's own mental health. In fact, previous research has focused more on the ways that culturally relevant stress negatively impacts adult mental health than child mental health (Paradies et al., 2015). Caregivers who report more immigration-related stress likely experience more symptoms of mental illness, and thus, they may be more likely to report symptoms in their children that are commonly associated with generalized anxiety disorder (e.g., frequent worrying), separation anxiety disorder (e.g., fearing separation from caregivers), and specific phobia (e.g., fearing specific stimuli).

When considering the present findings related to self-reported child anxiety and caregiver-reported child anxiety together, it is clear that researchers must select measures that are culturally and developmentally responsive and interpret findings through these lenses. It appears that immigration-related stress is related to children developing beliefs that reflect fear of interactions with others, and in addition, immigration-related stress is related to caregivers perceiving more anxiety-related symptoms in children. These findings highlight the importance of examining the impact of culturally relevant stress on mental health at both broad and narrow levels, as this enables researchers to understand the differential impact of this stress on distinct types of mental health problems.

In addition to the link between stress and child anxiety, overall immigration-related stress and several subscales of immigration-related stress were also linked to HCCs. However, this association was in the opposite of the hypothesized direction—stress was related to lower HPA axis activity. This finding may reflect the contradictory findings in previous research on HPA axis activity and chronic stress, as chronic stress in childhood has been associated with both hypercortisolism and hypocortisolism (Miller et al., 2007). The majority of research attention has been devoted to hypercortisolism as a result of acute and chronic stress, and hypercortisolism has been associated with both medical and mental health problems (Essex et al., 2011; McEwen & Tucker, 2011). However, it has become known that cortisol deviations in both directions (high and low) can indicate HPA axis dysregulation and can be harmful (Miller et al., 2007). Recent research with children has confirmed that hypocortisolism is a potential marker of HPA axis dysregulation, and blunted cortisol can result from growing up in a context of chronic stress (Badanes, Watamura, & Hankin, 2011). It may be that, prior to puberty, children facing significant and inescapable stress show attenuated cortisol, and this under-activation of the HPA axis puts children at risk, because when it is functioning typically, the HPA axis helps to regulate the immune system and protect against exaggerated immune responses (Badanes et al., 2011). Thus, similar to hypercortisolism, hypocortisolism is an indicator of HPA axis dysfunction that may be linked to physical and mental health consequences. Some previous research has shown that hypocortisolism does not act as a mechanism through which stress leads to internalizing symptoms in children—instead, hypocortisolism is a marker of the chronic stressfulness of a child's environment and a child's inability to adaptively address stress (Badanes et al., 2011). Thus, hypocortisolism is not the cause of internalizing symptoms, but it is the result of chronic stress and maladaptive coping. Assuming that hypocortisolism reflects a child's inability to cope with stress in a way that is adaptive, hypocortisolism may indicate a child's risk of developing mental health problems; however, that is because it results from growing up in a context of chronic stress, which is itself an indicator of risk for mental health problems. In the present sample, chronic HPA axis activity was not associated with children's self-reports of anxiety or caregivers' reports of child anxiety. Instead, there was a direct link between immigration-related stress and child anxiety. These results lend additional support for previous research indicating that hypocortisolism and internalizing symptoms may both result from growing up with chronic stress, yet they are not causally related (Badanes et al., 2011).

In addition, no significant mediation effect was found when testing whether Pre-T3 HPA axis activity is a mechanism through which T1 immigration-related stress causes T3 anxiety. This lack of a mediation effect is supported by past research in which attenuated HPA axis activity was found to be both a marker of chronic stress (e.g., financial strain), and a risk factor for depression in youth, but not a mechanism through which stress causes mental illness (Badanes et al., 2011). Thus, it is possible that in the present sample, most children displayed attenuated HPA axis activity as a result of the chronic stress faced by Mexican-origin children in the U.S., and this chronic stress also predicted higher rates of anxiety problems. Indeed, the HCCs found in this study are generally lower than those found in previous studies of hair cortisol in middle childhood (e.g., Dettenborn, Tietze, Kirschbaum, & Stalder, 2012; Noppe et al., 2014; Vanaelst et al., 2012). Future research is needed to determine whether attenuated HPA axis activity or heightened HPA axis activity is more strongly related to risk for mental health problems, and in which conditions. However, it appears that HPA axis activity is likely not a mediator of the effect of stress on mental health for all children, but rather, it is an indicator of risk that is context-dependent. In the present sample, stress was linked to blunted HPA axis activity in most children, but person-centered analyses may reveal that this was not the case for all children. It is also possible that the failure to detect this mediation effect was due to low sample size, as power analyses conducted prior to data analysis revealed that the present sample size was sufficient to detect mediation only if one or both of the pathways evidenced a large effect size. Thus, it is possible that this mediation effect does exist, but at a medium or small magnitude.

