The Influence of HIV and ART exposure on neonate brain volumes

By

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DECLARATION

I, *AISHAH OLAGUNJU*, hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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ABSTRACT

Introduction: There is growing evidence that maternal health in pregnancy influences the infant neurodevelopment. However, there are limited studies including broad metrics of maternal health when studying typical and atypical infant neurodevelopment.

Although the human immunodeficiency virus (HIV) is a global pandemic, South Africa has the biggest and most high-profile HIV epidemic. Over the past decade, South Africa has reduced vertical transmissions of HIV, from improvements in antiretroviral medicines and the widespread accessibility of prevention programs. An outcome of this achievement is an increasing population of HIV-exposed-uninfected (HEU) infants and children.

Despite improved outcomes compared to their peers living with HIV, HEU infants and children are at risk of neurodevelopmental delays in relation to HIV–unexposed–uninfected (HUU) children. As a result, there is a need to better understand the outcome of HIV/ART exposure on the fetal/infant/child brain. This study aimed to investigate the effect(s) of HIV and duration of ART exposure and the potential impact of additional maternal health factors during pregnancy on neonate brain volumes.

Methods: Using magnetic resonance imaging (MRI), T1-weighted brain images of neonates were acquired. Infants included those whose mothers initiated ART preconception (HEU-pre), those whose mothers initiated ART post-conception (HEU-post) and infants born to mothers living without HIV or HIV unexposed uninfected (HUU). The data were quality checked and volumes were determined using the infant FreeSurfer tool. Statistical analysis was done in R to identify maternal health factors related to neonatal volumes as well as volumetric group differences due to HIV and ART exposure.

Results: This analysis included 151 infants (49 HEU-pre; 48 HEU-post; 54 HUU; mean age 1.8 weeks; 50.3% male). Across all newborns, maternal Harvard Trauma Questionnaire (HTQ) score during pregnancy was associated with bilateral amygdala volumes. Within HEU infants, maternal CD4 count was associated with right thalamus and caudate volumes bilaterally.

Group analysis showed a significant decrease in mean caudate volume bilaterally in the HEU-post group (left hemisphere p=0.006; right hemisphere p=0.009), as well as reduced right amygdala volume after controlling for maternal HTQ assessed in pregnancy. There was also a significant increase in the left lateral ventricle (p=0.04) and a decrease in the left cerebral white matter of HEU-pre infants (p=0.03).

Conclusion: This study observed volumetric differences in HEU infants dependent on timing of maternal ART initiation, maternal immune health and maternal trauma assessed during pregnancy.

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ADHD - Attention deficit hyperactivity disorder

ART - Antiretroviral therapy

ASD - Autism spectrum disorder

ASL - arterial spin labelling

BAEP - Brainstem Auditory Evoked Potentials

CMV – cytomegalovirus

CNR - contrast to noise ratio

CNS - Central nervous system

CSF - cerebrospinal fluid

CT – computed tomography

CUBIC - Cape Universities Body Imaging Centre

DTI- diffusion tensor imaging

FA - Fractional Anisotropy

FDR – False discovery rate

fMRI - functional MRI

GA – Gestational Age

GABA - Gamma-aminobutyric acid

GM – Grey Matter

HEU - HIV-exposed-uninfected

HIV - human immunodeficiency virus

HTQ - Harvard Trauma Questionnaire

HUU- HIV–unexposed–uninfected

IL-6 - interleukin-6

IQ – Intelligence quotient

MD - Mean diffusivity

MRI - Magnetic resonance imaging

MRS - magnetic resonance spectroscopy

MTCT - Mother-to-child transmission

NIH – National Institutes of Health

OPC - Oligodendrocyte precursor cells

PCR- Polymerase chain reaction

PWLH – Pregnant women living with HIV

RF - Radio Frequency

RNA – Ribonucleic Acid

ROI – Region of interest

SIDS - Sudden infant death syndrome

SNR - signal-to-noise ratio

TE – Echo Time

TNF-α - Tumor necrosis factor-alpha

TR - Repetition Time

TS - Tourette's syndrome

TTE – Time to echo

VL - Viral load

WM – White Matter

INTRODUCTION

Although the human immunodeficiency virus (HIV) is a global pandemic, South Africa with an estimated 7.7 million people living with HIV in 2018 has the biggest and most widely recognized HIV epidemic in the world (UNAIDS 2019). Over the past decade, South Africa has made great progress in reducing mother-to-child transmission (MTCT) of HIV. lmproved antiretroviral medications and the widespread accessibility of the prevention MTCT (PMTCT) program have contributed significantly to the reduction of MTCT (SANAC, 2014). The rate of MTCT stood at 1.3% in 2017, down from 3.6% in 2011 (Department of Health, 2017). The success of reducing MTCT of HIV however led to an unexpected outcome of a growing population of HIV-exposed-uninfected (HEU) infants and children. At present, the long-term effects of perinatal HIV and ART exposure (and time of administration) on the developing infant/child are not well understood. It has been shown that despite improved outcomes compared to their peers living with HIV, HEU infants and children continue to exhibit neurodevelopmental delay compared to HIV–unexposed–uninfected (HUU) children, particularly in resource-poor settings. (Le Doare et al., 2012, Marina et al., 2019, Rana, 2021). As a result, there is a need to gain a deeper understanding of the outcome of HIV/ART exposure on the fetal/infant/child brain.

A common method to measure the possible effects of HIV/antiretroviral therapy (ART) exposure on the developing infant is the administration of neuropsychological tests. There are many neuropsychological studies published in HEU populations that examine a wide range of cognitive domains. These studies make use of different scales to measure developmental outcomes. The results from neurodevelopmental studies of HEU and HUU infants and children have been inconsistent. In some studies, HEU infants demonstrate deficits across domains - lower scores in language, cognition, motor functions and behavioral domains (Forehand et al., 1998, Dorsey et al., 1999, Sanmaneechai et al., 2005, Van Rie et al., 2009, Kerr et al., 2014). Other studies find no significant exposure difference across developmental domains (Williams et al., 2010; Sirois et al., 2013; Ngoma et al., 2014; Chaudhury, 2017). Combining the results of multiple studies is a useful way to better understand data across studies and pull out the most relevant outcomes. A recent meta-analysis of neurocognitive outcomes in HEU infants reported

this population was at a higher risk of deficits in gross motor and expressive language outcomes (Wedderburn et al., 2022).

Brain imaging using magnetic resonance imaging (MRI) is another tool to study the influence of HIV/ART exposure in infants and children. An advantage of neuroimaging techniques is the ability to understand the influence of ART and HIV exposure on the developing brain of HEU infants earlier than neurocognitive tests. This allows for earlier interventions. In addition, repeated brain imaging can be used to prospectively study changes in structure, function and metabolism unlike neurocognitive tests which are often biased if repeated. Disadvantages of brain imaging studies include a lack of direct relationships to specific brain functions or behaviors and the wide range of outcomes due to various modalities (such as brain volumes, functional/structural connectivity, and metabolite levels). This latter point makes combining the results of studies to draw broader conclusions challenging.

Of the few imaging studies in pediatric populations, the majority report subtle brain changes in HEU compared to HUU infants/children, echoing findings from neurocognitive studies. Altered microstructural integrity of major white matter tracts, altered localized metabolism as well as diffuse hyper intensity in the white matter and tegmentum have been reported in HEU infants/children compared to their HUU counterparts (Madzime et al., 2021; Jankiewicz 2017; Robertson, 2018; Graham et al., 2020; Tardieu et al., 2005; Tran et al., 2016; Yadav, 2020). Two studies report no difference in metabolic and structural outcomes between HEU and HUU children (Jahanshad et al., 2015, Holmes et al., 2017). Recent studies report reduced total grey matter (Wedderburn et al., 2022), smaller caudate (bilateral) and left putamen volumes in HEU infants (Wedderburn et al., 2022, Ibrahim et al., 2023).

The conclusion from neuropsychological and neuroimaging studies is that there are subtle effects of HIV/ART exposure on the developing brain across a range of ages and measures. However, it is not yet clear whether the exposure-related outcomes reported previously are directly linked to in utero exposure to HIV and/or ART, or indirectly influenced by familial and/or environmental factors. Or a combination of multiple factors. More research is needed

that includes the duration of ART exposure in utero, as well as other possible maternal/environmental factors when studying the brain of HEU infants/children.

Maternal depression and trauma have been found to have a significant impact on infant outcomes, including neurodevelopment measures. According to the American Psychiatric Association, trauma refers to a psychological or emotional reaction to a distressing or disturbing event. It can be the result of a single event, or it can be the result of ongoing experiences (American Psychiatric Association, 2013). While depression is defined as a mental state characterized by long-lasting emotions of sadness, despair, and a lack of enthusiasm or enjoyment in daily activities (American Psychiatric Association, 2013). Maternal depression and trauma can influence each other and co-occur. Maternal trauma can increase the risk of developing depression. Similarly, maternal depression can make it more difficult to cope with the effects of trauma and may exacerbate symptoms of post-traumatic stress disorder (National Institute of Mental Health, 2021).

MRI studies have shown that maternal depression is associated with changes in the brain structure of infants and young children (Rifkin-Graboi et al., 2013, Wen et al., 2017, Pellowski et al., 2023). Other studies have investigated the relationship between maternal depression and trauma and infant neurodevelopment, including cognitive, emotional, and behavioral outcomes. These studies showed that prenatal maternal depression were associated with low levels of motor, cognitive and socio-emotional skills (Nomura et al., 2019; Davis et al., 2015).

One study using MRI found that infants of mothers with depression had smaller brain volumes in several regions, including the hippocampus and amygdala, which are important for memory and emotion regulation. Additionally, these infants had reduced white matter integrity in the corpus callosum, which is a structure that serves to connect the left and right hemispheres of the brain (Rifkin-Graboi et al., 2013). Another study found that infants exposed to maternal depression in utero had lower scores on measures of cognitive development and increased risk for developmental delays (Canadian Paediatric Society, 2004). Studies have also shown that maternal depression and trauma can affect the quality of mother-infant interactions and the

emotional regulation of the child, leading to behavioral and emotional problems such as anxiety and aggression (Kim et al., 2009).

Overall, research suggests that maternal depression and trauma can influence infant brain development and are important factors to consider when studying neurodevelopmental outcomes.

In addition to the potential role of maternal mental health on the developing fetus or newborn, the maternal immune system may also affect the developing brain. Maternal CD4 count, which is a blood test that measures the number of CD4 cells (T helper cells) has been noted to impact fetal/infant neurodevelopment (Wedderburn et al., 2022). CD4 cells are a type of white blood cell that plays a key role in the immune system's response to infection, and it is used to monitor the progression of HIV infection and management of ART (WHO, 2021). A healthy CD4 count typically ranges between 500 and 1,500 cells/mm3 (Battistini-Garcia and Guzman, 2022). In people living with HIV, the virus attacks and kills CD4+ T cells, leading to a decline in CD4 count over time. This is a characteristic feature of HIV infection and a major contributor to the weakened immune system seen in individuals living with HIV (Vohra et al., 2020). As CD4 count declines, the possibility of developing infections caused by opportunistic pathogens and other complications increases.

