Examining the Effect of Adverse Childhood Experiences (ACEs) on Executive Functioning and Brain Plasticity

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Submitted in partial fulfillment of the requirements for degree of Masters' of Science in Applied Health Sciences

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Abstract

Adverse childhood experiences (ACEs) are exposures to experiences such as maltreatment, household dysfunction, and other traumatic or stressful events occurring in the first 18 years of life. Exposure to ACEs in childhood, a critical time for development, have been found to have enduring, negative effects on physical and mental health across the life course. Specifically, ACEs may influence the neurotransmitter systems in the brain and alter brain function leading to behaviour changes that can be observed in adulthood. Brain-derived neurotrophic factor (BDNF) is a brain plasticity factor involved in creating and maintaining connections and pathways in the brain. Pathways that utilize executive function (EF), higher order cognitive responses, may be influenced by exposure to ACEs. The purpose of the current study was to examine the relationship between ACEs and EF and to examine whether BDNF helped to explain that relationship. The current study conducted a cross-sectional analysis that used data from the Niagara Longitudinal Heart Study which was a follow up study conducted out of Brock University. The final sample size for the current analysis was n=236. Retrospective reporting of ACEs was collected using the Childhood Trust Events Survey. The Behaviour Rating Inventory of Executive Function Adult Version (BRIEF-A) was used to measure everyday EF and included the Inhibit and the Working Memory clinical measures and three composite measures, the Behaviour Regulation Index, Metacognition Index, and the Global Executive Composite. Finally, serum BDNF was used as a measure of current plasticity. The relationship between ACEs and BDNF was non-significant and therefore no indirect effects were explored. There was a significant relationship between accumulation of ACEs and all EF measures, and this effect was similar across males and females. The current study adds to the

literature finding that accumulation of ACEs was associated with low EF clinical and composite scores in young adulthood.

Acknowledgements

I would like to thank my supervisor, Dr. Terrance Wade, because without you this would not have been possible. Thank you for your guidance and for being the most amazing mentor. You have not only taught me the foundations of research, statistics, and writing but you have also provided me with many lessons in life. Most importantly, thank you for believing in me and pushing me when I did not believe in myself. I am excited to continue my journey in research and academia.

I would like to acknowledge and thank my committee members who have brought their expertise and taken the time to support me. Dr. MacPherson and Dr. Molnar, thank you for all the time and detailed feedback you provided on my project. Special thanks to Dr. McQuaid for agreeing to be my external examiner and for her interest in my project. I truly enjoyed defending my thesis with this amazing committee and discussing so many exciting avenues of research.

I would like to thank everyone involved in the Niagara Longitudinal Heart Study, but especially my lab friends: Kylie, Jessy, Kingston, Aindriu, Madison, and Nathan. Being part of this team truly made my grad school experience amazing. Thank you for helping me with my project and for all the laughs along the way.

Finally, I would like the thank my family and friends who have encouraged and supported me through my academic journey. I have to also give a special thanks to my Mom and Dad, I am so grateful for your endless love and encouragement. You have shaped me into the person I am today, I love you and hope to keep making you proud.

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Abbreviations

ACE – Adverse childhood experience

EF – Executive function

PFC – Prefrontal cortex

BDNF – Brain-derived neurotrophic factor

CDC – Centre for Disease Control

CRH – Corticotropin releasing hormone

ACTH – Adrenocorticotropin hormone

SES – Socioeconomic status

TrkB – Tropomysoin kinase B

NLHS – Niagara Longitudinal Heart Study

HBEAT – Health Behavioural and Environmental Assessment Team

PHAST – Physical Health and Activity Study Team

BAM – Brock Active Muscles study

BRIEF-A – Behaviour rating Inventory of Executive Function Adult Version

BRIEF – Behaviour rating Inventory of Executive

CTES – Childhood Trusts Events Survey

BRI – Behaviour Regulation Index

MI – Metacognition Index

GEC – Global Executive Composite

ELISA – Enzyme linked immunosorbent assay

PCA – Principal component analyses

Chapter 1: Introduction

The current study aim is to understand how exposure to traumatic experiences in childhood, often referred to as adverse childhood experiences (ACEs), are related to executive function (EF) in young adulthood. ACEs include a broad array of experiences ranging from maltreatment and abuse to living in a severely dysfunctional home to other exposures outside the family such as living through a natural disaster. ACEs are prevalent in Canada and the USA with approximately two thirds of individuals reporting at least one ACE[1–3]. Individuals who reported experiencing one ACE also had a higher probability of experiencing additional ACEs indicating that these experiences tend to cluster[1]. The accumulation of these stressful experiences produce a chronic physiological stress response that may influence the development of pathways in the brain responsible for EF[1,2]. The prefrontal cortex (PFC) is a main area of the brain involved in EF. The PFC starts developing after the first year of life and does not finish developing until young adulthood[4]. There are multiple areas that make up the PFC and development differs across time and sex[4,5]. The span of time during childhood and adolescence, when ACEs generally occur, is critical because the pattern of PFC development follows an inverted U shape[4,5]. Development of the PFC is dependent on the ability of the brain to adapt and form new neural connections, a process known as plasticity. Brain-derived neurotrophic factor (BDNF) is a protein neurotrophic factor involved in neuronal growth and maintenance and used as a measure of brain plasticity. Altered BDNF expression has been connected to various neurological conditions and mental disorders[6], these alterations may also be affecting EF.

Adverse Childhood Experiences (ACEs)

ACEs have been identified as prevalent, traumatic experiences occurring in the first 18 years of life that lead to long-term negative health outcomes[7]. There are three main domains in which ACEs are generally categorized including maltreatment, household dysfunction, and other stressful experiences. Child maltreatment includes physical, emotional, or sexual abuse, and severe physical and/or emotional neglect. Exposure to severe household dysfunction includes experiences such as witnessing severe threats and interpersonal violence, spousal violence, severe mental illness, substance or alcohol abuse in the family, and incarceration or the sudden death of a family member. The third domain of ACEs includes other highly stressful experiences such as severe bullying, accidents or injuries, neighbourhood violence and witnessing a death, and natural disasters. Accumulation of ACEs has been linked to negative health behaviours[8], mental illness[9–11], as well as chronic health conditions such as poor cardiovascular health[12], which all together attribute to early mortality[1,8,13].

Executive Function (EF)

Felitti and colleagues focused on maltreatment and household dysfunction in their original ACEs study and discussed how ACEs may not only be influencing physical and mental health outcomes and creating overall life instability[1]. ACEs may also affect brain development and the neurotransmitter systems[1]. These changes in development may be negatively influencing EF. Throughout life, but especially in childhood, both positive and negative experiences shape brain circuitry. Experiences create learned responses and lead to the development of higher-order executive functions[14]. EF is a broad term used to describe higher-order cognitive processing involved in emotional regulation, decision-making, problem solving and other high-level cognitive processes[15]. There are different constructs that fall under the umbrella term EF such as

inhibition, task shifting and working memory which begin developing around 3 years old[16]. Development of these constructs continues through middle childhood (i.e. 7-12 years of age) and into adolescence and early adulthood as the PFC develops[16,17]. EF pathways in the brain are not fully developed until early adulthood and are influenced by the internal and external environment[18].

In addition to the PFC, other areas of the brain also play a role in both behavioural and physiological responses to the environment. The current study focuses on the PFC, the hippocampus, and the amygdala as the primary areas of interest. These areas of the brain are involved in EF and other critical behavioural responses. The PFC is predominantly involved in decision-making and higher-level cognitive function involved in EF responses. Inputs from both the hippocampus and amygdala influence the PFC and EF. The PFC focuses attention to external stimuli and the hippocampus utilizes working memory to then consolidate this information. The hippocampus is primarily responsible for storing and recalling memories and using them to adapt to the current environment through behavioural and physiological responses. A key piece of information involved in memories is the emotions associated with an experience or stimuli. Emotions are experience-dependent for each individual and can be positive, negative or neutral. The stressful or traumatic nature of ACEs would lead to negative emotional associations and signal the fear response. The area of the brain that is associated with recognizing and responding to fear and threatening stimuli is the amygdala. Working almost opposite or counter to the PFC and higher-order EF responses, the amygdala, when activated, initiates drive-related, emotional responses[19]. As such, these three areas interact to produce behavioural responses to situations faced every day based, in part, on the memories and emotions elicited by past experiences. Each area informs the others and facilitates overall behavioural responses. These responses can be

either drive-related, emotional behaviours or higher-level EF behaviours. When EF is developed and functioning properly, there is an ability to regulate behaviours manifesting in appropriate social behaviours and suppression of drive-related, emotional behaviours.

When the brain is exposed to a toxic childhood environment, altered pathways are developed to combat the negative environment that may alter life course trajectories. It is possible that exposure to ACEs results in poorer EF in early adulthood and across the life span. The reasoning behind the link between ACEs and EF is that ACEs occur during what is generally considered a critical period of development of higher cognitive processes in the PFC. As the development of the PFC follows an inverted U shape with childhood and adolescence being the time points in which development is on an upward trajectory and are susceptible to toxic experiences[4,5]. Once fully developed in young adulthood, the mid 20's, PFC function can be maintained but onward through adulthood function declines[4,5]. Exposure to stressful or traumatic experiences in the first 18 years of life may induce a chronic physiological state of stress that alters development leading to changes in physiological and behavioural responses. These alterations may lead to the favouring of drive-related, highly emotional behavioural responses as opposed to appropriate social behaviours and self-regulation that are associated with EF. Further, this physiological stress reaction may decrease brain plasticity in the developing brain which would decrease the ability to create appropriate connections leading to poorer EF.

Brain-Derived Neurotrophic Factor (BDNF)

Plasticity is the brain's ability to learn and adapt to different experiences leading to the creation and survival of different neuronal pathways[19]. Plasticity has been known to rise and fall across the life span with sensitive periods of development being a time of increased neuronal growth and connections. BDNF is a plasticity factor that facilitates neuronal growth by

strengthening connections through synaptogenesis and dendritic arborisation[20]. Appropriate connections within and across brain areas are crucial during development. High expression of BDNF in the PFC and hippocampus have been shown to facilitate growth of neuronal pathways in these areas[21]. Specifically, expression of BDNF can differ across developmental time points, can be influenced by sex and is also experience-dependent[22]. These differences are due to number of unique genetic and environmental influences in each individual that create varying time points of rises and falls of plasticity. For example, when exposed to an ACE, a chronic stress response may occur which increases cortisol levels and decreases BDNF expression. Reduced BDNF levels in the PFC and hippocampus may lead to altered development and maladaptation through changes in neuronal pathways[20]. Moreover, when connectivity is increased in the amygdala as opposed to the PFC and hippocampus, the responses to stressors will reflect the fear system and not the higher order EF system[23]. These highly emotional behavioural responses are important and functional in acute survival situations, when the threat is outside the ability of the body and brain to handle, as in the case of some ACEs, the result is a chronically heightened stress response. This may create pathways, or learned responses, in the brain to utilize highly emotional responses as opposed to appropriate EF processes to deal with subsequent similar or dissimilar exposures. These learned responses could affect children both in the short term and in the longer term leading to poor EF across the life course.

The purpose of this research is to examine the relationship between the exposure to ACEs and EF and to examine how current BDNF levels may help to explain this relationship.

Chapter 2: Literature Review

Adverse Childhood Experiences (ACEs)

As introduced above, ACEs are highly stressful experiences or maltreatment occurring in the first 18 years of life that can alter the life course trajectory. It has been shown that ACEs are strongly linked with chronic disease, poorer quality of life, and early mortality among adults[1,8,9,13]. The three main categories of ACEs are child maltreatment, household dysfunction, and other highly stressful experiences generally occurring outside the home such as bullying, and natural disasters. ACEs studies examining prevalence and accumulation using different populations and measures have been conducted across Canada, the USA, and internationally.

Some of the original research in the area of ACEs conducted by Felitti et al.[1], in the Center for Disease Control (CDC) Kaiser Study known as the Adverse Childhood Experiences Study, demonstrated a significant relationship between the accumulation of ACEs and negative health outcomes in adult life. This study sampled a total of 17,337 adults across two waves and used retrospective reporting of ACEs. During the first wave of data collection researchers measured two domains of ACEs including various types of abuse and household dysfunction. In the second wave, physical and emotional neglect were included in addition to the previous ACEs categories of abuse and household dysfunction[7]. Based on the two waves of data collection in the ACEs study, 63.9% of participants reported at least one ACE[1,7]. Subsequent research on ACEs in North America have reported similar prevalence rates to the original ACEs study ranging between 33%[24] and 63.9%[1] of people experiencing at least one ACE.

As stated above, ACEs generally do not occur in isolation as various types of ACEs tend to cluster together. That is, an individual who reports one type of ACE has a high probability of experiencing additional types of ACEs and the accumulation is associated with greater

prevalence and severity of subsequent health problems[2,9,25,26]. Recognizing that the accumulation of ACEs is important because it creates a more complete understanding of the consequences of multiple exposure to stressful experiences in childhood and long-term health outcomes as well as the cost of ACEs on the economy. For example, a review and meta-analysis by Bellis and colleagues examined the impact of experiencing ACEs in Europe and North America on individual health outcomes and the economic burden of ACEs[27]. The meta-analysis found that, while the overall cost of ACEs was approximately $1.3 trillion per annum and accounted for 37.5 million disability adjusted life years, the majority of these costs were attributed to those who reported 2 or more ACEs compared to those reporting only 1 ACE[27]. Specifically, those with exposure to 2 or more ACEs have increased physical and mental health problems such as cancer, diabetes, heart disease, depression, and anxiety as well as higher rates of negative health behaviours including smoking, alcohol consumption, and risky sexual behaviours[1,7,8,27].

Identifying and measuring ACEs is not consistent across all studies and the way to categorize ACEs for analysis remains an open question. The original ACEs study combined ACEs across the maltreatment and household dysfunction items into a cumulative score then capitalizing on this clustering of experiences used a threshold of 4 or more as the highest category[1]. A threshold measure was used because the people with higher numbers of exposures became fewer and fewer, by using the threshold measure researchers were able to capture the impact of accumulation of these experiences. A variety of different categorizations of ACEs have been used subsequently to examine outcomes related to exposure. Studies have examined individual ACEs such as physical abuse or sexual abuse while others have examined categories of ACEs such as maltreatment or household dysfunctions either as present versus absent or as cumulative measures of exposure[10,27]. Interestingly, recent work has assessed the various ways to

measure ACEs and found little difference in outcomes across these different categorizations suggesting that the effects on health and behavioural outcomes are robust regardless of how ACEs are combined, constructed, or categorized[10,12]. Moving forward, a possible explanation for these outcomes is that exposure and accumulation of ACEs creates an allostatic overload leading to an ongoing physiological stress response and maladaptation.

Brain Physiology and the Stress Response

When examining the effect of ACEs on EF, it is important to understand the role of the brain in the physiological stress response. The brain is involved in both initiating and halting the stress response. Normally, when faced with an acute external threat, the brain initiates an acute stress response that is necessary to regain homeostasis. The hypothalamic-pituitary adrenal (HPA) axis is the physiological pathway involved in producing the stress response. A cascade of signalling through the HPA axis occurs when the brain detects a threat or imbalance. The hypothalamus releases corticotropin-releasing hormone (CRH) to act on the pituitary gland and signal the release of adrenocorticotropic hormone (ACTH)[28]. ACTH enters the blood stream and signals the adrenal glands, specifically the adrenal cortex[20], to produce cortisol which is commonly known as the stress hormone. Cortisol acts throughout the body and brain to produce effective acute responses.

