ABSTRACT

Title of Dissertation: ASSESSING THE IMPACT OF TYPICAL VARIATIONS IN STRESSFUL LIFE EVENTS ON HIPPOCAMPAL DEVELOPMENT IN CHILDHOOD

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The negative impact of extreme stress on early brain development is well-documented. An emerging body of work suggests that less extreme and more typical variations in stressful experiences (e.g., parental divorce, changing schools) may also exert an impact on the brain, especially in early childhood; however, more systematic research is needed. Across, three studies, this dissertation addressed this gap by exploring effects of typical variations in stressful life events on development of the hippocampus, a brain region highly susceptible to stress.

Study 1a assessed the impact of stressful life events on the development of hippocampal subfield volumes (i.e., CA1, CA2-4/dentate gyrus (DG), subiculum) in an accelerated longitudinal sample of 102 4- or 6-year-old children who were each followed for 3 years. Analyses revealed that experiencing more stressful life events was related to smaller CA1 and CA2-4/DG volumes in the 6- (but not 4-) year-old cohort. Study 1b used the same sample described in Study 1a to investigate the

impact of stressful life events on functional connectivity between the hippocampus and stress-related cortical regions. Analyses revealed a significant association in the 4- (but not 6-) year-old cohort, such that experiencing more stressful life events was related to greater connectivity between the hippocampus and the insula, a region important for emotional processing. Study 2 assessed moderating effects of sex and socioeconomic status (SES) on the association between stressful events and hippocampal subfield volumes using a large (n = 4,348), diverse subsample of 9-10-year-old adolescents from the Adolescent Brain and Cognitive Development Study. Analyses revealed that stressful life events were related to smaller subiculum volumes, but these associations did not vary by sex or SES.

Overall, these findings provide evidence of the impact of typical variations in stressful life events on both hippocampal structure and functional connectivity. Findings also highlight the complexity of stress effects on the brain as these experiences may impact the hippocampus in an age-dependent manner. These results advance our current understanding of how stress influences hippocampal development and pave the way for studies to assess the implications of findings both for cognitive processes and the development of stress-related disorders.

ASSESSING THE IMPACT OF TYPICAL VARIATIONS IN STRESSFUL LIFE EVENTS ON HIPPOCAMPAL DEVELOPMENT IN CHILDHOOD

by

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Dedication

To all of the people who supported me throughout this long journey, especially my parents Marilyn and William, my partner John, my sister Samantha, and my best friends. Your support has meant the world to me and I dedicate this to you.

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List of Abbreviations

CA1 = cornu ammonis 1

CA2 = cornu ammonis 2

CA3 = cornu ammonis 3

CA4 = cornu ammonis 4

CA2-4/DG = cornu ammonis 2-4/dentate gyrus

DG = dentate gyrus

GC = glucocorticoid

FD = framewise displacement

ICA = independent component analysis

eTICV = estimated total intracranial volume

LGM = latent growth modeling

MCC = midcingulate cortex

RMSEA = root mean squared error of approximation

ROI = region of interest

SEM = structural equation modeling

SES = socioeconomic status

SRMR = standardized root mean squared residual

STG = superior temporal gyrus

vmPFC = ventromedial prefrontal cortex

Chapter 1: General Introduction

Early brain development is characterized by both heightened plasticity and heightened susceptibility to variations in the environment (Casey et al., 2005; Fox et al., 2010; Gogtay et al., 2004; Nelson et al., 2019). Early life stress, resulting from neglect or maltreatment, is one factor that can have a highly detrimental impact on the developing brain relative to stress experienced during other periods of life (e.g., Belsky & de Haan, 2011; Bick & Nelson, 2015; McLaughlin et al., 2019). Although some studies have pointed to more global deficits in brain structure as a result of extreme stress (e.g., Sheridan et al., 2012), more controlled animal studies have highlighted certain regions in the brain that are highly sensitive to stress, and thus, disproportionately impacted by stress, including the hippocampus (McEwen, 1999; McEwen et al., 2016; Sapolsky et al., 1990).

The hippocampus is a brain region that is important for an array of behaviors including memory, spatial navigation, and learning (Chersi & Burgess, 2015; Eichenbaum, 1999; Tulving & Markowitsch, 1998). It is also a key limbic region that is implicated in emotional processing (Immordino-Yang & Singh, 2013). Importantly, stress-induced alterations in hippocampal structure and function have been linked to both behavioral deficits later in development in animals and humans (Bolton et al., 2017; Pechtel & Pizzagalli, 2011) and also risk for developing stress-related disorders in humans, including major depressive disorder (MDD) and post-traumatic stress disorder (PTSD; Carrión et al., 2010; Frodl et al., 2010; Jameison & Dinan, 2001; MacQueen & Frodl, 2010). Although, prospective research in human children is rare,

these outcomes from both animal and limited human research underscore the importance of investigating effects of stressful experiences on the hippocampus.

Conceptualization of Stress: Considering Typical Variations in Stressful Life Events

Although much research has focused on severe, traumatic psychosocial stress in relation to the brain (e.g., abuse or neglect; Belsky & de Haan, 2011; McLaughlin et al., 2019), recent work has suggested that more typical variations in stressors may also have a deleterious effect on the developing hippocampus (Blankenship et al., 2019; Humphreys et al., 2019). These more typical stressful events can be conceptualized as less traumatic and/or more common negative life events that may be related to the self (child) or others (parent, family, friends). Examples of such events include changing schools, parental divorce, experiences of hostile or harsh parenting practices, and lower socioeconomic status (SES). Some of these events may be acute in nature, such as changing schools, whereas others may be more chronic in nature, such as hostile parenting or lower SES. In this dissertation project, I focus mainly on acute events and will refer to these stressors as "typical variations in stressful events."

Although these typical stressful events may appear to be relatively benign in nature and span both events related to both the child and the child's family or environment, research suggests that the stress response system (i.e., hypothalamic-pituitary-adrenal (HPA) axis) can react in a similar way to a range of stressors (Loman & Gunnar, 2010). Emerging work shows that these stressors engage the stress response system and likely impact the hippocampus through similar mechanisms as extreme stress (i.e., accumulation of stress hormones; Blankenship et

al., 2019; Faravelli et al., 2012). Furthermore, the effects of such stressful events potentially persist beyond the event itself and may have long lasting impacts. Therefore, increased understanding of how typical variations in stressful events impact hippocampal development, and brain development in general, is important and should be investigated further.

In the remaining sections of this introduction, I briefly discuss how stress "gets under the skin" to impact the brain. Next, I discuss research assessing the relation between extreme stress and hippocampal structure and function in animals and humans to provide an idea of how typical variations in stressful events may impact hippocampal development. Finally, I discuss gaps in the literature, specifically in our understanding of the effects of typical variations in stressful events on hippocampal development.

How Does Stress "Get Under the Skin"?

Although the body's response to stress is complex, one of the major stress response systems in the body, the HPA axis, is activated in response to a stressor and a cascade of events occurs. Specifically, the hypothalamus releases corticotrophin releasing factor (CRF), which stimulates the pituitary glands to produce adrenocorticotropic hormone (ACTH), which then stimulates the adrenal glands to produce glucocorticoids (GCs; i.e., cortisol in humans). GCs bind with receptors in the hippocampus and other regions in the brain. Compared to other brain regions, the hippocampus has a relatively high density of GC receptors (Virgin et al., 1991), and has the ability to inhibit activity of the HPA axis through a negative feedback mechanism. Typically, the HPA axis activates in response to an acute stressor and

then returns to baseline functioning in response to feedback from the hippocampus (van Haarst et al., 1997). However, when stress becomes chronic, the stress response system does not have the opportunity to adequately return to baseline functioning, resulting in altered function of the HPA axis (Frodl & Keane, 2013). The number of GC receptors in the hippocampus is reduced and the negative feedback mechanism is impaired, exposing the brain, and the hippocampus in particular, to excessive GCs for long periods of time. Consequently, the structure and function of the hippocampus is impacted by this exposure to GCs (Bunea et al., 2017; van Haarst et al., 1997).

The idea of a cascade of events leading to altered neural structure and function (as can be observed in the hippocampus) is often referred to as the glucocorticoid cascade hypothesis and is supported by work in animal and human studies (Frodl & Keane, 2013; Sapolsky et al., 1986). Although the majority of research providing support for this hypothesis has focused on extreme variations in stress, one can hypothesize that typical variations in stress have a similar impact on the hippocampus through analogous mechanisms (i.e., altered HPA axis functioning, Loman & Gunnar, 2010). Some research has already suggested that more typical variations in GCs and stressful events are related to brain structure and function (Blankenship et al., 2019; Blankenship, Botdorf et al., 2019; Humphreys et al., 2019).

In addition to negative impacts on the brain, it is also possible that typical variations in stressful events have positive effects on the brain, such as supporting brain development, since some research suggests that low levels of stress are adaptive in nature (Chen & Miller, 2012). When children experience stressors, they are often comforted by their caregiver. This type of parental support has been related to

superior stress response systems and larger hippocampal volumes in rodents (Champagne et al., 2008) and young children (Luby et al., 2016). In this way, experiencing stressors may not always result in negative effects on the brain as they may simply aid in "tuning" the stress response system to respond to the environment the child will experience.

Relations Between Early Life Stress and the Hippocampus in Rodents

Given difficulties in assessing extreme stress in humans, animal studies have greatly informed our understanding of how stress experienced early in life influences the hippocampus. In rodents, research shows that hippocampal microstructure is impacted by alterations in GC levels resulting from stress, which is induced through paradigms, such as maternal deprivation (Champagne et al., 2008; Derks et al., 2016) or inconsistent maternal care (i.e., limited bedding and nesting; Naninck et al., 2015). These effects are particularly evident when stress is experienced early in the rodent's life while the hippocampus is still developing.

Specifically, impacts are observed in subfields of the hippocampus (e.g., cornu ammonis (CA) areas 1 and 3 and dentate gyrus (DG)), which are functional subunits that are disproportionately distributed throughout the longitudinal axis of the hippocampus (Insausti & Amaral, 2003). Impacts of stress on subfield structure include reductions in dendritic branching, synaptic plasticity, and spine density. Furthermore, reductions in developmental and adult neurogenesis in DG and delayed DG development resulting from stress have been reported (Naninck et al., 2015; Youssef et al., 2019). Compared to other subfields of the hippocampus, CA1 has a higher density of GC receptors, making this subfield particularly susceptible to early

life stress (Champagne et al., 2008). In addition to impacts in DG and CA1, reductions in pyramidal cells in CA3 have also been observed (Champagne et al., 2008). Intriguingly, some studies also suggest accelerated maturation of hippocampus in rodents exposed to early life stress (Bath et al., 2016). Collectively, these studies suggest structural deficits resulting from early life stress that disproportionately impact CA1, CA3, and dentate gyrus subfields in the hippocampus. Importantly, these stress-induced structural changes in the hippocampus are related to functional changes as well, including reduced cell activity in specific subfields of the hippocampus (Joëls & de Kloet, 1989; Okuhara & Beck, 1998).

Sex Differences in the Effects of Early Life Stress on the Hippocampus in Rodents

Animal research has also suggested sex differences in the impacts of stress on hippocampal subfields. Some research suggests that effects of maternal deprivation on hippocampal structure may be more prevalent in males than females (Derks et al., 2016). However, other studies suggest females may be more susceptible to stress (Loi et al., 2014). Although there are contradictory findings, this line of work underscores the idea that sex differences in the impacts of stress on hippocampal development are meaningful and warrant further study.

Findings from rodent studies have laid important foundations for understanding the impacts of both extreme early life stress (i.e., maternal deprivation) and less extreme early life stress (i.e., limited nesting and bedding) on hippocampal development in human samples. Although there are limitations in assessing impacts of early life stress on the hippocampus in humans, results of research using

hippocampal volume as a proxy for microstructural changes are largely similar to those observed in rodents.

Relations Between Early Life Stress and the Hippocampus in Humans

Severe Early Life Stress and the Hippocampus in Adults

Many studies have found that a history of extreme early life stress in the form of maltreatment or neglect is associated with reduced hippocampal volume in adulthood (for a review, see Calem et al., 2017), mirroring findings from animal studies. Although studies in humans commonly assess total hippocampal volume, some research has begun to show specificity in the impacts of early life stress, specifically maltreatment, on subfield volume in adulthood. Two studies showed reduced CA3, CA2-4, DG, and subiculum volumes in adults with a history of childhood maltreatment (Teicher et al., 2012; Whittle et al., 2017).

Research assessing early life stress and hippocampal function in adults is sparse and has largely focused on early life stress in the form of poverty. One study that utilized resting state functional connectivity, which is thought to reflect the intrinsic functional organization of the brain, showed relations between low socioeconomic status (SES) and reduced hippocampal functional connectivity with the default mode network, a network of brain regions activated at rest (Sripada et al., 2014). Another study assessed hippocampal activation during a memory task and also showed impacts of early life stress on hippocampal function. Specifically, poverty during childhood (at age 9), but not adulthood, was related to decreased hippocampal activation in adulthood (Duval et al., 2017).

Severe Early Life Stress and the Hippocampus in Children

In children, relations between extreme early life stress and hippocampal structure are less clear. Some of the studies examining this relation show negative associations between severe early life stress and hippocampal volumes (Hanson et al., 2015; Luby et al., 2013; Malhi et al., 2019; Whittle et al., 2017). However, other studies find no associations (Mehta et al., 2009; Sheridan et al., 2012). For example, Luby et al. (2013) found that poverty in early childhood (3-6 years old) was related to reduced total hippocampal volume at school age. However, two randomized control studies, which assessed impacts of institutionalization on brain development, showed no differences in total hippocampal volume between children who were institutionalized and those who were not (Mehta et al., 2009; Sheridan et al., 2012). Interestingly, studies that have assessed subfields in adolescents showed that childhood abuse and neglect were associated with reduced CA3 volume (Malhi et al., 2019) and CA1 and subiculum volume (Lee et al., 2018). Collectively, these studies suggest mixed findings in relation to early life stress and hippocampal structure in children.

As with adults, only limited research has assessed impacts of early life stress on hippocampal function in children. One study showed reduced hippocampal activation during a memory task in a sample of 10- to 17-year-old children and adolescents with a history of trauma and post-traumatic stress symptoms (Carrión et al., 2010).

Typical Variations in Stressful Events and the Hippocampus in Children

Limited research has begun to assess more typical variations in early life stress in children in relation to the hippocampus (Blankenship et al., 2019; Hanson et al., 2011; Humphreys et al., 2019; Merz et al., 2019; Saxbe et al., 2018; Yu et al., 2018). For example, Humphreys et al. (2019) used a retrospective interview method and found a negative relation between severity of stressful events prior to 5 years of age (but not after 5 years of age) and total hippocampal volume in a group of typically developing adolescents. Specifically, adolescents who experienced more severe stressors prior to 5 years of age had smaller left and right hippocampal volumes. Blankenship et al. (2019) assessed stress in the form of hostile parenting in a sample over-recruited for parental depression and showed that high levels of early negative parenting assessed at preschool age, but not concurrent parenting, were related to smaller hippocampal tail volume at school age via increases in cortisol levels. Finally, a study in 5-9-year-old children showed associations between increased hair cortisol levels, thought to reflect increased stress levels, and smaller volumes of CA3 and DG subfields (Merz et al., 2019). Although limited, these findings suggest typical variations in stressful events during childhood impact hippocampal structure.

With regards to hippocampal function, one study by Saxbe and colleagues (2018) showed that in a sample of adolescents, community violence was not only related to smaller hippocampal and amygdala volumes, but also greater resting state connectivity between hippocampus and frontolimbic regions 3-5 years later. These results further showed no effect of concurrent violence on connectivity. To date, no

research has assessed how typical variations in stressful events relate to hippocampal function during early childhood. However, a recent study assessed stress physiology (but not stressful events) in early childhood and showed that heightened cortisol after a stressful task was related to later alterations in hippocampal functional connectivity with precuneus and midcingulate cortex (MCC). Specifically, early, but not concurrent, cortisol release was related to greater connectivity between hippocampus and MCC and precuneus. It was hypothesized that this greater connectivity could represent accelerated development or simply altered connectivity resulting from a heightened stress response (Blankenship, Botdorf et al., 2019).

Importance of Considering Timing

Several of the studies described above suggest that timing should be considered when examining the impact of stressful events on hippocampal development. Research has accumulated suggesting that early experiences impact the hippocampus more than later experiences (Anderson et al., 2008; Pechtel & Pizzagalli, 2011; Teicher et al., 2018; Tottenham & Sheridan, 2010). Some research further suggests that impacts are greater when stress is experienced prior to 5 years of age vs. after 5 years of age (Humphreys et al., 2019; Luby et al., 2016). The findings outlined above provide support for this notion as they showed early, but not later, stressful life events and stress physiology related to hippocampal structure (Blankenship et al., 2019) and function (Blankenship, Botdorf, et al., 2019), respectively. Relatedly, some research also shows beneficial effects of positive experiences, such as maternal support, on hippocampal volume in preschool, but not school aged, children (Luby et al., 2012). Although it is possible that it simply takes

time for effects to be seen on hippocampal structure and function, research has hypothesized a period of heightened sensitivity in early childhood for the impact of stress on hippocampal development (Andersen et al., 2008; Humphreys et al., 2019; Tottenham & Sheridan, 2010). During this period, the hippocampus may exhibit greater plasticity and be particularly sensitive to early life stress.

The developmental trajectory of the hippocampus also highlights the importance of timing. Research in non-human primates suggests there are increased rates of synaptogenesis and dendritic development in the hippocampus until 5 years of age (Eckenhoff & Rakic, 1988; Josselyn & Frankland, 2012; Lavenex & Banta Lavenex, 2013). Research in humans suggests a more prolonged trajectory (Ghetti & Bunge, 2012; Lee et al., 2020) and that the hippocampus as a whole is likely continuing to mature throughout childhood and adolescence. Certain subfields, including CA1 and DG, may show greater development during early childhood relative to other subfields (Canada, et al., 2021; Jabès et al., 2010; Riggins et al., 2018).

Gaps in the Literature

Progress has been made in the study of the impacts of typical variations in stressful life events on the hippocampus, but important gaps in the literature remain. This set of dissertation studies aimed to increase understanding of the effects of typical variations in stressful life events on the structural and functional development of the hippocampus in children. More specifically, this work filled gaps in the literature related to longitudinal research, the heterogeneous nature of the hippocampus, sex effects, and the use of large, diverse datasets.

Longitudinal Research (Studies 1a and 1b)

One gap concerns limited and mixed findings of associations between early life stress and the hippocampus in childhood. Much of the research highlighted above, especially with regards to hippocampal structure, utilized cross-sectional research. Some studies that assessed typical variations in stress were longitudinal in nature, but only included MRI data at one time-point (Blankenship et al., 2019; Blankenship, Botdorf, et al., 2019; Humphreys et al., 2019), making it difficult to assess change in the hippocampus as a result of stressful events. More longitudinal research is needed with multiple time points to understand how stressful events relate to *changes* in hippocampal structure and function during early childhood when development of the hippocampus is still occurring. Utilizing longitudinal data will also facilitate the use of more robust quantitative methods, including latent growth modeling, which will allow for assessing whether stressful events alters the developmental trajectory of the hippocampus during an important period in development.

Considering Heterogeneity of the Hippocampus (Studies 1a, 1b, and 2)

Although the hippocampus is a heterogeneous structure, total hippocampal volume was typically assessed in the aforementioned structural studies. In functional studies, the hippocampus is often considered as a whole as well. Assessing the hippocampus in this way may obscure regionally specific impacts of early life stress on hippocampal subregions and subfields. The use of hippocampal subfields in assessing hippocampal structure also allows for comparisons to be made with animal research. Some limited research has begun to use subfields, but, to my knowledge, no research focused on stressful experiences in younger children has taken this approach.

Thus, assessing the impact of early life stress on subfields, especially in early childhood, is critical for advancing our understanding of stress on brain development (Study 1a & 2). The low resolution of functional data makes it difficult to assess the function of subfields. Therefore, dividing the hippocampus along the longitudinal axis into anterior and posterior divisions is important for functional studies to assess differences in connectivity between anterior and posterior hippocampus (Study 1b).

Assessing How Sex Influences Stress Effects on the Hippocampus (Study 2)

Another important gap that remains relates to sex differences in the impact of stress on the hippocampus. Research suggests that males and females respond differently to stress. For example, females may be more sensitive to stress than males, which is evident in higher rates of stress-related disorders, such as depression, among females (Bennett et al., 2005). Some recent rodent work has also suggested sex differences in the impact of early life stress on the hippocampus as outlined above.

Unfortunately, sex is often included as a control variable rather than a variable of interest in human studies assessing stressful events and the hippocampus. Given sex differences in both hippocampal development (Canada et al., 2020; Tamnes et al., 2018) and also in the effect of stress on the structure of the hippocampus in both humans and rodents (Derks et al., 2016; Loi et al., 2014), human research must consider and focus on investigating sex differences. It is important to understand how stress differentially impacts hippocampal development in males and females.