Viral load refers to the quantity of HIV RNA (viral genetic material) present in an individual's bloodstream. Viral load is an important indicator of how well HIV treatment is working, as effective ART can suppress viral replication and reduce viral load(Ford & Chiller, 2022). As viral load decreases, CD4 count tends to increase, indicating that the immune system is becoming stronger. There is a growing body of literature examining the effects of maternal CD4 count (or viral load) on the outcomes of HEU infants, particularly in terms of neurodevelopment.

Ramey et al., 2016 found that lower maternal CD4 count at delivery was associated with lower cognitive and motor development scores in HEU infants at 6 and 18 months of age (Ramey et al., 2016). Other studies observed that higher maternal viral load during pregnancy was associated with lower cognitive development scores in HEU infants at 12 and 18 months of age (Crume et al., 2019, Gustafson et al., 2019). The authors suggest that this association may be

due to the increased risk of maternal morbidity and mortality associated with higher viral load, which may negatively impact the infant's cognitive development.

Overall, these studies suggest that lower maternal CD4 count or higher viral load during pregnancy may be associated with negative neurodevelopmental outcomes in HEU infants. The association may be due to the increased health risks of a weakened immune system, which may in turn negatively impact the infant's cognitive and motor development. However, more research is needed to gain a complete understanding of the correlation between maternal HIV status and HEU infant neurodevelopmental outcomes.

Fetal brain development occurs throughout pregnancy, with different stages of development happening during each trimester. As a result, disruptions to the fetal environment – from maternal infections, mental health, or medications – at different time periods may result in different deficits or outcomes. Women aware of their HIV status who become pregnant may already be on treatment, meaning their newborn will have been exposed to antiviral medication for 9 months. However, women unaware of their HIV status will not begin treatment until later in their pregnancy. In this case, the developing fetus will have been exposed to ART for a shorter period. In both scenarios, we need to consider the potential influence of antiviral and HIV exposure in the appropriate time period.

During the first trimester (week 1-12) the neural plate undergoes a folding process to form the neural tube, which will eventually become the brain and spinal cord. The brain begins to divide into different regions, and by the end of the first trimester, the brain has three primary vesicles: the prosencephalon (forebrain), mesencephalon (midbrain), and rhombencephalon (hindbrain). Neural stem cells in the neural tube generate both neurons and glia, including oligodendrocyte precursor cells (OPCs) that will eventually mature into oligodendrocytes (Dubois et al., 2014)

In the second trimester (week 13-24), the cerebellum and cerebrum form. The cerebral hemispheres and the brainstem begin to take shape, and the brain's surface begins to fold, creating the characteristic wrinkles and furrows of the mature brain. Myelination of white matter also begins. The brain's neural connections also start to form, and by the end of the

second trimester, the fetal brain is capable of producing primitive reflexes such as sucking and grasping (Lebel and Deoni, 2018).

During the third trimester (week 25-40), the cerebral cortex and thalamus form. The cerebellum also undergoes significant development. Rapid growth and maturation of the cerebral cortex occurs, and myelination continues in subcortical white matter. By the end of the third trimester, the brain has reached approximately 80% of its adult weight (Kostovid et al., 2006, Moore et al., 2017)

This thesis adds to the small body of brain imaging literature in this population. It builds on published work by Ibrahim et al., which used manual segmentation to study a subset of volumes. Using a larger sample, this work will validate the results reported by Ibrahim et al. using an automated segmentation tool. As manual segmentation is time consuming, previous work was limited in the volumes examined. This work will also examine the whole brain and further examines the possible role of maternal trauma in pregnancy as a confounder. While Wedderburn et al. recently published a volumetric study of HEU infant volumes in a similar sized cohort, their work did not segment the entire brain and has a smaller population of HEU infants. The cohort presented by Wedderburn et al. included a heterogeneous ART regimen, making it harder to interpret findings around ART initiation. This study will add additional outcomes to the findings of both studies, and present complementary results.

This study determined anatomical volumes in a cohort of HEU and HUU neonates from the same community using an automated segmentation program (Infant FreeSurfer). The thesis aims to answer the following questions:

(1) Do maternal health factors during pregnancy (CD4 count in mothers living with HIV and trauma scores) influence neonate brain volumes?
(2) Does duration of ART in pregnancy influence brain volumes in neonates exposed to HIV in utero?
(3) Does HIV and ART exposure in utero influence neonate brain volumes?

In order to accomplish these aims, this thesis has the following associated hypotheses based on previous literature:

(1) Across all neonates, a higher level of trauma in pregnancy will result in larger amygdala volumes.
(2) Within HEU neonates, maternal CD4 count in pregnancy – a measure of immune system health – will negatively relate to basal ganglia volumes.
(3) Within HEU neonates, longer ART exposure in utero will be neuroprotective.
(4) After accounting for potential maternal and infant factors, HEU neonates will have reduced white matter and basal ganglia volumes compared to unexposed newborns.

These aims will be accomplished by working through the following objectives:

- Data curation and qualities checks,
- Segmentation using infant FreeSurfer,
- Quality check FreeSurfer outputs,
- Determine infant and maternal factors related to neonate volumes,
- Run correlations and build linear regression models to explore associations between neonate volumes and maternal CD4 count, duration of ART (in HEU infants) and maternal trauma scores in pregnancy (in all infants),
- Run correlations and build linear regression models to identify in utero HIV/ART exposure effects on volumes.

This dissertation comprises four chapters:

The first chapter reviews the relevant literature. This includes an overview of fetal brain development, the potential influence of maternal HIV/ART on fetal neurodevelopment and imaging studies on the effect(s) of maternal HIV/ART exposure in pediatric populations.

The second chapter recounts the study methodology, materials and design. This section includes the characteristics of the study population, inclusion/exclusion criteria, data acquisition, image processing, as well as statistical data analysis.

The third chapter presents the results of the study.

The fourth chapter discusses the results of the study and how they fit into the existing literature. It also includes study limitations and suggestions for future research.

1. LITERATURE REVIEW

1.1 HIV and Pregnancy

Fetal brain development

The central nervous system (CNS) starts to develop around the middle of the third week from the neural plate, which arises from the embryonic ectoderm thickening. The cranial two-thirds of the neural plate (which is flat and plate-like in structure) represents the future brain, while the caudal one third represents the future spinal cord. The neural plate begins to fold-in gradually, giving rise to the neural groove, which is bordered on both sides by the neural folds. During the fourth week, the neural folds on both sides begin to close up in the cranial and caudal directions, transforming the neural groove into the neural tube. The neural tube consists of a lumen (known as the neural canal), and the walls. The neural canal develops into the central canal of the spinal cord and the brain ventricular system, while the brain and spinal cord arise from the neural tube walls. Figure 1 below gives a diagrammatic representation of the neurulation process.

Fusion of the neural folds gives rise to three primary vesicles: prosencephalon (the forebrain), mesencephalon (the midbrain) and rhombencephalon (the hindbrain). By the fifth week, five secondary brain vesicles arise from the primary vesicles:

- The forebrain divides into the (i) telencephalon which is the future cerebrum, and (ii) diencephalon which gives rise to the thalamus, hypothalamus, epithalamus, subthalamus and pretectum

- The midbrain remains a single vesicle, and develops into the midbrain

- The hindbrain gives rise to the (i) metencephalon which becomes the pons and cerebellum, and (ii) myelencephalon which develops into the medulla oblongata.

Figure 2 gives a diagrammatic sketch of the brain vesicles as well as their adult derivatives.

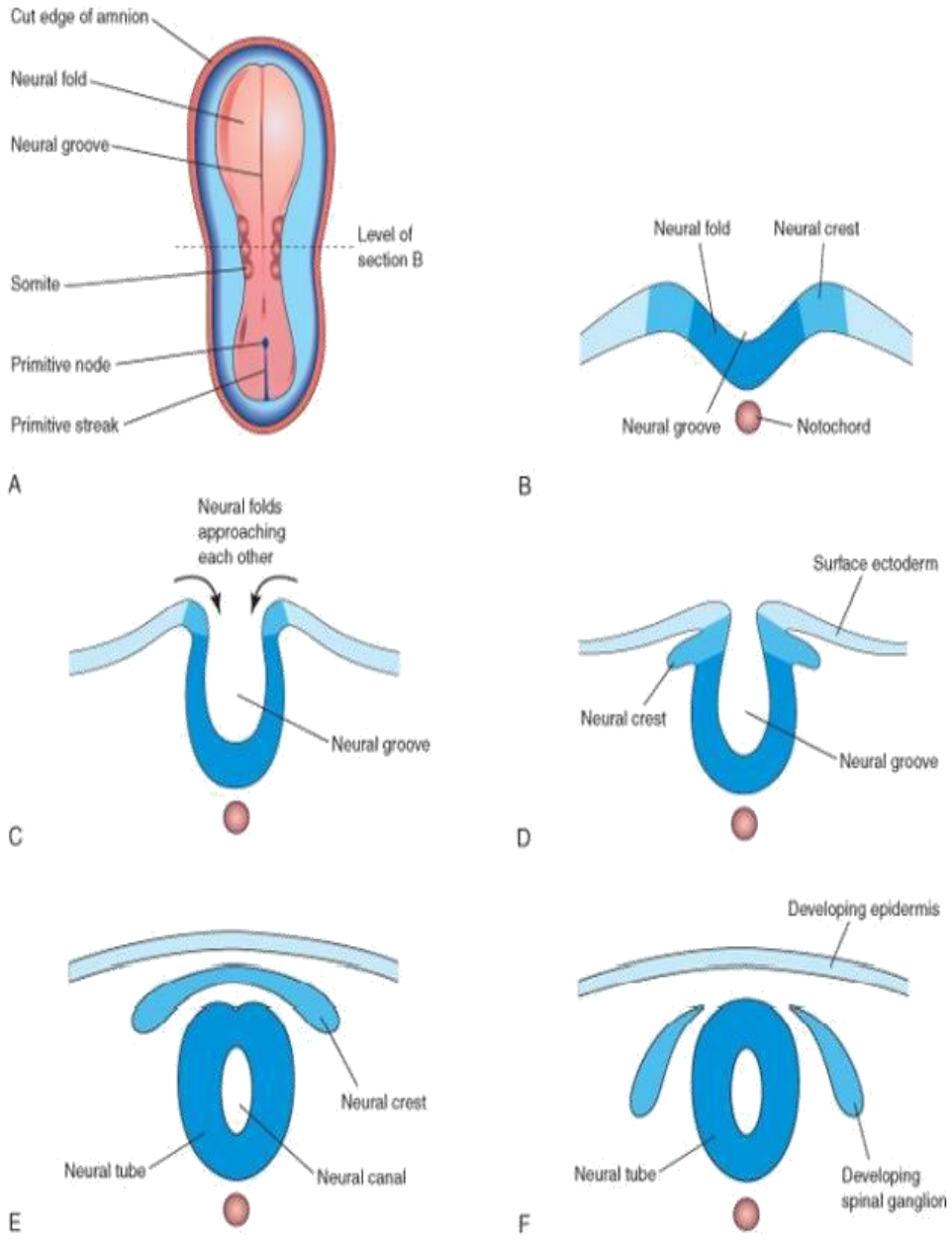


Figure.1 Diagrammatic Representation of Neurulation (Moore & Persaud, 2008).