Cortisol is a steroid hormone that is able to cross the blood brain barrier and act on different areas of the brain. The presence of cortisol in the brain signals a negative feedback mechanism to stop the stress response[20]. Glucocorticoid receptors detect cortisol and signal the hypothalamus and pituitary gland to halt the release of ACTH and CRH. Release and regulation of cortisol is important and serves a vital function in acute situations. The HPA axis response is most beneficial when activated in the short term. The activation of the HPA axis leads to

physiological changes in the body and brain which allows for adaptation to the environment; these responses are known as allostatic responses[18]. Allostatic load is the concept in which stress and changes in the environment provide a beneficial engagement of the allostatic responses[14,18]. There are limits to the extent in which the brain and body are able to deal with these environmental assaults. It is presumed that ACEs, being extremely stressful experiences, are outside the limits in which the body and brain are able to cope thus creating an allostatic overload. Prolonged allostatic overload leads to a chronic physiological stress response resulting in a pathological state[18,29].

When a threat or trauma is outside the limits that the body can handle, a chronic, toxic stress response is experienced. This lack of termination of the stress response and increased cortisol levels over prolonged periods of time lead to changes in development and brain physiology. Research in both animals and humans have provided evidence that prolonged, toxic exposure to stress hormones, specifically cortisol in humans and corticosterone in animals, alters normal physiological development in the brain[30–32]. When attempting to respond and adapt to allostatic overload, the brain is vulnerable to negative alterations that are observed in structural changes and functional outcomes as discussed further below[33]. Normal physiological development, while in part is dependent on genetics, is heavily influenced by experiences that stimulate the growth and consolidation of functional cells in the brain known as neurons.

Neurons are responsible for communication within and across areas of the brain and generally have four main components including the cell body, dendrites, axon, and axon terminals[19,34]. The function of dendrites is to collect information from external and internal environments. Information is also sent between neurons through signalling from the axon terminals detected by the dendrites of other neurons. As the brain grows and develops, the

organization of these neuronal pathways are formed. Pathways in different areas of the brain grow and develop based on the needs of the body in response to the environment. In the case of ACEs, the effects of a toxic, physiological stress response will differ across brain regions and result in altered behavioural responses compared to others where these noxious external stimuli are not experienced.

Both humans and animals utilize drive-related behavioural responses that are based on survival and are driven by emotion in the presence of external threats[19]. For example, the fight or flight response carried out by the HPA axis is utilized to prepare the body to protect itself from a current threat by either escaping or defending against it. These responses are important in acute, survival situations but are not appropriate responses in day-to-day life. In contrast to animals, humans have an ability to exert greater control over these impulses and possess the cognitive complexity to plan for long term outcomes. These higher-level cognitive responses are known as executive function (EF).

Executive Function (EF)

As mentioned in the introduction, EF is the umbrella term for higher-order cognitive functions such as working memory, emotional control, planning and organizing, and inhibition, to name a few. Increased connections in the PFC and hippocampus produce EF responses resulting in greater emotional regulation, cognitive use and rational behavioural responses[15]. The PFC selectively attends to stimuli and is involved in working memory; the hippocampus is involved in memory consolidation and retrieval[19]. Connections to other areas of the brain, such as the amygdala, are also involved in the carrying out the these complex processes of EF[15]. Attaching emotions to memory is incorporated via the amygdala and other limbic structures to create emotional meaning to experiences that will influence future behaviour. While these

memories can be positive, negative, or neutral, the amygdala is associated with fear and threatening stimuli and, when activated, initiates drive-related, emotional responses[19]. As such, the amygdala drives the physiological response to ACEs as they are highly stressful experiences. Recalling past memories via the hippocampus results in responses that can be either drive-related, emotional behaviours through the amygdala or higher-level EF behaviours through the PFC. Knowing when to use these responses is based on experience-dependent learning and ACEs would likely initiate a highly emotional behavioural fight or flight response as opposed to EF. As such, these areas produce responses to situations faced every day based, in part, on the memories and emotions elicited by past experiences. The PFC provides the ability to regulate one's behaviour based on both the current situation and the long-term impact of a decision which is a hallmark of EF. When EF is developed and functioning properly, there is an ability to regulate one's behaviours manifesting in appropriate social behaviours and suppression of drive-related, emotional behaviours.

In research on humans, the literature varies in measures of EF and examines the effect of individual ACEs and combinations of ACEs on EF. Examining each ACE domain and various EF measures will provide direction when assessing gaps in the literature. Focusing first on household dysfunction, a toxic home environment can certainly play a role in EF development. Stressful, chaotic, or impoverished environments which lack cognitive resources lead to altered pathways in the brain[35–37]. For example, one study compared university students who reported growing up in a stressful and unpredictable environment to students who reported growing up in a predictable home environment. The young adults exposed to the stressful, unpredictable environment as measured by both financial harshness and a chaotic home environment performed poorer on inhibition tasks such as the Stroop test, stop signal task, and antisaccade

task[35]. Further, a study compared children between 8 and 11 years of age in higher versus lower socioeconomic status (SES) groups based on parental education, household income, and a ratio between income and needs found that children in the lower SES group had lower working memory scores on a digit span test, lower cognitive flexibility scores on the Trial Making Test, and lower semantic fluency on the a verbal fluency test[36]. As such, it appears that exposure to socioeconomic hardship, household dysfunction, and chaotic and unpredictable environments is reflected in the inability to execute appropriate EF.

Other research has examined various childhood exposures to maltreatment including abuse and neglect. One study examined abuse and neglect as well as exposure to community violence among 13-17 year old's from a low SES catchment area finding that increased likelihood of experiencing a highly stressful environment predicted poorer inhibitory control and working memory in a delayed match-to-sample working memory task[37]. Further research examining a convenience sample of 110 children with an average age of 9 years from a large western city in the USA identified that children exposed to physical, emotional, and/or sexual abuse, and/or exposure to interpersonal violence within the family resulted in poorer EF compared to those exposed to non-familial trauma such as natural disasters, motor vehicle accidents or community/peer violence or those with no exposure[38]. In this study, EF was operationalized as both a composite score and specific dimensions including working memory, inhibition control, interference control, auditory attention, and processing speed. Further studies found similar results with respect to maltreatment in childhood resulting in poorer inhibitory control[39], and poorer attention and working memory[40,41].

Overall, the literature provides evidence that exposure to ACE domains, specifically maltreatment and household dysfunction, results in poor EF. This indicates a need to consider all

domains of ACEs in order to create a more informed understanding of how ACEs accumulate and influence EF. Human experiences are perceived by the brain and lead to alterations of allostatic responses to survive in both the short and long term. ACEs are impactful experiences from childhood that continue to impact individuals into later life. The enduring effects of these experiences continue to persist because of the biological embedding through the allostatic responses[18]. These underlying neuronal mechanisms in response to allostatic overload can be further examined via animal models.

Much of the research examining the underlying mechanisms of stress and neuron physiology has been conducted in animal models, specifically in rats and mice. Research using rat models has found that increased stress, induced through immobilization and restraint, and the resulting increased corticosterone levels leads to decreased connectivity as measured by fewer and shorter apical dendrite branches in the PFC neurons[42] and hippocampal neurons[30]. However, increased connectivity in the amygdala was observed when a chronically stressed state was created and increased corticosterone levels were associated with a prolonged fear responses[30].

The PFC has been shown in human studies to be involved in planning for the long term, inhibition, and task shifting as well as decision making and learning[15]. Therefore a reduction in connections in the PFC, as observed in the above animal studies, would suggest that chronic stress leads to impaired learning[30]. Further, impaired working memory was observed when rodents were exposed to chronic stress which can be attributed to the reduced connectivity in both the PFC and the hippocampus[30]. Human studies have also found, using MRI scans, that exposure to early life stress resulted in smaller PFC volume of adolescents and young adults when exposed to accumulation of early life stress[43]. These structural changes and exposure to

early life stress were also associated with poorer spatial working memory measured by the Cambridge Neuropsychological Test Automated Battery subtest of spatial working memory[43].

Alternatively, the amygdala is responsible for the fear response and driving highly emotional behaviours. Increased connectivity in the amygdala after exposure to chronic stress resulted in decreased fear extinction in animal models[30]. Corticosterone measures during a fear test remained elevated, indicating that the amygdala maintained the fear response and continued to drive the HPA axis[30]. The increase in the connectivity in the amygdala supports allostatic overload altering allostatic responses in the brain and body leading to maladaptive behaviour. This increased connectivity in the amygdala pushes the HPA axis to continue the "fight or flight" response and supports the lack of ability to regulate the fear response through EF.

In addition to these findings, one study examined whether working memory differed between male and female rats and found that spatial memory and object recognition were poorer in chronically stressed males compared to controls[44]. However, chronically stress females had significantly greater spatial memory and no differences in object recognition compared to controls[44]. The researcher attributed this to the presence of estrogen in female rats which was confirmed by further examining ovariectomized female rats with and without estrogen replacement[44]. This evidence suggests sex differences in chronic stress outcomes and should be considered when examining the effects of ACEs on EF.

Although EF in humans is complex and different from animals, animal models provide insight into how highly emotional behaviours may be heightened as a result of allostatic overload. Research in animal models examines changes in brain physiology and the behavioural outcomes as a result of these changes and the influence of sex hormones. As previously stated, in rats that were chronically stressed through forced restraint and immobilization, researchers

observed altered behaviour, poorer memory, and reduced fear extinction[30]. These findings can be further interpreted and used to explain altered brain function in terms of EF. Working memory, a dimension of EF, was found to be significantly poorer in chronically stressed rats while behavioural evidence shows the brain favouring the impulsive, highly emotional behavioural responses[30]. This also highlights the importance of examining sex differences and the influence of sex hormones along with the stress response.

Brain-Derived Neurotrophic Factor (BDNF)

Alterations in neuron physiology and brain organization are influenced by changes in brain plasticity factors, such as BDNF. BDNF is a neurotrophic factor that is involved in neuronal growth and survival. Examining differences and fluctuations in BDNF in relation to exposure to ACEs may identify a mechanistic link to the observed changes in neuronal connectivity. In the brain, BDNF is stored in neuronal vesicles and, when released, acts on the tropomysoin-related kinase b (TrkB) receptors of other neurons[20,21]. The activation of TrkB leads to several downstream signals that regulate gene transcription and lead to increased neuronal survival, growth and differentiation[21]. The expression of BDNF varies in different areas of the brain across developmental time points based on the experiences and needs of the individual. BDNF expression is also influenced by stress hormone and both play important roles in long-term adaptations.

Adding to the current understanding of allostatic overload and its effects on the stress response, changes in BDNF expression as a result of the chronic stress response has been examined using animal models. Animal models have provided evidence that BDNF is influenced by stress hormone in rats, corticosterone, and other glucocorticoids. These stress-induced reductions in BDNF may result in neuronal atrophy and reduction in hippocampal volume[45]. A

threatening stimulus could be used to engage the HPA axis and glucocorticoid secretion. For example, one study found that, when exposed to predator scent, an increase in corticosterone occurred followed by a decrease in expression of BDNF in the hippocampus[45]. The influence of BDNF and other biochemical changes in the brain can be used to understand altered connectivity and function[46]. When exposed to increased corticosterone and decreased BDNF, there were poorer outcomes in spatial learning and memory[47]. When chronically stressed rats were infused with BDNF, the influence of stress was attenuated resulting in spatial learning and memory similar to controls[47]. This provides evidence that BDNF plays a key role in EF and may have the ability to attenuate the effects of toxic stress[47]. Also highlighted is the importance of BDNF to hippocampal functions involved in EF[47]. When BDNF was decreased and unable to facilitate appropriate growth and consolidation of neurons, exposure to allostatic overload and chronic stress hormone are associated with negative behavioural responses observed[45].

There is also interplay between BDNF and stress hormones where appropriate levels of both are necessary for allostasis leading to appropriate development. Prolonged secretion of corticosterone has been linked to decreased BDNF levels and decreased neuronal growth in the animal models[45,47]. These physiological changes in BDNF and corticosterone were also associated with behavioural changes and lowered EF[45,47]. The literature linking stressful or traumatic experiences in childhood and differences in BDNF is not as consistent as the animal models. In a study examining the effects of child sexual abuse, children and adolescents who had experienced sexual abuse had significantly lower serum BDNF levels compared to age and sex matched controls[48]. Consistent with the ACEs literature, experiencing multiple sexual assaults in childhood was associated with lower BDNF levels[48]. Alternatively, in a sample of middle age adults, with a mean age of 36.9 years old, who had experienced child maltreatment there was no

significant relationship with current BDNF expression[49]. Further investigation of the relationship between ACEs and BDNF as well as the possible impact on EF is needed in young adults and across time. Other hormones, such as sex hormones, also influence circulating BDNF and may influence functioning. Circulating BDNF changes during female menstrual cycle indicating a sex-dependent relationship[50].

Possible sex differences will also be considered in the current study focusing on how allostatic overload influences the internal physiological environment leading to changes in allostatic responses of EF. Due to inconsistent findings in the literature, it is important to explore possible sex differences. Many animal models have been conducted in male rats or mice exclusively, leaving gaps in the literature when understanding stress and changes in physiological pathways. A review of animal models that did examine sex as well as the influence of stress and age found that BDNF expression varied based on sex as well as types and duration of exposures to stress[51]. For example, in early life when exposed to maternal separation a study found significant decreases in BDNF expression in areas of the brain such as the PFC and hippocampus, but that the expression of BDNF varied based on sex and developmental status[51]. Moreover, one study found differences in BDNF expression in different areas of the hippocampus in male and female rats[52]. Along with these sex differences, BDNF expression in areas of the hippocampus were differentially influenced when comparing females with intact ovaries, ovariectomized females and females receiving hormone replacement (estrogen or estrogen and progesterone)[52]. As such, sex differences and BDNF expression may be linked to the presence of estrogen, either naturally occurring or through hormone replacement therapy, requiring further exploration of the influence of female sex hormones[44,51,52].

Summary

In summary, research examining a variety of stressful exposures to child maltreatment and adversity, commonly referred to as ACEs, find that they are both prevalent in the population and influence multiple biological systems and health outcomes including brain development[1,8]. Physiological and behavioural changes have been observed in animal models providing supporting evidence of the damaging effects of chronic stress response on the brain. Changes in physiology include shorter and fewer dendrites in hippocampal and PFC neurons[42] and increased growth and activity in the amygdala[30,42]. These changes in physiology lead to changes in function in animal models. And among animals, poorer working memory and poorer emotional control were observed when exposed to chronic stress hormone[30,31,47]. These models provide insight into influences of continued stress response, highlighting the importance of recognizing extreme stressors as they may lead to maladaptation. Moreover, there is some animal evidence that changes in BDNF resulting from induced stress may influence neuronal physiology and brain development.

In humans, exposure to ACEs, which have been shown to be both prevalent and tend to occur in clusters, elicits physiological stress responses during a time that is considered critical in the development of various brain structures and functions[1,9,25]. The alterations and changes in neuronal pathways associated with exposure to ACEs has been shown to lead to poor EF for individuals. These alterations have been observed in childhood and adulthood suggesting lifelong consequences resulting from these exposures.