Assessing Large, Diverse Samples (Study 2)

Many of the studies outlined above used smaller samples consisting of less than 100 subjects, which makes it difficult to generalize results to a larger population. Some structural studies use samples with as few as 31 child participants (Yu et al., 2018). On the higher end of the spectrum, Hanson et al. (2011) included 317 children in their sample. Furthermore, many of the studies do not use highly diverse samples. More research is needed with large, diverse samples to clarify findings from previous work and also assess whether findings generalize to the larger population. Larger, more diverse samples also allow for testing how stress effects on the hippocampus may differ by variables related to the child's environment. For example, it allows for assessing the extent to which stress impacts hippocampal development in the same way for all children or whether effects vary by SES. Children of low SES are already under heightened levels of stress; therefore, low SES may compound the effects of stress on the brain. High SES may also reduce or provide a buffer against the impacts of stress on the brain. Therefore, assessing stress and hippocampal volume in a large, diverse sample is important.

Collectively Studies 1a, 1b, and 2 addressed the limitations laid out above. Specifically, through these three studies, I assessed how typical variations in stressful events experienced during childhood impact hippocampal development through assessing hippocampal structure (Study 1a); hippocampal function (Study 1b); and hippocampal structure in a large, diverse sample (Study 2). Studies 1a and 1b made use of the same sample of children. Given that certain aspects of the Methods section

were the same for Studies 1a and 1b (e.g., Participants), these sections are only included in Study 1a.

Chapter 2: Typical variations in stressful life events relate to hippocampal subfields in young children (Empirical Study 1a)

Study 1a assessed relations between typical variations in stressful life events and structural development of hippocampal subfields in young children (4- to 8-year-olds) using an accelerated longitudinal design. This study included a 4-year-old and a 6-year-old cohort of children from a larger study designed to assess memory and brain development. The two cohorts of children (although only 2 years apart in age) provided the opportunity to assess stressful events experienced prior to 5 years of age and after 5 years of age given that animal and human research has suggested birth through 5 years old to be a particularly important time for the impacts of stress on the hippocampus (Andersen et al., 2008; Humphreys et al., 2019; Luby et al., 2016).

Aim 1: The first aim was to assess the impact of stressful life events (as reported by parents) at 4-years old and 6-years old on hippocampal subfield development (CA1, CA2-4/DG, and subiculum).

Hypothesis 1_a: I hypothesized that greater reported stressful life events would impact development of CA1 and CA2-4/DG. However, given mixed findings in previous studies of rodents and humans (Champagne et al., 2008; Malhi et al., 2019; Naninck et al., 2015), no specific hypothesis was made regarding direction of effects.

Hypothesis 1_b: I hypothesized that greater reported stressful life events would not impact subiculum given that a) research in animals does not support a consistent association between early life stress and structural development of subiculum, and b) research in humans suggest that this subfield shows a less protracted developmental course than other subfields (Canada, et al., 2021; Riggins et al., 2018).

Aim 2: The second aim was to assess time-dependent effects of stressful life events on hippocampal subfield development by comparing effects of stressful life events in the 4 vs. 6-year-old cohorts.

Hypothesis 2: I hypothesized that reported stressful life events experienced at 4 years of age would impact development of CA1 and CA2-4/DG more than stressful life events experienced at 6 years of age given that a) rodent work suggests that earlier experienced events impact development more (Bath et al., 2016; Champagne et al., 2008; Naninck et al., 2015) and b) emerging work in humans suggesting that stressful events experienced prior to 5 years of age (compared to after 5 years old) may result in a greater impact on hippocampal development (Andersen et al., 2008; Humphreys et al., 2019; Luby et al., 2016).

Methods

Participants

Studies 1a and 1b used the same sample of children. A total of 200 children (100 females) between 4 and 8 years of age participated in the study. The study utilized an accelerated longitudinal design. Of the 200 total children, 102 (43 females) 4 and 6-year-old children were invited to return to the lab for two subsequent annual visits and were included in the longitudinal cohort. Participants and their families were recruited from the greater Baltimore-Washington Metropolitan area through the use of flyers and the Infant and Child Studies Consortium at the University of Maryland. The longitudinal sample is the focus of Studies 1a and 1b.

There were 61 subjects in the 4-year-old cohort, which was over recruited to account for missing MRI data due to the young age of the participants. Five subjects in the 4-year-old cohort were missing Stressful Life Events Score data, 10 subjects

were missing structural MRI data (i.e., did not have at least 1 MRI data point). Therefore, there are 46 subjects included in the structural MRI analyses for the 4-year-old cohort. There were 41 subjects in the 6-year-old cohort. Six subjects are missing Stressful Life Events Score data and two subjects are missing structural MRI data. Therefore, there are 33 subjects included in analyses for the 6-year-old cohort. Thus, there were 79 subjects total in the 4-year-old and 6-year-old cohort analyses. Descriptive statistics about the sample are presented in Table 1.1. The number of subjects with 3, 2, and 1 time points of MRI data is listed in Table 1.2

Table 1.1

Demographic characteristics of the accelerated longitudinal sample by cohort

	4-year-old Cohort	6-year-old Cohort
Time 1 Mean Age years (*n*)	4.22 (61)	6.46 (41)
Time 2 Mean Age years (*n*)	5.45 (50)	7.55 (35)
Time 3 Mean Age (*n*)	6.44 (48)	8.52 (34)
Sex, female [*n* (%)]	29 (48%)	15 (37%)
Race, [*n* (%)]		
Asian	2 (3%)	1 (2%)
Black/African American	5 (8%)	10 (24%)
Multi-Racial	11 (18%)	9 (22%)
White, European-American	37 (61%)	20 (49%)
Did Not Disclose	6 (10%)	1 (2%)
Ethnicity [*n* (%)]		
Hispanic/Latino descent	8 (14%)	4 (10%)
Family income [*n* (%)]		
<$25,000	4 (7%)	1 (2%)
$25,001 to $55,000	5 (8%)	2 (5%)
$55,001 to $105,000	14 (23%)	12 (29%)
>$105,001	36 (59%)	24 (59%)
Did not disclose	2 (3%)	2 (5%)
Parental education [*n* (%)]		
At least one parent with a four-year college degree	56 (89%)	36 (88%)

Table 1.2

Number of accelerated longitudinal subjects with 3, 2, and 1 time points of structural MRI data

Number of Time Points	Cohort	N of subjects
3	4-year-old	17
	6-year-old	16
2	4-year-old	14
	6-year-old	6
1	4-year-old	15
	6-year-old	11
Total	4-year-old	46
	6-year-old	33
	Sum	79

Children were screened to ensure they were not born premature and did not have developmental delays or disabilities. Children under the age of 7 years provided verbal assent and children over the age of 7 years provided written assent. Parents also provided written and verbal consent. Families received $20 for participating in each wave of the study and were able to select a prize of their choice.

Stressful Life Events Checklist

A stressful events checklist comprised of the Stressful Life Events Scale (Williamson et al., 2003) and Life Events Scale (Preschool Age Psychiatric Assessment; Egger et al., 2006) was used to provide an index of the number and severity of stressful life events the child experienced in the previous 12 months in both Studies 1a and 1b. This contained 52 items and was completed by the child's parent via a computerized survey. For each event, the parent indicated whether the event occurred in the child's life in the previous 12 months. If the event occurred, the parent was asked to indicate the impact it had on the child on a scale from 1 to 4,

where 1 represented “No Effect” and 4 represented a “Great Effect”. Data were recoded to be on a scale from 0 to 3 so that 0 indicated “No Effect”.

A Stressful Life Events Score was calculated by summing the number of stressors experienced by the child in addition to the severity indicated for each stressor. For example, if the parent reported that the child changed schools in the previous 12 months and that this event had a great effect on the child as indicated by a recoded score of 3, the event received a score of 4 (event occurrence and severity summed). Scores for each stressor were then summed across events to create the overall Stressful Life Events Score. Importantly, this summed score takes into account both the number of events and the impact on the individual.

Recent research has highlighted potential short comings with cumulative stress models that sum across stressors without taking into account the differential impact of the type of stressors on the individual (McLaughlin & Sheridan, 2016). Although it may be optimal to assess stress using a dimensional approach (i.e., assessing threat vs. deprivation), it is likely that the events that will be commonly endorsed in this sample will not fall along dimensions of threat or deprivation (which are often used in research on extreme stress). I expected these to be typical variations in stressful events rather than extreme variations, such as abuse or neglect. For that reason, I used a cumulative stress approach. Importantly, one benefit of the method used is that both the event and the impact on the child were taken into account.

MRI Assessment

Participants were scanned in a Siemens 3.0-T scanner (MAGNETOM Trio Tim System, Siemens Medical Solutions, Erlangen, Germany) using a 32-channel

coil. Children completed two structural scans: a high-resolution T1 magnetization-prepared rapid gradient-echo (MPRAGE) sequence scan and an ultra-high resolution T2-weighted scan of the medial temporal lobe. Scan parameters were as follows for the T1-weighted structural scan: 176 contiguous sagittal slices; 9 mm isotropic voxel size; 1900 ms TR; 2.32 ms TE; 900 ms inversion time, 9º flip angle, 256 X 256 pixel matrix. Scan parameters were as follows for the ultra-high resolution T2-weighted scan: 24 slices; .4 X .4 X 2mm voxel size; 4120 ms TR; 41 ms TE; 149º flip angle.

In addition to the structural scans, children completed a functional task-free T2*-weighted gradient-echo-planar imaging sequence scan. Scan parameters were as follows for the task-free T2*-weighted functional scan: 210 EPI volumes; 36 oblique interleaved slices; 3.0mm x 3.0mm x 3.5mm voxel size; 2000 ms TR; 24 ms TE; 3 mm slice thickness; 70 º flip angle; 192 mm field of view, and 64 x 64 voxel matrix. Data from the structural scans will be the focus of Study 1a while data from the functional scan will be the focus of Study 1b.

Prior to the MRI assessment, children completed a mock scan where they received motion feedback and could get acclimated to the scan environment. To reduce head motion during the scan, padding was placed around the child's head, and they received motion feedback via a research assistant who remained in the scanner room with them. Motion was also tracked in real time during the functional scan. If motion exceeded 2 mm in any direction, the scan was stopped and repeated. Children were monitored to ensure they were awake, and if they fell asleep, the scan was stopped and repeated. During the functional scan, children watched *Inscapes*, a video that shows a series of abstract shapes (Vanderwal et al., 2015). *Inscapes* was used to

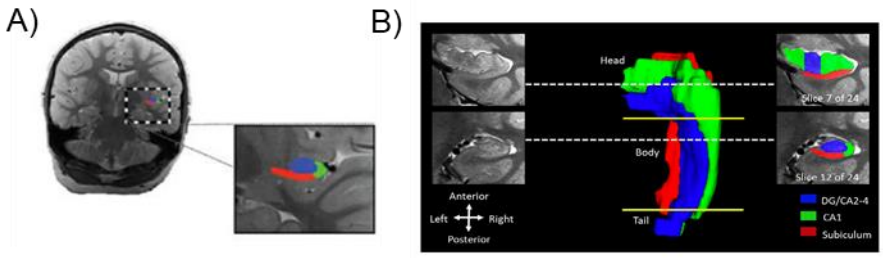
reduce motion, increase compliance, and also ensure that children did not fall asleep during the scan. During the two structural scans, children watched a movie of their choosing and were allowed to fall asleep. A quality check was done of the data following collection, and if the images were not deemed to be of low quality, children were asked to return to the lab for a rescan.

Hippocampal Subfields

Subfields were traced through a combination of manual and automated methods using the ultra-high-resolution scan of the medial temporal lobe. Specifically, an existing protocol was adapted for use with this sample (La Joie et al., 2010). Subfields were identified and traced in the head and body subregions of the hippocampus. The tracing protocol yields the following subfields: CA1, CA2-4/DG, and subiculum. CA2-4/DG is a combination region consisting of CA2, CA3, CA4, and DG as it is often difficult to accurately delineate each of these subfields. A visual depiction of the subfields is presented in Figure 1.1. Two tracers, who were blind to age and sex, completed ten cases each. Reliability between raters was assessed and Dice Similarity Coefficients (DSC) showed agreement between raters: CA1 = .73, CA2-4/DG = .85, subiculum = .74. A value of .7 or greater is considered acceptable (Zijdenbos et al., 1994). Reliability of volume measurements was also high as indicated by the Intra-class correlation (ICC; Shrout & Fleiss, 1979). A value of .9 or higher is considered highly reliable. Specifically, ICCs were as follows for each subfield: CA1 = .98; CA2-4/DG = .90; subiculum = .93

Figure 1.1

A) Cross-sectional slice of the brain with hippocampal subfields highlighted. B) 3D rendering of hippocampal subfields. Adapted from Riggins et al., 2020.



One tracer completed an additional 10 cases, which was then used to create a template for use in the Automatic Segmentation of Hippocampal Subfields software (ASHS; Yushkevich et al., 2015). Next, the training cases were used to identify subfields in the remaining subjects' data. Visual inspection was used to ensure quality was high; however, no manual edits were made. Although subfields can be delineated in the head and body of the hippocampus separately (Riggins et al., 2018), subfields were summed across head and body to provide larger and more stable estimates of each subfield (Canada et al., 2021). Finally, subfields were also collapsed across hemisphere given there are no specific hypotheses regarding hemispheric differences and similar trajectories are thought to be present. Subfield volumes that exceeded 3SD from the mean were omitted from analyses. This resulted in the removal of one subject from the 6-year-old cohort.

Thalamus volume and estimated total intracranial volume (eTICV) were extracted from the T1-weighted structural scan at each wave of data collection using Freesurfer 5.1 (Fischl, 2012). Thalamus was used as a control region to assess specificity of results with subfields. eTICV was used to adjust both hippocampus and

thalamus volumes using an ANCOVA approach to ensure that results were not driven by differences in head size (Raz et al., 2005). All analyses were run with raw and adjusted volumes to ensure the adjustment did not impact results. Raw and adjusted volumes were similar; thus, results using adjusted volumes are reported for simplicity.

Data Analysis Plan

Structural Equation Modeling

Structural equation modeling (SEM) was used to assess associations between stressful life events and hippocampal development. Importantly, this method can speak to critical developmental questions, such as modeling change over time, as it allows developmental researchers to assess both intra- and inter-individual changes. SEM is flexible enough to precisely model the longitudinal nature of the data and allows one to assess causal associations rather than simple correlations (Little, 2013). Furthermore, SEM has the capability of including both measured and latent variables, which reduces error in the model. Finally, SEM can handle missing data, which often accompanies longitudinal datasets (Enders, 2013).

Latent Growth Modeling

Within the SEM framework, a technique referred to as latent growth modeling (LGM) was used to analyze the data. LGM allows one to characterize change in a variable of interest over time through the use of latent factors (i.e., slope and intercept). More specifically, LGM allows for taking the raw observed data and using it to make inferences about the latent growth curve underlying the data, which cannot be directly observed. Specifically, one can use the raw measurement data to make

inferences about the starting point (i.e., intercept) and rate of change (i.e., slope). This is especially useful because it reduces measurement error in the model. Additionally, rather than pooling over all subjects in the sample to simply assess a mean score for individuals within a time point, LGM is a more rigorous method for assessing repeated measures data because it allows for looking at the same individual over multiple time points (assessing intra-individual change in addition to inter-individual change; Duncan & Duncan, 2009; Kievit et al., 2018; Lawrence & Hancock, 1998; McArdle, 2009).

In addition to modeling trajectories, conditional LGMs can help understand predictors that may impact the starting point or rate of change of the dependent variable of interest, making it an especially good technique to assess how stressful life events impact subfield development (Curran et al., 2010; Kievit et al., 2018). Specifically, there is a mean and variance associated with the slope and intercept, and one can use predictors to assess how they relate to variance in initial volumes (i.e., intercept) and how volumes change over time (i.e., slope). In other words, it can assess how predictors account for heterogeneity in change of observed variables. These conditional LGM models were used in Studies 1a and 1b.

In this study, multiple-group LGM was used, such that separate models were run for the 4-year-old and 6-year-old cohorts. This allowed for testing the impact of stressful life events on hippocampal subfield development before and after 5 years of age.

Models Assessing Development of Subfields

First, the development of hippocampal subfields was characterized for each cohort. Detailed developmental trajectories of subfield volumes (from 4 to 8 years) have been previously reported from this sample for hippocampal head and body subregions separately (Canada et al., 2021). However, in the current study, subfields were collapsed across head and body to provide larger and more stable estimates of each subfield; therefore, I characterized the trajectories first to better understand how stressful life events related to both the estimated intercept (i.e., initial volume) and slope factors (i.e., change in volume).

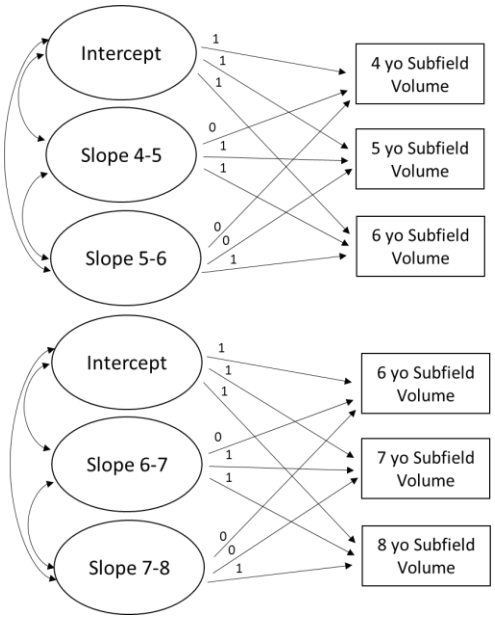
To assess the development of hippocampal subfields, three observed indicators of subfield volumes (1 at each time point) were entered in the latent growth model. Each subfield was assessed in a different model given the differential developmental trajectories of subfields in the hippocampus (Canada et al., 2021; Riggins et al., 2018). This resulted in 6 models. In each model, intercept loadings were fixed to 1 (i.e., Time 1 served as the reference point) as I was interested in assessing concurrent effects of stressful events on subfield volumes and also prospective associations with change in subfield volumes. Models were first assessed using a single slope. The loadings associated with the slope were specified as 0, 1, and 2 given that subfield volumes were assessed at approximately equally spaced time-points (i.e., 1 year between each assessment). Acceptable fit is typically indicated by a root-mean square error of approximation (RMSEA) less than .06 and a standardized root mean square residual (SRMR) less than .08 (see Hu & Bentler,

1999). Model fit indices exceeded these cutoffs suggesting poor model fit when a single slope was used.

Piecewise models were then used to improve model fit, which allowed for fitting two slopes to the data for each cohort. Specifically, one slope was fit from Time 1 to Time 2 and another slope was fit from Time 2 to Time 3 for each cohort. Loadings for the slope from Time 1 to Time 2 were 0, 1, and 1 and loadings for the slope from Time 2 to Time 3 were 0, 0, and 1. Intercept and slope factors were allowed to covary, and the error terms' covariances were set to zero. In this model, there were 9 parameters to be estimated: mean and variance associated with the intercept and each slope factor and covariance between the intercept and slope factors. Using piecewise models resulted in just-identified models with zero degrees of freedom. Therefore, no model fit indices are reported. Models are laid out in Figure 1.2 and were tested using Mplus (Muthén & Muthén, 1998–2017).

Figure 1.2

Models used to assess growth of hippocampal subfields in the 4- and 6-year-old cohorts



Note. yo = year old. Separate models were run for each cohort and each subfield volume (CA1, CA2-4/DG, and subiculum).

Models Assessing Stressful Life Events and Development of Subfields

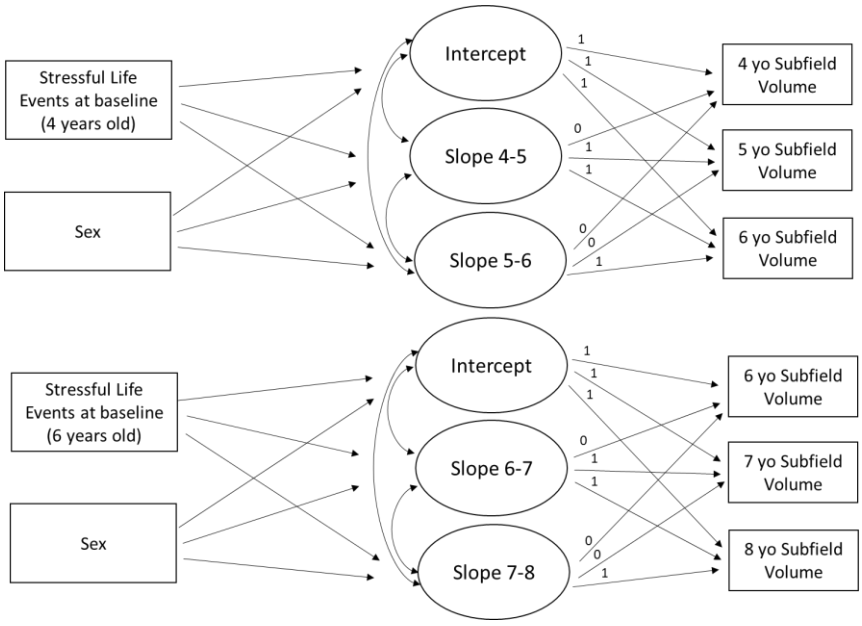
Once the latent intercept and slope factors were estimated for subfield volumes, the Stressful Life Events Score was entered as a predictor in the model to assess **Aim 1** (i.e., how stressful life events relate to subfield volumes in each cohort). Because typical stress is relatively stable over time (Cohen & Hamrick, 2003), and because I aimed to investigate impacts of early stress on later development, only stress at baseline was assessed. Therefore, stress was entered in the model as a time-invariant predictor. Parental education, which is a proxy for SES, and sex were investigated as potential covariates. There were no significant associations between parental education and the intercept or slope factors, so it was not included as a

covariate to preserve power. Subfield volumes did differ based on sex, so it was included as a time-invariant covariate. There were 2 residual error terms in the model that assessed the Stressful Life Events Score as a predictor: 1 associated with the predictor (i.e., Stressful Life Events Score) and 1 associated with the covariate (i.e., sex). Once again, error term covariances for the subfield volume indicators were set to zero. In this model, there were 11 parameters to be estimated: mean and variance associated with the intercept and each slope factor, covariance between the intercept and slope factors, and two error variances. Models were just-identified; therefore, no fit indices were estimated. Models are laid out in Figure 1.3

Hypothesis 1_a was tested by assessing how the Stressful Life Events Score related to the latent intercept and slope factors associated with CA1 and CA2-4/DG while **Hypothesis 1_b** was tested by assessing how the Stressful Life Events Score related to the latent intercept and slope factors associated with subiculum.