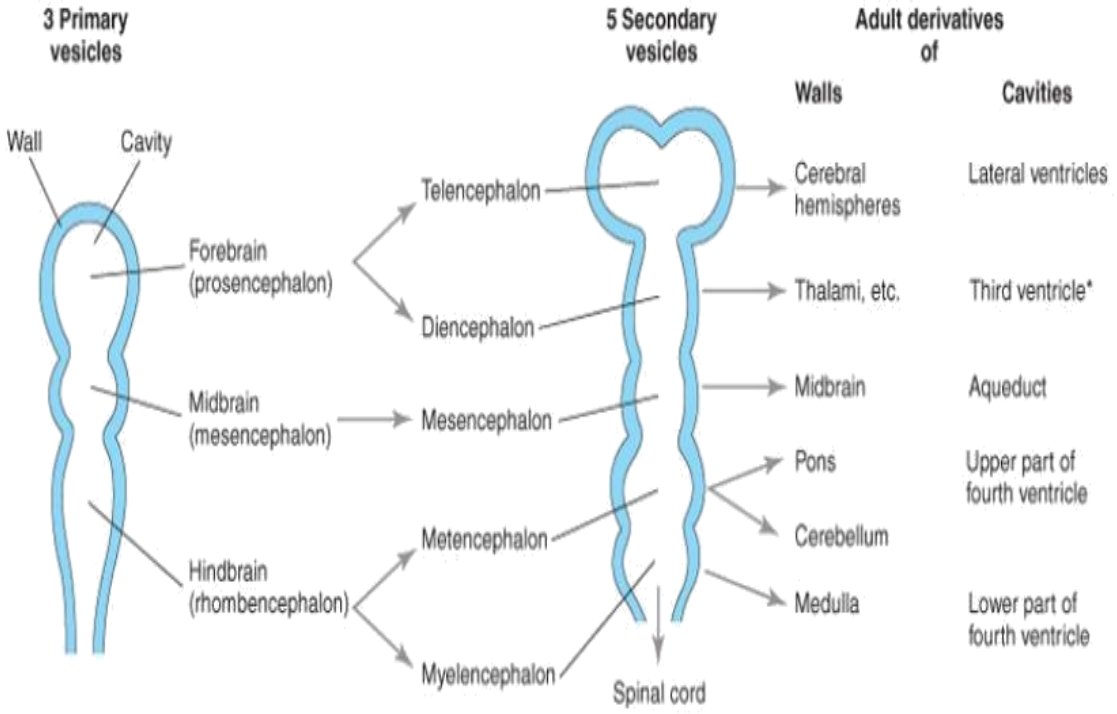


Figure 2. Brain Vesicles and adult derivatives (Moore & Persaud, 2008) *The anterior portion of the third ventricle is formed by the cavity of the telencephalon, while the majority of this ventricle is derived from the cavity of the diencephalon.

Neural stem cells in the ventricular zone of the neural tube give rise to both neurons and glia that will eventually differentiate into mature oligodendrocytes. Axons begin to grow and extend from newly formed neurons and oligodendrocyte precursor cells (OPCs) begin to differentiate into mature oligodendrocytes to produce myelin sheaths that insulate axons (Dubois et al., 2014). Myelination is most pronounced in the brainstem and spinal cord, but also occurs in the cerebellum and subcortical white matter. The corpus callosum, a major white matter tract that connects the two hemispheres of the brain, undergoes significant growth and myelination in the last trimester. Myelination of the prefrontal cortex, a brain region critical for executive function, does not fully mature until early adulthood (Lebel and Deoni, 2018).

Factors that can disrupt typical fetal development

Fetal development is a complex and intricate process that involves a multitude of factors that can affect the growth and development of the fetus. The typical fetal development process can be disrupted by various factors.

Genetic abnormalities are one of the main factors that can disrupt fetal development. They can occur spontaneously or be inherited from the parents, and can affect various aspects of fetal development, such as the formation of organs and tissues. Genetic abnormalities that can disrupt fetal development include chromosomal abnormalities, such as Down syndrome, and gene mutations, such as cystic fibrosis (American College of Obstetricians and Gynecologists, 2016).

Maternal health conditions can also disrupt fetal development. Conditions such as gestational diabetes, hypertension, and maternal infections can affect fetal growth and development. For example, gestational diabetes can cause excessive fetal growth, while maternal infections such as rubella can cause congenital abnormalities (American College of Obstetricians and Gynecologists, 2019).

Lifestyle factors, such as maternal smoking and alcohol consumption, can also disrupt fetal development. Maternal smoking can lead to low birth weight and respiratory problems, while alcohol consumption can cause fetal alcohol syndrome, which can result in cognitive and developmental problems (National Institute on Alcohol Abuse and Alcoholism, 2021).

Environmental toxins are another factor that can disrupt fetal development. Exposure to toxins such as lead, mercury, and pesticides can have adverse effects on fetal development. For example, exposure to lead can lead to cognitive impairment, while exposure to mercury can cause neurological and developmental problems (Grandjean & Landrigan, 2014).

In conclusion, fetal development can be disrupted by various factors, including genetic abnormalities, environmental toxins, maternal health conditions, and lifestyle factors.

Maternal infections and how they can disrupt fetal brain development

Maternal infections during pregnancy have been associated with adverse effects on fetal brain development. Infections in pregnancy can lead to inflammation and other immune system responses that can interfere with normal fetal brain development, potentially leading to long-term cognitive and behavioral problems in the child.

Maternal infections that have been associated with disruptions in fetal brain development include cytomegalovirus (CMV), rubella, toxoplasmosis, and Zika virus, among others (Gale & Berardo, 2017). These infections can cause inflammation and immune responses that affect the developing brain (Shimizu et al., 2023), leading to changes in neuronal migration, synaptic connectivity, and neural circuitry (Hwang et al., 2019, Elgueta et al., 2022). These changes can result in cognitive and behavioral problems later in life, such as learning disabilities, attention deficit hyperactivity disorder (ADHD), and autism spectrum disorder (ASD) (Brown, 2011).

The exact mechanisms through which maternal infections disrupt fetal brain development are not yet fully understood. However, studies have suggested that the immune response to these infections, including the production of cytokines and other inflammatory molecules, can contribute to the disruption of normal brain development (Meyer et al., 2012). These molecules can interfere with the migration of neurons and the formation of synapses, as well as disrupt the balance of neurotransmitters in the developing brain (Fatemi & Folsom, 2015).

In addition to the direct effects of maternal infections on fetal brain development, maternal stress and anxiety related to these infections can also affect fetal brain development. Stress hormones such as cortisol can cross the placenta and affect the developing brain, potentially leading to changes in brain structures and functions such as the hippocampus, prefrontal cortex and the amygdala (Sandman & Davis, 2012).

In conclusion, maternal infections during pregnancy can have adverse effects on fetal brain development. Understanding the mechanisms through which these infections disrupt brain development is essential for developing interventions to prevent or mitigate their effects.

Potential maternal mechanisms influencing HEU neurodevelopment

HEU infants have been reported to be at increased risk of morbidity, mortality and neurodevelopmental delays compared to HUU infants (Slogrove et al., 2018, Le Doare et al., 2012). These risks may begin in pregnancy or may be a result of events that occur postnatally. It is likely a combination of factors that occur across time. In addition to the factors outlined above, mothers living with HIV may be at an increased from additional factors which may affect the developing fetus or infant. The following are potential mechanisms through which maternal HIV status could impact the development of HEU infants:

1. Exposure to HIV: The negative impact of maternal HIV infection on the uninfected developing brain is well established, although the impacts are of lower intensity compared to their counterparts living with HIV (Le Doare et al., 2012; Hoare et al., 2014). Reduced maternal CD4 count and increased viral load have also been linked to increased mortality/morbidity in HEU infants (Brahmbhatt et al., 2006).

2. Exposure to ART: In utero exposure to ART regimens can also have an impact on the developing fetus, with studies suggesting that certain antiretroviral drugs may be associated with increased risk of adverse birth outcomes and neurodevelopmental delays in HEU infants (Zash et al., 2018). However, isolating the impact of ART alone is difficult because it involves differentiating between the effects of HIV and of ART on fetal development (Desmonde et al., 2016). Some studies have associated ART exposure with poor growth, prematurity and metabolic disturbance (Powis et al., 2011; Jao et al., 2014; Hofer et al., 2016). Other studies have found no adverse effect of ART on fetal development (Petra, 2002; Piske et al., 2018). Certain ART regimens have been found to potentially have solitary impacts (individual regimen having different side effects) on fetal development. Atazanavir and tenofovir have been associated with reduced language scores and lower bone mineral content respectively (Caniglia et al., 2016; Siberry et al., 2015).

3. Maternal inflammation: Maternal inflammation, which is common in pregnant women living with HIV, has been linked to adverse outcomes in HEU infants, including impaired growth and increased risk of infection (Krogh et al., 2015). Inflammatory agents can increase the permeability of the fetal blood–brain barrier, allowing infectious/inflammatory agents into the brain. Inflammatory agents in fetal blood can reach the fetal brain, triggering the release of cytokines which impacts cell migration and axonal growth. (Dalitz et al., 2003; Krogh et al., 2015).

4. Maternal immune activation (MIA): HIV infection causes chronic immune activation, and this activation can be transmitted from the mother to the fetus (Bbosa et al., 2021). Maternal immune activation during pregnancy has been linked to adverse outcomes in HEU infants, including impaired growth and increased susceptibility to infections (Stylianou 2009, Filteau, 2010). The proposed mechanism for MIA-induced alterations in fetal neurodevelopment is the presence of pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). These cytokines are produced by activated maternal immune cells and can cross the placenta to reach the developing fetus (Smith et al., 2007). Studies in animal models have shown that prenatal exposure to elevated levels of these cytokines can lead to behavioral and cognitive deficits in offspring, including impaired social interaction and memory function (Meyer, 2014). Studies have also shown that exposure to maternal cytokines can lead to changes in the expression of genes involved in neuronal development, synaptic plasticity, and neurotransmitter signaling (Estes et al., 2016).