The purpose of the current study was to examine the relationship between the exposure to ACEs and EF and to examine whether brain plasticity represented an indirect effect linking ACEs to EF. This study assessed whether there was a relationship between the accumulated exposure to ACEs and EF. To further the current research, the accumulation of ACEs was

examined in the analyses and assessed across multiple measures of everyday EF. Second, BDNF levels were examined to assess whether it helps to explain any relationship between ACEs and EF. Finally, potential sex differences across these relationships were examined to assess whether the relationship between ACEs and EF was different in males and females and whether this may be due to differences in hormones based on early or late menstrual cycle phases. There was no direction specified when examining potential sex and menstrual differences as previous literature is equivocal in its findings identifying varying EF outcomes based on both sex and hormones[44,51].

Hypothesis

1. Higher exposure to ACEs will be associated with lower EF among young adults.
2. Higher exposure to ACEs will be associated with lower BDNF levels.

2a. BDNF would indirectly affect the relationship between ACEs and EF.

3. The relationship between ACEs and EF will differ between males and females and/or across menstrual cycle.

In addition to the specified hypotheses, there were six covariates identified from the literature above that were included in the analyses, specifically: childhood household SES, respondent sex, menstrual cycle, use of antidepressants, mental illness diagnosis, and unhealthy eating. Childhood household SES was included to ensure that differences in EF were due to ACEs and not a lack of cognitive resources commonly associated with SES[36] as well as other factors associated with SES[53]. Respondent sex and menstrual cycle were included as previous literature has shown sex-dependent alterations in stress hormone secretion[44], behaviour outcomes[44], and BDNF expression[51,54]. Antidepressant use was included as a covariate as it has been shown to affect BDNF levels[14,18,55–57]. Mental illness diagnosis has also been associated

with changes in BDNF[6] and has been associated with ACEs[8–10]. Finally, unhealthy eating was included as there could be physiological influences on EF and circulating BDNF. Moreover, unhealthy eating could also be considered a negative health behaviour associated with ACEs[58,59].

Chapter 3: Methods

Sample

Using data from the Niagara Longitudinal Heart Study (NLHS), which began in 2017, the current study consisted of a cross-sectional analysis. The NLHS is a follow-up study using a prospective-retrospective cohort design that combined three baseline studies. The three baseline studies were conducted in the Niagara Region in Canada between 2007 and 2013. These three baseline studies collected data on cardiovascular health and development in childhood. The studies included were as follows: The Health Behavioural and Environmental Assessment Team (HBEAT), the Physical Health and Activity Study Team (PHAST), and the Brock Active Muscles Study (BAM). The first wave of the HBEAT (N=1836; ages 10-13 years) and PHAST (N=2278; ages 12-15 years) recruited participants from the Niagara Catholic District School Board and the District School Board of Niagara respectively. BAM (N=291; ages 8-18 years) was a community-based study that used a convenience sample. The three studies varied in study design and collection of demographics, psychosocial, lifestyle and biological measures. While these three studies varied at baseline, the data collected in the current NLHS follow-up is consistent and extensive.

The three baseline studies contained subsets of their initial samples within their respective studies that were further involved in a lab component that consisted of a detailed, non-invasive cardiovascular assessment. The participants from these initial lab components in HBEAT (N=334), PHAST (N=126), and BAM (N=104) were the target participants for recruitment in the NLHS longitudinal follow up study[60]. These lab component subsamples were recruited across different characteristics between studies to further understand developmental changes from childhood to adolescence specific to each study. HBEAT specifically targeted a

subsample stratified across blood pressure levels (<=95th percentile;90-95th percentile; below 90th percentile) while the PHAST lab-based study component was stratified across risk for Developmental Coordination Disorder. BAM utilized a convenience subsample from its full community sample to include in the lab-based component. Data from these three initial lab-based subsamples comprise the first wave of the NLHS (time=1). Data collection is currently ongoing for the NLHS follow-up with the sample containing N=237 with participants between the ages of 18-25 years old being used for the current study.

While the current data collection protocol for the follow-up NLHS builds on the detailed cardiovascular measures taken during all three of the baseline studies, it also includes a number of new measures collected through biological samples and a self-report questionnaire[60]. The NLHS testing was held in the Brock University Hemodynamics lab. It took approximately 3½ hours to complete the full protocol including a cardiovascular assessment, biological specimen collection, and completion of the self-report questionnaire. Before beginning testing, the lab researcher read through the consent form with the participant and ensured that informed consent was provided. Participants were aware that at any point during the testing they could decline providing samples or answering questions without penalty or loss of compensation they would receive for participating in the study ($100 CAD).

After receiving consent to participate, researchers collected a number of cardiovascular and anthropometric measures. Second, blood, saliva and hair samples were collected and further analysed for a number of biological markers. A short break was given after biological samples were collected where the participants were provided with a snack (i.e., a granola bar and juice box) to prevent fatigue due to the length of the testing. Finally, participants independently completed a self-report questionnaire that took approximately an hour to complete. Of specific

interest to this study, the self-report questionnaire included an ACEs inventory and the Behaviour Rating Inventory of Executive Functioning Adult version (BRIEF-A) which are discussed below. Participants also provided information on prescription drug use and indicated antidepressant drug use. As this retrospective questionnaire required participants to recall sensitive and traumatic events, participants were made aware that completion of the questionnaire was voluntary. After completing the questionnaire, all participants were provided with information on available supportive counselling resources if they felt the need for these services.

The current study conducted a cross-sectional analysis on the NLHS follow-up data (n=237). After preparing the data for analysis one participant was deleted from the dataset due to inconsistent reporting on the BRIEF-A and various portions of the NLHS questionnaire; therefore, the final sample size used for analysis included 236 participants. While the NLHS is a follow-up study and there are some aspects of longitudinal data, the current study did not conduct a longitudinal analysis. The use of measures across time and limited sample size were the main reasons for not conducting a longitudinal analysis. First, The BRIEF-A was used in wave 2 of the NLHS and measured adult EF (n=237). The Behaviour Rating Inventory of Executive Function (BRIEF) measured EF in childhood in wave 1. Due to ongoing testing restrictions from March 2020 due to the COVID-19 pandemic, there were a limited number of participants tested that had BRIEF data from wave 1 and the sample size was not sufficient to conduct longitudinal analysis (<50 cases). Blood samples were taken exclusively during the NLHS follow up and, therefore, serum BDNF cannot be measured over time. For these reasons, the current study was confined to a cross-sectional design.

Measures

The cross-sectional analysis examined measures of ACEs, EF, and brain plasticity. The variables operationally defined below were used to test the current study hypotheses. The Childhood Trust Events Survey (CTES) and BRIEF-A data from the NLHS follow-up was used to measure ACEs and EF respectively. Finally, serum levels of BDNF were used as a measure of current brain plasticity.

Adverse Childhood Experiences (ACEs)

Participants completed the self-report Childhood Trust Events Survey (CTES) that collected retrospective information on childhood physical, sexual, and emotional abuse, physical and emotional neglect, household dysfunction, and other traumatic experiences that occurred prior to 18 years of age. The CTES is a 26-item survey that was created by the Childhood Trust at the Cincinnati Children's Medical Center and is based on the original CDC-Kaiser ACEs study[1,61]. For the self-report version of the CTES, participants indicated "Yes" or "No" to each of the specific statements regarding exposure to an adverse event that occurred in childhood.

ACE scores were coded multiple ways to compare the data in the current study to previous work conducted including the original ACEs study[1] and subsequent studies[2]. This allowed for further understanding of the effects of exposure to accumulation of ACEs on EF and BDNF. The coding from the original ACE study focused on three types of maltreatment including sexual, physical, and emotional abuse and five types of household dysfunction including witnessing domestic violence, having someone in the household suffering from serious mental illness or suicidal, someone who was addicted to drugs or alcohol, a family member in prison, and separation from a parent or parents[1]. Marital separation and divorce was considered an ACE in the household dysfunction category in the original ACEs study[1], however was

excluded from analysis in the current study. Currently, divorce and separation of parents is seen as much more common and accepted among younger age cohorts compared to when the original ACEs study was conducted in the 1990's[1] among those who were middle aged and older. If divorce or separation were leading to a volatile environment, these experiences would be captured through the measures of household dysfunction and abuse.

Further, as there is no consistent practice in previous literature as to how to categorize ACEs for analysis. Two different measures of ACEs were created, a threshold measure and a continuous measure. To be consistent with the original work by Felitti and colleagues, the first measure used the maltreatment and household dysfunction items to create a threshold-type measure of ACEs. This threshold-type measure ranged from those reporting 0, 1, 2, 3, up to those reporting 4 or more exposures as the highest group to examine accumulation of ACEs. The threshold combing 4 or more ACEs was identified in the ACEs study conducted by Felitti and colleagues[1]. Second, a continuous measure of total ACEs was constructed summing the three types of abuse and the five types of household experiences and ranged from 0-8. Researchers have found support for the retrospective collection of ACEs in adulthood finding that individuals are willing to report ACEs when they are in a safe, comfortable environment with one study identifying test-retest kappa values 0.6-0.7 after one year[62,63].

Executive Function (EF)

The BRIEF-A is a self-report questionnaire completed by participants in the NLHS to measure everyday EF. The BRIEF-A contains 75 questions and uses a 3-point response scale for each question as follows: "Never (N)", "Sometimes (S)" or "Often (O)". The BRIEF-A items are grouped into nine clinical scales and these clinical scales can then be categorized into two higher-order composite scales and a total EF composite score. The nine individual scales include

Inhibit, Shift, Emotional Control, Initiate, Self Monitor, Working Memory, Plan/Organize, Organization of Materials, and Task Monitor. The first composite measure is the Behaviour Regulation Index (BRI) which combines the Inhibit, Shift, Self Monitor, and Emotional Control clinical scales . The Metacognition Index (MI) is made up of the Initiate, Working Memory, Plan/Organize, Organization of Materials, and Task Monitor clinical scales[64]. The BRI focuses on modulating behaviour and emotions whereas the MI focuses on self-managing and self-monitoring[64,65]. The sum of the BRI and the MI scores create the total composite EF score; the Global Executive Composite (GEC)[64,65]. Missing data is coded as "Never (N)" for the purpose of calculating raw clinical scores and raw composite scores in accordance with the BRIEF-A Manual[64]. All raw scores were converted into standardized T-scores to allow for comparison across scales and to normalize the data to the population.

For this study, the analysis was conducted using multiple measures of EF from the BRIEF-A. The GEC was used to compare overall EF and the two composite measures, the BRI and the MI, were examined to understand which areas of EF may have greater deficits. An analysis of the Inhibit and Working Memory clinical scales was also conducted to compare results from the current study to the literature[35–37,40]. The creators of the scale have run multiple validity and reliability tests on various populations to support the use of the BRIEF measures[64,66]. A factor analysis was conducted to ensure loading of factors in the current study was consistent with the literature. Based on the results of the factor analysis and the BRIEF-A manual[64], the individual clinical and composite scales were constructed using the BRIEF-A measures and examined as continuous measures. The measures of everyday functioning allow for the current analysis to be compared to the literature that examined constructs such as inhibition or working

memory. Further, the three composite scales were constructed to measure behaviour regulation, metacognition and global executive functioning[64].

Brain Plasticity

Brain-derived neurotrophic factor (BDNF) is a protein trophic factor involved in neuronal survival, growth and differentiation. Since BDNF is influential in the brain's ability to change pathways and learn from experiences, it was used as a marker of brain plasticity. The literature supports utilizing serum levels to measure BDNF[57] systemically because of the ability of BDNF to cross the blood-brain barrier[6,67]. During the NLHS lab visit, participants voluntarily provided a blood sample that was taken by a registered nurse. Blood samples were all processed in a systematic, consistent manner across each participant. First, samples were left at room temperate for 30 minutes to allow blood to clot and were then centrifuged at 3000 x g for 15 minutes at 4°C[60]. Serum was then separated and stored in aliquots at -80°C for further analysis to measure levels of BDNF.

A human Free BDNF Quantikine ELISA kit was run to measure BDNF in the current sample of NLHS participants and all samples were analysed in duplicate and averaged (R&D Biotechne Systems, Minneapolis, Minnesota, USA). The ELISA is an enzyme-linked immunosorbent assay, a technique that uses the binding of antibodies and antigen with an enzyme. When antigen and enzyme are present and a substrate is added a fluorescent colour is produced, when completed the concentration of a specific antigen is then measurable. All reagents were prepared before carrying out the assay procedure according to the manufacturer's lab manual (R&D Biotechne Systems, Minneapolis, Minnesota, USA). 100µL of Assay Diluent was added to each plate well, dividers that allow for the isolated sections on the plate for each sample, followed by 50 µL of standard, control, or sample to each. Duplicates of all standards,

controls and samples were carried out to ensure accuracy and reliability of assays. To run the assays, the prepared plates were covered and left to incubate at room temperature for two hours. Following the incubation period, 100µL of Human Free BDNF Conjugate was added to each well which were then covered and left to incubate for one hour. During this time, the conjugate would bind to BDNF in the samples. Next, a wash procedure was carried out three times. Once washed, 200µL of Substrate Solution was added to each well which were then covered in tinfoil to protect from light and left to incubate for 30 minutes. If antigen and enzyme were present, the substrate would lead to a reaction that would produce a fluorescent colour. Stop solution, 50µL, was added and the plate was gently tapped to thoroughly mix the Stop Solution to halt the reaction. Once a uniform colour change was observed the plate was ready to be analyzed.

To measure BDNF, the optical density for each well was measured in a microplate reader set to 450nm. Analysis of standards, controls, and samples will be carried out to compare BDNF levels, a continuous measure, in the cross-sectional analysis of NLHS participants.

Covariates

As previously mentioned, there were six covariates that were considered. These covariates included childhood household SES, respondent sex, menstrual cycle, use of antidepressants, mental illness diagnosis, and unhealthy eating. Chronic cortisol (n=148) was considered and would have been included as a covariate if significant correlations were observed with main outcome variables in preliminary analysis. Each covariate was constructed from the NLHS data and were created and selected intentionally while minimizing loss of degrees of freedom for the analysis where possible.

Childhood household SES covariate was the highest education level held by a parent in the household collected during the three baseline studies. Parent education was used because of

missing data and self-reporting bias with respect to household income reported by parents at baseline. Highest parent education status was coded as follows: grade 11 or less, grade 12, high school diploma or GED, partial college or training, college or university degree, and graduate degree or professional degree. Each education level was assigned a value from 1 (grade 11 or less) to 6 (graduate degree or professional degree) and was treated as an ordinal covariate in the regression analysis.

Respondent sex and menstrual cycle were included in analysis to explain sex differences observed between males and females. Cycle was categorized into an early follicular phase group (day 1-14), late luteal phase group (day 15-28), and an "other" group was constructed that included females who had irregular periods, an intrauterine device (IUD), did not menstruate, or individuals who were missing cycle data. To include both sex and menstrual cycle and avoid loss of cases in all analyses, a series of conditionally relevant variables were created to compare females in early follicular phase, females in the late luteal phase, and females in the other group to males as the reference group.