Figure 1.3

Models used to assess relations between stressful life events and hippocampal subfield development in the 4- and 6-year-old cohorts



Note. yo = year old. Separate models were run for each cohort and each subfield volume (CA1, CA2-4/DG, and subiculum).

Aim 2 was to assess whether effects differed between cohorts (i.e., timing-dependent effects of stressful life events). **Aim 2, Hypothesis 2** was tested by comparing effects of the Stressful Life Events Score on the intercept and slope factors in the 4-year-old and 6-year-old cohorts. Specifically, a bootstrapping approach was used to compare estimates and assess if the impact of stressful events on subfield intercept and slope factors significantly differed when stressful events were assessed at 4 vs. 6 years of age.

Specificity Analyses

To assess specificity of results obtained from the analyses discussed above, additional analyses were run with bilateral thalamus volume, a brain region that is not

thought to be greatly impacted by stress (Frodl et al., 2010; Humphreys et al., 2019; Sah et al., 2005).

Missing Data

Missing data were evident in the sample, and LGM provided an optimal way to handle this missing data without losing subjects. More specifically, it allowed for both incomplete and unbalanced datasets (Duncan et al., 2006). This is useful because if missing data are not handled appropriately, it can result in biased estimates and decreased precision. It is important to note though that assumptions about the "mechanism of missingness" must be met for models to provide valid results. The current study employed an accelerated longitudinal design, resulting in planned missingness in the data. In addition to planned missingness, missing data resulted from poor image quality (due to motion or issues with scanner) and attrition. Given these factors, the data are missing at random, which meets the assumptions for using full information maximum likelihood (FIML) to estimate missing data.

Results

Stressful Life Events Checklist

Descriptive statistics associated with the Stress Life Events Checklist are presented in Table 1.3 and Figure 1.4. The Stressful Life Events Score for the 4-year-old and 6-year-old cohorts did not significantly differ from one another ($t(90) = .58$, $p = .56$). Correlational analyses showed that the Stressful Life Events Score at Time 1 was correlated with that at Time 2 ($r(66) = .42$, $p < .001$), but not at Time 3 ($r(61) = .14$, $p = .27$) across cohorts. However, the Stressful Life Events Scores at Time 2 and Time 3 were correlated ($r(58) = .41$, $p < .001$).

Table 1.3

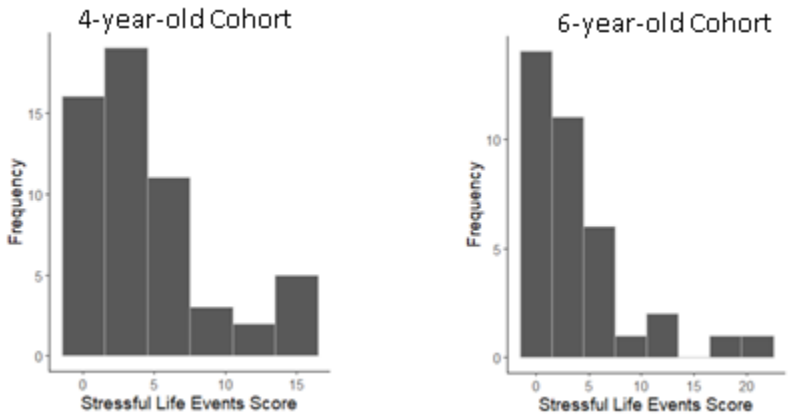
Descriptive statistics associated with the Stressful Life Events Checklist for the 4- and 6-year-old cohorts

	4-year-old Cohort				6-year-old Cohort			
	M	*SD*	Min	Max	*M*	*SD*	Min	Max
Cumulative number of events	2.29	2.46	0	15	1.78	1.81	0	8
Cumulative effect of events	2.72	2.64	0	11	2.89	3.72	0	13
Stressful Life Events Score	4.52	4.34	0	15	3.94	5.03	0	21

Note. *M* = mean. *SD*=standard deviation. Min=minimum value. Max=maximum value.

Figure 1.4

Histograms representing the frequency distribution of the Stressful Life Events Score for each cohort



The five most endorsed events included: the child changed schools or childcare providers, the parent started a new job, the child was separated from the parent for more than a week, the parents experienced high levels of stress, and the child moved to a new home (Table 1.4). Some events were not endorsed at all (e.g., child was victim of physical violence, parent was assaulted, child was poisoned).

When assessing specific types of events that were endorsed most, it appeared that parents did not endorse the most extreme events, which supported the idea that I was assessing more normative variations in stressful events.

Table 1.4

Frequencies and descriptive statistics for the most common stressful life events reported across cohorts

Event	Frequency	Mean Effect	Range
Child changed schools	37	1.37 (1.06)	0-3
Parent started a new job	26	0.71 (.91)	0-3
Child was separated from parent for more than a week	19	0.94 (.99)	0-3
Parent experienced high levels of stress	12	1.25 (.62)	0-3
Child moved to a new place	10	1.27 (1.01)	0-3

Note. Standard deviations are in parentheses.

Bivariate Correlations Between Variables

Bivariate correlations between variables included in the growth models for the 4- and 6-year-old cohorts are in Tables 1.5 and 1.6, respectively.

Table 1.5

Bivariate correlations for each variable in the growth models assessing subfield volumes for the 4-year-old cohort

	1	2	3	4	5	6	7	8	9	10	11
1. Sex											
2. Parental Ed	.21										
3. Stress Score	-.05	-.06									
4. CA1 W1	-.21	.04	.04								
5. CA1 W2	-.20	.13	.25	.85*							
6. CA1 W3	-.06	.02	-.02	.77*	.80*						
7. DG W1	-.41*	-.02	.25	.42*	.54*	.67*					
8. DG W2	-.20	.01	.27	.38	.52*	.57*	.78*				
9. DG W3	-.20	.09	.21	.58*	.69*	.65*	.81*	.81*			
10. Sub W1	-.17	.08	.17	.35*	.41	.37	.47*	.267	.28		
11. Sub W2	-.18	.15	.60*	.55*	.47*	.41*	.51*	.60*	.66*	.65*	
12. Sub W3	-.01	.16	.25	.28	.60*	.39*	.46*	.40*	.56*	.71*	.79*

Note. *$p < .05$. Parental Ed=Parental Education. Stress Score = Stressful Life Events Score. Sub = subiculum. W1= Wave 1. W2= Wave 2. W3= Wave 3.

Table 1.6

Bivariate correlations for each variable in the growth models assessing subfield volumes for the 6-year-old cohort

	1	2	3	4	5	6	7	8	9	10	11
1. Sex											
2. Parental Ed	.44*										
3. Stress Score	.003	.16									
4. CA1 W1	-.43*	-.30	-.32								
5. CA1 W2	-.52*	-.26	-.24	.80*							
6. CA1 W3	-.58*	-.56*	-.31	.84*	.86*						
7. DG W1	-.45*	-.15	-.40*	.55*	.76*	.72*					
8. DG W2	-.50*	-.22	-.36	.65*	.69*	.67*	.91*				
9. DG W3	-.40	-.29	-.37	.59*	.68*	.73*	.89*	.86*			
10. Sub W1	-.40*	-.02	-.06	.43*	.61*	.76*	.67*	.64*	.68*		
11. Sub W2	-.31	.09	-.31	.55*	.54*	.52*	.66*	.72*	.60*	.70*	
12. Sub W3	-.39	-.09	-.22	.47*	.44*	.63*	.56*	.57*	.59*	.79*	.89*

Note. *$p < .05$. Parental Ed=Parental Education. Stress Score = Stressful Life Events Score. Sub = subiculum. W1= Wave 1. W2= Wave 2. W3= Wave 3.

Development of Subfields

Results from analyses assessing the development of subfields are summarized below and statistics associated with the estimated mean and variance for each factor are reported in Table 1.7. Growth trajectories for each subfield and cohort are depicted in Figure 1.5.

CA1

No significant change in CA1 volume was observed for either cohort. However, there was significant variance associated with initial CA1 volume and change in CA1 volume for both cohorts.

CA2-4/DG

No significant change in CA2-4/DG volume was observed for the 4-year-old cohort. There was significant change in volume from 6 to 7 years old for the 6-year-old cohort. In addition, there was significant variance associated with initial CA2-4/DG volume and change in CA2-4/DG volume for both cohorts.

Subiculum

No significant change in subiculum volume was observed for the 4-year-old cohort. There was marginally significant change in subiculum volume from 6 to 7 years old for the 6-year-old cohort. Furthermore, there was significant variance associated with initial subiculum volume and change in subiculum volume for both cohorts.

Table 1.7

Growth parameters for each subfield model for the 4- and 6-year-old cohorts

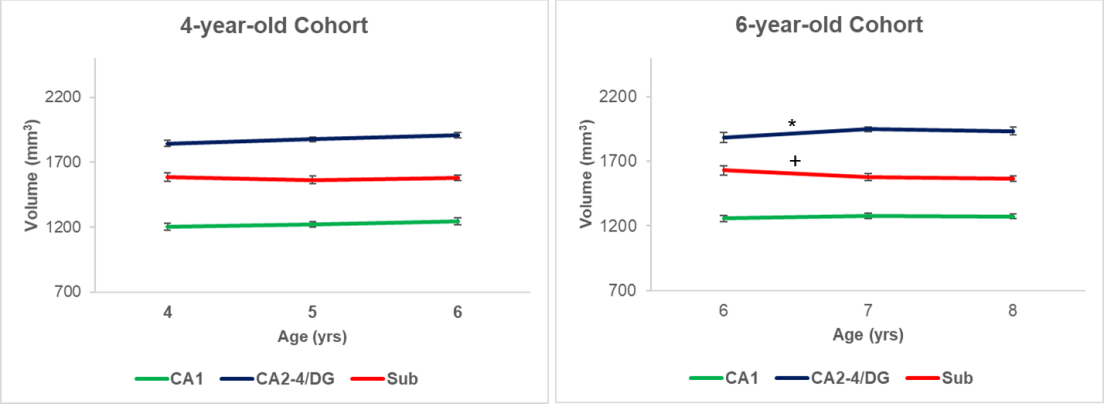
4-year-old cohort	Intercept Mean	Slope 4-5 Mean	Slope 5-6 Mean	Intercept Variance	Slope 4-5 Variance	Slope 5-6 Variance
CA1	1200.70 (23.19)**	18.97 (16.05)	25.13 (20.83)	25895.31 (4524.96)**	6323.68 (2199.38)**	8795.98 (3592.49)**
CA2-4/DG	1843.50 (26.86)**	32.508 (22.70)	30.65 (27.19)	34843.76 (7647.16)**	13893.44 (6170.76)*	18387.20 (4845.49)**
Subiculum	1585.84 (32.08)**	-21.509 (29.03)	17.38 (21.44)	46215.87 (9477.33)**	28827.26 (6379.47)**	13677.68 (3183.61)**

6-year-old cohort	Intercept Mean	Slope 6-7 Mean	Slope 7-8 Mean	Intercept Variance	Slope 6-7 Variance	Slope 7-8 Variance
CA1	1256.97 (22.13)**	19.85 (18.92)	-3.18 (18.43)	17603.87 (3833.75)**	8113.26 (2512.59)**	6881.46 (2238.90)**
CA2-4/DG	1883.26 (37.66)**	64.34 (20.37)**	-12.65 (31.24)	51142.43 (13852.50)**	9362.65 (2451.90)**	20971.63 (5670.80)*
Subiculum	1628.99 (37.75)**	-49.95 (27.58)++	-13.992 (20.87)	51808.71 (13223.42)**	20936.72 (5164.54)**	9789.99 (2656.70)**

Note. $^{**}p < .01$. $^{*}p < .05$. $^{+}p < .06$. $^{++}p < .07$. Coefficients are unstandardized. Standard errors are in parentheses.

Figure 1.5

Growth trajectories of each subfield for the 4- and 6-year-old cohorts



Note. $^{*}p < .05$; $^{+}p < .07$. Sub = subiculum.

Associations between Stressful Life Events and Development of Subfields

CA1

For the 4-year-old cohort, results showed that a higher Stressful Life Events Score was marginally significantly associated with less change in volume from 5-6 years old, but was unrelated to initial volumes (Table 1.8). In other words, a lower Stressful Life Events Score was marginally significantly related to greater change in CA1 volume from 5-6 years old. For the 6-year-old cohort, the Stressful Life Events

Score was negatively associated with initial volume, such that a greater Stressful Life Events Score was related to smaller initial CA1 volume (Table 1.8). The Stressful Life Events Score was unrelated to change in volume for the 6-year-old cohort.

CA2-4/DG

For the 4-year-old cohort, there were no significant relations between the Stressful Life Events Score and initial volume or change in volume. For the 6-year-old cohort, results showed a significant negative association between the Stressful Life Events Score and initial volume. Specifically, a greater Stressful Life Events Score was related to smaller CA2-4/DG volumes at Time 1. No associations emerged between the Stressful Life Events Score and change in volume for the 6-year-old cohort (Table 1.8).

Subiculum

There were no associations between the Stressful Life Events Score and initial volume or change in volume for either cohort (Table 1.8).

Table 1.8

Parameters for models assessing the relation between stressful life events and subfield development for the 4- and 6-year-old cohorts

4-year-old Cohort	**Intercept *b* (*SE*)**	**Slope 4-5 *b* (*SE*)**	**Slope 5-6 *b* (*SE*)**
CA1	4.65 (6.03)	-.68 (4.47)	-8.91 (4.73) ++
CA2-4/DG	11.14 (6.52)+	2.86 (6.56)	-2.19 (6.82)
Subiculum	10.90 (8.51)	10.30 (7.93)	-9.24 (5.65)+

6-year-old Cohort	**Intercept *b* (*SE*)**	**Slope 6-7 *b* (*SE*)**	**Slope 7-8 *b* (*SE*)**
CA1	-8.44 (3.78)*	-1.96 (3.24)	-2.00 (3.36)
CA2-4/DG	-17.55 (6.52)*	.90 (4.07)	-.67 (5.81)
Subiculum	-2.75 (7.37)	-4.83 (4.67)	.30 (.939)

Note. $**p < .01$. $*p < .05$. $++p < .06$. $+p < .10$. Coefficients are unstandardized. *SE* = standard error.

Comparing Effects Between Cohorts

To assess whether significant effects observed in Aim 1 differed between cohorts, a bootstrapping approach was used. Results showed that associations between the Stressful Life Events Score and initial CA2-4/DG volume significantly differed between the two cohorts. No differences were observed for the association between the Stressful Life Events Score and initial CA1 volume. This suggests that stressful events relate to subfields differently for the 4- (no association) and 6-year-old (significant negative association) cohorts for CA2-4/DG, but not CA1 volume (Table 1.9).

Table 1.9

Parameters for analyses comparing effects between the 4- and 6-year-old cohorts

Model	**Intercept *(SE)***	**95% Bootstrapped CI**
CA1	-13.09 (8.70)	[-30.15, 3.97]
CA2-4/DG	-27.60 (11.19)*	[-49.53, -5.68]

Note. $*p < .01$. Coefficients are unstandardized. SE = standard error.

Specificity Analyses

In an effort to assess specificity of associations with subfield volumes, relations between the Stressful Life Events Score and initial thalamus volume and change in thalamus volume were assessed. Development of thalamus was first assessed using piecewise growth models. Model specifications were the same as those used for models with subfield volumes. Results indicated that there was significant positive change for the 4-year-old cohort from 5-6 and for the 6-year-old cohort from 6-7 and 7-8 years old. Furthermore, there was significant variability associated with the intercept and slope factors for each cohort (Table 1.10, Figure 1.6).

Table 1.10

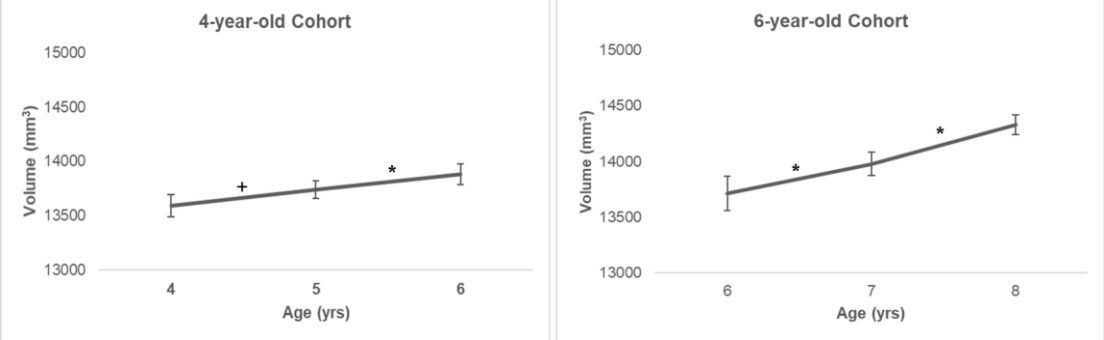
Parameters for growth models assessing total thalamus volumes for the 4- and 6-year-old cohorts

	Intercept Mean	**Slope 4-5 Mean**	**Slope 5-6 Mean**	**Intercept Variance**	**Slope 4-5 Variance**	**Slope 5-6 Variance**
4-year-old cohort	13591.70 (101.10)**	144.77 (81.01)+	420.39 (95.26)**	49471.00 (108748.80)**	27255.97 (64908.27)**	36477.30 (83362.71)**
	Intercept Mean	**Slope 6-7 Mean**	**Slope 7-8 Mean**	**Intercept Variance**	**Slope 6-7 Variance**	**Slope 7-8 Variance**
6-year-old cohort	13714.85 (154.58)**	264.40 (107.51)*	353.41 (89.09)**	95583.80 (265985.90)**	37333.30 (84523.74)**	21825.50 (49527.09)**

Note. $^{**}p < .01$. $^{*}p < .05$. $^{+}p < .08$. Estimates are unstandardized. Standard errors are in parentheses.

Figure 1.6

Growth trajectories of total thalamus volume for each cohort



Note. *p < .05. +p < .09.

Results from models including the Stressful Life Events Score as a predictor variable showed that there were no associations between the Stressful Life Events Score and initial volume or change in volume for the 4-year-old cohort. However, the Stressful Life Events Score was negatively related to change in volume from 6 to 7 years old for the 6-year-old cohort. In other words, a lower Stressful Life Events Score was related to greater change in thalamus volume from 6-7 years old (Table 1.11).

Table 1.11

Parameters for models assessing relations between the Stressful Life Events Score and total thalamus volume for the 4- and 6-year-old cohorts

	Intercept ***b (SE)***	**Slope 4-5** ***b (SE)***	**Slope 5-6** ***b (SE)***
4-year-old Cohort	-18.66 (23.68)	9.79 (19.25)	-19.47 (23.11)

	Intercept ***b (SE)***	**Slope 6-7** ***b (SE)***	**Slope 7-8** ***b (SE)***
6-year-old Cohort	13.42 (31.22)	-44.30 (19.64)*	2.39 (.88)

Note. *p<.05. **p<.01. *SE* = standard error.

Analyses comparing significant effects in the 4- vs. 6-year-old cohorts showed that the relation between the Stressful Life Events Score and change in volume from Time 1 to Time 2 (4 to 5 years old for the 4-year-old cohort and 6 to 7 years old for the 6-year-old cohort) significantly differed between cohorts (b = -55.46, SE = 24.85, p =.026; 95% CI [-104.17, -6.75]). This suggests that stressful events relate to change in thalamus volume differently for the 4- (no association) and 6-year-old (negative association) cohorts.

Discussion

This study aimed to assess relations between stressful life events and volume of hippocampal subfields both prior to and after 5 years of age, which is when the hippocampus is thought to be relatively mature (Serres, 2001). Aim 1 was to assess the impact of stressful life events at 4- and 6-years-old on hippocampal subfield volumes (CA1, CA2-4/DG, and subiculum) and change in volume over a 2-year period. Aim 2 was to assess the time-dependence of these stress-brain associations.

Aim 1: Associations between Stressful Life Events and Subfield Volumes

Based on work in older children and adults (Lee et al., 2018; Malhi et al., 2019; Merz et al., 2019; Whittle et al., 2017) and rodent samples (Champagne et al., 2008; Naninck et al., 2015), I hypothesized that greater reported stressful life events would impact development of CA1 and CA2-4/DG (Hypothesis 1a), but not subiculum (Hypothesis 1b) in both cohorts of children. I found partial support for my hypotheses. In the 4-year-old cohort, a greater number of stressful events (experienced the year prior/between 3 and 4 years of age) was marginally significantly related to more negative change in CA1 volume from 5 to 6 years of age.