5. Maternal microbiome: The maternal microbiome, which is the collection of microorganisms that live on and in the human body, has been shown to play a role in infant health (Yao, 2021). HIV infection and ART use during pregnancy can alter the maternal and infant microbiome, which may impact the development of the infant's immune system and metabolism (Bender, 2016). Alterations in the maternal

microbiome during pregnancy, such as bacterial vaginosis, have been associated with adverse outcomes in HEU infants, including increased risk of infection (Hsiao et al., 2019). However, Fouda and colleagues observed that certain vaginal bacteria, including Lactobacillus crispatus, were associated with better neurodevelopmental outcomes in HEU infants. They also found that HEU infants born to mothers with a higher diversity of vaginal microbiota had lower cognitive scores at 12 months (Fouda et al., 2021). Some studies have also suggested that the gut-brain axis may be involved in the relationship between the maternal microbiome, the infant gut microbiome, and neurodevelopmental outcomes in HEU infants (Ragonese et al., 2019, Denny et al., 2020, Caparros-Martin et al., 2019). One study noted that HEU infants with a higher abundance of Bifidobacterium in their gut microbiome had better neurodevelopmental outcomes at 1 year of age, including improved cognitive and motor development (Ragonese et al., 2019). Another study reported that HEU infants with a less diverse gut microbiome had higher levels of inflammatory markers in their plasma, suggesting that alterations in the gut microbiome may contribute to neuroinflammation in HEU infants (Caparros-Martin et al., 2019).

6. Maternal Infection: Mothers living with HIV have been found to be at increased risk for certain infections, CMV, rubella and syphilis (Lim et al., 2019), which have been documented to impact infant/child development. These infections could interfere with normal oxygen supply to the fetal brain, which has the potential to injure brain neurons as adequate oxygen is required for normal fetal brain development (Dalitz et al., 2003; Panchaud et al., 2016; Filteau and Rowland-Jones, 2016).

7. Maternal mental health: Maternal HIV infection is associated with higher rates of depression and anxiety, which can have negative effects on infant development through disruptions to the mother-infant relationship and caregiving behaviors (Mellins et al., 2019).

8. Maternal micronutrient deficiencies: Maternal nutrition plays a crucial role in infant brain development (Chaparro & Suchdev, 2019). Micronutrient deficiencies, such as of vitamin A, zinc, and selenium, are common in pregnant women living with HIV and have been associated with poor outcomes in their infants, including increased risk of infection and mortality (Kawai et al., 2010). Micronutrients such as iron, zinc, and vitamin B6 are essential for the synthesis and metabolism of neurotransmitters such as dopamine, serotonin, and gamma-aminobutyric acid (GABA), which are critical for brain development and function (Georgieff et al., 2011). Deficiencies in these micronutrients during pregnancy may lead to reduced neurotransmitter synthesis and function, which could impair neurodevelopment in offspring (Gernand et al., 2016).

9. Socioeconomic factors: Maternal HIV infection is often associated with poverty, food insecurity, and other socioeconomic factors that can impact infant development through their effects on maternal health, stress, nutrition and access to resources (McCoy DC et al., 2018). Studies have found an association between low household income, maternal education and lower cognitive, motor and language scores in HEU infants at 12 and 18 months of age (Goga et al., 2015, Fitzgerald et al., 2019). Another study also observed that low socioeconomic status is associated with higher levels of exposure to environmental toxins such as lead and air pollution, which can have negative effects on neurodevelopment (Guxens et al., 2012).

1.2 Regions of Interest

This study looked at cerebral grey and white matter, subcortical structures, brain ventricles and the brain stem.

Grey matter

Like the rest of the brain, grey matter develops from the ectoderm and increases in volume until about the age of eight (Staudt et al., 2019). After year eight, volume decreases as grey

matter density increases which allows for high processing and more complex cognitive abilities (Mercadante and Tadi, 2020).

Grey matter refers to the collection of neuronal cell bodies within the CNS, and it gets its grey color tone (and hence its name) from the high concentration of neuronal cell bodies. The cerebral cortex refers to the grey matter that surrounds the cerebrum, while those that lie deep in the brain are known as nuclei. Due to the high number of neurons present, the brain can process and transmit new information using axon signaling in its white matter (Chiao et al., 2020). Grey matter also contains glial cells, axon tracts, neuropil (glia, dendrites, and unmyelinated axons), as well as capillary blood vessels (Jiang et al., 2015). Because this part of the brain is involved in movement, memory and emotions, injury/abnormalities (including volume reduction) may affect these functions (Mercadante and Tadi, 2020).

White Matter

White matter is the collection of neuronal axons within the CNS. White matter contains myelinated axons (which has a high lipid fat content), giving it a whitish color from which its name is derived. Fatty myelin insulates axons, allowing faster signal transport. White matter fibers are distributed in tracts or bundles that extend out from the neuron bodies and connect the brain cells. The three classifications of WM fibers are:

(1) Association fibers connect different parts of the cerebral cortex of the same hemisphere to each other.

(2) Commissural fibers connect identical parts of the two hemispheres

(3) Projection fibers connect the cerebral cortex to other regions of the CNS.

Since white matter is responsible for axon protection and conducting nerve signals, damage to it can affect sensory and motor functions, as well as reactions to external stimuli (Han et al., 2017).

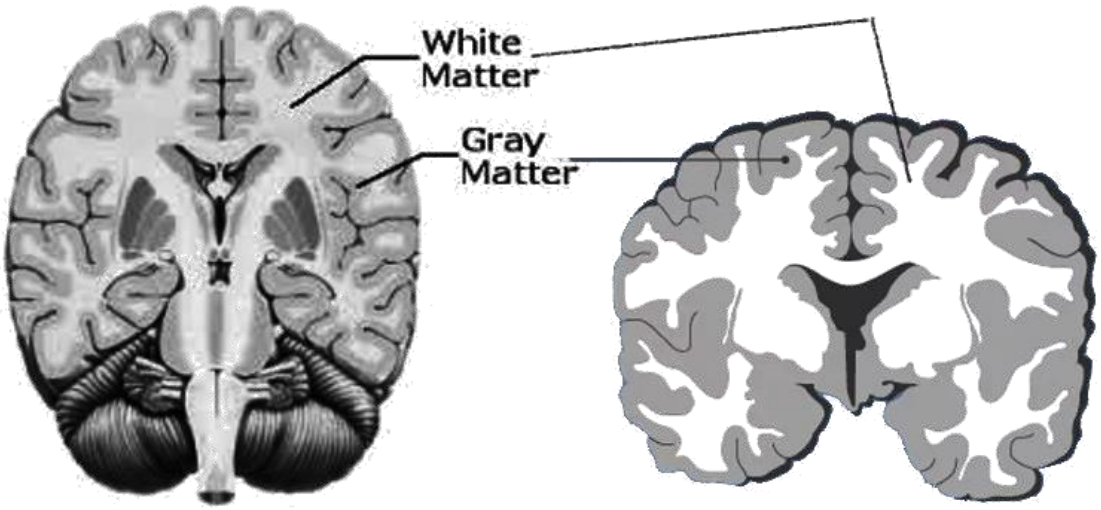


Figure1-3 White and Gray Matter (Mercadante and Tadi 2020)

Subcortical Structures

These are a group of diverse structures that lie deep within the cerebral hemispheres and are closely related to the ventricular system. These structures play a critical role in regulating a wide range of physiological and cognitive processes, including movement, emotion, motivation and autonomic functions. Subcortical structures have been found to undergo notable volumetric changes (increase) in children, and structural abnormalities have been associated with psychiatric disorders (Raznahan et al., 2014, Koshiyama, 2018). The subcortical structures of the brain include the thalamus, hypothalamus, basal ganglia, brainstem and amygdala.

The thalamus is located in the diencephalon and is a relay station for sensory information going to the cerebral cortex (Torrico, 2022). The hypothalamus is located below the thalamus and is involved in the regulation of various bodily functions such as hunger, thirst, and temperature regulation (Anand and Dhikav, 2012). The thalamus and hypothalamus are situated above the third ventricle. Abnormal thalamus and hypothalamus volumes in infants have been associated

with a range of negative effects on development and behavior. Research has shown that infants with abnormal thalamus and hypothalamus volumes may be at an increased risk for a variety of developmental disorders, including autism spectrum disorder, ADHD, and intellectual disability (Hazlett et al., 2017). Furthermore, research suggests that abnormal thalamus and hypothalamus volumes may be linked to problems with regulation of bodily functions and emotions in infants. For example, one study found that infants with abnormal thalamus volumes had difficulties with the regulation of body temperature, while another study found that infants with abnormal hypothalamus volumes had difficulties with the regulation of appetite and sleep (Shaw et al., 2007, Smith et al., 2012).

The basal ganglia, which include the caudate nucleus, putamen, and globus pallidus, are located deep within the cerebral hemispheres and surround the lateral ventricles. They are involved in the control of movement and coordination. The caudate nucleus is particularly known to control voluntary skeletal movement, and also play a role in learning, memory and reward (Driscoll et al., 2022). One study found that infants with abnormal basal ganglia volumes were at a higher risk for developmental delays and cognitive impairment (Girard et al., 2011). Additionally, another study found that infants with larger basal ganglia volumes were more likely to develop ASD later in life (Wolff et al., 2012). Also, abnormalities in the basal ganglia have been linked to other neurodevelopmental disorders such as ADHD and Tourette's syndrome (TS) (Kates et al., 2016).

The brainstem, which includes the midbrain, pons, and medulla oblongata, connects the brain to the spinal cord and is involved in the control of various functions such as breathing, heart rate, and blood pressure. The brainstem surrounds the fourth ventricle. Abnormal brainstem volumes in infants can have a significant impact on their development and overall health. One study found that infants with smaller brainstem volumes were at a higher risk for developmental delays and neurological disorders, such as cerebral palsy (Limperopoulos et al., 2008). Another study found that infants with abnormal brainstem volumes were more likely to have lower cognitive scores and developmental milestones compared to infants with normal brainstem volumes (Limperopoulos et al., 2009). Furthermore, abnormal brainstem volumes

have been associated with an increased risk of sudden infant death syndrome (SIDS) (Kinney et al., 1994).

The amygdala is an almond-shaped structure that is located deep within the temporal lobes of the brain. It is known to regulate negative emotions such as anxiety, fear, aggression and emotional memory (AbuHasan et al., 2022). Abnormalities in amygdala volume in infants can have significant effects on their emotional development and behavior. One study found that infants with larger amygdala volumes at 6 months of age were more likely to display fearful behavior at 12 months of age (Fox et al., 2007). Another study found that infants with smaller amygdala volumes at 6 months of age were more likely to display less social engagement and less positive emotion (Gunnar, 1996). Additionally, research has found that infants with abnormal amygdala volumes may be at a higher risk for developing anxiety and mood disorders in later childhood and adolescence (Monk, 2008).