Specific Aim #2

The second aim of this study was to determine whether attentional bias to threat moderated the association between HPA axis activity and child anxiety. Support for the hypothesized moderating effect was not found. This was surprising, given that attentional bias to threat has been theorized to be a causal and maintaining factor in anxiety disorders (Bar-Haim et al., 2007; Shechner et al., 2012), and in past research, attentional bias to threat has distinguished between the at-risk children who develop anxiety and the at-risk children who do not develop anxiety (Pérez-Edgar, Bar-Haim, et al., 2010; Pérez-Edgar, McDermott, et al., 2010; Pérez-Edgar et al., 2011). It may be that, instead of being a moderator that determines which children develop anxiety in a context of HPA axis dysregulation, attentional bias serves as a causal mechanism through which anxiety disorders develop for some children. Indeed, attentional bias to threat has been shown to determine the trajectory of children's anxious behavior later in life (Shechner et al., 2012). Thus, attentional bias could be a cognitive style that increases the likelihood that anxiety will develop. Alternatively, attentional bias to threat may be a cognitive style that is likely to develop in the presence of anxiety, thereby maintaining an anxiety disorder. It is not necessarily the case that attentional bias is the determining factor that distinguishes anxious children from nonanxious children, but instead, this may be a trait that commonly appears among children who have developed anxiety disorders. In fact, attentional bias to threat has been observed across anxiety disorders in previous research (Bar-Haim et al., 2007; Shechner et al., 2012). Finally, it is possible for this null finding of moderation to be the result of low power. This study was powered to detect a moderation effect as long as it was between a medium and large effect size (a minimum of $f^2 = .305$), based on power analyses. Thus, it may be that attentional bias to threat does impact the link between HPA axis activity and anxiety, but at a smaller magnitude. Given that attentional bias was a significant moderator within the longitudinal model of moderated mediation, perhaps the inclusion of an additional variable in that model created a suppression effect, thereby increasing the magnitude of this moderation effect to statistical significance when the suppressor variable was present.

Specific Aim #3

The third aim and final aim of this study was to evaluate an integrated longitudinal model of the development of anxiety symptomatology in low-income Mexican-origin children. Four models evidenced significant moderated mediation. Specifically, it was found that overall immigration-related stress and three subscales of immigration-related stress were related to lower Pre-T3 HPA axis activity, and the association between Pre-T3 HPA axis activity and T3 social anxiety differed depending on T3 attentional bias to threat. For children who had no T3 attentional bias or T3 attentional bias *toward* threat, there was no association between Pre-T3 HPA axis activity and T3 social anxiety, whereas for children who had T3 attentional bias *away* from threat, Pre-T3 HPA axis activity was associated with greater T3 social anxiety. In post-hoc analyses examining children's social anxiety based on their attentional bias to threat (among those with HCC data), although the difference in social anxiety scores was not statistically significant, children with attentional bias *away* from threat had somewhat higher social anxiety than children with no attentional bias or attentional bias *toward* threat.

When interpreting these significant moderated mediation models, there are two main points to make. First, immigration-related stress was tied to lower HPA axis activity. As previously discussed, this finding lends support to the research suggesting that both hyper- and hypocortisolism reflect HPA axis dysregulation, and in particular, this finding replicates previous research showing that growing up in a context of chronic stress can be associated with blunted cortisol (Badanes et al., 2011). As under-activation of the HPA axis may place children at risk for medical and psychological problems, it is important to understand the associations between this physiological state and key outcomes.

The second main point to interpret is that the association between HPA axis activity and social anxiety differed depending on attentional bias to threat. There was no relation for children who had no attentional bias or attentional bias *toward* threat, whereas HPA axis activity was tied to greater social anxiety for children who had attentional bias *away* from threat. This finding lends support for research examining the discrete components of anxiety separately. It appears that HPA axis activity is only related to social anxiety when accompanied by attentional bias *away* from threat. This finding is in contrast to a priori hypotheses focusing on attentional bias *toward* threat; however, there is support for this finding in previous research that separates social anxiety from other anxiety components. Specifically, the vigilance-avoidance hypothesis of social anxiety posits that social anxiety includes both hypervigilance and avoidance of the threatening stimulus (e.g., Mogg, Bradley, de Bono, & Painter, 1997). Within realistic social-evaluative situations, there is evidence for both brief hypervigilance to social threat cues and more sustained avoidance of social threat cues (Bögels & Mansel, 2004). When individuals are actively avoiding stimuli associated with social threat, they may be engaging in self-focused attention—attending to internal activities, such as one's own arousal, behavior, thoughts, or emotions (Bögels & Mansel, 2004). Although self-focused attention was not measured in the present study, it may be that the dot-probe task in this study captured the avoidance behavior that is characteristic of social anxiety (as similar dot-probe tasks have done in previous research), rather than the hypervigilance that occurs much more briefly in social anxiety (Bögels & Mansel, 2004).