In the 6-year-old cohort, a greater number of stressful events (experienced the year prior/between 5 and 6 years of age) was related to smaller CA2-4/DG and CA1 volumes at 6 years of age. As hypothesized, stressful events did not relate to subiculum volume in either cohort.

These results support work that has begun to assess effects of stress on subfields in children and adolescents. Specifically, results from this study are in line with findings that showed that adolescent girls exposed to emotional trauma had smaller CA3 volumes (CA3 is within our CA2-4/DG delineation; Malhi et al., 2019) and findings in 5- to 9-year-old children that showed that hair cortisol (a proxy for cumulative stress) was related to smaller CA3/DG volumes (Merz et al., 2019). The negative associations with CA2-4/DG and CA1 in the 6-year-old cohort fit with the larger literature in a consistent manner as negative effects are often reported. Findings observed in the 6-year-old cohort also align with rodent literature, which typically shows that early life stress is associated with smaller subfield volumes by impacting dendritic branching, synaptic growth, and neurogenesis (Champagne et al., 2008; Derks et al., 2016; Naninck et al., 2015). Findings also align with work in adults, which shows that childhood maltreatment is associated with smaller CA1, CA2-4, and DG volume during adulthood (Lee et a., 2018; Teicher et al. 2012; Whittle et al., 2017).

Aim 2: Assessing Time-Dependence of Effects

Aim 2 was to assess the time-dependence of effects detected in Aim 1. It was hypothesized that there would be a greater effect of stressful life events in the younger vs older cohort on CA1 and CA2-4/DG volumes (Hypothesis 2) given work

that has highlighted birth through 5 years of age as being particularly important for the impacts of extreme stress on the hippocampus (Andersen et al., 2008; Serres, 2001). However, some work in humans has revealed a more protracted developmental course for hippocampal subfields (Ghetti & Bunge, 2012; Lee et al., 2020). The current study showed that the 6-year-old cohort demonstrated a negative relation between stressful events and Time 1 CA2-4/DG and CA1 volumes, which is more consistent with the idea of prolonged development of subfields and a prolonged period during which subfields can be impacted by stress. In contrast, the 4-year-old cohort showed no significant associations with Time 1 volumes. Effects associated with CA2-4/DG Time 1 volumes statistically differed by cohort (CA1 did not), which provides evidence of time dependence of effects. In particular, the relation between stressful events and CA2-4/DG volume for the 6-year-old cohort was significantly different than that observed for the 4-year-old cohort even though both cohorts experienced similar levels of stress.

These time-dependent findings are somewhat in contrast to previous work, which showed a greater impact of stress experienced prior to 5 years of age compared to stress experienced after 5 years of age (Andersen et al., 2008; Humphrey et al., 2019; Luby et al., 2016). However, many of these studies assessed the hippocampus as a whole during adolescence and often in relation to more extreme stress. In contrast, this study assessed subfields during childhood and in relation to typical variations in stress. Although effects were observed mainly after 5 years of age, they still provide support for the idea of age-dependent associations and for the idea that stress experienced during childhood does have effects that can be observed during

childhood. Importantly, they also provide more specificity than the extant literature by assessing subfields.

Specificity of Findings

To investigate specificity of findings within the brain, thalamus was assessed, which is a structure not thought to be greatly implicated by variations in stress (Frodl et al., 2010; Humphreys et al., 2019; Sah et al., 2005). Stressful events were not related to initial volumes, but were associated with more negative change in volume from 6-7 years old, suggesting that findings may not be specific to hippocampal subfields. Significant associations with thalamus were only observed in the 6-year-old cohort and significantly differed from the 4-year-old cohort providing additional evidence of time-dependence of stress effects on the brain. Although these findings with thalamus may be spurious in nature, stress has wide ranging impacts on the brain, and some work does suggest that thalamus may be impacted by stress (Philip et al., 2016). Furthermore, the hippocampus and thalamus are structurally and functionally connected (Aggleton et al., 2010; Blankenship et al., 2017; Goldstone et al., 2018) so detrimental impacts on the hippocampus could extend to other structures as well.

Strengths

Strengths of this study include the assessment of hippocampal subfields, which provide more precision than using total hippocampus. The manual tracing of hippocampal subfields is also a strength of the study. Other strengths include the use of longitudinal data with multiple time points and cohorts that allows for assessing stress effects at different points of hippocampal development. In addition, the

longitudinal SEM methods used to analyze the data are a strength as they allowed for multiple time points of data to be included and for handling missing data. The narrow age range is a strength in that it provides the ability to home in on specific ages where greater impacts of stress on the developing hippocampus may be apparent. Many studies focus on preschool to adolescence, but there is a great deal of change occurring even in early childhood within age groups. The differential effects at 4 and 6 years of age underscores the importance of assessing stress in limited age ranges as large age ranges may obscure findings.

Limitations and Future Directions

One limitation of this study is the small sample size, which made it impractical to assess moderating effects of sex. This is an important question to investigate as research shows that males and females experience and process stress differently and females are at a higher risk for developing stress-related disorders (Bennett et al., 2005; Derks et al., 2016; Loi et al., 2014). Another limitation of this study is that it assessed stress using parent reported stressful events, which required the parent to assess the perceived impact of the event on the child. As with any parent report measure, this is a limitation as there may be bias associated with it. Future research should utilize a variety of stress-related measures, including physiological measures, for a more robust assessment of stress.

This is a relatively high SES sample so findings cannot be generalized to low SES samples and moderating effects of SES could not be assessed. Future work should examine these associations in more diverse samples to understand how low SES and other forms of stress may compound the impact of stressful life events on

subfield development. Assessing moderating effects of SES with stress or indirect effects of SES and stress on subfields will provide some answers to this question.

Conclusion

Results show that childhood is a period of plasticity where even typical variations in stress can influence brain development. However, the impacts of stress on the hippocampus are not uniform across development or even across subfields. Instead, stress impacts the brain differently at different developmental time points. These findings are only one part of the puzzle, but add to our knowledge of how childhood experiences can shape the brain.

Chapter 3: Associations between typical variations in stressful life events and hippocampal functional connectivity in young children (Empirical Study 1b)

Study 1b aimed to assess how variations in stressful life events impact hippocampal functional connectivity in early childhood both prior to and after 5 years of age. Specifically, these analyses explored variations in functional connectivity between the hippocampus and other regions of the brain during a task-free state. Such measures of functional connectivity are thought to reflect the intrinsic functional organization of the brain (Fox & Raichle, 2007). Studies show that coupling of activity between the hippocampus and various regions in the brain is related to an array of cognitive and behavioral outcomes, such as memory, spatial navigation, and emotional processing (Geng et al., 2020; Fastenrath et al., 2014; Chersi & Burgess, 2015; Immordino-Yang & Singh, 2013). In addition, variations in hippocampal functional connectivity may be related to risk for psychopathology (Hao et al., 2020; Li et al., 2020; Malivoire et al., 2018). However, few studies have examined stress in relation to hippocampal functional connectivity, especially in this age group. To fill this gap in the literature, this study used the same accelerated longitudinal sample of 4- to 8-year-old children from Study 1a. Specific aims are laid out below.

Aim 1: The first aim of the study was to characterize development of hippocampal functional connectivity with several stress-related regions of interest (ROIs) in 4 and 6-year-old children across a 2-year period.

Hypothesis 1: Given research suggesting linear increases in functional connectivity of the hippocampus in this age range (Geng et al., 2021), I hypothesized that there

would be increases in hippocampal functional connectivity between 4 and 6 years of age and 6 and 8 years of age. Because these increases may be small in magnitude (Blankenship et al., 2017), I did not use a whole brain approach, and instead focused on a priori ROIs related to stress in previous research in older children.

Aim 2: The second aim was to assess whether greater stressful life events (as reported by parents) relate to the development of hippocampal connectivity with each ROI.

Hypothesis 2: I hypothesized that greater reported stressful life events would be associated with initial hippocampal connectivity and change in connectivity with each ROI. However, no hypotheses were made regarding whether stress would relate to greater or weaker connectivity.

Aim 3: The third aim was to assess time-dependent effects of greater stressful life events on the development of hippocampal functional connectivity with each ROI.

Hypothesis 3: Given research suggesting that the hippocampus may be more susceptible to stress prior to 5 years of age (Andersen et al., 2008; Humphreys et al., 2019), I hypothesized that there would be greater associations between stressful life events and hippocampal connectivity with each ROI in the 4 vs. 6-year-old cohort.

Methods

Participants

See Study 1a for specific information on the full sample of children. Of the sample of 79 children from Study 1a (46 in Cohort 1 and 33 in Cohort 2), 4 subjects were missing functional data (2 from each cohort). Thus, there were 75 subjects who

had both stress data and at least one time point of functional MRI data, including 44 subjects in the 4-year-old cohort and 31 subjects in the 6-year-old cohort (Table 2.1)

Table 2.1

Number of subjects with 3, 2, and 1 time point of functional MRI data in the 4- and 6-year-old cohorts

Number of Time Points	Cohort	N of subjects
3	4-year-old	24
	6-year-old	22
2	4-year-old	13
	6-year-old	8
1	4-year-old	7
	6-year-old	1
Total	4-year-old	44
	6-year-old	31
	Sum	75

Stressful Life Events Checklist

See Study 1a for specific information regarding this questionnaire.

MRI Assessment

See Study 1a for information relating to the MRI scans.

fMRI Preprocessing

Task-free functional data was processed using the Data Processing Assistant for Resting State fMRI toolbox (DPARSF, version 3.1; Yan et al., 2016). Preprocessing steps included slice timing correction, realignment of functional data, registration of structural data to functional data, and regression of nuisance variables (i.e., white matter, CSF timeseries data). MELODIC FSL was used to perform independent component analysis (ICA) to remove motion and non-brain components (e.g., artifacts related to respiration and pulsation; Griffanti et al., 2017). Censoring

was applied by removing volumes that exceeded .5mm framewise displacement (FD), including the volume prior to and the volume after the offending volume. Brain extraction was performed using 6 toolboxes, including ANTs, AFNI, Brainsuite, FSL, Robex, & SPM (Tillman et al., 2018). Functional data was normalized to a 4.5- to 8.5-year-old symmetrical MNI child template using ANTs (Fonov et al., 2011). Temporal bandpass filtering (0.01-0.1 Hz), spatial smoothing (5 mm FWHM Gaussian kernel), and head motion correction was performed using AFNI (Cox, 1996). Only subjects that had greater than 5 min of usable task-free data were included in analyses (Geng et al., 2019; Blankenship, Botdorf et al., 2019). In cases where the child completed multiple runs due to issues with data quality, the runs were concatenated to meet the 5-minute cutoff for inclusion in analyses.

Structural T1-weighted images were previously processed using Freesurfer 5.1 (Fischl, 2012). Hippocampal seed masks were created to allow for assessing functional connectivity of anterior hippocampus (Blankenship, Botdorf, et al., 2019; Poppenk et al., 2013). This report focuses on the anterior portion of hippocampus, as this subregion has been shown to be functionally related to a network of brain regions important to episodic memory (Vincent et al., 2006). Furthermore, in a prior study, this subregion showed connectivity with more of the ROIs included in this study than posterior hippocampus (Blankenship, Botdorf, et al., 2019). Anterior hippocampus was defined using anatomical landmarks. Specifically, the anterior hippocampus was defined as all slices prior to the uncal apex until the outer boundary created by Freesurfer was reached (Weiss et al., 2005). Two independent coders, who were blind to age and sex, noted slice boundaries of the hippocampus, which were used to divide

hippocampus into anterior and posterior portions. Reliability was assessed between the two coders and showed 94.60% agreement within 1 slice and 99.99% agreement within two slices. When there was disagreement between raters on the correct slice location, the more experienced rater's slice was used. Automatic segmentation adapter tool (ASAT; Wang et al., 2011) was then used to refine the hippocampal boundaries. If an error was present and lasted for seven slices or more, manual edits were applied to correct the error. Refined hippocampal segmentations were used to create subject-specific hippocampal masks, which were used for the seed-based connectivity analyses.

Functional Connectivity Data

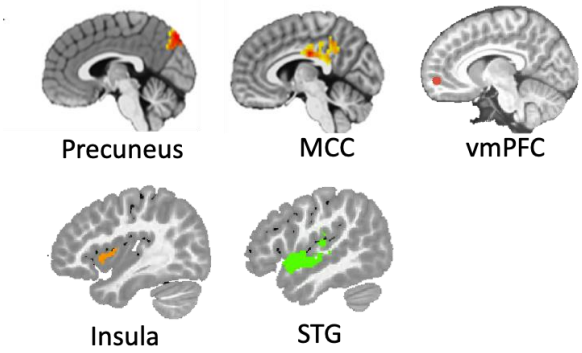
An ROI-based approach was used to assess connectivity between the anterior hippocampal seed region and five predefined ROIs. These regions came from previous research assessing stressful events and functional connectivity (Blankenship, Botdorf et al., 2019; Saxbe et al., 2018; Sripada et al., 2014). They include frontolimbic regions, such as superior temporal gyrus (STG) and insula (Saxbe et al., 2018), and regions in default mode network, including ventromedial prefrontal cortex (vmPFC; Sripada et al., 2014), precuneus, and midcingulate cortex (MCC; Blankenship, Botdorf et al., 2019). Posterior cingulate cortex (PCC) was originally proposed as an ROI but given that there was a high degree of spatial overlap between MCC and PCC, PCC was excluded as an ROI and was not included in analyses.

Functional masks were used for precuneus, MCC, insula, and STG. These masks were resampled to match the functional resolution of the data in the current study. A structural mask was used for vmPFC. After unsuccessful attempts to acquire

the functional mask from the authors of the original work, I opted to create a structural ROI by placing a 5mm radius sphere around the coordinates listed in the paper for vmPFC (Sripada et al., 2014). Although it is ideal to use the same type of ROI for all regions, it was not possible in this case. Using both structural and functional ROIs was deemed to be the best option. ROIs are shown in Figure 2.1.

Figure 2.1

Regions assessed in relation to the hippocampus in functional connectivity analyses



3dDeconvolve, a command within the AFNI toolbox, was used to create correlation maps of functional connectivity between hippocampus and all voxels in the brain. Separate maps were created at each time point. Next, *r* values (representing correlations between the hippocampal and ROI timeseries) were extracted for all three waves of data for further analysis in latent growth models. These models will be discussed in further detail in the next section.

Data Analysis Plan

Latent Growth Modeling

See Study 1a for information related to the latent growth modeling approach used in this study.

Description of Models

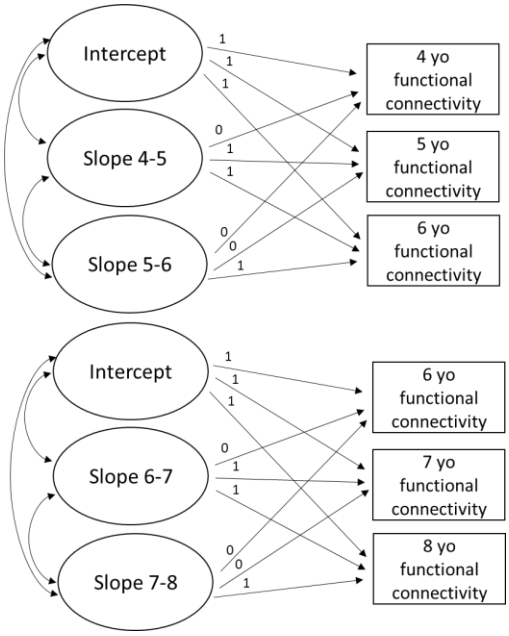
As in Study 1a, multiple-group LGM was used such that each cohort was examined in a separate model. This allowed for testing the effects of stressful events at different points in childhood on development of hippocampal functional connectivity. In each model, observed indicators were functional connectivity between the hippocampus and each ROI (i.e., correlations between the hippocampal and ROI timeseries) at each time point. Separate models were run for each ROI, which resulted in 10 models.

Models investigating development of hippocampal connectivity were first assessed (Figure 2.2). **Aim 1, Hypothesis 1** (i.e., assessing growth of hippocampal connectivity with each ROI) was tested by estimating the latent intercept and slope factors for hippocampal-ROI connectivity in each model. Intercept loadings were constrained to 1. As in Study 1a, Time 1 was used as the reference point as I was interested in assessing concurrent effects of stressful events on connectivity and also prospective associations with change in connectivity. I assumed change in connectivity between the hippocampus and each ROI was linear in nature (Geng et al., 2021). However, poor model fit resulted from fitting one slope to the data as the RMSEA exceeded .06 and SRMR exceeded .08 (Hu & Bentler, 1999). Therefore, piecewise models were used, which allowed for fitting multiple slopes to the data. Specifically, one slope was fit from Time 1 to Time 2 (Slope 1) and another slope was fit from Time 2 to Time 3 (Slope 2) for each cohort. Loadings for Slope 1 were 0, 1, and 1 and loadings for Slope 2 were 0, 0, and 1. The intercept and slope factors

were allowed to covary. Covariances of error terms were set to zero. The models were just-identified; therefore, no fit indices were estimated.

Figure 2.2

Models used to assess growth of hippocampal functional connectivity in the 4- and 6-year-old cohorts



Note. yo = year old. Separate models were run for each cohort and each region of interest (insula, precuneus, MCC, STG, vmPFC).

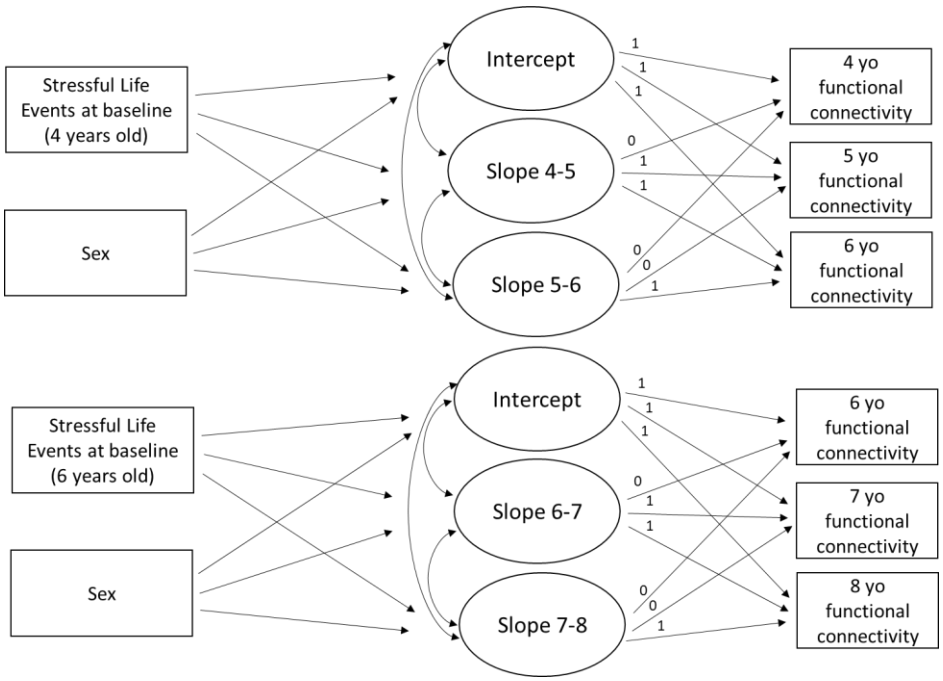
Next, the Stressful Life Event Score was entered as a predictor in the models (Figure 2.3). Because typical stress is relatively stable over time and because I was interested in looking at impacts of early stress on later development, only stress at baseline was assessed (consistent with Study 1a). **Aim 2, Hypothesis 2** (i.e., assessing how stressful life events relate to hippocampal connectivity with each ROI) was tested by assessing how Stressful Life Events predicts variability in the intercept

and slope factors of hippocampal connectivity with each ROI. Models were just-identified; thus, fit indices were not estimated.

Aim 3 focused on the time-dependent effects of Stressful Life Events on connectivity. **Hypothesis 3** was tested by comparing the impact of Stressful Life Events on connectivity in the 4- and 6-year-old cohorts. A bootstrapping approach was used to assess whether there were differences in the relation between Stressful Life Events and hippocampal connectivity for each model where there was a significant association between stressful events and the intercept or slope factors. Unstandardized estimates are reported for all models described above. Models were tested using Mplus (Muthén & Muthén, 1998–2016).

Figure 2.3

Models used to assess relations between stressful life events and the development of hippocampal functional connectivity for the 4- and 6-year old cohorts



Note. Separate models were run for each region of interest.

Missing Data

See Study 1a.

Covariates

Parental education was assessed as a potential time-invariant covariate in relation to hippocampal connectivity values. However, given that there was little variability in parental education in this sample (88% had at least one parent with a 4-year college degree) and parental education did not relate to connectivity values, it was not utilized as a covariate. Sex was included as a time-invariant covariate given known sex differences in the development of the hippocampus in this age range (Canada et al., 2020; Tamnes et al., 2018).

Results

Bivariate correlations between the Stressful Life Events Score, potential covariates, and functional connectivity data for each ROI at each time point are presented in Tables 2.2 and 2.3.