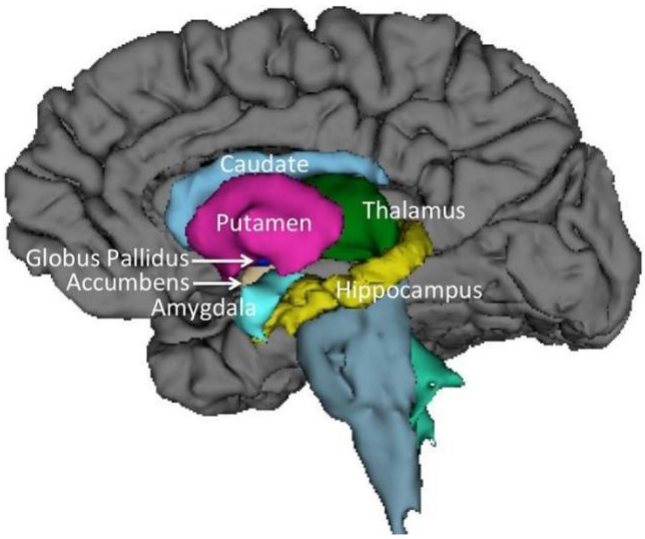


Figure 1-4 Subcortical brain structures (wikipedia)

1.3 HIV exposure and neurocognitive outcomes

HIV exposure can have a negative impact on neurocognitive development in infants and children, leading to risks of impairments across various domains. Research studies are typically focused on specific age ranges, in order to look at different aspects of brain development. Neurocognitive studies in young children report risks of deficits across a range of domains. A study in Kenya compared neurocognitive function in HEU children aged 5-12 years, observing that children exposed to HIV have significantly lower mean scores in cognitive ability, short-term memory, delayed memory, attention, and processing speed compared to their unexposed counterparts (Benki-Nugent et al., 2022). Another study found that HEU children showed deficits in executive function, visual-spatial processing, and visual-motor integration compared to unexposed children (Richardson et al., 2017). Bisiacchi et al. (2018) reported that HEU children showed lower scores in cognitive and language development compared to unexposed children. In children it is often difficult to determine if deficits are related to HIV exposure, or due to environmental factors such as parenting, nutrition and socioeconomics. To account for this, some studies control for potential confounding variables giving some indication that the consequences of HIV exposure on cognitive development are independent of environmental factors. After controlling for factors such as socioeconomic status and maternal IQ, studies found that HEU children had lower scores in memory and learning tests, cognitive processing speed and working memory (Bisiacchi et al., 2006, Smith et al., 2012). A 2018 meta-analysis reported that HEU children had poorer cognitive and motor scores as compared to their HUU counterparts (Mc Henry et al., 2018). In addition, the study found HEU children with exposure to ART had lower cognitive and motor scores than their HEU peers who were not exposed to ART (Mc Henry et al., 2018).

While environmental factors may play a role in infant neurodevelopment, they are potentially minimized. As a result, studies focused on infants may provide more insight into domains linked to HIV exposure. Similar to children exposed to HIV, prenatal exposure to HIV can affect a wide range of domains in infants. The following is a summary of domains reported by studies as being most affected in HEU infants:

-Attention and Executive Functioning: Infants exposed to HIV are at a higher risk of developing attention deficits and impairments in executive functioning such as planning, decision-making, and problem-solving compared to those who are unexposed (Smith et al., 2017). At 7-9 months of age, Laughton et al. (2013) noted that HEU infants had poorer scores on measures of attention.

-Language and Communication: HIV exposure can have negative effects on infants' language, leading to language and communication delays. A study of HEU infants at 18 months found that they had lower language scores (LeBlanc et al., 2015). Another study also observed that HEU infants at 12 months had lower receptive language (ability to understand, follow directions and respond) scores (Filteau et al., 2018).

-Motor Development: Infants exposed to HIV are more likely to experience motor delays, including delays in reaching, crawling, and walking, compared to infants who are not exposed. One study found that HEU infants had lower scores on standardized motor development tests at 6, 12 and 24 months of age (Llorente et al., 2013). This suggests that prenatal HIV exposure is associated with delays in achieving motor milestones and deficits in fine motor and coordination skills.

-Memory and Learning: HIV exposure has been linked to memory and learning impairments in infants, including difficulties in recalling information and retaining new information (Strathdee et al., 2010).

-Social-Emotional Development: Infants exposed to HIV are at a higher risk of developing social-emotional difficulties, such as decreased ability to form strong attachments, and a higher likelihood of developing behavioral problems (Wiener et al., 2010). Prenatal HIV exposure has also been associated with an increased risk of mental health problems such as anxiety and depression in HEU children (Mellins and Malee, 2013).

Although studies have reported risks of deficits across a wide range of domains, a recent systematic review and meta-analysis identified the cognitive areas in which HEU infants are at

the highest risk. The meta-analysis reported lower scores in expressive language and gross motor function (Wedderburn et al., 2022). Most mothers in the study were on ART, and they found no evidence of a treatment regimen effect (Wedderburn et al., 2022). Interestingly, both the meta-analysis in children (McHenry et al., 2018) and infants (Wedderburn et al., 2022) find motor function to be at high risk of deficit in relation to HIV exposure.

1.4 Neuroimaging Studies on HEU Infants

MRI

Magnetic resonance imaging (MRI) is a non-invasive investigative procedure for the safe assessment of the human brain. It was developed in 1976 by Dr. Raymond Damadian and colleagues. MRI is able to produce real-time high quality 3D-images from all planes (axial, coronal and sagittal) with the use of large magnetic fields.

The important components of MRI include a bore, coil, powerful computer system and patient table. The bore is a horizontal tube capable of producing stable, large magnetic fields. Modern MRI systems can create magnetic fields from 0.5-10.5 Tesla. The coil transmits radiofrequency waves, which the body absorbs and reflects back to be translated into an image on the computer system. MRI can be used to study various aspects of the brain based on the specific technique implemented. Both structural MRI and diffusion tensor imaging (DTI) provide structural information of the brain whereas functional MRI (fMRI), magnetic resonance spectroscopy (MRS) and arterial spin labelling (ASL) provide different measures of dynamic physiological information (Symms et al., 2004).

The difference in the water and fat content of white and grey matter enables their differentiation through visual or automated analysis using structural MRI. Structural MRI outcomes are frequently based on T2-and T1-weighted images. The contrast in MR images is determined by two parameters that are specific to the tissue being imaged - T1, which is the longitudinal relaxation time, and T2, which is the transverse relaxation time. T1 and T2 are two distinct time constants that govern the behavior of excited protons in a magnetic field. T1 is

indicative of the time required for spinning protons to align themselves with the external magnetic field and return to equilibrium, while T2 represents the time taken for the loss of phase coherence among the nuclei spinning perpendicular to the main field and the attainment of equilibrium or out-of-phase status for the excited protons.

The Repetition Time (TR) is the duration between the applications of successive pulse sequences on the same slice in MRI. On the other hand, the Echo Time (TE) or Time to Echo (TTE) refers to the interval between the delivery of the Radio Frequency (RF) pulse and the detection of the echo signal. Manipulation of TR and TE parameters enables the modification of MR image characteristics to highlight various tissue types. Short TE and TR durations are utilized in the production of T1-weighted images.

T1-weighted images are produced by using short TE and TR times while T2-weighted images are generated through extended TE and TR durations, where the T2 properties of the tissue largely dictate the contrast and brightness of the image. To differentiate between T1- and T2-weighted images, the brightness of cerebrospinal fluid (CSF) is assessed, as it appears dark in T1-weighted and bright in T2-weighted imaging (Preston, 2006).

Spatial resolution and signal-to-noise ratio (SNR) are the fundamental parameters that govern image quality. The resolution of an MRI image within a plane is primarily dictated by the number of pixels (picture elements) present in the frequency and phase encoding directions, while through-plane resolution is influenced by the thickness of the slice being imaged. SNR of the image is affected by various factors, including pixel size, slice thickness, scan time, and the imaging sequence utilized. Since the scan time is ultimately determined by the patient's ability to remain still during the procedure, it is a key determinant of the overall image quality (Symm et al., 2004).

In infant MRI images, however, the contrast is the reverse of that of children and adults (which is described above). On T1-weighted images, the intensity of white matter is lower than gray matter, while the intensity of the white matter is higher than gray matter on T2-weighted images (Oishi et al., 2013). This is because the majority of white matter is unmyelinated, and its water content is closer to adult gray matter (Weisenfeld and Warfield, 2009). Also, the smaller

size of the infant brain results in a smaller-scaled region of interest, making it necessary to create higher resolution images in order to appreciate details of the infant brain. The relatively reduced contrast in infant images leads to reduced contrast to noise ratio (CNR) in images (Mewes et al., 2006; Xue et al., 2007).

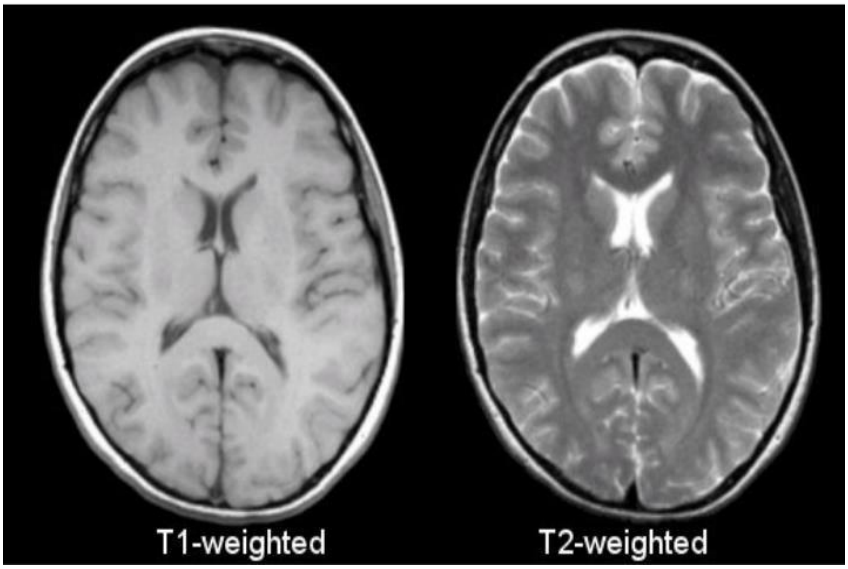


Figure 1-4 T1 and T2-weighted images (Preston 2006)

Imaging studies

Table 1 below summarizes imaging studies including HEU infants and children as well their HUU counterparts. Twelve studies examined HEU compared to HUU children using MRI. Eight of these studies were performed in South Africa (Tran et al., 2016, Jankiewicz et al., 2017, Holmes et al., 2017, Robertson et al., 2018, Graham et al., 2020, Madzime et al., 2021, Wedderburn et al., 2022, Ibrahim et al., 2023), one in Thailand (Jahanshad et al., 2015), one in the USA (Cortey et al., 1994), one in France (Tardieu, 2005) and one from India (Yadav et al., 2020). Five of these studies used DTI (Jahanshad et al., 2015; Tran et al., 2016; Jankiewicz et al., 2017, Yadav et al., 2020, Madzime et al., 2021) while four used MRS (Cortey et al., 1994, Holmes et al, 2017, Robertson et al., 2018, Graham, 2020) and three utilized used structural MRI (Tardieu et al., 2005, Wedderburn et al., 2022, Ibrahim et al.,2023).