It is difficult to interpret these seemingly contradictory findings, wherein stress is linked to lower HCCs, but higher HCCs are linked to higher social anxiety for children who have attentional bias *away* from threat. Based on a strong history of research demonstrating the negative impact of stress on mental health, it seems unlikely that greater immigration-related stress was linked to less social anxiety in the present sample. Indeed, in this study, it was found that immigration-related stress was positively associated with children's social anxiety. Thus, the interpretation that stress was related to lower anxiety appears unfounded. Instead, it is important to consider the conditional effect of HPA axis activity on social anxiety for low-income Mexican-origin children. In the present sample, only when children displayed attentional bias *away* from threat, HPA axis activity was linked to worse social anxiety. This finding makes sense in the context of the vigilance-avoidance hypothesis of social anxiety, which inspired a body of research that demonstrated both hypervigilance and avoidance of threatening stimuli in individuals with social anxiety (e.g., Mogg et al., 1997; Bögels & Mansel, 2004).

However, some confusion remains, because in the present sample, higher immigration-related stress was associated with lower HPA axis activity, yet higher HPA axis activity was associated with higher social anxiety in children with attentional bias *away* from threat. These findings are difficult to reconcile based on the research literature and based on other results that were found with the present sample. Thus, it appears likely that person-centered analyses might have gleaned different, more meaningful, results. Although the variable-centered analyses that were conducted revealed a number of interesting associations among different values of this study's key constructs, variable-centered analyses can be problematic, as these approaches assume a certain degree of homogeneity across people in the ways variables impact each other. In contrast, person-centered analyses focus on categories of individuals with distinct patterns of responding, thereby assuming heterogeneity across people in the ways variables influence each other (Bergman & Wangby, 2014). Person-centered analyses may be particularly important in research on HPA axis activity, as individuals differ in their expression of glucocorticoid receptor genes, and these differences can be impacted by stressful experiences early in life, further complicating the effect of HPA axis activity on mental health (Badanes et al., 2011; Klengel & Binder, 2015). In the present study, person-centered analyses were not conducted; thus, it is not possible to determine whether these results would change when examining distinct patterns of responding. However, based on the other results of this study, it seems likely that some low-income Mexican-origin children experience high levels of immigration-related stress, leading to an abnormally attenuated HPA axis stress response, whereas other children experience less dysregulation of the HPA axis, and in these children, high HPA axis activity leads to social anxiety when accompanied by neurocognitive styles that are typical of anxiety (such as avoidance of feared stimuli). Future research should include larger samples of low-income Mexican-origin children in order to examine these individual differences.

Exploratory Analyses

Some of the exploratory analyses that were run to further explore these data were nonsignificant. For example, an immigrant parent's time living in the U.S. was not related to child HPA axis activity or child anxiety. There is research literature suggesting a population-level effect called the *immigrant paradox*, which states that as immigrants spend more time in the U.S., their physical and mental health suffers (Marks, Ejesi, & García Coll, 2014). However, this finding was not replicated with the variables in the current study. In addition, all exploratory analyses testing the curvilinear effects of Pre-T3 HPA axis activity on T3 anxiety were nonsignificant. Despite past research finding associations between both hypo- and hypercortisolism and negative mental health outcomes (Miller et al., 2007), the present null finding suggests that the significant results linking HPA axis activity to anxiety better describe this association in this sample. Finally, T3 attentional bias was not a significant moderator of the association between T1 immigration-related stress and Pre-T3 HPA axis activity.