Table 2.2

Bivariate correlations between each variable in the growth models assessing hippocampal functional connectivity for the 4-year-old cohort

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Sex																	
2. Parental Ed.	-.13																
3. Stress Score	-.02	.29															
4. HC-Prec W1	-.07	.33	.25														
5. HC-Prec W2	.08	.23	-.06	.58*													
6. HC-Prec W3	.07	.13	.14	.34	.31												
7. HC-MCC W1	.04	.09	.11	.81*	.42*	.36											
8. HC-MCC W2	-.01	.16	.05	.45*	.83*	.08	.31										
9. HC-MCC W3	-.03	.18	.22	.34	.34	.90*	.42*	.12									
10. HC-Insula W1	.13	-.03	.34	.36*	.43*	.29	.30	.32	.30								
11. HC-Insula W2	.33*	-.07	-.09	.08	.41*	.14	.07	.33*	.17	-.09							
12. HC-Insula W3	-.13	.03	.07	-.18	.10	.46*	-.13	-.07	.49*	.15	.06						
13. HC-STG W1	.04	.11	.09	.44*	.34	.44*	.40*	.14	.49*	.67*	.01	.23					
14. HC-STG W2	.37*	.09	-.15	.21	.59*	.10	.06	.60*	.17	.02	.60*	-.16	.10				
15. HC-STG W3	-.12	.08	.17	.04	.26	.68*	.13	.08	.75*	.21	.11	.75*	.36	.06			
16. vmPFC W1	.03	.00	.00	.36*	.30	.47*	.52*	.13	.60*	.27	.01	-.07	.38*	.15	.30		
17. vmPFC W2	-.01	-.09	.27	.21	.43*	.25	.19	.51*	.25	.393*	.26	-.14	.19	.42*	.14	.28	
18. vmPFC W3	-.14	.30	.13	.15	.32	.68*	.19	.13	.82*	.12	.15	.52*	.29	.21	.75*	.42*	0.19

Notes. **p* < .05. Parental Ed. = Parental Education. Stress Score = Stressful Life Events Score. HC = hippocampus. Prec = precuneus. MCC = midcingulate cortex. STG = superior temporal gyrus. vmPFC = ventromedial prefrontal cortex. W1 = Wave 1. W2 = Wave 2. W3 = Wave 3.

Table 2.3

Bivariate correlations between each variable in the growth models assessing hippocampal functional connectivity for the 6-year-old cohort

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Sex																	
2. Parental Ed.	.11																
3. Stress Score	.05	.14															
4. HC-Prec W1	-.16	-.02	.14														
5. HC-Prec W2	.12	-.25	-.09	.20													
6. HC-Prec W3	-.09	-.19	-.20	.34	.41*												
7. HC-MCC W1	-.34*	.02	.16	.77*	.23	.39*											
8. HC-MCC W2	.02	-.21	-.02	.26	.91*	.45*	.31										
9. HC-MCC W3	-.18	.01	-.15	.27	.22	.92*	.35	.25									
1. HC-Insula W1	.02	-.04	-.25	.40*	.37	.62*	.57*	.29	.53*								
11. HC-Insula W2	.10	-.22	-.34	-.04	.77*	.24	.16	.66*	.12	.43*							
12. HC-Insula W3	-.12	.13	-.12	.26	.27	.70*	.38*	.22	.72*	.66*	.20						
13. HC-STG W1	-.08	-.01	-.18	.32	.30	.48*	.61*	.24	.39*	.74*	.51*	.57*					
14. HC-STG W2	.157	-.12	-.31	-.08	.85*	.31	.07	.76*	.17	.40*	.92*	.27	.46*				
15. HC-STG W3	-.07	.14	-.15	.12	.25	.75*	.28	.17	.81*	.59*	.19	.90*	.55*	.29			
16. vmPFC W1	-.17	-.13	-.08	.57*	.18	.38*	.67*	.20	.22	.51*	.25	.32	.56*	.13	.25		
17. vmPFC W2	.04	-.11	-.18	.03	.57*	.49*	.20	.57*	.39*	.40*	.66*	.17	.43*	.65*	.22	.20	
18. vmPFC W3	-.21	.08	-.04	.14	.05	.66*	.45*	.08	.74*	.55*	.11	.64*	.42*	0.08	.72*	0.32	0.25

Notes. *$p < .05$. Parental Ed. = Parental Education. Stress Score = Stressful Life Events Score. HC = hippocampus. Prec = precuneus. MCC = midcingulate cortex. STG = superior temporal gyrus. vmPFC = ventromedial prefrontal cortex. W1 = Wave 1. W2 = Wave 2. W3 = Wave 3.

Aim 1: Development of Hippocampal Functional Connectivity

Table 2.4 summarizes model estimates from piecewise models assessing growth and Figure 2.4 offers a visual depiction of growth trajectories for hippocampal connectivity with each ROI. There was significant variance in initial connectivity (i.e., intercept) and change in connectivity (i.e., slope) for all regions for each cohort. Furthermore, for all models, initial connectivity was negatively associated with change from Time 1 to Time 2 suggesting that those who have greater connectivity values at Time 1, change less over the course of one year (from 4-5 years of age for the 4-year-old cohort and 6-7 years of age for the 6-year-old cohort). Initial

connectivity values were not related to change in connectivity from Time 2 to Time 3 for either cohort (4-year-old cohort: 5-6 years old; 6-year-old cohort: 7-8 years old). Overall, there was only limited change in connectivity observed. Specific findings are described below.

Insula

Results showed no change in hippocampal-insula connectivity in either cohort.

Precuneus

Results showed no change in hippocampal-precuneus connectivity in either cohort.

MCC

In the 4-year-old cohort, there was significant positive change in connectivity from 4-5 years of age and marginally significant change from 5-6 years old. There was no significant change in connectivity for the 6-year-old cohort.

vmPFC

For the 4-year-old cohort, there was marginally significant positive change in connectivity from 5-6 years of age. There was no significant in connectivity for the 6-year-old cohort.

STG

There was no significant change in connectivity for the 4-year-old cohort. For the 6-year-old cohort, there was significant positive change in connectivity from 6-7 years of age.

Table 2.4

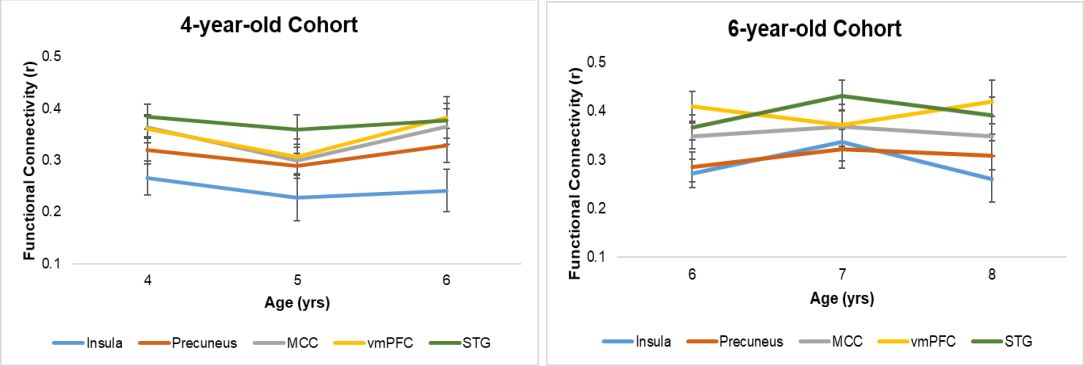
Parameters from models assessing growth of hippocampal functional connectivity for the 4- and 6-year-old cohorts

4-year-old cohort	Intercept Mean	Slope 4-5 Mean	Slope 5-6 Mean	Intercept Variance	Slope 4-5 Variance	Slope 5-6 Variance	Intercept-Slope 4-5 corr.	Intercept-Slope 5-6 corr.
Insula	.27 (.03)**	-.04 (.05)	.01 (.04)	.04 (.01)**	.08 (.02)**	.06 (.02)**	-.04 (.01)**	.01 (.01)
Precuneus	.32 (.03)**	-.03 (.02)	.04 (.03)	.03 (.01)**	.02 (.01)**	.04 (.01)**	-.01 (.004)**	-.003 (.01)
MCC	.36 (.02)**	-.07 (.03)**	.07 (.04)+	.02 (.004)**	.02 (.01)**	.05 (.01)**	-.01 (.004)**	.01 (.01)
STG	.38 (.02)**	-.03 (.03)	.02 (.03)	.02 (.01)**	.03 (.01)**	.04 (.01)**	-.02 (.01)**	.01 (.01)
vmPFC	.36 (.03)**	-.05 (.04)	.08 (.04)+	.03 (.01)**	.04 (.01)**	.06 (.02)**	-.02 (.01)**	.01 (.01)
6-year-old Cohort	**Intercept Mean**	**Slope 6-7 Mean**	**Slope 7-8 Mean**	**Intercept Variance**	**Slope 6-7 Variance**	**Slope 7-8 Variance**	**Intercept-Slope 6-7 corr.**	**Intercept-Slope 7-8 corr.**
Insula	.27 (.03)**	.07 (.04)++	-.08 (.05)	.03 (.01)**	.04 (.01)**	.07 (.02)**	-.01 (.01)+	.01 (.01)
Precuneus	.29 (.03)**	.04 (.04)	-.01 (.03)	.03 (.01)**	.05 (.01)**	.04 (.01)**	-.03 (.01)**	.004 (.01)
MCC	.35 (.03)**	.02 (.03)	-.02 (.04)	.03 (.01)**	.04 (.01)**	.05 (.01)**	-.02 (.01)**	.003 (.01)
STG	.37 (.03)**	.07 (.03)*	-.04 (.04)	.02 (.01)**	.03 (.01)**	.04 (.01)**	-.01 (.01)*	.003 (.01)
vmPFC	.41 (.03)**	-.04 (.04)	.05 (.05)	.03 (.01)**	.06 (.02)**	.06 (.02)**	-.03 (.01)**	.01 (.01)

Note. *$p < .05$. **$p < .01$. +$p < .06$. ++$p < .09$. Corr.=correlation. Estimates are unstandardized. Standard errors are in parentheses.

Figure 2.4

Growth trajectories for each region of interest in functional connectivity analyses for the 4- and 6-year-old cohorts



Aim 2: Stressful Life Events and Functional Connectivity

Insula

The Stressful Life Events Score significantly related to initial connectivity for the 4-year-old cohort, suggesting that higher levels of stress were related to greater hippocampal-insula connectivity at Time 1 (4 years of age). No associations were observed between the Stressful Life Events Score and change in connectivity for either cohort or initial connectivity for the 6-year-old cohort (Table 2.5).

Precuneus, MCC, STG, & vmPFC

There were no associations between the Stressful Life Events Score and functional connectivity with any other regions in either cohort. See Table 2.5 for statistics associated with each model.

Table 2.5

Parameters from growth models assessing stressful life events and hippocampal functional connectivity for the 4- and 6-year-old cohorts

4-year-old Cohort	**Intercept** ***b (SE)***	**Slope 4-5** ***b (SE)***	**Slope 5-6** ***b (SE)***
Insula	.016 (.008)*	-.018 (.011)	.005 (.001)
Precuneus	.006 (.007)	-.006 (.006)	.010 (.009)
MCC	.001 (.006)	.002 (.007)	.012 (.010)
STG	.002 (.007)	-.005 (.007)	.011 (.009)
vmPFC	-.001 (.007)	.013 (.008)	-.003 (.011)
6-year-old Cohort	**Intercept** ***b (SE)***	**Slope 6-7** ***b (SE)***	**Slope 7-8** ***b (SE)***
Insula	-.008 (.006)	-.011 (.090)	.014 (.011)
Precuneus	.005 (.006)	-.012 (.009)	-.001 (.008)
MCC	.005 (.005)	-.008 (.008)	-.003 (.010)
STG	-.005 (.005)	-.008 (.007)	.007 (.008)
vmPFC	-.002 (.006)	-.007 (.010)	.008 (.010)

Note. *$p < .05$. +$p < .08$. *SE*=standard error.

Aim 3: Time Dependence of Effects

Model estimates were compared between the 4- and 6-year-old cohorts for models showing significant effects (i.e., insula). Results showed a marginally significant difference between the effect observed with initial hippocampal-insula connectivity in the 4- and 6-year-old cohorts (b = -.025, SE=.013, p = .065, 95% bootstrapped CI [-.051,.002]).

Discussion

This study investigated how stressful life events experienced during early childhood relate to the development of hippocampal functional connectivity. The first aim was to assess how functional connectivity changes over the age span assessed and the second aim was to assess how stressful events were related to functional connectivity. It was hypothesized that there would be an association between stressful life events and connectivity for both cohorts; however, the direction of effects was not hypothesized given mixed findings in the literature (e.g., Duval et al., 2017; Saxbe et al., 2018; Sripada et al., 2014). Results from Aim 1 analyses showed that, overall, there was not a great deal of change occurring in hippocampal functional connectivity with any of the ROIs assessed. Results from Aim 2 analyses showed that stressful events did predict connectivity with insula in the 4-year-old cohort. In particular, greater stressful events experienced between 3 and 4 years of age was related to greater connectivity between hippocampus and insula at 4 years of age. This association was not observed in the 6-year-old cohort. Furthermore, stressful events did not predict change in hippocampal-insula connectivity for either cohort, and no significant associations emerged for any other ROI. Results from Aim 3 analyses

indicated that the effects of stressful events on hippocampal-insula connectivity were marginally significantly different in the 4- vs. 6-year-old cohort.

Aim 1: Development of Hippocampal Functional Connectivity

Only minor changes in connectivity were observed between the hippocampus and the ROIs assessed. Specifically, significant change was observed in MCC and vmPFC in the 4-year-old cohort and STG in the 6-year-old cohort, but the mean change in connectivity was small in magnitude. For example, the correlation representing hippocampal-STG connectivity only increased by .065. These low levels of change align with previous research reporting similar findings of small magnitudes of change for hippocampal connectivity (Blankenship et al., 2017). Some research has suggested greater linear increases in connectivity between hippocampus and several regions important to memory (Geng et al., 2021). However, the regions assessed in the current study are not typically engaged during memory processing. This may explain the difference between the findings. Overall, there was also a great deal of variance associated with the slope for each ROI, which could be due to true individual differences among subjects but could also be due to artifacts in the data. This is discussed in more detail in the limitations section.

Aim 2: Stressful Life Events and Functional Connectivity

The current findings align with previous research showing associations between stress and hippocampal connectivity (Blankenship, Botdorf, et al., 2019; Duval et al., 2017; Saxbe et al., 2018). For example, this finding is similar to previous research from our lab in a different sample of children (older children who were oversampled for depression) which showed that increased cortisol release between 3

and 5 years old was related to greater hippocampal functional connectivity with cortical regions (Blankenship, Botdorf, et al., 2019). It is also similar to research in older children, which showed that stress in the form of community violence was related to greater functional connectivity between hippocampus and insula (Saxbe et al., 2018). Thus, despite differences in how stress was measured, greater stress was associated with greater connectivity in each of the aforementioned studies (cf. Sripada et al., 2014).

Although hippocampal-insula connectivity did not show much change in the 4-year-old cohort, older children have greater hippocampal-insula connectivity in this sample than younger children. Furthermore, insula showed a marginally significant positive change in the 6-year-old cohort suggesting that there is some evidence of strengthening of connectivity across childhood. Therefore, greater connectivity between hippocampus and insula is likely indicative of more mature connectivity, which potentially lends support to the idea of accelerated maturation in relation to stress (Bath et al., 2016; Callaghan & Tottenham et al., 2016). However, it is difficult to know whether greater connectivity is considered more or less mature and if more mature connectivity is always superior. Thus, additional research is needed on the development of hippocampal functional connectivity during childhood.

Associations between stressful events and hippocampal functional connectivity were specific to Time 1, which suggests that higher levels of stress associated with such events are related to greater connectivity and provides evidence that stress can impact brain connectivity in a semi-rapid fashion. In this study, stressful events experienced in the year prior related to greater connectivity at 4 years

old. Although these findings are encouraging, they were not observed in the 6-year-old cohort and there was no association between stressful events and change in connectivity. This is somewhat puzzling and suggests that more research needs to be done to assess impacts of stressful experiences on functional connectivity at different points of development.

Why Insula?

Significant findings only emerged for insula and not for the other ROIs. Insula is a part of the salience network of brain regions. Such an association between greater stressful life events and increased connectivity could potentially point to hypervigilance to threat (Rabinak et al., 2011). This region is also often thought of as part of limbic system, along with hippocampus, and is important for emotion processing. Hippocampus and insula are both functionally and structurally connected (Ghaziri et al., 2018) and research has shown enhanced hippocampal-insula coupling in response to acute stress (Chang & Yu, 2019). Some work has suggested that in childhood, there is a tradeoff between a mature emotional processing system and a less mature cognitive control system (Herzberg et al., 2021). Given that no associations emerged with regions important to cognitive control, such as vmPFC, stronger coupling of hippocampus and insula could suggest a more mature emotional processing system. Therefore, these typical variations in stress may actually be advantageous to development and help support the development of the emotional processing system during early childhood.

Aim 3: Time-Dependence of Effects

Significant associations were only observed for the 4-year-old cohort even though both cohorts experienced similar levels of stress, which is consistent with the idea that the hippocampus may show greater plasticity in younger children (Andersen et al., 2008; Humphreys et al., 2019). However, the analysis assessing whether results differed between cohorts showed only a marginally significant difference so results related to time-dependence should not be over interpreted. Regardless, these findings still do provide support for the impact of early stress, within the typical range, on hippocampal development.

Strengths

One strength of this study is the longitudinal nature of the data, which includes multiple time points of MRI data. Much of the stress literature in relation to the brain is cross-sectional in nature or only includes one MRI time point. Although stress was only related to the brain at Time 1 in this study, there are still benefits associated with including multiple time points as it allows for assessing how stress impacts *maturation* of the brain. Importantly, the use of functional connectivity allowed for moving beyond structure to assess how stressful events impact function. This is some of the first research in this age range focused on stress and hippocampal functional connectivity.

The analytic approach used in this study is a strength. The latent growth modeling approach allowed including multiple time points and for handling missing data. Also, the use of piecewise models allowed for fitting different slopes to the data to enhance model fit. Regarding processing of MRI data, a strict motion correction

was used to account for movement in the functional data. Volumes that exceeded .5mm framewise displacement were censored along with the volume before and after the offending volume. Furthermore, ICA was used as an additional method to remove any artifacts from the data.

Limitations and Future Directions

The small sample size is a limitation as it precluded a thorough investigation of whether effects differ for males vs. females. Relatedly, this is a high SES sample with little variability in this variable. Future research should assess whether the current findings extend to more diverse samples given that SES, stress, and brain development are often intricately connected variables.

Another limitation is the use of both functional and structural ROIs. Although these are both acceptable ways to create ROIs, it would be preferable to have all structural or all functional ROIs. The use of functional ROIs is often ideal because it provides an exact map of voxels showing significant correlations. Structural ROIs may miss some of the voxels that would or would not be included in the functional mask.

Although multiple steps were taken to ensure high quality of functional data, assessing brain function in young children still has challenges. Resting state fMRI is inherently more variable and tends to have small signal-to-noise ratio even in adult samples (Power et al., 2014). The high levels of variance associated with the connectivity measures in this study highlight these challenges. Steps were taken to ensure that motion was not driving effects, but the data is simply messy, which is evident in the variance associated with the intercept and slope factors for each ROI.

Having external reliability measures or connectivity data from an independent dataset would be helpful to understanding what correlation levels one would expect in this age range. Given that only limited findings emerged with the ROIs, it is possible that stress is not related to connectivity with the remaining regions. However, it is also possible that these variables are related but that the variability in the data impeded the detection of additional effects.

Exploratory bivariate correlations between stress measures at Time 2 and 3 showed some associations with connectivity at different time points. Therefore, a next step could be assessing how stress at each time point relates to connectivity at each time point using a cross-lagged panel model. This model will allow for assessing both concurrent and lagged effects of stress on connectivity (e.g., does stress at Time 2 relate to connectivity at Time 3?).

Conclusions

In sum, findings suggest that typical variations in stressful events impact hippocampal functional connectivity in young children. Future research should continue to explore both the timing dependent and region dependent nature of these associations when assessing stress effects on the brain.

Chapter 4: Typical variations in stressful life events and hippocampal subfields in the Adolescent Brain and Cognitive Development Study (Empirical Study 2)

This study builds on Studies 1a and 1b by assessing stress life events and hippocampal volume in the Adolescent Brain and Cognitive Development (ABCD) sample, a large, population-based sample of children. The children in this sample are older in age (9-10 years old) than in Studies 1a and 1b, which allowed for testing the impacts of stressful events on the hippocampus at a different point in development, particularly since human studies suggest prolonged maturation beyond age 5 (Ghetti & Bunge, 2012; Lee et al., 2020; Tamnes et al., 2018). Study developers attempted to match the ABCD sample with that of the United States population on key demographic variables (e.g., sex, race; Dick et al., 2020; Garavan et al., 2018). Therefore, a benefit of using this sample was that it was more diverse and representative of the United States population.

Thus far, research that has investigated stressful events in relation to hippocampal structure has used relatively small datasets making it difficult to assess factors that may moderate these associations. Due to its size and diversity, this sample specifically allowed for testing whether stressful events impact hippocampal subfield volumes in all children in the same way or whether certain individuals show greater impacts of stress on the brain (i.e., males vs. females; individuals with low vs. high SES). Therefore, the goal of Study 2 was to investigate how variations in stressful life events may impact hippocampal subfield volumes in a large, diverse sample of

children and whether these stress-brain impacts are moderated by specific variables (i.e., sex and SES).