Most MRI studies focused on or included white matter regions of the brain. In comparison to unexposed control participants, the HEU group demonstrated abnormal white matter properties in the middle cerebellar peduncles and right posterior corona radiata (Tran et al., 2016; Jankiewicz 2017). Altered white matter microstructural integrity of major white matter tracts, altered localized metabolism as well as diffuse hyperintensity in the white matter and tegmentum was also noted in the HEU group compared to their HUU counterparts (Tardieu et al., 2005, Tran et al., 2016). Yadav and colleagues also observed altered white matter integrity in the right fusiform, left postcentral gyrus, left pulvinar, left inferior temporal gyrus, left angular, right corpus callosum, left insula, left hypothalamus, right parietal lobe, right post central gyrus and right parietal lobe in the HEU group. They also noted this in the left extra nuclear, right superior frontal gyrus, left middle temporal gyrus, right inferior temporal gyrus, left cerebellar tonsil and right culmen in the HEU group. Altered neurometabolism was reported in both gray (basal ganglia and frontal lobe) and white matter regions of the brain in exposed children (Cortey et al., 1994; Robertson et al., 2018 Graham et al., 2020). Madzime et al (2021) noted altered white matter integrity within resting state functional networks, specifically the visual, motor and posterior default mode networks. Wedderburn et al., (2022) reported smaller caudate as well as reduced total grey matter volumes in the exposed group, while Ibrahim et al. (2023) observed reduced left putamen volume across HEU infants and smaller caudate volume in HEU-post infants only. Two studies also found an association between maternal immune health (CD4 count) with grey matter (Wedderburn et al.,2022) and caudate volumes (Ibrahim et al., 2023).

Both Jahanshad et al. (2015) and Holmes et al. (2017) reported no exposure related differences. All studies presented were cross sectional with the exception of Holmes et al. 2017, which studied metabolite levels longitudinally in young children (Holmes et al. 2017). The longitudinal design may account for the lack of HIV exposure results, as cross sectional outcomes may represent developmental delays that resolve with age.

One non-MRI neuroimaging study from Mexico looked at Brainstem Auditory Evoked Potentials and noted significant delay of wave I and I-III interwave intervals in HEU children, suggesting a negative impact on lower brainstem function (Poblano et al., 2004).

While 11 of the 13 studies report HIV exposure effects on the infant/child brain, they do not provide a consensus on the regions and specific effects of neonatal HIV exposure. This might be due to various factors such as difference in study design and imaging modalities, varying maternal viral load, socioeconomic factors as well as limited sample size mostly below 100. In addition, only two of the above studies included in utero ART duration measures and other factors in pregnancy that may influence the developing brain (Wedderburn et al., 2022, Ibrahim et al., 2023).

Table 1: Imaging studies on HEU and HUU infants

Reference	Country	Study Design	Imaging modality	Region(s) of Interest	Age at scan	Sample size	Conclusion
(Cortey et al., 1994)	USA	Cross-sectional	MRS	Parieto-Occipital white matter (WM)	1-10 days	HEU=5 HUU=5	Choline-to-creatine ratio is ↑ in HEU; NAA/creatine ratio is lower in HEU
(Poblano et al., 2004)	Mexico	Cross-sectional	BAEP	Brainstem	34-46 weeks	HEU=37 HUU=37	Latencies showed significant delay of wave I and I-III interwave interval in HEU
(Tardieu et al., 2005)	France	Cross-sectional	MRI	Supratentorial WM, pontine tegmentum, and BG	10-44 months	HEU=49	Diffuse hyperintensity in WM and tegmentum
(Jahanshad et al., 2015)	Thailand	Cross-sectional	DTI	Corpus callosum, whole brain WM	5-15 years	HEU=30 HUU=33	No observed group difference
(Tran et al., 2016)	South Africa	Cross-sectional	DTI	Cerebral, brainstem and cerebellar white matter	2-4 weeks	HEU=15 HUU=22	↑ FA in middle cerebellar peduncles
(Holmes et al., 2017)	South Africa	Longitudinal	MRS	Voxels in the frontal gray matter (GM), WM and (BG)	5, 7 and 10 years	HEU=29 HUU=35	No observed group difference
(Jankiewicz et al., 2017)	South Africa	Cross-sectional	DTI	Whole brain white matter	7 years	HEU=19 HUU=27	Cluster in the right posterior corona radiate with ↑ FA in HEU
(Robertson et al., 2018)	South Africa	Cross-sectional	MRS	Voxel in the BG	7 and 9 years	HEU=14 HUU=21	No group differences at age 7; HEU had ↓ NAA,

							glutamate/ choline and creatine at age 9.
(Yadav et al., 2020)	India	Cross-sectional	DTI	Cerebral GM and WM	8-12 years	HEU=12 HUU=18	↓ FA, ↑ MD in the right parietal lobe
(Graham et al., 2020)	South Africa	Cross-sectional	MRS	Voxels in BG, GM, WM	11 years	HEU= 30 HUU=30	Lower NAA in WM
(Madzime et al., 2021)	South Africa	Cross-sectional	DTI	WM tracts in resting state functional networks	7 years	HEU=19 HUU=27	↑ WM integrity in visual, motor and posterior default mode networks
(Wedderburn et al., 2022)	South Africa	Cross-sectional	MRI	Whole brain	2-6 weeks	HEU= 40 HUU= 106	↓ Caudate and total grey matter
(Ibrahim et al., 2023)	South Africa	Cross-sectional	MRI	Thalamus, BG, cerebellum, and vermis	2 weeks	HEU =79 HUU= 41	↓ Caudate and left putamen

***MRS** = Magnetic Resonance Spectroscopy, **MRI** = Magnetic Resonance Imaging, **BAEP** = Brainstem Auditory Evoked Potentials, **DTI** = diffusion tensor imaging, **FA =** Fractional Anisotropy, **MD** = mean diffusivity.

1.5 AntiRetroviral Therapy (ART)

ART refers to a combination of medications used to treat HIV infection. ART works by reducing the virus replication and suppressing the virus. This gives the immune system time needed to self-repair, thereby preventing the progression of the disease. ART has significantly improved the prognosis and quality of life of people with HIV since its introduction in the mid-1990s. According to UNAIDS, as of 2021, 28.7 million people were accessing ART globally, and the therapy had contributed to a 65% reduction in AIDS-related deaths since 2000 (UNAIDS, 2021). Although the therapy is not a cure for HIV, it has been shown to help people with HIV live longer, healthier and reduce the risk of transmitting the virus to others, including vertical transmission (WHO, 2016).

The effect of ART on newborn outcomes

ART is a critical component in lowering vertical transmission of HIV. One study observed that the use of ART during pregnancy was associated with a significant reduction in vertical transmission of HIV, from 28% to about 1% (Brown et al., 2015). It can further protect the infant from the negative consequences of maternal HIV. For example, ART use during pregnancy was associated with improved maternal health and a lower risk of preterm birth and low birth weight (Tincani et al., 2019, Patel et al., 2015).

While ART is generally safe during pregnancy and reduces the risk of vertical HIV transmission, it may also have adverse effects on the development of the uninfected infant. Studies have shown that ART exposure during pregnancy and infancy can have negative effects on cognitive development and IQ scores in HEU infants. Specifically, ART such as Efavirenz has been associated with decreased attention, motor skills and language development in infants (Nucifora et al., 2010; Squires et al., 2014, Fenton et al., 2011). One study found an increased risk of neural tube defects associated with ART use during the first trimester of pregnancy (Williams et al., 2015). Another study found an increased risk of preterm birth and neonatal

death in infants exposed to efavirenz, a common component of ART regimens (Zash et al., 2018).

The probable mechanism for these adverse effects is that ART may alter the developing brain's ability to produce, process, and respond to neurotransmitters and hormones critical for brain development (Squires et al., 2014). Additionally, ART exposure can lead to oxidative stress, which has been shown to have negative effects on cognitive development (Mgutshini et al., 2018).

Although some studies have reported neurocognitive risks associated with ART exposure, the overall beneficial effects of ART outweigh the risks. However, there is a need for continued research on the impacts/effects of ART on infant neurodevelopmental outcomes.

The effects of different ART initiation timings on infant outcomes

The initiation timing of ART during pregnancy and infancy can have a significant impact on infant neurocognitive outcomes. Early initiation (before conception or during the first trimester) of ART during pregnancy has been shown to reduce the risk of vertical transmission of HIV and to have positive effects on infant neurocognitive outcomes. In a study by Kiarie et al. (2010), early initiation of ART during pregnancy was associated with improved developmental scores and reduced behavioral problems in infants.

A study published in 2018 by Shapiro et al. found that starting ART before conception or during the first trimester of pregnancy resulted in better infant neurocognitive outcomes compared to starting ART later in pregnancy. The study followed 1,746 HEU infants born to mothers living with HIV in the United States and Puerto Rico. The researchers assessed neurodevelopmental outcomes at 1 year of age using the Bayley Scales of Infant and Toddler Development, 3rd edition. They found that infants whose mothers started ART before conception or during the first trimester had higher cognitive, language, and motor scores compared to infants whose mothers started ART later in pregnancy. Another study by Lahuerta et al. published in 2020 followed 1,996 HEU infants born to mothers in Africa. The study found that starting ART earlier

in pregnancy was associated with better neurocognitive outcomes at 1 year of age. The researchers used the Malawi Developmental Assessment Tool to assess neurodevelopmental outcomes. They found that infants whose mothers started ART during the first trimester had higher developmental scores compared to infants whose mothers started ART after the first trimester. Delayed initiation of ART during pregnancy has been shown to have negative effects on infant neurocognitive outcomes. In a study by Okpo et al. (2014), delayed initiation of ART was associated with decreased scores on tests of attention, fine motor skills, and language development in infants.

In conclusion, starting ART before conception or during the first trimester of pregnancy is associated with better infant neurocognitive outcomes compared to starting ART later in pregnancy.

2. METHODOLOGY

2.1 STUDY DESIGN

Recruitment Criteria

This project is part of the Healthy Babies Study (HBS) which is an NIH grant to study the influence of ART exposure in utero (PIs: E Meintjes, B Laughton and A van der Kouwe). 212 Xhosa-speaking women (18 years and older) attending the antenatal clinic at the Michael Mapongwana Community Health Centre (MMCHC) in Khayelitsha were recruited before 29 weeks gestation. The Gestational Age (GA) is determined by the last menstrual period. Women with chronic disorders such as diabetes, hypertension etc., less than 18 years of age or on any medication different from pregnancy supplements or ART, were excluded from the study. Mothers living with HIV who do not adhere to prescribed medication or receive non-standard ART were also excluded. Infant inclusion criteria included: infants delivered between 36-42 weeks GA, birth weight >2500 g, negative on HIV-1 PCR and physically/developmentally healthy.

Pregnant women were divided into three groups based on HIV status and treatment. The groups included women living with HIV on ART at preconception (HEU-pre), women living with HIV on ART at post-conception (HEU-post) and HIV negative (HUU). All recruited pregnant women living with HIV (PWLH) were on ART combination of Tenofovir/Efavirenz/Emtricitabine. Newborns of PWLH were administered Nevirapine if their risk of contracting the virus was considered low, while Zidovudine was administered alongside Nevirapine if considered to be at high risk (maternal VL > 1000 copies/mL at 32 weeks GA). In mothers living with HIV, CD4 counts from clinical visits within 6 months of delivery were recorded. The physical and mental health of all mothers was also monitored and they were interviewed regarding smoking, alcohol intake and drug use. The Harvard Trauma Questionnaire (HTQ) for Posttraumatic Stress Symptoms was used for to assess the exposure to trauma in the mothers during pregnancy.