The questionnaire in the NLHS follow up study collected data on current prescription drug use and mental illness diagnosis. Antidepressant use was defined as the use of any of the following: escitalopram, citalopram, duloxetine, venlafaxine, desvenlafaxine, amitryptiline, paroxetine, fluoxetine, buproprion, sertraline, lorazepam, clonazepam and alprazolam. The antidepressant covariate was a dichotomous variable coded as "yes" or "no", if participants reported using any of the previously mentioned medications then they were coded as "yes". A dichotomous variable to assess the presence or absence of mental illness diagnosis was constructed and included the following: Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive

Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD). Autism was also included in the mental illness diagnosis covariate to preserve degrees of freedom as these individuals also have alterations in neuronal pathways leading to differences in function.

Finally, individuals provided information on dietary intake and types of foods/beverages they consume. The following items on the Food and Eating Habits questionnaire were used to create the unhealthy eating choices covariate: if participants eat French fries or fried potatoes, drink pop or flavoured drinks that are not diet, eat fast food like hamburgers or pizza or chicken nuggets, eat sweets like chocolate bars or candy or cookies or pie or cake, and eat salty snacks like potato chips or corn chips or taco chips or crackers. The frequency of consumption for each item was reported as never, less than 1 per week, 1 to 6 per week, 1 per day, or more than 1 per day for each of the above unhealthy food choices. A factor analysis identified these 5 items loaded together and were distinct from the other items in this questionnaire, Cronbach's alpha for the 5 items was α=0.77. Based on these results, the 5 items were summed to create a continuous variable that measures unhealthy eating and was used as a covariate.

Statistical Analysis

Statistical analyses were conducted in two stages. First, analyses were conducted to provide descriptive information. Second, regression analyses were performed. Two ACEs measures were created and used in the analysis. Comparing ACE with a threshold measure of 0, 1, 2, 3, or $\geq$ 4 ACEs, based on the findings of Felitti and colleagues[1], allowed the current analysis to be compared to past ACEs literature. ACEs were also treated as a continuous variable, 0-8, to examine the effect of accumulation of ACEs on EF and current serum BDNF levels. Two clinical

measures of EF, Inhibit and Working Memory, and three composite measures of EF including BRI, MI, and GEC, were used as continuous measures in analyses. These measures of EF were standardized and normalized to population data, based on the BRIEF-A manual higher T-scores indicated lower EF[64]. Further, current brain plasticity, measured using serum levels of BDNF, was examined as a continuous variable in all analyses. Current BDNF levels were examined as an outcome of ACEs and also a possible mediating factor in EF as an outcome of ACEs based on the specified hypotheses. Statistical Analysis Software (SAS) University Edition was used to run all statistical analysis.

Regression Analysis

The regression analysis was conducted using the NLHS follow-up study data and examining the variables ACEs, EF and BDNF. First, multiple linear regressions were run using each of the BRIEF-A measures regressed on each of the ACEs measures and covariates as previously discussed. Separate models for each of the BRIEF-A measures and ACEs measures were run to determine if ACEs, the predictor variable, and EF, the outcome variable, were related. Next, the relationship between ACEs and BDNF was examined. Two models were created, the first model run was BDNF regressed on the ACEs threshold measure. The second model regressed BDNF on ACEs threshold measure and all covariates. If significant findings were reported for the models examining BDNF and ACEs, then it would indicate the need to examine indirect effects of BDNF. The indirect effects model would have ACEs as the predictor, BDNF as an indirect effect, and EF as the outcome to determine if current BDNF levels affected the relationship between EF and ACEs. A final multiple regression analysis was run to explore whether the association between ACEs and EF varied as a function of respondent sex (sex X ACEs interaction). To further explore any possible differences across sex and menstrual cycle,

additional models were examined that were stratified by sex with BRIEF-A measures and regressed on ACEs and all covariates included in the model except for menstrual cycle. Lastly, an additional model for females only (n=128) was tested, in which BRIEF-A measures were regressed on ACEs threshold measure and all covariates including menstrual cycle using early cycle phase as the reference group. All analyses were run on SAS University Edition and reported parameter estimates, standardized estimates, 95% confidence intervals at an alpha set to $p<0.05$ using two-tailed tests.

Chapter 4: Results

Attrition Analyses

Individuals with missing BDNF data, as a result of either declining to provide a blood sample during testing or inability of the phlebotomist to secure a blood sample, were not significantly different from individuals with BDNF data for all main outcome variables (Table 1). An adjusted sample size of n=226 was used when examining the effects of BDNF on main outcome variables.

Highest education level held by a parent/guardian at time=1 was used to measure socioeconomic status (SES) in childhood. There were 17 individuals in the current dataset, time=2, that had missing parent reports from time=1. There were no significant differences in main outcome variables, using independent sample T-tests and chi squared tests where appropriate, between participants with and without childhood household SES data (Table 1). Due to parents not completing the questionnaire at time=1, a missing data imputation using other variables from household status was not possible. For this reason, participants' education status at time=2 was used for missing SES data for the 17 cases. This was based on the fact that, among those with highest parent education at time=1, there was no significant difference between participant's education status at time=2 and highest education level in the childhood home at time=1 (results not shown).

Table 1. Attrition analysis comparing main outcomes of participants with and without childhood household SES data and participants with and without BDNF data

	Total (n = 236)	With childhood household SES data (n = 219)[a]	Missing childhood household SES data (n = 17)	*P* value	BDNF (n = 226)	No BDNF sample (n = 10)	*P* value
Sex (%)							
Males	108 (45.76)	98 (90.74)	10 (9.26)	0.38	103 (95.37)	5 (4.63)	0.24
Females	128 (54.24)	121 (94.53)	7 (5.47)		123 (96.09)	5 (3.91)	
ACEs Measures							
ACEs total, mean (SD)[b]	2.49 (2.16)	2.54 (2.19)	1.82 (1.74)	0.19	2.49 (2.15)	2.50 (2.51)	0.98
ACEs threshold (%)[c]							
0	41 (17.37)	38 (17.35)	3 (17.65)	0.58	38 (16.81)	3 (30.00)	0.74
1	57 (24.15)	50 (22.83)	7 (41.18)		56 (24.78)	1 (10.00)	
2	46 (19.49)	43 (19.63)	3 (17.65)		44 (19.47)	2 (20.00)	
3	27 (11.44)	26 (11.87)	1 (5.88)		26 (11.50)	1 (10.00)	
$\geq$ 4	65 (27.54)	62 (28.31)	3 (17.65)		62 (27.43)	3 (30.00)	
BRIEF-A Scores, mean (SD)							
Inhibition	55.43 (9.91)	55.44 (9.86)	55.35 (10.94)	0.97	55.28 (9.74)	58.80 (13.55)	0.27
Working memory	55.64 (10.95)	55.50, 10.99)	57.41 (10.60)	0.49	55.59 (10.99)	56.70 (10.53)	0.76
BRI	53.82 (9.60)	53.98 (9.68)	51.82 (8.39)	0.37	53.60 (9.44)	58.00 (12.06)	0.09
MI	52.92 (9.61)	52.84 (9.72)	54.00 (8.09)	0.63	52.83 (9.56)	54.90 (10.91)	0.51
GEC	53.57 (9.47)	53.59 (9.61)	53.35 (7.62)	0.92	53.41 (9.37)	57.10 (11.59)	0.23
Serum Measures, mean (SD)							
BDNF	24535.92 (6924.63)	24645.64 (7070.84)	23095.82 (4500.52)	0.39	-	-	-

Abbreviations: ACE, adverse childhood experience. BDNF, brain derived neurotrophic factor. BRI, Behaviour Regulation Index. BRIEF, Behaviour Rating Inventory of Executive Function. GEC, Global Executive Composite. MI, Metacognition Index. SES, socioeconomic status.

[a] Childhood household SES was defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1.
[b] ACEs total was the sum of experiences that occurred before the age of 18 collected via a retrospective, self-report questionnaire. Experiences included the following: physical abuse, emotional abuse, sexual abuse, witnessing domestic violence, living with an individual with drug or alcohol abuse, living with an individual with mental illness, living with an individual who had been incarcerated and/or experiencing separation from parents. The sum could range from 0-8.
[c] ACEs threshold included the same experiences as the ACEs sum; however, ranged from 0-4. Individuals who reported 0, 1, 2, or 3 ACEs were put in their respective categories. Individuals who experienced 4, 5, 6, 7, or 8 experiences were grouped together in the $\geq$ 4 category.
*$p<0.05$ (two tailed)

Factor Analysis of Executive Function

Principal component analyses (PCA) were run to determine if the EF data collected in the NLHS loaded onto a single factor consistent with the constructs defined by the BRIEF-A manual[64]. This analysis was used to support the use of the BRIEF-A moving forward.

First, the items for the Inhibit clinical scale and the Working Memory clinical scale were run separately (Table a1). All BRIEF-A items used to construct the Inhibit clinical scale significantly loaded onto one factor with an eigenvalue of 2.72 with the next highest value of 1.19. The eigenvalue for factor one of Working Memory was 3.49 and the next highest value was 0.85. The specific items for the Inhibit clinical scale and the Working Memory clinical scale loaded onto single factors and these results support the use of these BRIEF-A clinical measures in the current analysis.

Moving forward, higher order factor analyses were conducted to examine the BRI and MI composite subscales and the GEC full composite scale (results not shown). This was done by examining the construction of the composite scores at two-levels including the clinical scale level and the individual item level. When PCA for all clinical scales included in the BRI were run, including the Inhibit, Shift, Self Monitor, and Emotional Control clinical scales, it gave a one-factor solution with all items loading significantly onto the first factor with an eigenvalue of 2.41 compared to the next highest factor with an eigenvalue of 0.80. The remaining clinical scales that construct the MI, including the Initiate, Working Memory, Plan/Organize, Organization of Materials, and Task Monitor clinical scales also gave a one-factor solution significantly loaded onto a single factor (3.51) with all other eigenvalues below 1.0. To further support these findings each item for the clinical scales were examined. When the individual BRIEF-A items that comprises each of the clinical scales to create the BRI were examined, all

but one item significantly loaded onto the first factor with an eigenvalue of 8.34 compared to the next highest factor with an eigenvalue of 2.90. Consistent with these results and the clinical scale results, every individual BRIEF-A item that were used to create the clinical scales included in the MI all significantly loaded onto one factor with an eigenvalue of 11.78 compared to the next highest eigenvalue of 2.60.

Finally, a higher order factor analysis was conducted for the GEC, which was constructed using the nine clinical scales and then the full 75 BRIEF-A items. PCAs were run for both the clinical scales and individual items to assess the GEC. At the clinical scale level, the PCA gave a one-factor solution with all items loading significantly onto the first factor with an eigenvalue of 5.11 compared to the next highest value of 0.98. When examining all 75 individual BRIEF-A items used to create the GEC, the first factor had an eigenvalue of 16.70 with the next highest value of 4.87. All PCAs involved in this higher order analysis significantly loaded onto one factor and support the use of the overall GEC composite measure with the current data.

To summarize, the analysis of the multiple and high order PCAs demonstrated a significant loading of Inhibit, Working Memory, BRI, MI, and GEC scales onto single factors consistent with reported results from the BRIEF-A manual[64]. This supports the use of the BRIEF-A measures proposed in the original scale development moving forward in the analysis.

Demographic, covariate and main outcome characteristics

All main outcome variables and covariates are presented below in Table 2 and were stratified across males and females. The mean reporting of ACEs was 2.5 experiences and was similar across males and females. When reporting ACEs there were 17.7% of individuals who reported experiencing zero ACEs. Alternatively, 27.5% of individuals in the sample reported experiencing 4 or more ACEs and were similar across sex. There were significant differences

between males and females for age in years determined using an independent sample T-test with females being significantly younger than males. Females were also significantly more likely to use antidepressants than males using a chi squared test. An independent sample T-test resulted in the composite MI score being significantly different between males and females. Females had significantly lower scores for the MI measure compared to males. There was no significant difference between mean serum BDNF for males and females based on the available, adjusted sample (n=226) using independent sample T-test. There was also no significant difference, using independent sample T-test, for chronic cortisol levels for males and females based on the available, adjusted sample size (n=148).

Table 2. Descriptive values for all main outcome variables and covariates for the total sample, males and females

	Total (n=236)	**Males (n=108)**	**Females (n=128)**	***P* value**
Age in years, mean (SD)	22.62 (1.51)	22.85 (1.45)	22.43 (1.53)	0.04*
Childhood Household SES[a], mean (SD)	4.51 (1.14)	4.53 (1.64)	4.50 (1.2)	0.85
Highest Parent Education (%)				0.38
Less than grade 11	3 (1.27)	2 (1.85)	1 (0.78)	
Grade 12	14 (5.93)	4 (3.70)	10 (7.81)	
Highschool diploma or GED	28 (11.86)	16 (14.81)	12 (9.38)	
Partial college or training	40 (16.95)	18 (16.67)	22 (17.19)	
University degree/ college diploma	116 (49.15)	49 (45.37)	67 (52.34)	
Graduate degree or professional degree	35 (14.83)	19 (17.59)	16 (12.50)	
Mental Illness Diagnosis[b] (%)				0.29
Yes	76 (32.20)	31 (28.70)	45 (35.16)	
No	160 (67.80)	77 (71.30)	83 (64.84)	
Antidepressant Use[c] (%)				0.01*
Yes	25 (10.59)	5 (4.63)	20 (15.63)	
No	211 (89.41)	103 (95.37)	108 (84.38)	
Unhealthy Eating[d], mean (SD)	7.21 (2.86)	7.19 (2.83)	7.22 (2.90)	0.95
Female Cycle, No. (%)	-	-	Females (n=128)	
Early cycle phase[e]	-	-	49 (38.28)	-
Late cycle phase[f]	-	-	52 (40.63)	
No Cycle[g]	-	-	27 (21.09)	
ACEs Measures				
ACEs Total[h], mean (SD)	2.49 (2.16)	2.28 (2.12)	2.66 (2.18)	0.17
ACEs Threshold[i] (%)				0.26
0	41 (17.37)	25 (23.15)	16 (12.50)	
1	57 (24.15)	23 (21.30)	34 (26.56)	
2	46 (19.49)	22 (20.37)	24 (18.75)	
3	27 (11.44)	11 (10.19)	16 (12.50)	
$\geq$ 4	65 (27.54)	27 (25.00)	38 (29.69)	
BRIEF-A Scores, mean (SD)				
Inhibit	55.43 (9.91)	56.82 (10.15)	54.26 (9.60)	0.05
Working Memory	55.64 (10.95)	56.33 (11.11)	55.05 (10.82)	0.37
BRI	53.82 (9.60)	52.62 (9.31)	54.84 (9.75)	0.08
MI	52.92 (9.61)	54.49 (9.55)	51.59 (9.49)	0.02*
GEC	53.57 (9.47)	54.02 (9.44)	53.20 (9.52)	0.50

Serum, mean (SD)	**Total (n=226)**	**Males (n=103)**	**Females (n=123)**	**p-value**
BDNF	24535.92 (6924.63)	24275.22 (6581.54)	24754.23 (7218.89)	0.61
Inflammatory Marker, mean (SD)	**Total (n=148)**	**Males (n=39)**	**Females (n=109)**	**p-value**
Cortisol	40.54 (116.87)	30.09 (50.45)	44.28 (132.82)	0.52

Abbreviations: ACE, adverse childhood experience. BDNF, brain derived neurotrophic factor. BRI, Behaviour Regulation Index. BRIEF-A, Behaviour Rating Inventory of Executive Function Adult version. GEC, Global Executive Composite. MI, Metacognition Index. SES, socioeconomic status.