Aim 1: The first aim was to assess concurrent associations between greater reported stressful life events and hippocampal subfield (i.e., CA1, CA3, DG, subiculum) volumes.

Hypothesis 1$_a$: Based on previous literature (Champagne et al., 2008; Malhi et al., 2019; Naninck et al., 2015), I hypothesized that there would be associations between greater stressful life events and volume of CA1, CA3, and DG. However, given mixed results in both the rodent and human literature, the direction of effects was not hypothesized.

Hypothesis 1$_b$: I hypothesized that there would be no association between stressful life events and volume of subiculum.

Aim 2: The second aim was to assess potential moderating effects of sex on the relation between stressful life events and subfield volumes. Specifically, sex was included as a moderator and interactions between stressful life events and sex predicting each subfield volume were assessed.

Hypothesis 2$_a$: Based on findings in rodent samples (Derks et al., 2016; Loi et al., 2014), I hypothesized that there would be an interactive effect between stressful life events and sex predicting CA1, CA3, and DG volume, such that males and females would show different relations between stressful life events and subfield volumes. The specific direction of the effects was not hypothesized given mixed findings in studies assessing how sex influences the effects of stress on the hippocampus

Hypothesis 2_b: I hypothesized that there not be an interactive effect between stressful life events and sex predicting subiculum volume.

Aim 3: The third aim was to examine potential moderating effects of SES on the relation between stressful life events and hippocampal subfield volumes. Specifically, SES was included as a moderator and interactions between stressful life events and SES predicting each subfield volume were assessed.

Hypothesis 3_a: Based on work suggesting that individuals with low SES experience greater levels of stressful events (e.g., Lantz et al., 2005), and thus may be disproportionately impacted by stress and also work suggesting that high SES can provide a buffer against stress effects on the brain, I hypothesized that there would be an interactive effect between stressful life events and SES predicting CA1, CA3, and DG volume. However, no hypotheses were made regarding whether low or high SES individuals would show greater effects of stressful life events on hippocampal structure.

Hypothesis 3_b: I hypothesized that there would not be an interactive effect between stressful life events and SES predicting subiculum volume.

Methods

Participants

This project utilized data from the Adolescent Brain Cognitive Development Study (ABCD) release 2.0 (Volkow et al., 2018). This is a large, diverse sample of 11,878 participants designed to assess substance abuse in adolescents. Participants were 9-10 years old at study entry. Children and their parents completed a series of questionnaires, and children completed a structural MRI scan. Of the full sample,

4,598 children provided both Life Events data and structural MRI data as of May 2020 and were included in the current study. MRI data was collected at baseline (Time 1, 9-10 years old) and retrospective Life Events data was collected at the 1 year follow up (Time 2; 10-11 years old).

Of the initial 4,598 subjects, 191 were missing either a T1 or T2 scan and 59 subjects were not included because they did not pass the quality control screening or it was deemed the MRI scan was not protocol compliant as defined by ABCD researchers (Hagler et al., 2019). Thus, 4,348 subjects remained. Descriptive statistics specific to the subsample used in this study are reported in Table 3.1.

Table 3.1

Demographic characteristics of the subsample with MRI data and Life Events Scale data (N=4,348)

Demographic Variable	
Age (yrs), Time 1 [*M* (sd)]	10.00 (.62)
Age (yrs), Time 2 [*M* (sd)]	11.01 (.64)
Child sex, female [n (%)]	2086 (48%)
Child race/ethnicity, [n (%)]	
Asian	97 (2%)
Black	376 (9%)
Hispanic	815 (19%)
Multi-Racial/Other	436 (10%)
White	2624 (60%)
Family income [n (%)]	
<$50,000	989 (23%)
$50,001 to $100,000	1211 (28%)
>$100,001	1828 (42%)
Did not disclose	320 (7%)
Parental education [n (%)]	
At least one parent with a 4-year college degree	2848 (66%)

Tasks and Questionnaires

Life Events Scale

Adolescents completed the Life Events Scale (Hamilton et al., 2011), which inquired about events occurring in the previous year and in the adolescent's lifetime. Specifically, they provided responses for 25 life events. For each event, the participant was asked if the event occurred, if it happened in the last year, if it was a good or bad experience, and the effect it had on them. In response to whether it was a good or bad experience, they responded "mostly good", "mostly bad", "don't know", or "not applicable". They rated the effect the event had on them on a scale from 0 to 3 where 0 had no effect and 3 had a large effect. Events that were marked as "mostly good" were excluded from the score as the focus was on negative, stressful events. Events that occurred in the last year were also excluded (as this survey was completed 1 year after the MRI data). This resulted in a final sample of 3,528 subjects who had Stressful Life Events data matching these criteria.

Similar to Study 1a, a Stressful Life Events Score were calculated by summing the number of stressors experienced by the child in addition to the severity indicated for each stressor. For example, if a child indicated that a family member died and rated the effect it had on them as a 3, that event received a score of 4 as both the event and severity were added together. Importantly, this summed score takes into account both the cumulative number of events and the cumulative impact the effects have on the individual. This is important as research has shown that both the event occurring and the individual's appraisal of the event can contribute to negative outcomes (Danese & Widom, 2020).

Sociodemographic Variables

Several variables from the demographics survey were used, including the participant's age, sex, race, and parent's highest level of education. Sex was coded as a dichotomous variable where males received a value of 0 and females a value of 1. Parental education was created by taking the highest level of education between the child's parents. Participants with at least 1 parent with a 4-year college degree were coded as 1, whereas participants with no parent with a 4-year college degree were coded as 0. Parental education was used as a proxy for SES in analyses assessing interactive effects. Race/ethnicity was coded as a 5 level dummy-coded variable (Asian, Black, Hispanic, Multi-racial/Other, White) with White serving as the reference group given that it was the largest group.

Pubertal Status

Pubertal status was assessed using the Pubertal Development Scale and Menstrual Cycle Survey (Petersen et al., 1988), which includes 5 questions that were specific to males or females. Scores were averaged across the 5 questions. Pubertal status was included as a covariate given research suggesting that puberty is influenced by stress and is related to maturation of the hippocampus (Herting & Sowell, 2017).

Memory Assessment

Episodic memory ability, which is a cognitive process heavily reliant on hippocampus, was used in exploratory analyses to assess the potential implications of stress-related effects on the hippocampus for memory. The NIH Toolbox Picture Sequence Memory Task was used to provide an index of episodic memory (Dikmen et al., 2014). Participants were shown a series of 15 pictures presented on the

computer and asked to remember the order of the images. After two learning trials, they were asked to reproduce the sequence that was presented to them. The number of adjacent pairs served as the variable of interest as this is an indicator of the ability to retain details (i.e., temporal order) in addition to item information. This variable was age-corrected for use in analyses.

MRI Assessment

Data was collected at 22 sites across the United States using one of three different scanners (Siemens (57.6%), GE (29.3%), and Philips (13.1%)). Participants first completed a "prescan" during which they were trained to ensure motion would not impact results and they were screened for MR contraindications. They then completed a series of structural and functional scans. The T1-weighted and T2-weighted structural scans were the focus of this study. Motion was tracked in real time for the structural scans. Scan parameters differed by scanner type for the T1-weighted and T2-weighted scan and are described in detail in Casey et al. (2018) and Hagler et al. (2019).

Raw T1-weighted and T2-weighted data were acquired from the ABCD database (https://nda.nih.gov/abcd), and data was processed using Freesurfer v7.1 (Fischl et al., 2002). Specifically, a series of preprocessing steps, including skull stripping, motion correction, normalization, and cortical and subcortical segmentation, were applied to the data among other steps. T2-weighted data was included with T1-weighted data to potentially improve processing of structural data, including cortical and subcortical segmentations. Data was processed using both a local server and supercomputing resources at the University of Maryland

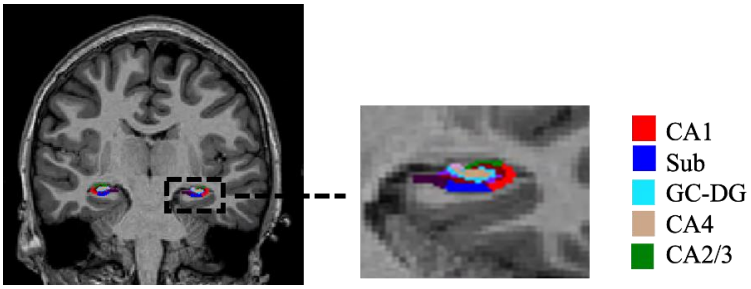
(http://hpcc.umd.edu), which took several months to complete given the magnitude of the processing and the large number of participants.

Hippocampal Subfields

Freesurfer v7.1 was then used to segment hippocampal subfields and generate volumes. Freesurfer has the capability of segmenting a number of subfields in the hippocampus using different parcellations, but the current report will focus on the parcellation that includes subiculum, CA1, CA3 (which also includes CA2), CA4, and DG (FS360 parcellation; Iglesias et al., 2015). As was done in Study 1a, subfields in the head and body subregions of the hippocampus were summed to create single volumes for each subfield. To limit the number of dependent variables in the model, CA4 and DG were combined, as is often done in manual tracing due to their close proximity to each other. This reduced the complexity of the model and number of parameters that needed to be estimated but allowed for CA3 to remain separate in order to make connections with the rodent literature, which assesses CA3 and DG separately. Figure 3.1 shows an example segmentation for one subject.

Figure 3.1

Example segmentation of hippocampal subfields from Freesurfer v7.1.



In addition to subfield volumes, estimated total intracranial volume (eTICV) and thalamus volume were extracted using Freesurfer. Thalamus is a brain region that

is not thought to be impacted as much by the effects of stress as hippocampus; therefore, it was extracted to use as a control region to assess specificity of stress effects on hippocampal subfields in the brain (Frodl et al., 2010; Sah et al., 2005). Brain volumes were adjusted to control for differences in eTICV using an analysis of covariance approach (Raz et al., 2005; Riggins et al., 2018), which ensured that differences in brain size were not driving effects. Age and sex interactions with eTICV were assessed and were nonsignificant. Therefore, the same adjustment was conducted for all subfields and subjects. Because results using raw vs adjusted volumes did not differ, only results from analyses using adjusted volumes are reported for simplicity.

Data Analysis Plan

Covariates

Site and scanner type (i.e., Siemens, Philips, GE) were included as random effects given differences that may arise from geographic location and different scan parameters between scanner types.

Fixed effect covariates included age, sex, parental education, and pubertal status. Age at baseline was included as a covariate in all analyses. Sex was included as a covariate in main effect analyses. Parental education was also included as a covariate in main effect analyses to ensure results were specific to stressful life events and not more general differences in SES. In analyses assessing interactive effects, parental education and sex were variables of interest and not covariates.

Analytic Methods

All aims of this study were assessed using measured variable path analysis in Mplus (Muthén & Muthén, 1998–2017). Path analysis is an extension of multiple regression that allows for assessing more complex models, such as those with multiple dependent variables (Streiner et al., 2005). Using a multilevel version of this allows for taking into account the hierarchical structure of the data. Furthermore, path analysis can use robust maximum likelihood estimation, which allows for nonnormality in the data and for estimation of missing data (Gibson & Ninness, 2005).

To test **Aim 1, Hypothesis 1** (i.e., associations between stressful life events and volume of CA1, CA3, and CA4/DG subfields), the Stressful Life Events Score was entered as the independent variable, subfield volumes were entered as the dependent variables, and parent education, sex, age, and pubertal status were entered as covariates. Scanner site and type were included as cluster variables. All subfields were run in the same model. This model is just-identified as the number of parameters included equals the number of parameters that must be estimated. Therefore, fit indices were not estimated.

To test **Aim 2, Hypothesis 2_a and 2_b** (i.e., interactive effects between stressful life events and sex), the interaction between the Stressful Life Events Score and sex was added to the model to assess whether the relation between Stressful Life Events and each subfield volume differs for males vs. females. Finally, **Aim 3, Hypothesis 3_a and 3_b** (i.e., interactive effects between stressful life events and SES) was assessed by adding the interaction between the Stressful Life Events Score and SES to the model to assess how interactive effects between stressful life events and SES impact

each subfield volume. The three-way interaction between sex, SES, and Stressful Life events was also assessed. All interactions were assessed in the same model.

Preregistration of Analyses

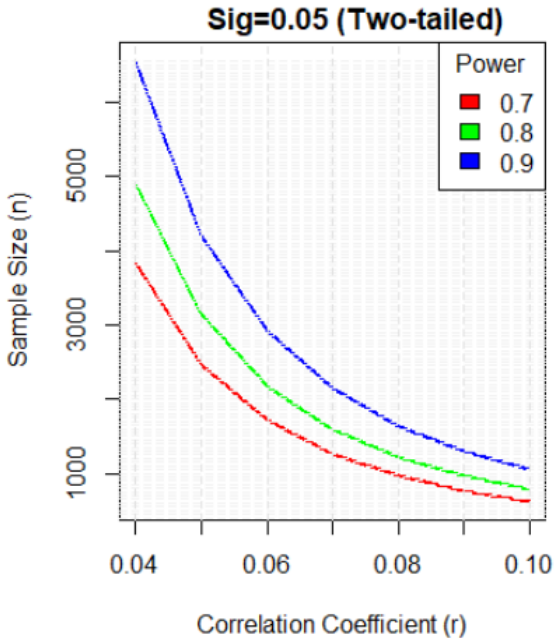
Analyses addressing Aims 1-3 were preregistered on Open Science Framework (OSF; https://osf.io/27yc3). In the preregistration, CA4 and DG subfields were proposed to be assessed separately. This was followed in preliminary analyses. However, these analyses showed associations with the Stressful Life Events Score were similar for these subfields. Because these subfields are often considered together in manual tracing protocols, they were summed to create a single CA4/DG subfield. Therefore, this resulted in a slight deviation from the preregistration.

Interpreting Effects in Large Datasets

Effects in this study will be smaller in magnitude than those often observed in smaller studies (e.g., studies with 100 subjects, such as Study 1a and 1b). Large datasets provide the opportunity to detect small effects with more precision that may not be detectable in a small dataset (Dick et al., 2020). Specifically, in a sample this size (~4000 subjects), a power analyses assessing 80% power and significance of .05 indicated that a bivariate correlation of ~.05 could be detected. This is illustrated in Figure 3.2.

Figure 3.2

Sample size estimation to detect effects with different power levels



Results

Associations with Covariates

Bivariate correlations are presented in Table 3.2. There was a positive correlation between age and subiculum volumes. Sex was related to all subfield volumes, such that males had significantly larger volumes compared to females. Pubertal status was negatively related to all subfield volumes, such that higher scores on the Pubertal Development Scale and Menstrual Cycle Survey were related to smaller volumes. Parental education was related to the Stressful Life Events Score and subfield volumes, such that those who had at least one parent with a 4-year degree experienced more stressful events and had larger subfield volumes (CA1, CA4/DG, subiculum) compared to those without a parent with a 4-year degree.

Table 3.2

Bivariate correlations between variables included in the path analyses

	1	2	3	4	5	6	7	8	9	10	11	12	13
1. Age (T1)													
2. Age (T2)	.99**												
3. Sex[1]	-.02	-.02											
4. Pubertal Status	.05**	.06**	.17**										
5. Parental Education[2]	.06**	.06**	.02	-.09**									
6. Events-Sum	-.02	-.03	-.03	.05**	-.15**								
7. Events-Effect	-.03	-.04*	-.01	.05**	-.16**	.80**							
8. Life Events Score	-.03	-.04*	-.02	.05**	-.16**	.90**	.98**						
9. Memory	.03	.03	.09**	-.04*	.12**	-.05**	-.06**	-.06**					
10. CA1	.03	.03	-.36**	-.09**	.06**	-.02	-.006	-.01	.03				
11. CA3	.02	.02	-.27**	-.03*	-.003	.012	.001	.005	-.02	.65**			
12. CA4	.03	.03	-.37**	-.08**	.04**	-.006	-.008	-.008	.02	.79**	.83**		
13. DG	.03	.02	-.38**	-.08**	.04**	0	-.001	0	.02	.83**	.80**	.99**	
14. Subiculum	.04**	.04*	-.30**	-.06**	.05**	-.03	-.02	-.03	.03	.67**	.18**	.52**	.57**

Note. *$p < .05$; **$p < .01$. Volumes are adjusted for eTICV. T1=Time 1; T2=Time 2; Sex, parental education, income, and race are coded 0, 1. [1]0 = Male, 1 = Female; [2]0 = At least one parent with a 4-year college degree, 1 = Neither parenting with a 4-year college degree. Memory was assessed using the Picture Sequence task from the NIH Toolbox.

Life Events Scale

Descriptive statistics regarding cumulative number of stressors, cumulative effect of stressors, and Stressful Life Events Score variables are presented in Table 3.3. As expected, stressful life events data was positively skewed, which can be seen in the histogram of the Stressful Life Events Score presented in Figure 3.3.

Table 3.3

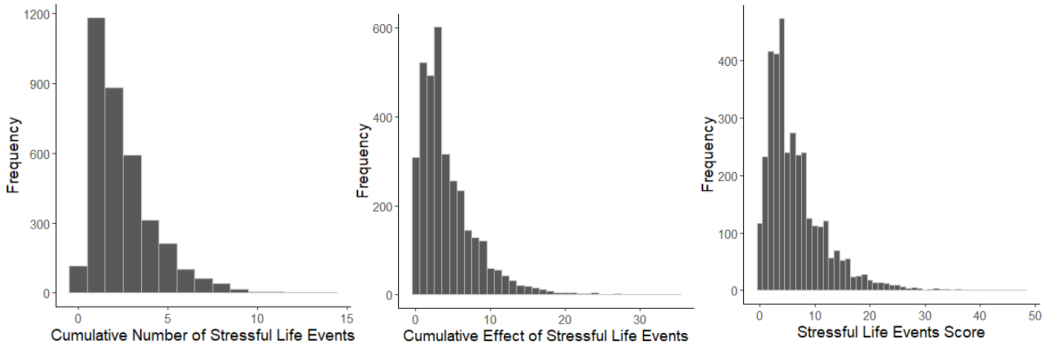
Descriptive statistics of measures from the Life Events Scale

	M (SD)	Minimum Value	Maximum Value
Number of events	2.55 (2.77)	1	14
Effect of events	4.19 (3.83)	0	35
Stressful Life Events Score	6.75 (5.35)	1	48

Note. *M* = mean. *SD*=standard deviation.

Figure 3.3

Histograms representing the frequency distribution of variables from the Life Events Scale



Results of one-way ANOVAs indicated that the Stressful Life Events Score did not vary by sex, $F(1, 3529) = 1.91$, $p=.167$. However, the Stressful Life Events Score did vary by SES, $F(1, 3526) = 90.08$, $p<.001$, and racial/ethnic group, $F(3, 3529)=8.36$, $p<.001$. Descriptive statistics for each group are presented in Table 3.4.

Table 3.4

Descriptive statistics for the Stressful Life Events Score by sex, SES, and race/ethnicity

	M	*SD*	Min	Max
Sex				
Males	6.65	5.37	0	48
Females	6.39	5.43	0	44
SES				
Low SES	7.68	6.29	0	48
High SES	5.90	4.74	0	33
Race				
Asian	4.22	3.36	0	16
Black	7.15	5.66	0	32
Hispanic	7.23	5.95	0	48
Multi-racial	6.59	5.51	0	44
White	6.29	5.18	0	36

The most commonly endorsed negative events were a family member died, a family member was seriously injured, the parents separated or were divorced, the child moved, and the child witnessed a crime or accident (Table 3.5). Extreme events, such as being the victim of assault or violence, were only endorsed for 1% of the children included in this sample**.**

Table 3.5

Frequency and descriptive statistics for the most commonly reported negative stressful life events

Event	Frequency	*M*	*SD*	Effect Range
Family member died	2271	1.78	1.01	0-3
Family member was seriously injured	880	1.75	1.02	0-3
Parents separated or divorced	851	1.98	1.12	0-3
Family moved	631	1.31	1.17	0-3
Child witnessed a crime/accident	542	.91	.98	0-3

Note. M = mean effect. *SD* = standard deviation.

Subfield Volumes

All subfield volumes were normally distributed. One outlier was removed as the standard deviation exceeded 3 SD for DG volume. Descriptive statistics for subfield volumes and histograms of the distribution of each subfield are presented in Table 3.6 and Figure 3.4.

Table 3.6

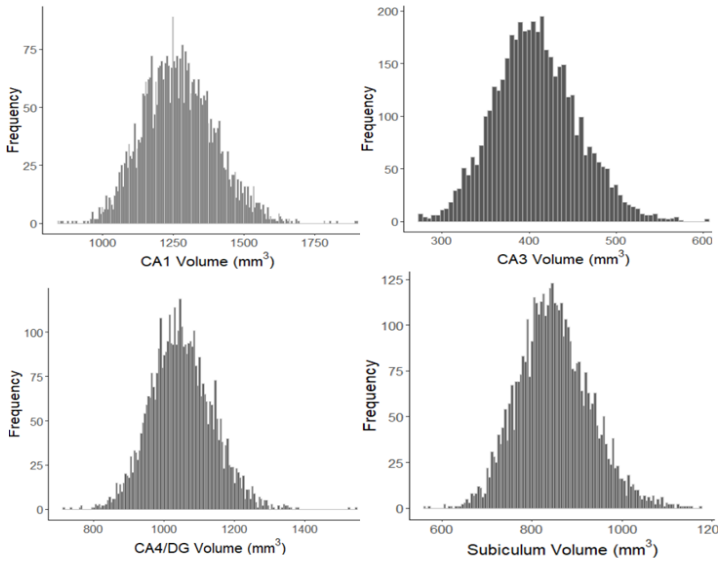
Descriptive statistics for hippocampal subfields volumes

	M	*SD*	Minimum Value	Maximum Value
CA1	1270.63	126.621	843	1898
CA3	407.80	47.852	273	607
CA4/DG	1048.80	87.90	716	1546
Subiculum	849.08	80.679	558	1174

Notes. *M* = mean. *SD* = standard deviation. Volumes are adjusted and are in mm^3 units.