Pre-T3 HPA Axis Activity as a Moderator

Exploratory analyses were run testing HPA axis activity as a moderator, instead of a mediator, of the association between T1 immigration-related stress and T3 anxiety. Although no interactions were found when examining self-reported child anxiety, there was a significant interaction between HPA axis activity and parenting stress when examining caregiver-reported child anxiety. For children with very low levels of Pre-T3 HPA axis activity, T1 stress was followed by marginally higher T3 caregiver-reported child anxiety, whereas for children with very high levels of Pre-T3 HPA axis activity, T1 stress was followed by marginally lower T3 caregiver-reported child anxiety. Although this moderation effect was statistically significant, simple slopes analyses revealed that simple slopes were only marginally statistically different from zero. Thus, the results of simple slopes analyses should be interpreted with caution. This moderation finding is particularly interesting, because it appears that for children with different levels of hair cortisol accumulation, parenting stress and caregiver-reported child anxiety may be associated in opposite directions. Past research suggests that hypercortisolism and hypocortisolism are malleable indicators of risk, as the stress response system is dynamic and context-dependent (Badanes et al., 2011). Thus, it is possible that the HPA axis functions differently across children in the present research. In fact, research is beginning to document the context-dependent nature of associations between HCCs and mental health—one study found that the direction of the association between HCCs and mental health outcomes was opposite in their “low risk group” compared to their “high risk group” (Fuchs et al., 2018). The field of epigenetics examines how genetic and environmental factors interact to shape risk for mental health problems, and many of these studies highlight the need for further research to achieve greater specificity in our understanding of these complex interactions (Klengel & Binder, 2015). Future research should include person-centered analyses to examine distinct patterns of associations among these variables across children (Bergman & Wangby, 2014). Person-centered analytic methods are particularly well-suited to research with HPA axis activity, given the differences among individuals in expression of glucocorticoid receptor genes, and resulting different interactions with early stressful experiences (Badanes et al., 2011; Klengel & Binder, 2015).

Limitations and Future Directions

The present study used longitudinal methods to examine immigration-related stress, HPA axis activity, attentional bias to threat, and anxiety among low-income Mexican-origin children. This research may have been underpowered to detect small to medium effects, in particular when analyses included HPA axis activity. The problematic hair samples included in the present research reduced the amount of usable HCC data. Future research should pay particular attention to the integrity of hair sample collection and storage, given the difficulty in analyzing samples that are low-weight or very tangled. This limitation was particularly salient in the present study, given the need to adapt methods of hair sample collection to families’ homes in the community. Future research should take care to implement rigorous quality-control procedures when adapting lab-based data collection procedures for community research.

This study used a computerized dot-probe task to measure attentional bias to threat, based on a large body of previous research (see Bar-Haim et al., 2007, for a meta-analytic review). In the present study, the dot-probe task was optimized for data collection within families' homes in the community. However, in addition to the dot-probe task, other lab-based methods have been used successfully to assess perceptions of threat in past research, such as designing an experimental protocol in which participants interact with a confederate who behaves in a threatening manner (Cole, Balcetis, & Dunning, 2013). Future community research could include threatening stimuli that more closely approximate day-to-day life than an image on a computer screen.

In addition to this limitation, the facial stimuli used in the present dot-probe task did not contain adequate racial/ethnic diversity to reflect the population of the United States. The present facial stimuli set was selected because, at the time when this dot-probe task was designed, these were the most standardized facial stimuli when choosing among color photographs of adult faces. However, future research should endeavor to use newly developed facial stimuli that more accurately reflect the racial/ethnic diversity of the general population from which the sample is drawn (e.g., the Multi-Racial Mega-Resolution database [MR2]; Strohminger, Gray, Chituc, Heffner, Schein, & Heagins, 2016).

In the present study, the measurement of immigration-related stress included caregiver-report of stress experienced by children. Future research should include children's reports of culturally relevant stress as well, as different associations were found in this research when examining child-report of anxiety vs. caregiver-report of child anxiety. Although most measures of immigration-related stress are developed for use with adults and adolescents, culturally responsive measures of stress for children are needed in order to advance understanding of the negative impacts of immigration-related policies in the U.S. for children of Mexican-origin immigrants.

This study was designed to examine risk factors implicated in the development of anxiety in low-income Mexican-origin children. However, the present methods and analytic approaches do not prove causality. Future research should recruit larger samples with which to examine the associations that were found with the present sample, in order to determine whether factors such as immigration-related stress, attenuated vs. heightened HPA axis activity, and attentional bias *away* from threat cause increases in anxiety symptoms.