[a] Childhood household SES was defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1 and ranged from 1-6.
[b] Mental illness diagnosis includes Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD).
[c] Antidepressant use includes selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, norepinephrine/dopamine reuptake inhibitor (NDRIs) and benzodiazepines.
[d] Unhealthy eating includes eating French fries or fried potatoes, drinking pop or flavoured drinks that are not diet, eating fast food like hamburgers or pizza or chicken nuggets, eating sweets like chocolate bars or candy or cookies or pie or cake, and eating salty snacks like potato chips or corn chips or taco chips or crackers.
[e] Early cycle phase included females who indicated they were on or between day 1 and day 14 of their menstrual cycle.
[f] Late cycle phase included females who indicated they were on or between day 15 and day 28 of their menstrual cycle.
[g] No cycle includes females who had an irregular cycle, had an IUD, were missing cycle data, or did not menstruate.
[h] ACEs total was the sum of experiences that occurred before the age of 18 collected via a retrospective, self-report questionnaire. Experiences included the following: physical abuse, emotional abuse, sexual abuse, witnessing domestic violence, living with an individual with drug or alcohol abuse, living with an individual with mental illness, living with an individual who had been incarcerated and/or experiencing separation from parents. The sum could range from 0-8.
[i] ACEs threshold included the same experiences as the ACEs sum; however, ranged from 0-4. Individuals who reported 0, 1, 2, or 3 ACEs were put in their respective categories. Individuals who experienced 4, 5, 6, 7, or 8 experiences were grouped together in the $\geq$ 4 category.
*$p<0.05$ (two tailed)

Correlation of ACEs, BRIEF measures and covariates

Correlations were run to examine the possible relationships between the proposed main outcome variables and covariates (Table 3). ACEs total and ACEs threshold measures were significantly correlated to each other as expected, and to all the BRIEF-A measures including Inhibit, Working Memory, BRI, MI, and GEC measures. Childhood household SES was also significantly correlated with ACEs measures and the BRIEF-A scores. Chronic cortisol was not significantly correlated with any of the ACEs measures, the BRIEF-A scores, or BDNF and therefore was not included in the regression analysis moving forward due to a significant loss of cases. BDNF was not significantly correlated with any of the ACEs measures or any of the BRIEF-A measures, and as a result, BDNF was not tested for potential indirect effects.

Table 3. Correlation matrix with all main outcome variables and covariates

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1. ACEs total[a]	-																	
2. ACEs threshold[b]	**0.93***	-																
3. Inhibit	**0.21***	**0.25***	-															
4. Working memory	**0.25***	**0.29***	**0.59***	-														
5. BRI	**0.29***	**0.33***	**0.74***	**0.61***	-													
6. MI	**0.25***	**0.26***	**0.62***	**0.82***	**0.66***	-												
7. GEC	**0.29***	**0.32***	**0.74***	**0.80***	**0.88***	**0.93***	-											
8. BDNF[c]	-0.07	-0.04	0.10	0.06	0.07	0.03	0.05	-										
9. Cortisol[d]	-0.08	-0.11	-0.10	-0.04	-0.07	-0.06	-0.07	-0.02	-									
10. Sex[e]	0.09	0.09	**-0.13***	-0.06	0.12	**-0.15***	-0.04	0.03	0.05	-								
11. Age	0.10	0.07	-0.02	0.06	-0.00	0.05	0.03	**-0.16***	0.07	**-0.14***	-							
12. Childhood household SES[f]	**-0.24***	**-0.26***	**-0.17***	**-0.16***	**-0.17***	**-0.19***	**-0.20***	0.08	0.06	-0.01	-0.12	-						
13. Antidepressant use[g]	0.10	0.11	**0.13***	**0.16***	**0.25***	0.10	**0.19***	-0.03	0.02	**0.18***	-0.06	0.03	-					
14. Mental Illness diagnosis[h]	**0.27***	**0.29***	**0.27***	**0.29***	**0.36***	**0.29***	**0.35***	-0.04	-0.06	0.07	-0.02	-0.08	**0.47***	-				
15. Unhealthy eating[i]	0.08	**0.13***	**0.26***	**0.23***	**0.31***	**0.27***	**0.32***	0.03	-0.02	0.00	-0.01	-0.04	0.01	0.08	-			
16. Early cycle phase[j]	-0.06	-0.03	-0.17	**-0.21***	**-0.20***	**-0.21***	**-0.22***	0.05	0.06	-	-0.13	0.04	0.02	-0.14	-0.07	-		
17. Late cycle phase[k]	-0.08	-0.08	0.07	0.05	0.11	0.10	0.12	0.01	-0.00	-	0.03	-0.09	-0.05	0.06	0.01	**-0.65***	-	
18. No cycle[l]	**0.18***	0.14	0.11	**0.18***	0.11	0.13	0.13	-0.07	0.07	-	0.12	0.06	0.04	0.10	0.06	**-0.41***	**-0.43***	-

Abbreviations: ACE, adverse childhood experience. BDNF, brain derived neurotrophic factor. BRI, Behaviour Regulation Index. GEC, Global Executive Composite. MI, Metacognition Index. SES, socioeconomic status.

[a] ACEs total was the sum of experiences that occurred before the age of 18 collected via a retrospective, self-report questionnaire. Experiences included the following: physical abuse, emotional abuse, sexual abuse, witnessing domestic violence, living with an individual with drug or alcohol abuse, living with an individual with mental illness, living with an individual who had been incarcerated and/or experiencing separation from parents. The sum could range from 0-8.

[b] ACEs threshold included the same experiences as the ACEs sum; however, ranged from 0-4. Individuals who reported 0, 1, 2, or 3 ACEs were put in their respective categories. Individuals who experienced 4, 5, 6, 7, or 8 experiences were grouped together in the $\geq$ 4 category.

[c] BDNF sample size was n=226 due to missing data.

[d] Cortisol sample size was n=148 due to missing data.

[e] Sex reference group was males.

[f] Childhood household SES is defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1.

[g] Antidepressant use includes selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, norepinephrine/dopamine reuptake inhibitor (NDRIs) and benzodiazepines.

[h] Mental illness diagnosis includes Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD).

[i] Unhealthy eating includes eating French fries or fried potatoes, drinking pop or flavoured drinks that are not diet, eating fast food like hamburgers or pizza or chicken nuggets, eating sweets like chocolate bars or candy or cookies or pie or cake, and eating salty snacks like potato chips or corn chips or taco chips or crackers.

[j] Early cycle phase included females who indicated they were on or between day 1 and day 14 of their menstrual cycle.

[k] Late cycle phase included females who indicated they were on or between day 15 and day 28 of their menstrual cycle.

[l] No cycle includes females who had an irregular cycle, had an IUD, were missing cycle data, or did not menstruate.

*$p < 0.05$, two tailed

Regression of BDNF on ACEs

Two models were tested to examine the relationship between BDNF and ACEs (Table 4). Model 1 regressed BDNF on the ACEs threshold measure and was not statistically significant. These results correspond with the null findings for BDNF in the correlation matrix (Table 3). Model 2 regressed BDNF on the ACEs threshold measure and all covariates; this model was also not statistically significant. Thus, tests of indirect effects were not conducted as BDNF was not a suitable candidate for a pathway linking ACEs to EF (Table 4).

Table 4. OSL regression of BDNF on ACEs threshold followed by BDNF on ACEs and covariates (N=226)

	BDNF					
	MODEL 1			MODEL 2		
	b	**β**	**95% CI**	**b**	**β**	**95% CI**
Intercept	24938***	0***	[23354, 26521]***	21451***	0***	[16289, 26613]***
ACEs threshold[a]	-193.17	-0.04	[-816.51, 430.17]	-88.98	-0.02	[-780.32, 602.37]
Childhood household SES[b]				508.05	0.08	[-341.74, 1357.85]
Antidepressant use[c]				-643.90	-0.03	[-4084.08, 2796.28]
Mental illness diagnosis[d]				-146.43	-0.01	[-2488.25, 2195.39]
Unhealthy eating[e]				103.88	0.04	[-223.24, 431.00]
Early phase cycle[f]				1023.57	0.06	[-1434.06, 3481.19]
Late phase cycle[g]				797.86	0.05	[-1603.30, 3199.01]
No cycle[h]				-453.55	-0.02	[-3605.00, 2697.91]
	R^2= 0.0017			R^2= 0.0149		

Abbreviations: ACE, adverse childhood experience. BDNF, brain derived neurotrophic factor. CI, confidence interval. SES, socioeconomic status.

[a] ACEs threshold included the same experiences as the ACEs sum; however, ranged from 0-4. Individuals who reported 0, 1, 2, or 3 ACEs were put in their respective categories. Individuals who experienced 4, 5, 6, 7, or 8 experiences were grouped together in the $\geq$ 4 category.
[b] Childhood household SES is defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1.
[c] Antidepressant use includes selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, norepinephrine/dopamine reuptake inhibitor (NDRIs) and benzodiazepines.
[d] Mental illness diagnosis includes Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD).
[e] Unhealthy eating includes eating French fries or fried potatoes, drinking pop or flavoured drinks that are not diet, eating fast food like hamburgers or pizza or chicken nuggets, eating sweets like chocolate bars or candy or cookies or pie or cake, and eating salty snacks like potato chips or corn chips or taco chips or crackers.
[f] Early phase cycle included females who indicated they were on or between day 1 and day 14 of their menstrual cycle. The reference group was males.
[g] Late phase cycle included females who indicated they were on or between day 15 and day 28 of their menstrual cycle. The reference group was males.
[h] No cycle includes females who had an irregular cycle, had an IUD, were missing cycle data, or did not menstruate. The reference group was males.
*p<0.05, **p<0.01, ***p<0.001

Regression of EF measures on ACEs

The regression analyses of BRIEF-A measures and ACEs followed three steps examining ACEs threshold measure, the total ACEs, and finally an interaction model with sex and the ACEs threshold measure. Stratified analyses across sex using the ACEs threshold measure and BRIEF-A measures were also conducted to identify any potential underlying differences. Assumptions for normality were checked and outliers were individually examined. First, the BRIEF-A clinical measures, Inhibit and Working Memory, were each regressed onto the ACEs threshold measure and all covariates (Table 5). In the next table, the BRIEF-A composite measures BRI, MI, and GEC were each regressed onto the ACEs threshold measure and all covariates (Table 6). All overall test of the full models regressing BRIEF-A measures onto the ACEs threshold measure along with covariates were statistically significant at $p<0.001$ (Table 5 & 6). ACEs threshold remained statistically significant in all models ($p<0.05$) and had a positive relationship with all BRIEF-A scores with higher ACEs score predicting higher BRIEF-A scores in all cases.

Second, the BRIEF-A measures were also regressed onto the ACEs total measure. The BRIEF-A clinical measures Inhibit and Working Memory were each regressed onto the ACEs total measure and all covariates (Table 7). Then the BRIEF-A composite measures BRI, MI, and GEC were each regressed onto the ACEs total measure and all covariates (Table 8). Similar to the threshold ACEs models, all models regressing BRIEF-A measures onto the ACEs total measure and covariates were statistically significant at a $p<0.05$ (Table 7 & 8). As well, the ACEs total measure remained statistically significant in all models except the model for the BRIEF-A Inhibit which was approaching significance. Across all BRIEF-A measures there was a positive relationship between BRIEF-A scores and ACEs total.

The Working Memory clinical measure and all models of BRIEF-A composite measures regressed onto the ACEs measures, the threshold measure and total measure, were statistically significant (Table 5-8). Similar R^2 values were found in all fully adjusted models using ACEs threshold measure and ACEs total measure. Model fit ranged from 0.19-0.20 for the BRIEF-A clinical measure models and 0.23-0.29 for the BRIEF-A composite measure models (Table5-8). In models using the BRIEF-A clinical measures, similar covariates were significant regardless of which ACEs measure was used (Table 5 & 7). Significant covariates in the BRIEF-A clinical measures models included unhealthy eating choices, mental illness diagnosis which were positively related with BRIEF-A clinical scores and early phase menstrual cycle which was negatively related to the BRIEF-A clinical scores (Table 5 & 7). Similar covariates were significant in the BRIEF-A composite measure models for both ACEs threshold and ACEs total measures (Table 6 & 8). Further, both ACEs measures remained statistically significant and had a positive relationship with BRIEF-A measures as hypothesized. When examining the parameter and standardized estimates, the ACEs threshold measure had slightly higher values for all BRIEF-A scores compared to the ACEs total measure (Table 5-8). Moving forward, due to the similar findings in models and the relationship between BRIEF-A scores and ACEs measures, only the ACEs threshold measure will be used in the sex-stratified regression analysis.

Table 5. OSL regression of BRIEF-A inhibition and working memory clinical scales on ACEs threshold and covariates (N=236)

	BRIEF-A					
	Inhibit			Working Memory		
	b	**β**	**95% CI**	**b**	**β**	**95% CI**
Intercept	52.67***	0***	[46.32, 59.06]***	51.21***	0***	[44.20, 58.22]***
ACEs threshold[a]	1.00*	0.15*	[0.13, 1.87]*	1.46**	0.20**	[0.50, 2.41]**
Childhood household SES[b]	-0.95	-0.11	[-2.01, 0.11]	-0.86	-0.09	[-2.03, 0.31]
Antidepressant use[c]	2.20	0.07	[-2.15, 6.55]	2.69	0.08	[-2.11, 7.48]
Mental illness diagnosis[d]	3.62*	0.17*	[0.67, 6.57]*	4.02*	0.17*	[0.77, 7.27]*
Unhealthy eating[e]	0.75***	0.22***	[0.34, 1.16]***	0.69**	0.18**	[0.23, 1.14]**
Early phase cycle[f]	-4.81**	-0.20**	[-7.93, -1.69]**	-4.46*	-0.17*	[-7.90, -1.02]*
Late phase cycle[g]	-2.62	-0.11	[-5.65, 0.41]	-1.56	-0.06	[-4.90, 1.78]
No cycle[h]	-2.17	-0.07	[-6.07, 1.74]	0.31	0.01	[-4.00, 4.61]
	R^2=0.1982***			R^2=0.2025***		

Abbreviations: ACE, adverse childhood experience. BRIEF-A, Behaviour Rating Inventory of Executive Functioning Adult Version. CI, confidence interval. SES, socioeconomic status.