Figure 3.4

Histograms representing frequency distributions of each subfield assessed.



Preregistered Analyses

Aim 1: Main Effects Analyses – Stressful Life Events Score and Hippocampal Volumes

Results showed a significant negative association between the Stressful Life Events Score and subiculum volume (Table 3.7; Figure 3.5). No other main effects were observed with CA1, CA3 or CA4/DG volumes.

Table 3.7

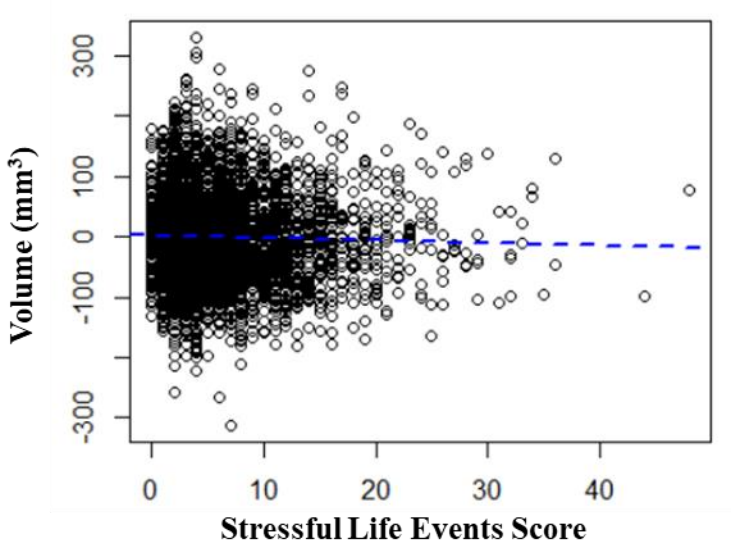
Results from the path analysis assessing main effects of stressful life events on subfield volumes

CA1		*b*	*SE*	*t*	*p*
	Stress Score	-.09	0.28	-0.32	0.751
	Sex	-89.56*	4.02	-22.30	<.001
	Parental Ed.	17.19*	7.18	2.39	0.017
	Age	4.82	3.00	1.61	0.108
	Puberty	-5.33*	2.41	-2.21	0.027
CA3		*b*	*SE*	*t*	*p*
	Stress Score	0.02	0.14	0.14	0.892
	Sex	-25.78*	1.51	-17.11	<.001
	Parental Ed.	0.26	1.67	0.16	0.877
	Age	1.12	1.23	0.91	0.361
	Puberty	1.11	0.91	1.22	0.223
CA4/DG		*b*	*SE*	*t*	*p*
	Stress Score	-0.02	0.2	-0.09	0.922
	Sex	-66.16*	2.91	-22.78	<.001
	Parental Ed.	8.58*	3.86	2.22	0.026
	Age	2.57	2.31	1.11	0.266
	Puberty	-2.01	1.39	-1.44	0.15
Subiculum		*b*	*SE*	*t*	*p*
	Stress Score	-0.38*	0.14	-2.60	0.009
	Sex	-48.95*	3.15	-15.56	<.001
	Parental Ed.	8.29*	4.04	2.01	0.04
	Age	4.32*	1.51	2.86	0.004
	Puberty	-0.63	1.68	-0.38	0.706

Note. Significant effects are denoted by *$p < .05$. Stress Score = Stressful Life Events Score. Parental Ed. = Parental Education.

Figure 3.5

Partial residual plot of analysis assessing effects of stressful life events on subiculum volume



Aims 2 and 3: Interaction Analyses – exploring moderating effects of sex and SES

Results from analyses assessing interactive effects are presented in Table 3.8. No two-way interactions with sex or SES emerged. There was a marginal three-way interaction between the Stressful Life Events Score, sex, and SES predicting CA4/DG volume (p<.06). No other significant interactions emerged with any subfield volumes.

Table 3.8

Results from the path analysis assessing interactive effects of stressful life events, sex, and SES on subfield volumes

CA1		*b*	*SE*	*t*	*p*
	Stress Score	0.68	0.63	1.08	0.281
	Sex	-93.99*	7.18	-13.09	<.001
	Parental Ed.	14.15	10.61	1.34	0.182
	Age	4.74	3.06	1.55	0.12
	Puberty	-5.04*	2.43	-2.08	0.038
	SexXScore	-0.52	0.74	-0.71	0.476
	SESXScore	-1.40	0.89	-1.57	0.115
	SexXSES	7.21	9.41	0.77	0.443
	SexXSESXScore	0.77	1.04	0.75	0.456
CA3		*b*	*SE*	*t*	*p*
	Stress Score	0.11	0.32	0.36	0.721
	Sex	-25.72*	2.04	-12.58	<.001
	Parental Ed.	0.08	2.08	0.04	0.971
	Age	1.09	1.22	0.89	0.373
	Puberty	1.08	0.90	1.19	0.233
	SexXScore	-0.43	0.37	-1.17	0.242
	SESXScore	-0.16	0.41	-0.39	0.694
	SexXSES	0.34	2.58	0.13	0.894
	SexXSESXScore	0.75	0.57	1.32	0.188
CA4/DG		*b*	*SE*	*t*	*p*
	Stress Score	0.55	0.66	0.83	0.404
	Sex	-66.69*	4.08	-16.36	<.001
	Parental Ed.	7.76	5.33	1.46	0.145
	Age	2.49	2.33	1.07	0.285
	Puberty	-1.92	1.39	-1.39	0.166
	SexXScore	-0.83	0.75	-1.11	0.265
	SESXScore	-1.45	0.94	-1.54	0.125
	SexXSES	2.13	4.63	0.46	0.645
	SexXSESXScore	2.15+	1.12	1.93	0.054
Subiculum		*b*	*SE*	*t*	*p*
	Stress Score	-0.02	0.39	-0.05	0.962
	Sex	-56.19*	5.97	-9.41	<.001
	Parental Ed.	3.06	5.92	0.52	0.605
	Age	4.23*	1.54	2.74	0.006
	Race	-0.38	1.68	-0.23	0.822
	SexXScore	-0.18	0.59	-0.29	0.768

	SESXScore	-0.89	0.83	-1.08	0.28
	SexXSES	11.42+	6.71	1.70	0.088
	SexXSESXScore	0.69	0.73	0.94	0.34

Note. Significant effects are denoted by *p<.05. +p<.09. Stress Score = Stressful Life Events Score. Parental Ed. = Parental Education.

Exploratory analyses

Specificity Analyses

Analyses assessing specificity of stress effects to subfields in the brain showed that there were no main effects or interactive effects of stressful events predicting thalamus volume (Table 3.9).

Table 3.9

Results from the path analysis assessing effects of stressful life events on thalamus volumes

Independent Variable	*b*	*SE*	*t*	*p*
Stress Score	5.63	4.51	1.25	0.212
Sex	-173.44*	67.23	-2.58	0.010
Parental Ed.	-38.21	61.06	-0.63	0.531
Age	90.43*	28.58	3.16	0.002
Puberty	-58.48*	29.36	-1.99	0.046
SexXScore	3.42	8.17	0.42	0.676
SESXScore	-11.92+	6.76	-1.76	0.078
SexXSES	41.42	78.49	0.53	0.598
SexXSESXScore	4.55	10.19	0.45	0.655

Note. Significant effects are denoted by *p<.05. +p<.09. Stress Score = Stressful Life Events Score. Parental Ed. = Parental Education.

Exploratory Analysis: Assessing Cumulative Number of Events and Effect of Events Separately

Research has suggested that both the cumulative number of events and a person's subjective appraisal of the events are important with regards to how stressful

events may impact an individual. Exploratory analyses assessed these variables separately to understand which one was a better predictor of subfield volumes. Results showed that only the cumulative number of events showed a significant negative effect of stressful events on subiculum volume (b = -1.62, SE = .48, p <.001). No other main effects were observed with CA1 (b= -1.2, SE = 1.04, p = .24), CA3 (b = .083, SE =.46, p=.86) or CA4/DG (b = -.284, SE = .82, p=.73) volumes. Interaction analyses showed that there was a three-way effect of cumulative number of events, sex, and SES on CA4/DG volume (b=5.94, SE=2.97, p=.045). Follow-up simple slopes analyses showed that this interaction was driven by males with high SES (b = -1.57, SE=.80, t=-1.97, p =.049). Specifically, in this group, a greater number of events was related to smaller volumes. Females with high SES did not show a significant effect (b = .71, SE=.82, t=.87, p =.39). Furthermore, neither males (b = .41, SE=.81, t=.50, p =.62) nor females (b = -.57, SE=.89, t= -64, p =.52) with low SES showed a significant effect. No significant main effects or interactive effects were observed when cumulative effect of events was assessed.

Exploratory Analysis: Assessing Whether Results Vary by Race/Ethnicity

Given the diversity of this sample and the differential levels of stress experienced across racial and ethnic groups, moderating effects of race and ethnicity were explored. Interaction terms were created between the Stressful Life Events Score and each dummy coded race variable. As indicated previously, White served as the reference group given that it was the largest group. No significant interactions emerged between the Stressful Life Events Score and race variables (Table 3.10). However, there were main effects of race on subfield volume that were independent

of SES and stressful events, suggesting that additional environmental variables are driving these effects and should be explored in further detail.

Table 3.10

Results from the path analysis assessing moderating effects of race/ethnicity and stressful life events on subfield volumes

CA1		*B*	*SE*	*t*	*p*
	Stress Score	-0.33	0.95	-0.34	0.732
	Sex	-89.67*	3.96	-22.64	<.001
	Parental Ed.	12.17+	6.93	1.76	0.079
	Age	4.83	3.05	1.59	0.113
	Puberty	-2.73	2.25	-1.21	0.225
	Asian	20.49	27.12	0.76	0.45
	Black	-59.15*	14.56	-4.06	<.001
	Hispanic	-1.12	17.63	-0.06	0.949
	Multiracial	-22.79	52.18	-0.44	0.662
	Asian X Stress	-1.52	4.04	-0.38	0.706
	Black X Stress	1.89	1.66	1.15	0.252
	Hispanic X Stress	0.28	1.19	0.23	0.817
	Multiracial X Stress	0.36	4.99	0.07	0.943
CA3		*B*	*SE*	*t*	*p*
	Stress Score	0.04	0.45	0.09	0.923
	Sex	-25.79*	1.50	-17.17	<.001
	Parental Ed.	1.98	1.81	1.09	0.273
	Age	1.27	1.22	1.04	0.298
	Puberty	0.63	0.96	0.66	0.512
	Asian	8.67	12.8	0.68	0.498
	Black	11.22*	4.84	2.32	0.02
	Hispanic	6.61	5.97	1.11	0.268
	Multiracial	-7.39	21.42	-0.35	0.73
	Asian X Stress	0.74	1.96	0.38	0.705
	Black X Stress	-0.12	0.53	-0.23	0.816
	Hispanic X Stress	-0.39	0.57	-0.68	0.496
	Multiracial X Stress	0.76	2.02	0.38	0.708
CA4/DG		*B*	*SE*	*t*	*p*
	Stress Score	-0.17	0.79	-0.22	0.828
	Sex	-66.26*	2.93	-22.62	<.001

	Parental Ed.	7.45	4.71	1.58	0.114
	Age	2.59	2.36	1.09	0.272
	Puberty	-1.32	1.51	-0.88	0.379
	Asian	25.11	19.58	1.28	0.2
	Black	-12.99	10.33	-1.26	0.209
	Hispanic	1.41	12.91	0.11	0.913
	Multiracial	-22.64	41.99	-0.54	0.59
	Asian X Stress	-2.24	2.79	-0.80	0.423
	Black X Stress	0.27	1.13	0.24	0.812
	Hispanic X Stress	0.09	0.98	0.09	0.929
	Multiracial X Stress	1.40	3.82	0.37	0.713
Subiculum		*B*	*SE*	*t*	*p*
	Stress Score	-1.38	0.97	-1.42	0.155
	Sex	-49.28*	3.08	-15.98	<.001
	Parental Ed.	3.42	4.20	0.81	0.416
	Age	4.33*	1.63	2.66	0.008
	Puberty	1.12	1.91	0.59	0.558
	Asian	17.09+	9.52	1.79	0.073
	Black	-44.05*	14.25	-3.09	0.002
	Hispanic	-12.97	14.63	-0.89	0.375
	Multiracial	-63.53	52.96	-1.2	0.23
	Asian X Stress	-0.93	1.30	-0.72	0.474
	Black X Stress	2.24	1.39	1.61	0.107
	Hispanic X Stress	1.34	1.13	1.18	0.239
	Multiracial X Stress	4.89	4.42	1.11	0.268

Note. Significant effects are denoted by *$p < .05$. +$p < .09$. Stress Score = Stressful Life Events Score. Parental Ed. = Parental Education.

Exploratory Analysis: Assessing Potential Behavioral Implications for Memory

In an initial attempt to assess the behavioral implications of these findings, memory was assessed in relation to stressful life events and the hippocampus using the NIH Toolbox Picture Sequence Memory Task (Dikmen et al., 2014). Specifically, the age-corrected score (i.e., number of adjacent pairs recalled) was used as a dependent variable in analyses. These were exploratory analyses, which were not preregistered, and were only conducted with subfields that showed significant

associations with stress in Aims 1, 2, or 3 (i.e., subiculum). Results from regression analyses showed that there was a marginally significant negative association between the Stressful Life Events score and memory (*b*= -.11, *SE*=.056, *p*=.051). When the cumulative number of results was used instead of the Stressful Life Events Score, there was a significant association between stress and memory (*b*= -.28, *SE*=.14, *p*=.036). Furthermore, results showed significant positive associations between subiculum and memory (*b*=.01, *SE*=.004, *p*=.013). This suggests that both stressful events and subfield volumes relate to memory. Future analyses will assess mediation between stressful events, memory, and hippocampal volume.

Discussion

The goal of this study was to assess the impact of negative stressful life events on hippocampal subfield volumes during adolescence. Aim 1 was to assess main effects of stressful life events on subfield volumes. Given research in animal (Champagne et al., 2008; Naninck et al., 2015) and human samples (Malhi et al., 2019; Merz et al., 2019), it was hypothesized that there would be an association between stressful life events and CA1, CA3, and CA4/DG subfield volumes, but not with subiculum volumes. Aims 2 and 3 were to assess moderating effects of sex and SES on the relation between stressful life events and subfield volumes.

Results from the Aim 1 analysis showed that contrary to hypotheses, greater stressful life events were related to smaller subiculum volumes. There were no associations between stressful life events and any of the other subfield volumes. Results from Aims 2 and 3 analyses showed that results did not vary by sex or SES. Findings are discussed in more detail below.

Aim 1: Main Effects of Stressful Life Events on Subfield Volumes

It was not hypothesized that there would be an association between stress and subiculum, but some work in adults has shown similar findings. Specifically, research has shown that experiencing early life stress in the form of childhood maltreatment was related to smaller subiculum volumes during adulthood (Lee et al., 2018; Teicher et al., 2012; Whittle et al., 2007). As described in the introduction, higher levels of cortisol typically released during stress may impact the hippocampus because of its large number of cortisol receptors. Interestingly, subiculum does not have as high a density of such receptors as other subfields, such as CA1 (Champagne et al., 2008). Nonetheless, it can still be impacted by stressful experiences through variations in GC levels as evidenced by some animal studies (Bath et al., 2016). Specifically, stress can result in reductions in dendritic branching and spine density in addition to cell death in subiculum.

Stressful events appear to negatively impact subiculum as adolescents who experience higher levels of stress have smaller subiculum volumes. Age was positively related to subiculum volume, and research has suggested a cubic pattern of development of subiculum from 10 to 30 years old, with larger increases in volume between about 10 and 15 years of age (Tamnes et al., 2018). This suggests that this subfield is still growing in size during this age range and a larger subiculum is more mature. Interestingly, puberty was negatively associated with subiculum volume, in addition to other subfield volumes. It is unclear what is driving this association; however, research has shown that age and puberty can exert independent influences on the development of the hippocampus (Selmeczy et al., 2018). It may be that

stressful events delay or prolong maturation of subiculum, but this is a question for future longitudinal data

Aims 2 and 3: Interactive Effects of Stressful Life Events, Sex, and SES on Subfield Volumes

No moderating effects of sex or SES were observed suggesting that stressful events impacted subiculum similarly across the sample. It was somewhat surprising that more associations were not seen with CA1, CA3, and DG given that these subfields are often implicated with stress in animal samples (Champagne et al., 2008; Naninck et al., 2015; Youssef et al., 2019). However, low SES, which can be thought of as a chronic type of stressor, was related to smaller CA1 volumes, which provides some support for findings from animal samples. Furthermore, CA2 was included in the CA3 parcellation so it is possible that the segmentation method obscured findings as well.

Implications of Findings

The effects of stress on subiculum volumes were small in magnitude; however, it is common to find small, yet significant effects, in datasets of this size. These effects likely have implications for behavioral processes related to hippocampal subfields, including memory. Exploratory analyses suggested that both stressful events and subfield volumes relate to memory. Given these associations, there is reason for future longitudinal research to assess indirect effects of stress on memory via subfield volumes. Mediation was not tested in this study because there was no time lag between the "cause and effect" variables, and for mediation models, there must be temporal precedence between variables.

SES and Subfield Volumes

Importantly, parental education and stressful events are intricately connected variables. Living in a family with low SES can be thought of as a chronic stressor, itself, and is often associated with a host of additional stressful experiences. In this sample, adolescents with low SES experienced more stressful events and had smaller subfield volumes than adolescents with high SES. Much work has shown that SES relates to brain development and hippocampal development in particular (Noble et al., 2012; Yu et al., 2018), and these findings extend that research to include subfield volumes.

Exploratory analyses revealed no interactions with race. However, stressful event levels varied across different race/ethnicity groups and race did show associations with certain subfield volumes. These associations were independent of SES, suggesting that there are additional environmental stressors (e.g., chronic discrimination) that are driving this effect and should be included in future research on this topic.

Strengths

One strength of this study is the use of "big data", which allows for detection of small associations that may not be detected in smaller datasets. Observed effects in the current study may be small, but given the large sample size, they are well-powered. Furthermore, the diversity of the sample allowed for assessing interactions with sex, SES, and race and for considering the role these variables play in the relation between stressful events and hippocampal volumes.

Another strength is the use of hippocampal subfields. By looking at functionally significant subunits of hippocampus, we can move beyond looking at the hippocampus as a whole and start to understand what specific subfields are driving associations often observed between stress and the hippocampus. The analytic approach is another strength as it allowed for assessing multiple dependent variables (i.e., multiple subfields) in the same analysis while also taking into account the hierarchical structure of the data. It also allowed for handling missing data so that data was not removed if it was missing one variable as is done with standard multiple regression.

Limitations and Future Directions

This study used an automated software package, Freesurfer 7.1, to segment hippocampal subfields, which could be viewed as a limitation. It is possible that using Freesurfer introduced some form of bias when compared to manual tracing (Schmidt et al., 2018; Weiss et al., 2014; Wisse et al., 2021). However, previous research has shown that the potential bias introduced in segmenting hippocampus, at least as a whole, using Freesurfer is consistent across subjects. Therefore, any potential bias may not be of much concern (Schoemaker et al., 2016). In a sample this large, using an automated toolbox, like Freesurfer, not only saves resources (Schmidt et al., 2018), but makes it possible to use big datasets in a way that may not be possible otherwise.

The ABCD data is a diverse sample, but it is not fully representative of the United States population (Heeringa & Berlund, 2020). Therefore, post-stratification weighting is a next step to ensure results are generalizable to the population. This can be done using a fixed population reference, such as the American Community Survey,

and creating weights for each subject using various demographic variables. All analyses would then take this weighting into account to ensure the sample is representative and results are generalizable to the population.

Conclusions

Results from this study showed that stressful life events negatively impact hippocampal subfield volumes in adolescents and that effects do not vary with sex or SES. These findings illustrate the impact that stress can have on the brain and provide avenues for future research to assess implications of findings.

Chapter 5: General Discussion

This group of papers examined associations between typical variations in stressful life events and the hippocampus. This was done across two levels of analysis (i.e., structure and function) and at different points in development using both a longitudinal sample focused on early childhood and a large, diverse sample of adolescents. Overall, these findings provide evidence that typical variations in stressful events can impact the hippocampus both in young children and adolescents.

By examining stressful events and the structure of the hippocampus, Study 1a found that parent-reported stressful life events related to subfield volumes in a region and age-dependent manner. Specifically, greater stressful life events scores were related to smaller CA1 and CA2-4/DG volumes in 6-year-old children. By assessing stressful life events and hippocampal functional connectivity, Study 1b found that parent-reported stressful life events related to greater hippocampal-insula functional connectivity in 4-year-old, but not 6-year-old, children. Finally, by assessing a large, diverse sample, Study 2 showed that youth-reported stressful life events related to smaller subiculum volume, which did not vary by sex or SES.