Further, although it was the intent of the present study to focus on the experiences of low-income Mexican-origin children, the specificity of this population may limit the generalizability of these results to other groups. Even within Mexican-origin children, there is variability in the experiences of children who are first generation vs. 1.5 generation (children who immigrated to the U.S. during early childhood), vs. second generation vs. later generations living in the U.S. (Cervantes, et al., 2013). The differences between experiences of Mexican-origin children of immigrants and children of immigrants from other countries of origin should also be recognized—the social attitudes in the U.S. associated with immigration are different for distinct racial/ethnic groups, and the discrimination that accompanies these attitudes has a large impact on children of immigrants (Torres et al., 2018).

Finally, future research should examine the questions that can be generated based on the results of the present study. This research found that there were significant differences within the present sample. For example, there was an association between HPA axis activity and social anxiety—but only in the context of attentional bias *away* from threat, and there was an association between parenting stress and caregiver-reported child anxiety that changed direction depending on the child's chronic HPA axis activity. These moderation results reveal that there are differences across this sample in the way that variables relate to each other; thus, future research should examine these constructs using person-centered methods. For example, latent profile analysis would allow for the detection of distinct subgroups of children who differ based on their patterns of variable attributes (Bergman & Wangby, 2014). This is particularly important in research examining HPA axis activity, as this stress response system functions differently in distinct people because of the difference across people in the expression of glucocorticoid receptor genes (Klengel & Binder, 2015). If latent profile analysis were used to identify groups of children who tended to display similar levels of variables such as stress, HPA axis activity, and attentional bias to threat, then membership in those subgroups could be used to differentially predict change in anxiety symptoms over time. Based on the results of this research, one subgroup of children may display high immigration-related stress, blunted HCCs, and no attentional bias, whereas another subgroup of children may display high immigration-related stress, heightened HCCs, and attentional bias *away* from threat. Future research should include an examination of additional variables to help explain the differences between subgroups of children (e.g., expression of glucocorticoid receptor genes).

Conclusions

Despite this study's limitations, it has some implications for the mental health of low-income Mexican-origin children. These children demonstrate disproportionately high rates of anxiety disorders, and it is important to document the reasons for this health disparity. This study documented a link between immigration-related stress and anxiety in these children—with both social anxiety and a composite of anxiety that reflects multiple *DSM*-defined anxiety disorders. Further, immigration-related stress was tied to low HPA axis activity in the present sample, and HPA axis activity was tied to social anxiety for children with attentional bias *away* from threat. Some of these findings were in contrast to hypotheses based on previous research, but there is some research reporting mixed findings related to hyper- vs. hypocortisolism and attentional bias *toward* vs. *away* from threat. This underscores the importance of documenting processes preceding the development of mental health problems in the population of interest, rather than extrapolating from findings in other populations. In this sample of low-income Mexican-origin children, results replicated previous research supporting the development of hypocortisolism in a context of chronic stress (Badanes et al., 2011), the vigilance-avoidance hypothesis of social anxiety (Bögels & Mansel 2004; Mogg et al., 1997), and the numerous harmful effects of immigration-related policies and associated discrimination against immigrant families in the U.S. (Torres et al., 2018). These findings highlight the importance of advocacy efforts advancing the dissemination of knowledge related to the mental health of low-income Mexican-origin children.

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VITA

In 2010, Stephanie Brewer graduated with honors from Washington University in St. Louis after double majoring in Psychology and Spanish. During her undergraduate studies, Dr. Brewer completed an independent research project in psychology, winning a Summer Undergraduate Research Award. In addition, she served as the university's chapter president for Psi Chi, The International Honor Society in Psychology. After graduating, Dr. Brewer worked for two years as a clinical research assistant at Columbia University Medical Center, contributing to research and gaining valuable clinical skills. Dr. Brewer then pursued graduate studies at Loyola University Chicago, where she was a member of Dr. Catherine DeCarlo Santiago's Children Adapting to Stress and Adversity (CASA) Lab. Dr. Brewer's graduate work focused on understanding the contextual stress and cultural strengths that impact psychological wellbeing for low-income Latino children and families. In addition, Dr. Brewer contributed to research identifying culturally relevant evidence-based treatments in schools. During graduate school, Dr. Brewer was awarded research funding from the American Psychological Foundation (APF) / Council of Graduate Departments of Psychology (COGDOP), the Hispanic Neuropsychological Society (HNS), and Loyola University Chicago. In addition, Dr. Brewer was awarded Loyola University Chicago's Graduate Student Mentor Award. In 2014, Dr. Brewer earned her Master of Arts in Clinical Psychology, and in 2018, her Doctor of Philosophy in Clinical Psychology (Specialization in Child Clinical Psychology). Dr. Brewer will dedicate her career to promoting equitable mental health services for historically underserved youth.