[a] ACEs threshold ranged from 0-4. Individuals who reported 0, 1, 2, or 3 ACEs were put in their respective categories. Individuals who experienced 4, 5, 6, 7, or 8 experiences were grouped together in the $\geq$ 4 category.
[b] Childhood household SES is defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1.
[c] Antidepressant use includes selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, norepinephrine/dopamine reuptake inhibitor (NDRIs) and benzodiazepines.
[d] Mental illness diagnosis includes Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD).
[e] Unhealthy eating includes eating French fries or fried potatoes, drinking pop or flavoured drinks that are not diet, eating fast food like hamburgers or pizza or chicken nuggets, eating sweets like chocolate bars or candy or cookies or pie or cake, and eating salty snacks like potato chips or corn chips or taco chips or crackers.
[f] Early phase cycle included females who indicated they were on or between day 1 and day 14 of their menstrual cycle. The reference group was males (n=108).
[g] Late phase cycle included females who indicated they were on or between day 15 and day 28 of their menstrual cycle. The reference group was males (n=108).
[h] No cycle includes females who had an irregular cycle, had an IUD, were missing cycle data, or did not menstruate. The reference group was males (n=108).
*p<0.05, **p<0.01, ***p<0.001

Table 6. OSL regression of BRIEF-A behaviour regulation index, metacognition index, and global executive composite on ACEs threshold and covariates (N=236)

	BRIEF-A								
	BRI			MI			GEC		
	b	β	95% CI	b	β	95% CI	b	β	95% CI
Intercept	46.25***	0***	[40.44, 52.06]***	50.73***	0***	[44.70, 56.76]***	48.72***	0***	[42.97, 54.46]***
ACEs threshold[a]	1.23**	0.19**	[0.44, 2.02]**	1.01*	0.15*	[0.19, 1.83]*	1.18**	0.18**	[0.40, 1.96]**
Childhood household SES[b]	-0.81	-0.10	[-1.78, 0.16]	-1.06*	-0.13*	[-2.06, -0.05]*	-1.03*	-0.12*	[-1.99, -0.08]*
Antidepressant use[c]	4.04*	0.13*	[0.06, 8.01]*	0.67	0.02	[-3.45, 4.80]	2.43	0.08	[-1.50, 6.36]
Mental illness diagnosis[d]	4.06**	0.20**	[1.37, 6.76]**	4.29**	0.21**	[1.50, 7.09]**	4.53***	0.22***	[1.87, 7.20]***
Unhealthy eating[e]	0.88***	0.26***	[0.51, 1.25]***	0.74***	0.22***	[0.35, 1.13]***	0.87***	0.26***	[0.50, 1.24]***
Early phase cycle[f]	-0.74	-0.03	[-3.59, 2.11]	-5.50***	-0.23***	[-8.45, -2.53]***	-3.76**	-0.16**	[-6.58, -0.94]**
Late phase cycle[g]	2.46	0.11	[-0.31, 5.22]	-2.52	-0.11	[-5.39, 0.35]	-0.51	-0.02	[-3.24, 2.23]
No cycle[h]	1.94	0.06	[-1.62, 5.51]	-2.13	-0.07	[-5.83, 1.57]	-0.55	-0.02	[-4.07, 2.97]
	R^2=0.2868***			R^2=0.2336***			R^2=0.2844***		

Abbreviations: ACE, adverse childhood experience. BRIEF-A, Behaviour Rating Inventory of Executive Functioning Adult Version. CI, confidence interval. SES, socioeconomic status.

[a] ACEs threshold included the same experiences as the ACEs sum; however, ranged from 0-4. Individuals who reported 0, 1, 2, or 3 ACEs were put in their respective categories. Individuals who experienced 4, 5, 6, 7, or 8 experiences were grouped together in the $\geq$ 4 category.
[b] Childhood household SES is defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1.
[c] Antidepressant use includes selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, norepinephrine/dopamine reuptake inhibitor (NDRIs) and benzodiazepines.
[d] Mental illness diagnosis includes Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD).
[e] Unhealthy eating includes eating French fries or fried potatoes, drinking pop or flavoured drinks that are not diet, eating fast food like hamburgers or pizza or chicken nuggets, eating sweets like chocolate bars or candy or cookies or pie or cake, and eating salty snacks like potato chips or corn chips or taco chips or crackers.
[f] Early phase cycle included females who indicated they were on or between day 1 and day 14 of their menstrual cycle. The reference group was males (n=108).
[g] Late phase cycle included females who indicated they were on or between day 15 and day 28 of their menstrual cycle. The reference group was males (n=108).
[h] No cycle includes females who had an irregular cycle, had an IUD, were missing cycle data, or did not menstruate. The reference group was males (n=108).
*p<0.05, **p<0.01, ***p<0.001

Table 7. OLS regression of BRIEF inhibition and working memory clinical scales on ACEs total and covariates (N=236)

	BRIEF-A					
	Inhibit			Working Memory		
	b	**β**	**95% CI**	**b**	**β**	**95% CI**
Intercept	53.43***	0***	[47.12, 59.74]***	52.32***	0***	[45.34, 59.29]***
ACEs total[a]	0.54	0.12	[-0.04, 1.13]	0.78*	0.15*	[0.14, 1.43]*
Childhood household SES[b]	-1.02	-0.12	[-2.08, 0.04]	-0.97	-0.10	[-2.14, 0.21]
Antidepressant use[c]	2.19	0.07	[-2.18, 6.56]	2.68	0.08	[-2.15, 7.51]
Mental illness diagnosis[d]	3.83*	0.18*	[0.88,6.78]*	4.33**	0.19**	[1.07, 7.51]**
Unhealthy eating[e]	0.78***	0.22***	[0.37, 1.19]***	0.73**	0.19**	[0.28, 1.18]**
Early phase cycle[f]	-4.70**	-0.19**	[-7.83, -1.57]**	-4.30*	-0.16*	[-7.75, -0.84]*
Late phase cycle[g]	-2.61	-0.11	[-5.65, 0.43]	-1.55	-0.06	[-4.91, 1.81]
No cycle[h]	-2.15	-0.07	[-6.08, 1.78]	0.34	0.01	[-4.01, 4.69]
	R^2=0.1919***			R^2=0.1910***		

Abbreviations: ACE, adverse childhood experience. BRIEF-A, Behaviour Rating Inventory of Executive Functioning Adult Version. CI, confidence interval. SES, socioeconomic status.

[a] ACEs total was the sum of experiences that occurred before the age of 18 collected via a retrospective, self-report questionnaire. Experiences included the following: physical abuse, emotional abuse, sexual abuse, witnessing domestic violence, living with an individual with drug or alcohol abuse, living with an individual with mental illness, living with an individual who had been incarcerated and/or experiencing separation from parents. The sum could range from 0-8.

[b] Childhood household SES is defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1.

[c] Antidepressant use includes selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, norepinephrine/dopamine reuptake inhibitor (NDRIs) and benzodiazepines.

[d] Mental illness diagnosis includes Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD).

[e] Unhealthy eating includes eating French fries or fried potatoes, drinking pop or flavoured drinks that are not diet, eating fast food like hamburgers or pizza or chicken nuggets, eating sweets like chocolate bars or candy or cookies or pie or cake, and eating salty snacks like potato chips or corn chips or taco chips or crackers.

[f] Early phase cycle included females who indicated they were on or between day 1 and day 14 of their menstrual cycle. The reference group was males (n=108).

[g] Late phase cycle included females who indicated they were on or between day 15 and day 28 of their menstrual cycle. The reference group was males (n=108).

[h] No cycle includes females who had an irregular cycle, had an IUD, were missing cycle data, or did not menstruate. The reference group was males (n=108).

*p<0.05, **p<0.01, ***p<0.001

Table 8. OSL regression of BRIEF-A behaviour regulation index, metacognition index, and global executive composite on ACEs total and covariates (N=236)

	BRIEF-A								
	BRI			MI			GEC		
	b	**β**	**95% CI**	**b**	**β**	**95% CI**	**b**	**β**	**95% CI**
Intercept	46.95***	0***	[41.18, 52.71]***	50.95***	0***	[45.00, 56.89]***	49.15***	0***	[43.47, 54.83]***
ACEs total[a]	0.72**	0.16**	[0.19, 1.26]**	0.69*	0.16*	[0.14, 1.25]*	0.76**	0.17**	[0.24, 1.29]**
Childhood household SES[b]	-0.87	-0.10	[-1.85, 0.10]	-1.07*	-0.13*	[-2.07, -0.07]*	-1.07*	-0.13*	[-2.04, -0.11]*
Antidepressant use[c]	4.04*	0.13*	[0.05, 8.04]*	0.70	0.02	[-3.42, 4.82]	2.45	0.08	[-1.48, 6.38]
Mental illness diagnosis[d]	4.25**	0.21**	[1.56, 6.95]**	4.33**	0.21**	[1.55, 7.11]**	4.64***	0.23***	[1.98, 7.29]***
Unhealthy eating[e]	0.91***	0.27***	[0.54, 1.29]***	0.77***	0.23***	[0.38, 1.15]***	0.90***	0.27***	[0.54, 1.27]***
Early phase cycle[f]	-0.61	-0.03	[-3.47, 2.25]	-5.42***	-0.23***	[-8.37, -2.46]***	-3.66*	-0.16*	[-6.48, -0.84]*
Late phase cycle[g]	2.47	0.11	[-0.31, 5.25]	-2.51	-0.11	[-5.38, 0.36]	-0.49	-0.02	[-3.23, 2.24]
No cycle[h]	1.90	0.06	[-1.69, 5.50]	-2.26	-0.08	[-5.97, 1.45]	-0.65	-0.02	[-4.19, 2.89]
	R^2=0.2800***			R^2=0.2344***			R^2=0.2822***		

Abbreviations: ACE, adverse childhood experience. BRIEF-A, Behaviour Rating Inventory of Executive Functioning Adult Version. CI, confidence interval. SES, socioeconomic status.

[a] ACEs total was the sum of experiences that occurred before the age of 18 collected via a retrospective, self-report questionnaire. Experiences included the following: physical abuse, emotional abuse, sexual abuse, witnessing domestic violence, living with an individual with drug or alcohol abuse, living with an individual with mental illness, living with an individual who had been incarcerated and/or experiencing separation from parents. The sum could range from 0-8.

[b] Childhood household SES is defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1.

[c] Antidepressant use includes selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, norepinephrine/dopamine reuptake inhibitor (NDRIs) and benzodiazepines.

[d] Mental illness diagnosis includes Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD).

[e] Unhealthy eating includes eating French fries or fried potatoes, drinking pop or flavoured drinks that are not diet, eating fast food like hamburgers or pizza or chicken nuggets, eating sweets like chocolate bars or candy or cookies or pie or cake, and eating salty snacks like potato chips or corn chips or taco chips or crackers.

[f] Early phase cycle included females who indicated they were on or between day 1 and day 14 of their menstrual cycle. The reference group was males (n=108).

[g] Late phase cycle included females who indicated they were on or between day 15 and day 28 of their menstrual cycle. The reference group was males (n=108).

[h] No cycle includes females who had an irregular cycle, had an IUD, were missing cycle data, or did not menstruate. The reference group was males (n=108).

*p<0.05, **p<0.01, ***p<0.001

Regression of EF on ACEs and covariates stratified by sex

The tests for a sex x ACEs interaction in the full models which revealed no significant effect moderation (not shown), were taken to infer no statistically significant relationship between ACEs and EF across sex. However, sex-stratified regression models were still explored to assess any possible underlying differences in EF outcomes between males and females and between menstrual cycle among females. First, sex-specific models were created by regressing BRIEF-A measures on the ACEs threshold measure and all covariates except menstrual cycle to directly compare models. An additional set of regression models for each of the BRIEF-A measures were run again for females only including the menstrual cycle covariate with early cycle phase as the reference category. Note that the stratified samples were reduced samples thereby reducing the power to detect statistical significance and, as such, the focus will be on examining differences in coefficients across models with the full models presented above.

The overall models of BRIEF-A clinical measures Inhibit and Working Memory for males, females, and females with inclusion of menstrual cycle covariate were all statistically significant $p<0.01$ (Table 9-11). All models had similar coefficients for BRIEF-A clinical measures and ACEs threshold measure as well as BRIEF-A clinical measures and all covariates except for antidepressant use. Antidepressant use was significant in the Working Memory model for males with an average increase of 10.02 points. In the Working Memory model for females, antidepressant use had a negative relationship with a score very close to 0 ($b=0.05$) but was not statistically significant. Further, the difference in relationship between sex and antidepressant use in Working Memory score can be investigated by examining other covariates such as mental illness diagnosis. For both males and females, mental illness diagnosis resulted in significant

increase in the BRIEF-A Working Memory score, 4.85 and 4.78, indicating no difference between sex-specific models.

Additional stratified models across sex were examined using the three BRIEF-A composite measures, MI, BRI, and GEC, and the ACEs threshold measure along with covariates. All models using the BRIEF-A composite measures were statistically significant for both males and females and demonstrated a similar result $p<0.001$ (Table 12-14). For males, ACEs threshold measure was significant with the MI score only but the coefficient size for both male and female models was very similar to the full regression models above. The ACEs threshold measure remained significant in the BRI and GEC models for females regardless of inclusion of menstrual cycle covariate in the models and similar to the full models above (Table 13 & 14). The findings were consistent across sex and with the full regression analyses reported above.

Table 9. OSL regression of BRIEF-A inhibition and working memory clinical scales on ACEs threshold and covariates for males (N=108)

	BRIEF-A					
	Inhibit			Working Memory		
	b	**β**	**95% CI**	**b**	**β**	**95% CI**
Intercept	54.28***	0***	[44.45, 64.11]***	49.90***	0***	[39.58, 60.22]***
ACEs threshold[a]	0.97	0.14	[-0.39, 2.34]	1.89*	0.26*	[0.46, 3.33]*
Childhood household SES[b]	-1.00	-0.11	[-2.68, 0.68]	-0.25	-0.03	[-2.02, 1.51]
Antidepressant use[c]	4.15	0.09	[-5.01, 13.32]	10.02*	0.19*	[0.39, 19.65]*
Mental illness diagnosis[d]	3.70	0.17	[-0.79, 8.19]	4.80*	0.20*	[0.09, 9.51]*
Unhealthy eating[e]	0.55	0.15	[-0.12, 1.21]	0.29	0.07	[-0.41, 0.99]
	R^2=0.1412**			R^2=0.2101***		

Abbreviations: ACE, adverse childhood experience. BRIEF-A, Behaviour Rating Inventory of Executive Functioning Adult Version. CI, confidence interval. SES, socioeconomic status.

[a] ACEs total was the sum of experiences that occurred before the age of 18 collected via a retrospective, self-report questionnaire. Experiences included the following: physical abuse, emotional abuse, sexual abuse, witnessing domestic violence, living with an individual with drug or alcohol abuse, living with an individual with mental illness, living with an individual who had been incarcerated and/or experiencing separation from parents. The sum could range from 0-8.

[b] Childhood household SES is defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1.

[c] Antidepressant use includes selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, norepinephrine/dopamine reuptake inhibitor (NDRIs) and benzodiazepines.

[d] Mental illness diagnosis includes Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD).

[e] Unhealthy eating includes eating French fries or fried potatoes, drinking pop or flavoured drinks that are not diet, eating fast food like hamburgers or pizza or chicken nuggets, eating sweets like chocolate bars or candy or cookies or pie or cake, and eating salty snacks like potato chips or corn chips or taco chips or crackers.

*p<0.05, **p<0.01, ***p<0.001

Table 10. OSL regression of BRIEF-A inhibition and working memory clinical scales on ACEs threshold and covariates for females (N=128)

	BRIEF-A					
	Inhibit			Working Memory		
	b	**β**	**95% CI**	**b**	**β**	**95% CI**
Intercept	47.76***	0***	[39.54, 55.98]***	49.82***	0***	[40.50, 59.15]***
ACEs threshold[a]	1.05	0.16	[-0.08, 2.18]	1.23	0.16	[-0.05, 2.51]
Childhood household SES[b]	-0.94	-0.11	[-2.32, 0.45]	-1.42	-0.15	[-2.99, 0.15]
Antidepressant use[c]	1.00	0.04	[-4.14, 6.14]	0.05	0.00	[-5.78, 5.88]
Mental illness diagnosis[d]	4.40*	0.22*	[0.40, 8.39]*	4.66*	0.21*	[0.14, 9.19]*
Unhealthy eating[e]	0.93***	0.28***	[0.40, 1.46]***	1.01**	0.27**	[0.41, 1.60]**
	R^2=0.2202***			R^2=2114***		

Abbreviations: ACE, adverse childhood experience. BRIEF-A, Behaviour Rating Inventory of Executive Functioning Adult Version. CI, confidence interval. SES, socioeconomic status.