Findings from these three studies fill important gaps in the literature by using longitudinal data, assessing heterogeneity of the hippocampus, assessing moderating effects of sex, and exploring effects in diverse samples. First, the use of longitudinal data allowed for probing whether stress impacts *development* of structure and function. Results revealed limited associations between stress and change in volume. However, these results are encouraging because although limited, these effects were detected in a small sample over just a few years of time. These findings provide proof of concept – that typical variations in stress can impact hippocampal development and

provide support to conduct larger longitudinal samples over wider spans of time in the future. Second, this project examined subfields of the hippocampus, which took into account the heterogeneity of this structure. This specificity is an improvement over studies that examine the hippocampus as a whole. Study 2, which used a large sample of adolescents, allowed for probing moderating effects of sex and SES. Results showed no interactions with sex, which was somewhat surprising given sex differences that often arise in rodent studies (Derks et al., 2016; Loi et al., 2014). However, it is still possible that sex effects could be observed at other periods in development. Finally, the use of a more diverse sample in Study 2 also allowed for assessing moderating effects of SES. Results showed that effects with subfields were similar across individuals with low and high SES.

Below, I compare findings across studies and discuss potential implications of findings for behavior, such as memory, and risk for psychopathology. Finally, I address avenues for future research.

Comparing Structural Findings from Study 1a and 2

Studies 1a and 2 assessed hippocampal structure and showed that stressful life events related to smaller subfield volumes in older children and adolescents (i.e., Study 1a: CA1 & CA2-4/DG, and Study 2: subiculum). This is consistent with adult samples, which often show that heightened levels of psychosocial stress experienced during childhood are related to smaller hippocampal volumes (Calum et al., 2017). The direction of effects also suggests variations in cortisol levels may be mediating effects. As described in the introduction, findings from rodent samples show

reductions in subfield volumes related to variations in GC levels in rodents exposed to stressors early in development (Champagne et al., 2008; Naninck et al., 2015).

Although there were similarities in findings, there were differences in the subfields that were related to stressful events in Study 1a (i.e., CA1, CA2-4/DG) and Study 2 (i.e., subiculum). It is unclear why the same subfields did not consistently show associations with stressful life events across both studies. This discrepancy could be due to methodological differences between the two studies (i.e., stress measures used, sample size, subfield tracing method) or differences in subfield development.

Study 1a and 2 both used self-reported stress measures; however, the survey questions differed slightly and the respondent differed between studies. Specifically, the parent responded to the questions for Study 1a and the adolescent responded for Study 2. Furthermore, the timing of events differed such that Study 1a focused on events that occurred in the previous year whereas Study 2 focused on events that occurred in the individual's lifetime. Regarding sample size, having a larger sample may have allowed for detecting associations that were obscured by a small sample. This may help explain why Study 2 found associations with subiculum, but Study 1a did not. However, small samples are still informative. In particular, they allow for robustly testing hypotheses generated by large samples. Future research should assess stressful life events and subfield volumes in 9-10-year-old adolescents (the age of participants in Study 2) using a smaller longitudinal sample to further assess the relation between stressful events and subiculum.

Studies 1a and 2 also had differences in subfield tracing protocols that should be considered. Study 1a used subfields that were identified using a combination of manual and automated methods and Study 2 used an automated method (i.e., Freesurfer). The gold standard for segmenting subfields is manual tracing (Pardoe et al., 2009; Rodionov et al., 2009). However, this is not always feasible with a large sample and with lower resolution scan data. Therefore, it is important to take into account the tradeoffs that come with using large datasets and automated procedures that do not require as much human intervention. One of the main differences between the tracing protocol from Study 1a and the protocol used by Freesurfer is the delineation of DG. In Study 1a, CA2-4 and DG were considered together as a combination region. However, Freesurfer's automated protocol allows for DG to be identified as a separate region from the CA subfields. Another difference is how subiculum is defined in the manual vs automated approach. In the manual approach, the delineation of subiculum includes pre-subiculum and para-subiculum, whereas in the automated approach, these additional subfields are not included in the subiculum delineation.

These methodological differences may contribute to differences in findings across the studies. However, given that differences emerged between 4- and 6-year-old children in the same study (i.e., no methodological differences), it is likely that the development of the hippocampal circuitry also provides an explanation for findings. In particular, certain subfields are likely more susceptible to environmental effects at certain points in development.

Findings from animal work suggests that the hippocampal circuitry is relatively mature around the age of 5 in human children (Lavenex & Banta Lavenex, 2013). Therefore, there is an emphasis on birth to 5 years of age when assessing stress effects on the hippocampus (Andersen & Teicher, 2004). However, recent studies in humans have shown more prolonged development of the hippocampus (Canada et al., 2021; Ghetti & Bunge, 2012; Lee et al., 2020). Thus, it is likely that stressful events continuously impact the hippocampus throughout childhood while subfields are still maturing. The findings from this current set of studies provide support for that idea and suggest that effects may vary at different points in development. Study 1a showed change in CA2-4/DG volume for the 6-year-old cohort, and this same subfield showed associations with stressful events. Study 2, which showed associations between stressful events and subiculum in 9-year-old children, did not assess growth of subfields in adolescents but research suggests that subiculum shows accelerated rates of change during this age range (Tamnes et al., 2018). Therefore, the different ages assessed in Studies 1a and 2 provide an explanation for findings as subfields are likely impacted when more change is occurring and they are more susceptible to outside influences.

Although stress-related variations in cortisol levels is the likely mechanism linking stressful events and hippocampal structure and functional connectivity, there were associations between stressful events and regions without a high density of GC receptors. In particular, Study 1a showed relations with thalamus and Study 2 with subiculum. This suggests that additional factors may be contributing to findings, such as differences in parental support following stressful events or developmental

differences in the ability to cognitively appraise stressful events. These and other factors should be explored further.

Comparing Structural and Functional Findings from Study 1a and 1b

Findings from Study 1a indicated functional connectivity differences in 4-year-old children and structural differences in 6-year-old children related to stressful life events. Structural and functional development of brain regions is a bidirectional, interactive process so it is possible that differences in function lead to differences in structure (Johnson, 2001). Therefore, greater functional connectivity at 4 years old could be a precursor to structural differences in the hippocampus at 6 years old. In particular, it is possible that stressful events impact connectivity with the hippocampus earlier in childhood. These functional alterations could then lead to structural differences in specific subfields of the hippocampus. The analyses used in Study 1a and 1b did not allow for testing structure and function in the same analysis, but future research should assess structure and function in the same model to further understand how these functional and structural differences relate to one another.

In humans, it is likely that these stressful life events build up over time and lead to alterations in cortisol levels, which impact the hippocampus. Research using rodent samples shows that stress-related structural differences in the hippocampus have functional implications (Joëls & de Kloet, 1989; Okuhara & Beck, 1998), which is likely what was observed using hippocampus functional connectivity in Study 1b. However, effects of stress (especially more typical variations in stress) can be both positive and negative in nature. These typical variations in stressful events can potentially be supportive of brain development as low levels of stress have been

shown to be adaptive in nature (Chen & Miller, 2012). Stress-related differences in functional connectivity between hippocampus and insula in Study 1b may potentially be indicative of more mature connectivity supported by these typical variations in stressful experiences. Because the implications for cognition and behavior were not tested, it is difficult to know for sure if this hyperconnectivity is negative or positive in nature, but it is possible greater connectivity is related to superior emotional processing ability or even memory ability.

Implications of Findings

Behavior

The downstream effects of stress-related differences in hippocampal structure and function are unknown. Study 2 provided some initial evidence that stressful events, hippocampal subfields, and memory may be related. Given the role of CA1, CA3 and DG in pattern separation and pattern completion, one can assume memory, in particular, may be impacted by volumetric differences in structure (Neunuebel & Knierim, 2014; Rolls, 2013). Subiculum is also important for memory in children and adults so alterations in this structure likely relate to memory (Canada et al., 2021; Zheng et al., 2018). Hippocampal functional connectivity is also associated with memory outcomes (Geng et al., 2021; Riggins et al., 2016) so it would be informative to explore whether stress-related variations in connectivity relate memory.

The hippocampus also serves a role in an array of cognitive processes beyond memory, including emotional processing (Chersi & Burgess, 2015). It will be important to assess impacts on these processes as well. For example, impacts of stress

on hippocampal-insula functional connectivity may have implications for emotional processing, given its role in this process (Immordino-Yang & Singh, 2013).

Psychopathology

These findings have potential implications for understanding risk for psychopathology. Hippocampal structural and functional differences have been implicated as a potential risk factor for developing stress-related disorders, such as depression and PTSD (Carrión et al., 2010; Frodl et al., 2010; Jameison & Dinan, 2001; Postel et al., 2019). Stressful life events also contribute to the development of such disorders (Dohrenwood, 2000; Grant et al., 2003). Therefore, alterations in brain structure and function represent a potential mechanism linking stressful events and development of stress-related disorders. Much previous work on this topic has assessed total hippocampus so it will be important for work to assess whether there are implications of stress-related differences in subfield volume on psychopathology. Some work shows that individuals with major depressive disorder (Hao et al., 2020) and PTSD show atypical connectivity with hippocampus (Malivoire et al., 2018). Given functional findings with insula, a key limbic region, it would also be interesting to see if stress-related variations in connectivity relate to risk for psychopathology.

Future Directions

Future research should aim to include multiple indices of stress (i.e., physiological, self-report) in the same study. Stress physiology data (i.e., cortisol levels), in particular, would help to further understand mechanisms linking stressful events to the hippocampus. By having both self-report data and stress physiology data

in the same study, one could assess how stressful events impact cortisol levels and then assess how alterations in cortisol levels impact hippocampal structure and function. This would also provide more robust measures of stress as there are limitations associated with using self-report or parent-report data (Monroe et al., 2008). A multi-informant approach could also be used in future research to assess both the parent and child's subjective appraisal of the event.

This study focused on one aspect of the child's environment (i.e., stressful life events). However, the impact of stressful events on the brain are not deterministic in nature, but rather probabilistic, such that other factors can increase or decrease the impact of stress on the brain. Factors that may buffer against stress effects on the brain should be the focus of future research. These factors include positive familial bonds with parents and siblings, secure attachment, and having a large social network (Luby et al., 2012; Rao et al., 2010). Support from caregivers, in particular, during times of stress, like the loss of a loved one, can result in immensely different outcomes on development.

Future research should also aim to assess whether these effects of stressful events on the structure and function hippocampus persist beyond childhood. There is a great deal of plasticity in the brain throughout development. Puberty has been suggested to be a period of reorganization in the brain where effects of early stress on the brain may be reversed (Gunnar et al., 2019). Therefore, it will be informative for future research to assess whether effects persist after pubertal development is complete. Additional data points from the ABCD study will be useful in examining this question.

Conclusions

Previous research has shown associations between extreme stressful life events (e.g., abuse or neglect) and the hippocampus. This series of studies extends that body of work to illustrate how typical variations in stressful experiences may shape hippocampal structure and functional connectivity. Changing schools or moving to a new home can be thought of as almost universal events that most children experience. Although these experiences may not be long in duration, their impacts may be felt long after the event has ended. Results from this set of studies suggest that impacts of such stressful events on the brain are age and region dependent (both with regards to subfields and functional connectivity with cortical regions), which future research should take into consideration and explore further. Overall, the current findings underscore the complexity of the effects of stress on the brain and generate more questions for research to pursue.

Appendices

Appendix A

Stressful Life Events Checklist used in Empirical Studies 1a and 1b

Please indicate whether any of the following stressful life events occurred to **your child/family** in the **past year.** If the event occurred more than once, please list all dates on which the event(s) occurred.

1. **New child(ren)** living in home (may be newborn or adopted child, foster child, or child(ren) of a previous relationship).
O Yes O No

MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

2. **Child's parental figures** (married or co-habitating) **separated** (split up).
O Yes O No

MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

3. **Child's parental figures divorced.**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

4. **Child's parental figure moved out of the household.**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

5. **New parental figure moved into the child's home** due to remarriage or establishment of apparently permanent relationship. New parental figure has been present for at least 1 month.
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

6. **The child moved to a new place**, with or without change of family structure.
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

7. **Child changed school or childcare provider(s).** Reasons for change include: Started school, return of primary parent to work, family choice, need for special class, expulsion from previous school, changing schools or classrooms in the middle of the year, as well as other reasons.
O Yes O No

MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

8. **Death of a pet** to which the child was closely attached.
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

9. **Move by child or significant figure resulted in the end of a close relationship**, with significant figure no longer available for friendship and companionship. Do not include friendships or relationships maintained after move through regular phone calls, letters, and/or visits.
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

10. **Noticeable reductions of family standard of living** as evidenced by inability to pay bills, need to sell things, need to move (including moving in with relatives), going on welfare or food stamps, inadequate food, clothing, heat. May be result of changes in household status and needs such as parental separation or divorce, death, taking in additional dependents, high medical bills or loss of household income due to cutback in hours, layoff or loss of job, inability to find employment, loss of employment benefits, depletion of savings, etc.
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____
MM/DD/YY:

11. **Loss of home** without separation from family. Child and family loses home because of eviction, end of lease, damage to home by a fire or natural disaster, or other reason and are not resettled in a home for at least one month. Do not include intentional moves to a new setting.
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

12. **Parent was arrested.**
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

13. **Child's parental figure is hospitalized** for more than 24 hours.
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

14. **Child separated from parent for week or more.**
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

15. **Child was in a serious car accident.**
OYes O No

MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

16. **Child was struck by a moving vehicle or bicycle.**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

17. **Child poisoned**: Child ingested an agent capable of producing an acute morbid, noxious, or deadly effect.
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

18. **Child was accidentally burned**: Accidentally, child suffers an injury by fire or excessive or intense heat. Exclude first-degree burns which are red, somewhat painful, similar to a sunburn, and non-blistering.
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

19. **Child experienced near drowning**: To be nearly suffocated in water or other fluid; to come close to perishing in water or other fluid. To be coded, the event must be a serious accident that had the potential to be life threatening.
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

20. **Child had an accidental serious fall.**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

21. **Child was mauled and/or bitten by an animal.**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

22. **Child fractured a bone(s).**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

23. **Child was diagnosed with an illness** carrying **current** risk of death or chronic disability (e.g. cancer, AIDS, cystic fibrosis, diabetes). Include asthma if it requires more than 24-hour hospitalization.
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

24. **Child admitted to a medical or psychiatric hospital** for more than 24 hours or spent more than 24 hours in a hospital emergency room.
O Yes O No
MM/YYYY: _____ MM/YYYY: _____

MM/YYYY: _____ MM/YYYY: _____

25. **Death of someone close** to the child: biological parent, sibling, peer, other parental figure, other relative with whom child has close ties, other adult who has played a significant role in the child's life.
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

26. **Child witnessed an event** that caused, or had potential to cause death or severe injury.
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

27. **Child experienced a natural disaster**: events not caused by intentional human actions (e.g. floods, hurricanes, tornadoes, earthquakes) in which people died or were badly injured or property was extensively damaged, or there was a risk of these outcomes.
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

28. **Child was removed from home** because of physical abuse or neglect.
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

29. **Child experienced a fire**: either accidentally or deliberately set, in which people actually dies or were badly injured or property was extensively damaged, or there was a serious risk of these outcomes.
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

30. **Child was a victim of physical violence** by non-family member. Child has been the victim of physical violence, with one or more people using force against the child with the potential to cause death or serious injury. Force may have been used in order to get something (e.g. mugging, robbery), or to intimidate or frighten the children, or for its own sake (assault, fight, torture). Victim may have been threatened with a weapon.
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

31. **Parent experienced high levels of stress** (include relationship/marital problems, job related problems, financial problems).
OYes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

32. **Poor social support provided to caregiver**.
OAbsent; I have enough social support
OPresent; I do not have enough social support

In the next section we are interested in events that took place in **your** life (i.e., the parent). Please indicate which of the following events **you** have experienced **in the past year.** If the event occurred more than once, please indicate all dates on which the event(s) occurred.

33. **Parent failed school, training program**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

34. **Parent changed jobs or started new job**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

35. **Parent laid off**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

36. **Parent fired**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

37. **Parent retired**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

38. **Parent stopped working, not retirement, for an extended period**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

39. **Infidelity in your relationship**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

40. **Death of parent's partner**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

41. **Parent had an abortion**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

42. **Parent had a miscarriage or stillbirth**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____

MM/YYYY: _____ MM/YYYY: _____

43. **Parent's child died**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

44. **Parent's relative became seriously ill**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

45. **Parent's family member other than spouse or child died**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

46. **Parent was assaulted or robbed**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

47. **Parent went to jail**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

48. **Parent was convicted of a crime**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

49. **Parent experienced significant financial loss**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

50. **Parent went on welfare**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

51. **Parent's close friend died**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

52. **Parent had a physical illness**
O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

53. **Parent experienced an injury**

O Yes O No
MM/YYYY: _____ MM/YYYY: _____
MM/YYYY: _____ MM/YYYY: _____

Appendix B

Life Events Scale used in Empirical Study 2

1. Someone in family died
    [ ] Yes
    [ ] No
If "Yes",
Did this occur in the past year?
    [ ] Yes
    [ ] No
1.1. Was this a good or bad experience?
    [ ] Mostly good
    [ ] Mostly bad
    [ ] Not applicable
    [ ] Don't know
1.2. How much did the event affect you?
    [ ] Not at all
    [ ] A little
    [ ] Some
    [ ] A lot
    [ ] Not applicable
    [ ] Don't know

2.Family member was seriously injured
    [ ] Yes
    [ ] No
If "Yes",
Did this occur in the past year?
    [ ] Yes
    [ ] No
2.1. Was this a good or bad experience?
    [ ] Mostly good
    [ ] Mostly bad
    [ ] Not applicable
    [ ] Don't know
2.2. How much did the event affect you?
    [ ] Not at all
    [ ] A little
    [ ] Some
    [ ] A lot
    [ ] Not applicable
    [ ] Don't know

3. Saw crime/accident
    [ ] Yes
    [ ] No
If "Yes",
Did this occur in the past year?
    [ ] Yes
    [ ] No

3.1. Was this a good or bad experience?
    [ ] Mostly good
    [ ] Mostly bad

[ ] Not applicable
[ ] Don’t know

3.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

4. Lost a close friend
[ ] Yes
[ ] No

If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

4.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

4.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

5. Close friend was seriously sick/injured
[ ] Yes
[ ] No

If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

5.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

5.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

6. Negative change in parent’s financial situation
[ ] Yes

[ ] No

If "Yes",

Did this occur in the past year?

[ ] Yes

[ ] No

6.1. Was this a good or bad experience?

[ ] Mostly good

[ ] Mostly bad

[ ] Not applicable

[ ] Don't know

6.2. How much did the event affect you?

[ ] Not at all

[ ] A little

[ ] Some

[ ] A lot

[ ] Not applicable

[ ] Don't know

7. Family member had drug/alcohol problem

[ ] Yes

[ ] No

If "Yes",

Did this occur in the past year?

[ ] Yes

[ ] No

7.1. Was this a good or bad experience?

[ ] Mostly good

[ ] Mostly bad

[ ] Not applicable

[ ] Don't know

7.2. How much did the event affect you?

[ ] Not at all

[ ] A little

[ ] Some

[ ] A lot

[ ] Not applicable

[ ] Don't know

8. Got seriously sick or injured

[ ] Yes

[ ] No

If "Yes",

Did this occur in the past year?

[ ] Yes

[ ] No

8.1. Was this a good or bad experience?

[ ] Mostly good

[ ] Mostly bad

[ ] Not applicable

[ ] Don't know

8.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

9. Parents argued more than previously
[ ] Yes
[ ] No

If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

9.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

9.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

10. Mother/father figure lost job
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

10.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

10.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

11. One parent was away from home more often
[ ] Yes
[ ] No

If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

11.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don't know

11.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don't know

12. Someone in the family was arrested
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

12.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don't know
12.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don't know

13. Close friend died
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

13.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don't know

13.2. How much did the event affect you?
[ ] Not at all

[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

14. Family member had mental/emotional problem
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

14.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

14.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

15. Brother or sister left home
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

15.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

15.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

16. Being a victim of crime/violence/assault
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes

[ ] No

16.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

16.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

17. Parents separated in last 12 months
[ ] Yes
[ ] No
If "Yes",

Did this occur in the past year?
[ ] Yes
[ ] No

17.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

17.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

18. Parents got into trouble with the law
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

18.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

18.2. How much did the event affect you?
[ ] Not at all
[ ] A little

[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

19. Attended a new school
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

19.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

19.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

20. Family moved
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

20.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

20.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

21. Parents got divorced
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

21.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

21.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

22. One of the parents went to jail
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

22.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

22.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

23. Got new stepmother or stepfather
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

23.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

23.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot

[ ] Not applicable
[ ] Don’t know

24. Parent got a new job
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

24.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

24.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

25. Got new brother or sister
[ ] Yes
[ ] No
If "Yes",
Did this occur in the past year?
[ ] Yes
[ ] No

25.1. Was this a good or bad experience?
[ ] Mostly good
[ ] Mostly bad
[ ] Not applicable
[ ] Don’t know

25.2. How much did the event affect you?
[ ] Not at all
[ ] A little
[ ] Some
[ ] A lot
[ ] Not applicable
[ ] Don’t know

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