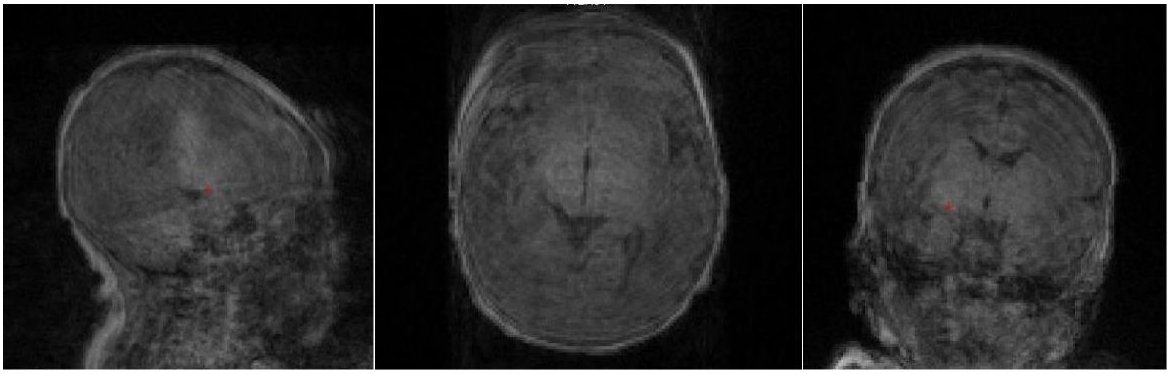


Figure 2- 2 Sagittal, axial and coronal views of poor quality images from one of the subjects excluded from the study before segmentation

Neuroimaging data

Infants were scanned at 40-41 weeks GA at the Cape Universities Body Imaging Centre (CUBIC), Groote Schuur Hospital. The infants were fed about 30 minutes prior to the time of scan and gently rocked to sleep. After which they were swaddled to prevent movement of any kind. The infants were placed supine on a special pillow containing styrofoam beads, sponge ear plugs were inserted in the ears, and foam ear pads designed for infants will be placed over the ears to help diminish noise. Once deeply asleep, the infant's head was positioned within the pediatric coil that maximizes SNR and then secured with rolled towels to limit head and body movements and provide additional sound protection. They were also visually monitored for any sign of distress such as movement and crying. The MRI protocol was completed in approximately 45 minutes on a Siemens 3 T Skyra scanner at CUBIC-UCT. The protocol included a high-resolution T1-weighted 3D echo-planar imaging (EPI) navigated multi echo magnetization prepared rapid gradient echo (MEMPRAGE) acquisition (FOV 192x192 mm2 , TR 2540 ms, TI 1450 ms, TE's = 1.69/3.55/5.41/7.27 ms, bandwidth 650 Hz/px, 144 sagittal slices, 1.0x1.0x1.0 mm3).

2.2 Image Processing

Due to the differences in contrast compared to the adult brain, special segmentation tools have been developed for infant Images. The images were processed using the infant FreeSurfer tool which is an automated segmentation and surface extraction pipeline designed for T1-weighted images of 0-2yrs infant brain developed by Zollei et al. The algorithm produces automated segmentations of cortical and subcortical areas of the brain, including full-brain volume and surface. It has achieved >90% overlap with expert-delineated brain masks despite difficulty in infant brain skull-stripping (Zollei et al., 2020).

Infant FreeSurfer uses a multi-stage pipeline process in segmenting infant images. Before inputting the T1-weighted image of the infant, the quality of the image is examined. After the images to be segmented are input, the intensity of the images is normalized and brought to a common scale by intensity normalization. Brain tissue is then extracted by the exclusion of skull and extra-meningeal tissues (skull-stripping) before the volumetric segmentation. Volumetric segmentation is a very important stage because it helps to measure brain volumes as well as visualize the anatomical structures of the infant brain. The last step which involves tessellation of gray-matter white-matter boundary is known as surface extraction (Zollei et al., 2020).

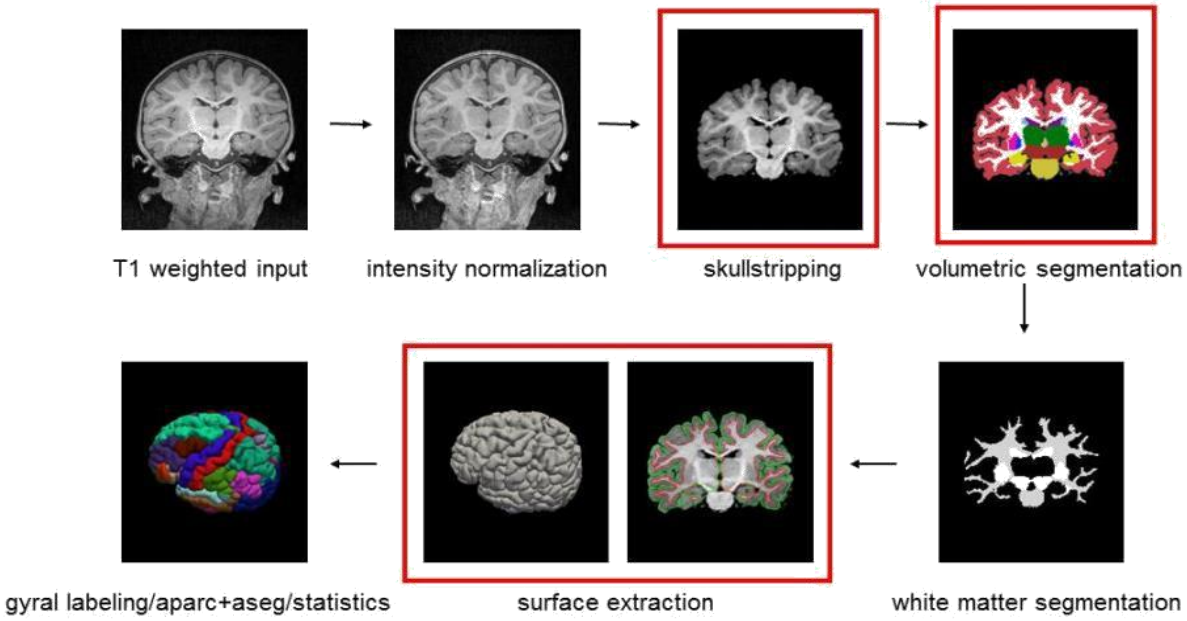


Figure 2-3 Multi-stage pipeline of the Infant Freesurfer tool

The quality of the images was assessed before the volumetric outputs were determined to ensure that the images were properly segmented and the borders are well defined. The images were quality checked after segmentation by visually inspecting the segmented images to identify any obvious errors, such as misalignment, tissue misclassification, or artifacts. I also ensured that the skull and non-brain tissues have been correctly removed, leaving only the brain tissue in the segmented images. All 151 images passed the post-segmentation quality check. The quantitative outputs of the ROIs were then processed. The ROIs include: the bilateral hippocampus, amygdala, thalamus, caudate, putamen, pallidus, cerebellum cortex, lateral ventricles, cerebral cortex and cerebral white matter, along with the vermis, midbrain, pons, medulla, 3rd and 4th ventricles.

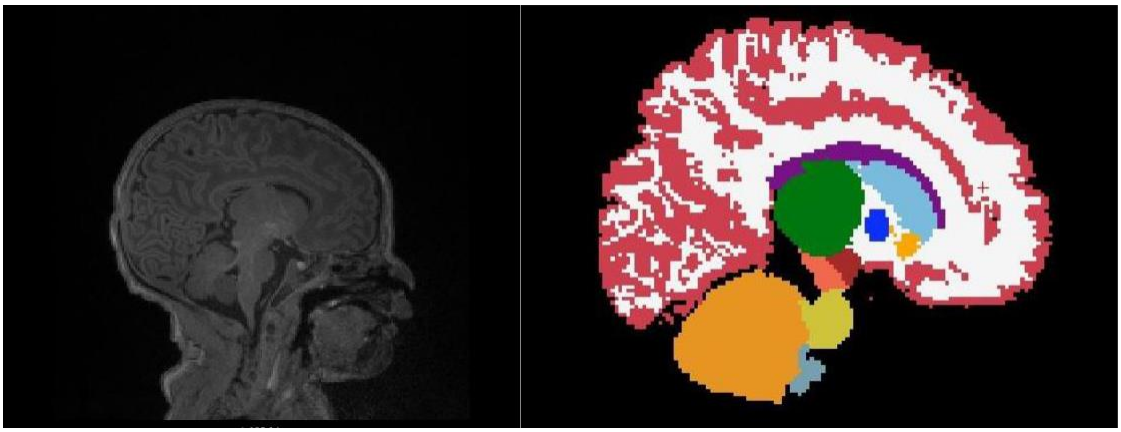


Figure 2-4 Sagittal view of T1- weighted Infant MRI before and after volumetric segmentation

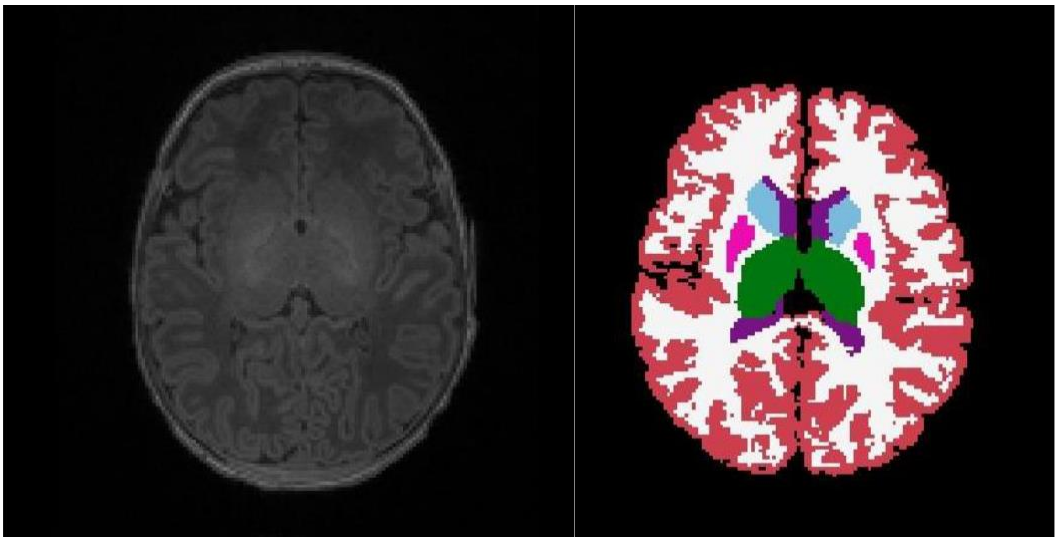


Figure 2-5 Axial view of T1-weighted Infant MRI before and after volumetric segmentation