[a] ACEs total was the sum of experiences that occurred before the age of 18 collected via a retrospective, self-report questionnaire. Experiences included the following: physical abuse, emotional abuse, sexual abuse, witnessing domestic violence, living with an individual with drug or alcohol abuse, living with an individual with mental illness, living with an individual who had been incarcerated and/or experiencing separation from parents. The sum could range from 0-8.

[b] Childhood household SES is defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1.

[c] Antidepressant use includes selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, norepinephrine/dopamine reuptake inhibitor (NDRIs) and benzodiazepines.

[d] Mental illness diagnosis includes Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD).

[e] Unhealthy eating includes eating French fries or fried potatoes, drinking pop or flavoured drinks that are not diet, eating fast food like hamburgers or pizza or chicken nuggets, eating sweets like chocolate bars or candy or cookies or pie or cake, and eating salty snacks like potato chips or corn chips or taco chips or crackers.

*p<0.05, **p<0.01, ***p<0.001

Table 11. OSL regression of BRIEF-A inhibition and working memory clinical scales on ACEs threshold and covariates for females including cycle phase(N=128)

	BRIEF-A					
	Inhibit			Working Memory		
	b	**β**	**95% CI**	**b**	**β**	**95% CI**
Intercept	46.65***	0***	[38.16, 55.14]***	48.61***	0***	[39.07, 58.14]***
ACEs threshold[a]	1.04	0.16	[-0.10, 2.18]	1.16	0.15	[-0.12, 2.44]
Childhood household SES[b]	-0.93	-0.11	[-2.33, 0.46]	-1.47	-0.15	[-3.04, 0.10]
Antidepressant use[c]	1.43	0.05	[-3.75, 6.61]	0.66	0.02	[-5.16, 6.47]
Mental illness diagnosis[d]	3.87	0.19	[-0.19, 7.93]	3.87	0.17	[-0.69, 8.44]
Unhealthy eating[e]	0.90**	0.27**	[0.38, 1.43]**	0.96**	0.26**	[0.37, 1.56]**
Late phase cycle[f]	2.10	0.11	[-1.37, 5.58]	2.66	0.12	[-1.25, 6.56]
No cycle[g]	2.50	0.11	[-1.69, 6.69]	4.86*	0.18*	[0.17, 9.56]*
	R^2=0.2329***			R^2=0.2397***		

Abbreviations: ACE, adverse childhood experience. BRIEF-A, Behaviour Rating Inventory of Executive Functioning Adult Version. CI, confidence interval. SES, socioeconomic status.

[a] ACEs total was the sum of experiences that occurred before the age of 18 collected via a retrospective, self-report questionnaire. Experiences included the following: physical abuse, emotional abuse, sexual abuse, witnessing domestic violence, living with an individual with drug or alcohol abuse, living with an individual with mental illness, living with an individual who had been incarcerated and/or experiencing separation from parents. The sum could range from 0-8.

[b] Childhood household SES is defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1.

[c] Antidepressant use includes selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, norepinephrine/dopamine reuptake inhibitor (NDRIs) and benzodiazepines.

[d] Mental illness diagnosis includes Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD).

[e] Unhealthy eating includes eating French fries or fried potatoes, drinking pop or flavoured drinks that are not diet, eating fast food like hamburgers or pizza or chicken nuggets, eating sweets like chocolate bars or candy or cookies or pie or cake, and eating salty snacks like potato chips or corn chips or taco chips or crackers.

[f] Late cycle phase included females who indicated they were on or between day 15 and day 28 of their menstrual cycle. The reference group was females in early cycle.

[g] No cycle includes females who had an irregular cycle, had an IUD, were missing cycle data, or did not menstruate. The reference group was females in early cycle.

*p<0.05, **p<0.01, ***p<0.001

Table 12. OSL regression of BRIEF-A behaviour regulation index, metacognition index, and global executive composite on ACEs threshold and covariates for males (N=108)

	BRIEF-A								
	BRI			MI			GEC		
	b	β	95% CI	b	β	95% CI	b	β	95% CI
Intercept	47.29***	0***	[38.58, 56.00]***	48.76***	0***	[39.96, 57.55]***	47.98***	0***	[39.37, 56.60]***
ACEs threshold[a]	0.69	0.11	[-0.52, 1.91]	1.34*	0.21*	[0.11, 2.56]*	1.15	0.18	[-0.05, 2.35]
Childhood household SES[b]	-0.78	-0.10	[-2.28, 0.71]	-0.46	-0.06	[-1.96, 1.05]	-0.64	-0.08	[-2.11, 0.84]
Antidepressant use[c]	6.51	0.15	[-1.61, 14.64]	6.87	0.15	[-1.33, 15.08]	7.17	0.16	[-0.87, 15.20]
Mental illness diagnosis[d]	3.51	0.17	[-0.46, 7.49]	4.93*	0.23*	[0.91, 8.94]*	4.63*	0.22*	[0.70, 8.56]*
Unhealthy eating[e]	0.87**	0.26**	[0.28, 1.45]**	0.48	0.14	[-0.11, 1.08]	0.70*	0.21*	[0.12, 1.28]*
	R^2=0.1988***			R^2=0.2226***			R^2=0.2391***		

Abbreviations: ACE, adverse childhood experience. BRIEF-A, Behaviour Rating Inventory of Executive Functioning Adult Version. CI, confidence interval. SES, socioeconomic status.

[a] ACEs total was the sum of experiences that occurred before the age of 18 collected via a retrospective, self-report questionnaire. Experiences included the following: physical abuse, emotional abuse, sexual abuse, witnessing domestic violence, living with an individual with drug or alcohol abuse, living with an individual with mental illness, living with an individual who had been incarcerated and/or experiencing separation from parents. The sum could range from 0-8.
[b] Childhood household SES is defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1.
[c] Antidepressant use includes selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, norepinephrine/dopamine reuptake inhibitor (NDRIs) and benzodiazepines.
[d] Mental illness diagnosis includes Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD).
[e] Unhealthy eating includes eating French fries or fried potatoes, drinking pop or flavoured drinks that are not diet, eating fast food like hamburgers or pizza or chicken nuggets, eating sweets like chocolate bars or candy or cookies or pie or cake, and eating salty snacks like potato chips or corn chips or taco chips or crackers.
*p<0.05, **p<0.01, ***p<0.001

Table 13. OSL regression of BRIEF-A behaviour regulation index, metacognition index, and global executive composite on ACEs threshold and covariates for females (N=128)

	BRIEF-A								
	BRI			MI			GEC		
	b	β	95% CI	b	β	95% CI	b	β	95% CI
Intercept	46.52***	0***	[38.82, 54.22]***	49.01***	0***	[40.92, 57.09]***	47.70***	0***	[40.08, 55.32]***
ACEs threshold[a]	1.71**	0.25**	[0.66, 2.77]**	0.81	0.12	[-0.30, 1.92]	1.27*	0.19*	[0.22, 2.31]*
Childhood household SES[b]	-0.99	-0.11	[-2.29, 0.31]	-1.67*	-0.20*	[-3.03, -0.31]*	-1.50*	-0.18*	[-2.78, -0.21]*
Antidepressant use[c]	1.68	0.06	[-3.13, 6.49]	-1.66	-0.06	[-6.71, 3.39]	-0.03	-0.00	[-4.79, 4.73]
Mental illness diagnosis[d]	5.76**	0.28**	[2.02, 9.50]**	4.86*	0.25*	[0.93, 8.78]*	5.68**	0.29**	[1.98, 9.38]**
Unhealthy eating[e]	0.94***	0.28***	[0.44, 1.43]***	0.95***	0.29***	[0.43, 1.47]***	1.03***	0.31***	[0.54, 1.52]***
	R^2=0.3379***			R^2=0.2291***			R^2=0.3191***		

Abbreviations: ACE, adverse childhood experience. BRIEF-A, Behaviour Rating Inventory of Executive Functioning Adult Version. CI, confidence interval. SES, socioeconomic status.

[a] ACEs total was the sum of experiences that occurred before the age of 18 collected via a retrospective, self-report questionnaire. Experiences included the following: physical abuse, emotional abuse, sexual abuse, witnessing domestic violence, living with an individual with drug or alcohol abuse, living with an individual with mental illness, living with an individual who had been incarcerated and/or experiencing separation from parents. The sum could range from 0-8.
[b] Childhood household SES is defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1.
[c] Antidepressant use includes selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, norepinephrine/dopamine reuptake inhibitor (NDRIs) and benzodiazepines.
[d] Mental illness diagnosis includes Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD).
[e] Unhealthy eating includes eating French fries or fried potatoes, drinking pop or flavoured drinks that are not diet, eating fast food like hamburgers or pizza or chicken nuggets, eating sweets like chocolate bars or candy or cookies or pie or cake, and eating salty snacks like potato chips or corn chips or taco chips or crackers.
*p<0.05, **p<0.01, ***p<0.001

Table 14. OSL regression of BRIEF-A behaviour regulation index, metacognition index, and global executive composite on ACEs threshold and covariates for females including cycle phase (N=128)

	BRIEF-A								
	BRI			MI			GEC		
	b	β	95% CI	b	β	95% CI	b	β	95% CI
Intercept	44.79***	0***	[36.89, 52.68]***	47.57***	0***	[39.28, 55.86]***	46.04***	0***	[38.25, 53.83]***
ACEs Threshold[a]	1.76**	0.26**	[0.70, 2.82]**	0.80	0.12	[-0.32, 1.91]	1.28*	0.19*	[0.24, 2.33]*
Childhood Household SES[b]	-0.94	-0.11	[-2.23, 0.36]	-1.67*	-0.20*	[-3.03, -0.30]*	-1.47*	-0.17*	[-2.75, -0.19]*
Antidepressant Use[c]	2.25	0.08	[-2.57, 7.07]	-1.08	-0.04	[-6.14, 3.97]	0.57	0.02	[-4.18, 5.33]
Mental Illness[d]	5.10**	0.25**	[1.32, 8.88]**	4.15*	0.21*	[0.19, 8.12]*	4.96**	0.25**	[1.23, 8.69]**
Unhealthy Eating[e]	0.90***	0.27***	[0.41, 1.40]***	0.92***	0.28***	[0.40, 1.44]***	1.00***	0.30***	[0.51, 1.48]***
Cycle day 15-28[f]	3.03	0.15	[-0.20, 6.27]	2.77	0.14	[-0.62, 6.16]	3.05	0.16	[-0.14, 6.24]
No cycle[g]	2.29	0.10	[-1.60, 6.18]	3.47	0.15	[-0.62, 7.55]	3.09	0.13	[-0.75, 6.93]
	R^2=0.3573***			R^2=0.2528***			R^2=0.3428***		

Abbreviations: ACE, adverse childhood experience. BRIEF-A, Behaviour Rating Inventory of Executive Functioning Adult Version. CI, confidence interval. SES, socioeconomic status.

[a] ACEs total was the sum of experiences that occurred before the age of 18 collected via a retrospective, self-report questionnaire. Experiences included the following: physical abuse, emotional abuse, sexual abuse, witnessing domestic violence, living with an individual with drug or alcohol abuse, living with an individual with mental illness, living with an individual who had been incarcerated and/or experiencing separation from parents. The sum could range from 0-8.
[b] Childhood household SES is defined as the highest level of education attained by a parent or guardian living in the same household as the participant at NLHS time=1.
[c] Antidepressant use includes selective serotonin reuptake inhibitors (SSRIs), serotonin/norepinephrine reuptake inhibitors (SNRIs), tricyclic antidepressants, norepinephrine/dopamine reuptake inhibitor (NDRIs) and benzodiazepines.
[d] Mental illness diagnosis includes Attention Deficit Disorder/ Attention Deficit Hyperactivity Disorder (ADD/ADHD), Anxiety, Conduct Disorder (CD), Depression, Obsessive Compulsive Disorder (OCD), Panic Disorder, Eating Disorder, Anorexia Nervosa, Avoidant Personality Disorder, Borderline Personality Disorder (BPD), Post Traumatic Stress Disorder (PTSD), Chronic PTSD, Oppositional Defiant Disorder (ODD).
[e] Unhealthy eating includes eating French fries or fried potatoes, drinking pop or flavoured drinks that are not diet, eating fast food like hamburgers or pizza or chicken nuggets, eating sweets like chocolate bars or candy or cookies or pie or cake, and eating salty snacks like potato chips or corn chips or taco chips or crackers.
[f] Late cycle phase included females who indicated they were on or between day 15 and day 28 of their menstrual cycle. The reference group was females in early cycle.
[g] No cycle includes females who had an irregular cycle, had an IUD, were missing cycle data, or did not menstruate. The reference group was females in early cycle.
*p<0.05, **p<0.01, ***p<0.001

Chapter 5: Discussion

The results of the current study found that the accumulation of ACEs was associated with poorer EF but not lower serum BDNF levels in young adulthood. The first hypothesis was supported with higher exposure to ACEs associated with higher BRIEF-A scores and therefore lower EF among young adults. For every ACE experienced, the BRIEF-A standardized scores increased by a value of approximately 1 point adjusting for other covariates. The BRIEF-A measures used in analysis were standardized T-scores and values that are 1.5 standard deviations (i.e., 15 points) above the mean (i.e., 50) are defined as abnormally elevated[64]. Based on these values, each ACE exposure is contributing to allostatic overload and that, as these exposures accumulate, it leads to alterations that are observable in young adulthood. While the relationship between ACEs and EF was found to be statistically significant, there were no significant findings in relation to ACEs or BRIEF-A measures and BDNF. Serum BDNF was measured at a single time point when participants were between the ages of 18 and 25 years old. There was a significant, inverse correlation between age and BDNF (Table 3) suggesting that younger individuals in the sample may still be cognitively developing at the time of testing[68]. Although BDNF cannot be used to explain the association between ACEs and EF at this time it may be more important earlier[48] or later in the life course which is discussed further below.

The prevalence of ACEs in the current study was higher than prevalence rates reported in previous studies[1,24,26]. In the current sample, 83% of participants reported experiencing at least one ACE which was higher than the Alberta ACEs study that reported 55.8%[2], and the original ACEs study by Felitti and colleagues that reported 63.9%[1]. There were 8 possible ACEs an individual could report experiencing in the current study and were defined as any of the three types of abuse, physical, sexual, or emotional, and/or any of the five types of household

dysfunction previously discussed. Felitti and colleagues found that as ACEs accumulated the number of people with higher exposures became fewer and fewer and led them the create a threshold measure of ≥ 4[1]. However, this threshold measure is not consistently used across all studies and for that reason the total sum of ACEs, ranging from 0-8, and the threshold measure ≥ 4 were both used in the current study. Based on the results from the correlation table and the regression models, these measures were very similar and both ACEs measures resulted in consistent, similar findings. This was expected as previous literature has also found no difference in outcomes regardless of using a continuous ACEs measure or ACEs threshold measures further supporting that ACEs have a robust effect on various health outcomes[10,12]. This is important to note because, regardless of measure used in the current study, the results similarly found that the accumulation of ACEs resulted in poorer EF.

These findings are important because they link poor EF among young adults to reported experiences occurring in childhood suggesting a long-term effect of ACEs. Consistent with the literature in both children and adults, exposure to accumulation of abuse and household dysfunction was associated with generally poorer EF[37,38] and specifically poorer inhibitory control[37,39] and working memory[37,40,41]. Inhibition was poorer when exposed to ACEs, suggesting that, when faced with stressful situations, individuals will struggle to inhibit impulses and may act on more highly emotional behavioural responses compared to those with greater inhibitory control[39]. Exposure to ACEs also lead to poorer BRI scores, which includes the Inhibit clinical scale, as well as Shift, Self Monitor, and Emotional Control clinical scales, indicating that individuals with accumulation of ACEs are less able to regulate behaviour and emotions. An explanation for this greater difficulty to regulate emotions could be due to greater connectivity in the amygdala, which has been associated with chronic exposure to stressful experiences and

inability to halt the fear response in animal models[30]. The Working Memory clinical scale and the MI are involved in self-management and self-awareness which is important for planning for the future. The main areas involved in these EF constructs are the prefrontal cortex (PFC) and hippocampus. Structural changes in the PFC due to early life stress have previously been linked to poorer spatial working memory[43]. Decreased connections in these brain areas have been associated with exposure to chronic stressful experiences in animal models and structural changes observed in human studies may explain the behavioural changes observed[30,31]. Based on the animal models and supporting evidence in human models, changes due to allostatic overload caused by ACEs in childhood may negatively impact development and EF in the long term as observed in the current sample of young adults.

The consequences of ACEs on poor EF further presents in deficits in other areas of life such as one's personal and social life. Psychosocial outcomes could be an inability to maintain relationships, act appropriately in social settings, or manifest as the reduced ability to cope with stressful situations. Linking these deficits to poorer EF and back to ACEs creates a more holistic understanding of why individuals are having negative health outcomes later in life. Mental illness, for example, has been strongly associated with exposure to ACEs and the inability to regulate emotions and behaviour through EF may further manifest and amplify these symptoms[9,11]. Individuals with mental illness diagnoses would already be experiencing difficulty with domains of EF such as emotional regulation and inhibition. Further, negative health behaviours such as eating, smoking and higher alcohol use have been identified as coping mechanisms to deal with the enduring effects of ACEs[1]. An explanation may be that a reduced ability to regulate emotions lead to a greater likelihood of seeking out these risky health-

behaviours that provide immediate, positive emotions regardless of the future, long-term negative effects[8].

Unhealthy eating choices was a highly significant covariate across all models in the current study. This relationship could have a physiological explanation or a psychological explanation. Specifically, unhealthy eating choices have a physiological impact on functioning, but it could also be another health-risk behaviour whereas consuming unhealthy foods serve as a psychosocial coping mechanism as a result of both ACEs and poor EF. The positive emotions felt when eating these unhealthy foods may serve as a coping mechanism due to lack of ability to regulate emotions[8] and the nutrition, or lack of nutritional value in these foods, also play a role in brain function[59]. However, there was no significant relationship between unhealthy eating choices and BDNF (see Correlations in Table 3) or BMI (results not shown), so these potential physiological pathways do not appear to be present in this young adult cohort. Through a public health lens, unhealthy eating is considered a negative health behaviour and leads to negative health outcomes such as obesity. Felitti has provided insight on how "negative" behaviours including smoking and alcohol and drug use may be an attempt at a personal solution as a coping mechanism for an individual who has experienced ACEs[69]. Seeking out positive emotional and physiological responses to things such as eating, smoking and alcohol use to stimulate the release of neurotransmitters, is an example of what Felitti discussed in this "public health paradox"[69]. The public health paradox describes that, at a population health level, these health behaviours are viewed as negative, but at an individual level, these health behaviours are positive, coping mechanisms, providing some relief from traumatic or stressful experiences[69]. Importantly, the current public health focus of targeting change in these "negative" health behaviours without examining the underlying context as to why these behaviours occur in the face of ACEs does not

resolve the deep-rooted trauma and may lead individuals to just substitute other potentially harmful behaviours to provide similar immediate, positive emotional stimuli or physiological responses.

The literature has also linked ACEs to mental disorders[1,53] which may relate to poor EF. In this analysis, mental illness diagnosis and antidepressant use were self-reported by participants in the NLHS and these variables were included as covariates in the analysis. The effect of having a mental illness diagnosis on BRIEF-A scores was significantly associated with poorer EF across all models independent of the relationship between ACEs and BRIEF-A scores. With the exception of the ACEs threshold and BRIEF-A Working Memory model for males, there was no significant relationship between antidepressant use in any of the BRIEF-A models. The inclusion of antidepressant use was to consider the possible enhancements or benefits of mood regulating medications on behavioural outcomes in EF measures and the physiologically impact on BDNF[6,56,57]. However, there was no significant relationship between mental illness or antidepressant use and BDNF. Based on these findings, it appears that presence of mental illness was an important factor in the models and was related to EF as well as to ACEs (see correlation table, Table 3). However, as these data are cross-sectional, it is not possible to examine whether mental illness is a concomitant outcome of ACEs associated with and connected with EF or whether it is somehow involved in the causal process either as an intervening outcome or a final outcome affected by EF.

The dichotomous variable for mental illness collapses all reported mental illness diagnoses as opposed to examining each diagnosis individually. As such, the measure combined several different diagnoses ranging from depression, anxiety, and eating disorders to ADD/ADHD and autism. This was due to the limited sample size and reporting of mental illness

diagnosis for the entire sample and the stratified sex samples. There was no statistically significant association between sex and self-report of mental illness diagnosis, but there was a significant difference in antidepressant use between females (15.6%) and males (4.6%). The differences in antidepressant use and the small sample size for males reporting antidepressant use may be the reason for the highly significant relationship between poorer working memory and antidepressant use among males. Although no physiological differences were found between antidepressant use and BDNF, potential sex differences may need to be considered when examining longer term outcomes.

The relationship between BDNF and ACEs is not well understood and there are currently no longitudinal studies measuring changes in BDNF over time in relation to exposure. There have been cross-sectional studies that have examined different age groups and exposures. The current study examined young adults and adds to this literature with significant differences in BDNF levels reported among children and adolescents but not among adults. Researchers examined serum BDNF in children and adolescents (2-17 years) who were exposed to sexual abuse compared to matched controls[48]. The participants who had experienced sexual abuse, regardless of severity, had significantly lower BDNF than controls[48]. Another important finding in this study was that multiple experiences of sexual assault, or an accumulation of exposures, was also significantly associated with lower BDNF[48]. The results run counter to the current study that did not find a significant relationship between BDNF and ACEs in young adults (mean age= 22.6 years). But similar to results reported here, a sample of middle age adults (mean age 37.7 years) who were exposed to child maltreatment also showed no significant differences in BDNF nor in brain morphology[49]. The significant findings in children may be due to either the proximity of measures taken to the time of exposure or due to the developmental stage where the

PFC is on the upward developmental trajectory in the life-course inverted U-shaped developmental curve. The lack of differences across ACE exposure detected among both young and middle-aged adults could be due to BDNF and plasticity levels varying over time in relation to this inverted U-shaped trajectory where development has slowed, reached an apex, or started to recede[49]. As such, there may be significant differences in how individual's BDNF levels vary over time which may also be experience-dependent but would not be captured in a cross-sectional analysis. That is, current life stress and changes in stress may also be influencing BDNF measures taken in adulthood and genetic polymorphisms in the BDNF gene play a further role in expression[70]. Sex differences across the lifespan would also be important to highlight and changes in hormonal regulation, specifically in females before menarche and after menopause which could influence BDNF[51].

In addition to BDNF, the influence of sex hormones and sex differences in brain developmental outcomes when exposed to accumulation of stressful experiences has also been identified in the animal models[44,54]. In the current study, females who were in the first 14 days of their cycle had significantly lower BRIEF-A scores, therefore better EF, compared to males while those in the late cycle and the other group were not statistically different. As a result, spikes in estrogen in the early phase of the female cycle may explain the significant difference in function compared to males[50]. And while there were no overall sex differences found in the relationship between ACEs and EF, there could be potential differences depending upon the cycle stage and estrogen levels which warrants further investigation as estrogen has been linked to BDNF expression and function[44]. The direction of each interaction in the models were the same indicating no difference in how ACEs and other covariates influenced BRIEF-A scores

between males and females. Moreover, there was also no significant relationship between sex and BDNF or menstrual cycle phase and BDNF.

Strengths and Limitations

The main strengths of the current study were the use of detailed, consistent data collected in the NLHS and the combination of questionnaire measures with biological samples to investigate the relationship between ACEs and EF. Although the current study was cross-sectional, the data collection process was extensive and included a number of measures such as mental illness diagnosis, medication use, and health behaviours which were used as covariates in the current analysis. The inclusion of these covariates in the full models provided supporting evidence as to the strength of the connection between the accumulation of ACEs and poorer EF. Further, a measure of serum level BDNF was also used in an attempt to examine one potential biological mechanism that could account for the relationship between ACEs and EF in young adults.

The current wave of the NLHS collected data during young adulthood and the measures of the main outcome variables, ACEs and EF, were unique to the current study. Previous studies examining outcomes of ACEs in adulthood focused on middle adulthood or old age[1,2,26]. The current sample consisted of individuals 18-25 years old allowing these young adults to recall experiences earlier and more proximal to the time of exposure to ACEs. The ACEs measure included maltreatment and household dysfunction and captures a variety of stressful exposures during the first 18 years of life. The measures of EF in young adulthood were constructed from the BRIEF-A questionnaire as opposed to participants completing lab tests to measure EF. The BRIEF-A scores provide a snapshot of individuals in their everyday life and how well they are able to utilize EF. Support for using the BRIEF-A in the current analysis was the successful

loading of all clinical and composite measures in the factor analyses along with supporting validity assessments conducted by the creators of the scale[64,66].

There are several limitations that need to be considered in the current study. First, data collected using self-report questionnaires are subject to self-report bias and recall bias. ACEs data relied on individuals' retrospective self-reports of ACEs in the first 18 years of life. These highly sensitive questions regarding ACEs were in the latter part of the self-report questionnaire as recommended in work assessing response bias in ACEs[60]. Even though these data were collected from young adults who should not be prone to recall bias to the same extent of older adults, this information is still subject to recall bias and self-report bias. With respect to self-reporting bias, the researchers worked to create a comfortable atmosphere and positive relationship during testing prior to individual and confidential completion of the questionnaire, but individuals may still be reluctant to provide information on ACEs. However, there is previous work supporting that when researchers create a safe, comfortable environment, people are willing to report these experiences[63].

The cross-sectional study design and the sample size were also limitations as conclusions drawn from the data are correlational in nature. There were no significant relationships between BDNF and ACEs or EF. The single time point for sample collection was during young adulthood and participants may have not yet fully developed cognitively but we were unable to examine change. The time when ACEs were experienced was not collected in the current study and this may have also been a limitation when analyzing BDNF. Lower BDNF was found in children and adolescents, 8-17 years old, who were exposed to child sexual abuse[48]. And the data were collected at a mean time of 22.72 months since the first occurrence of sexual abuse[48].

Finally, the sample size also limited the ability to conduct the fully powered, sex-stratified models. While the statistical interaction between sex and ACEs was nonsignificant, the main goal of the stratified analysis was to examine if there were any anomalies across sex and across menstrual cycle. An ad hoc analysis of the necessary sample size for adequate statistical power was n=300 which was not available for the stratified sex models. In fact, the full sample available for analysis was only 80% of what would be an ideally powered test of the relationship between ACEs and EF lending greater confidence to the strength of the overall findings observed in this study.

Future Directions

Moving forward, researchers should continue to study the impact of ACEs on EF over time and how these changes may influence psychosocial outcomes and early mortality. Preferably, researchers and clinicians should focus on longitudinal studies to measure changes in EF over time. A large, prospective cohort study including individuals ranging from high to low risk of exposure to ACEs with multiple data collection time points including multiple data sources during childhood, adolescence, young adulthood, middle adulthood and old age would capture changes in EF development and function across the lifespan and allow for a better understanding of the potential underlying mechanisms. ACEs need to be recognized and identified as proximal to exposure as possible. Identifying initial exposures would allow researchers to measure the timing and severity of ACEs. More importantly, informing clinicians would allow them to intervene and provide support to prevent long term, negative health outcomes as well as prevent continued exposure to ACEs.

The current study used the BRIEF-A questionnaire to measure EF in everyday life and had consistent findings with the literature and studies using performance on EF lab tests. For this

reason, researchers should continue to use the BRIEF-A questionnaire in future studies along with EF lab tests. Measures of brain function, such as electroencephalogram and brain imaging, such as functional magnetic resonance imaging, should also be collected during EF tests. Combining measures of EF performance and corresponding brain function would allow us to better examine the changes in structure and function that has been observed in the animal models. Finally, although no differences were observed in this cross-sectional study, serum BDNF should be measured across multiple time points as well as other biological markers such as cortisol to understand changes in stress response and plasticity due to exposure to ACEs and, ideally, serum collected proximal to ACE exposures to support previous studies[48]. Continuing to collect serum BDNF levels over time would explain how ACEs could influence EF and neurodegeneration across the life course and in old age.

Conclusion

In conclusion, higher accumulated ACE exposure was associated with lower EF in young adulthood across both clinical and composite constructs. Exposure to stressful experiences or stressful environments creating allostatic overload in childhood play a role in development and lead to maladaptation to structure and function. These structural and functional maladaptation become embedded which is why negative outcomes, such as poor EF, are being observed into adulthood. Specifically, poorer inhibition, working memory, behaviour regulation, metacognition, and overall functioning were all associated with higher exposure to ACEs. Moreover, there were no sex differences in the relationship between ACEs and EF, indicating that the accumulation of ACEs leading to poorer EF affects males and females similarly. Finally, there was also no association between ACEs, EF, or sex with BDNF in the current sample of young adults. However, further work should examine BDNF over time to assess changes. To

conclude, the current study adds to the growing literature that not only are ACEs occurring, but they are also having a lasting, long-term impact on individual health and development.

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Appendix

Table a1. Factor loadings from the principal component analyses (PCA) for the BRIEF-A Inhibit and the Working Memory clinical measures

Inhibit	
Items (BRIEF-A item number)	**Factor Loading**
I have trouble sitting still (16)	67
I am impulsive (73)	65
People say I am easily distracted (55)	65
I rush through things (58)	64
I make decisions that get me into trouble (legally, financially, socially) (43)	53
I have problems waiting my turn (29)	50
I make inappropriate sexual comments (36)	43
I tap my fingers and bounce my legs (5)	56
Eigenvalue	2.72[1]
Working Memory	
Items (BRIEF-A item number)	**Factor Loading**
I have a short attention span (35)	72
I forget instructions easily (46)	72
I have trouble doing more than one thing at a time (68)	67
I forget what I'm doing in the middle of things (17)	65
I have trouble remembering things, even for a few minutes (such as directions, phone numbers) (56)	65
I have trouble with jobs or tasks that have more than one step (11)	63
I have trouble staying on topic when talking (26)	63
I have trouble concentrating on tasks (such as chores, reading or work) (4)	61
Eigenvalue	3.49[2]

[1]The second highest eigenvalue for the Inhibit clinical scale items was 1.19.
[2]There was no second factor in the for the Working Memory clinical scale items.