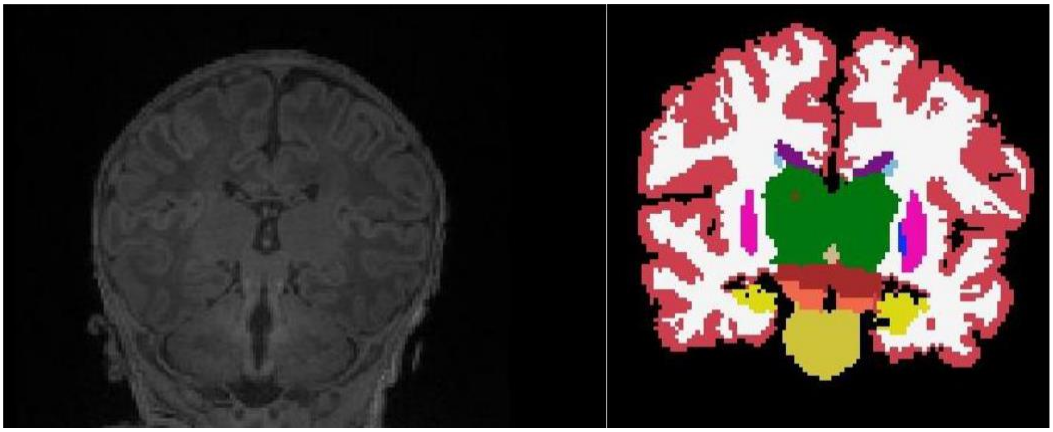


Figure 2-6 Coronal view of T1-weighted Infant MRI before and after volumetric segmentation

2.3 Statistical Analysis

Analysis was conducted using R with p-value at ≤0.05 as a statistically significant level. A one-way ANOVA was carried out to explore the difference of maternal/infant indices across groups. An unpaired Student's t-test was used to explore group differences of the ROIs in HIV-exposed and unexposed infants (exploring HIV exposure), as well as pre and post-conception ART initiation (exploring ART exposure).

To build linear models that included potential covariates, a Pearson's correlation was used to identify associations between the ROIs and relevant infant and maternal confounders. Variables associated with the outcome variable with a p-value of ≤0.05 were included in the linear regression model. Exploration of volumetric variation in all infants with HTQ scores and HEU infants with CD4 count was also done with linear regression.

In the event of data that may be considered an outlier, which we define as more than three times the standard deviation, we will check if the outlier is influential. This involves re-running the analysis without this data point to determine if the significance level changes.

The statistical technique of multiple comparison correction was implemented in this study to control for the rate of false positives, or type I errors, that may arise when conducting multiple statistical tests. Conducting multiple tests on the same data increases the chance of finding a significant result by chance. A common way to reduce type I errors is to apply a correction taking into account the number of tests conducted. There are many different methods for multiple comparison corrections. In this study, False Discovery Rate (FDR) correction was used to correct for type I errors, as the method controls for the expected proportion of false positives among all significant results (Chen et al., 2017). However, this approach may also increase the rate of false negatives, or type II errors, where a significant effect is missed.

We present uncorrected and corrected p-value results at the traditional significance level of p≤0.05. We also include results at the less strict level of p≤0.10 in order to highlight potential patterns.

3. RESULTS

3.1 Participants

Figure 7 summarizes participants recruited, excluded and data used for this study. Of the 212 enrolled mothers, 186 infants met the inclusion criteria (66 HEU-pre; 52 HEU-post; 68 HUU) with 26 infants excluded (13 had low birth weight, 3 had birth defects, 2 due to consent withdrawal and the remainder were lost to follow-up). Only 167 of these infants successfully remained in the scanner long enough for a T1-weighted image to be acquired, 16 of which were visually assessed to be of poor quality due to low signal-to-noise ratio and motion artifacts. The analysis of this study is based on the 151 participants with high quality T1-weighted images.

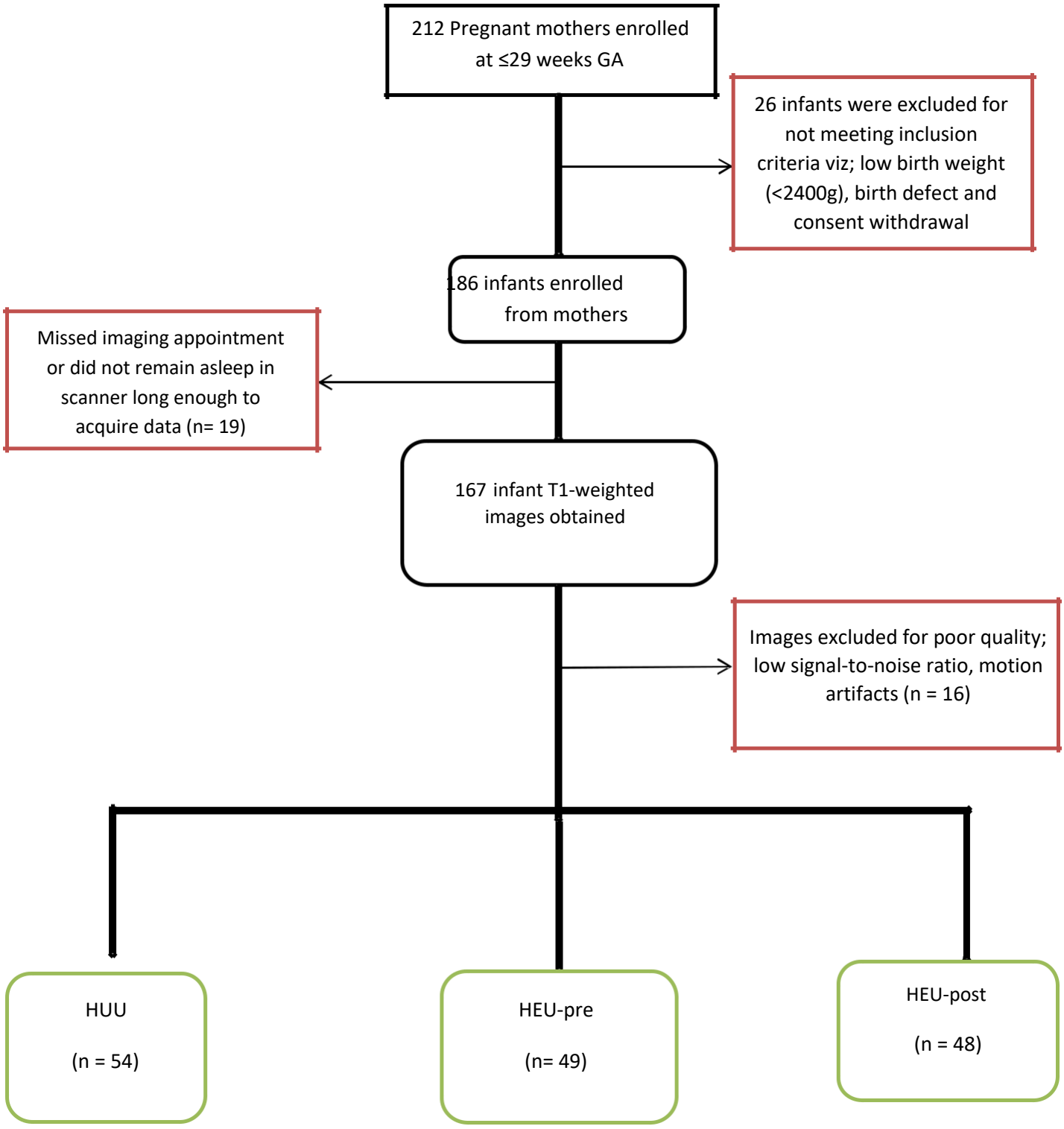


Figure 7: Enrolment summary of study participants

Table 2 below shows the demographic and health data for participants of this study. There was a significant difference in infants' head circumference at birth ($p=0.06$) and at the time of MRI acquisition ($p=0.01$) with the HEU-post group having a smaller head circumference. Mothers that initiated ART preconception have a significantly lower intake of Absolute Alcohol per day ($p=0.04$), while mothers that initiated ART postconception have a significantly lower weight change ($p<0.01$). Mothers who initiated ART preconception also had a significantly higher CD4 count compared to mothers who initiated ART post-conception ($p<0.01$).

	HUU (n=54)		HEU (n=97)				p-value
	mean±SD	Range	HEU-pre(n=49)		HEU-post(n=48)		
			mean±SD	Range	mean±SD	Range	
BIRTH INDICES							
GA (weeks)	39.83 ± 1.17	36.71 - 42.14	39.98 ± 1.36	36.57 - 42.14	39.51 ± 1.91	36.53 – 42.29	0.29
Weight (kg)	3250 ± 383.88	2575 – 4180	3240.41± 409.49	2500 - 4255	3225.52 ± 340.62	2500 – 3890	0.95
Length (cm)	50.38 ±2.95	45–58	49.65 ± 2.24	44-55	49.87 ± 2.72	43–56	0.37
HC (cm)	34.06 ±1.32	32-37	34 ± 1.43	30-37	33.49 ± 1.14	31-37	**0.06**
MRI INDICES							
GA	41.57 ± 0.85	39.71-43.71	41.64 ± 1.14	39–45	41.31 ± 1.67	32.43 - 43.43	0.39
Age	1.74 ± 1.12	0.43 - 4.86	1.68 ± 0.97	0.43 – 4.57	1.80 ± 0.98	0.29 – 5.43	0.85
Weight	3495.19 ± 481.62	2500 - 4550	3448.06 ± 487.27	2550 – 4700	3431.92 ± 375.53	2750 – 4450	0.77
Length	51.39 ±1.97	47 - 55.5	51.40 ± 2.04	47–56	52 ± 1.69	48.0 – 55.5	0.20
HC	35.08 ±1.24	32 - 37.5	35.55± 1.27	33.5 – 38.0	34.82 ± 1.16	31.0 – 36.5	**0.01**
ICV	398,648 ± 36,148	335 ,245–488,431	403 017 ± 35720.6	326 568 – 486 512	392 375 ± 29790	334 895 – 462 269	0.31
Sex Male N (%)	29 (53.7%)		25 (51.0%)		22 (45.8%)		0.73
MATERNAL INDICES							
Weight change	0.43 ± 0.19	-0.05 – 1.01	0.31 ± 0.26	-0.64 – 0.87	0.28 ± 0.25	-0.25 – 1.09	**<0.01**
Alcohol (AADxp)	0.02 ± 0.03	0 – 0.10	0.01 ± 0.02	0 – 0.05	0.02 ± 0.02	0 – 0.08	**0.04**
CD4 count	_	_	556.21 ±151.48	108 - 839	423.31 ± 202.21	52 - 913	**<0.01**
	Number of mothers that report use		Range				
Cannabis	4		0-1				
Meth	6		0-1				
Tobacco	1		0 -0.2				

Table 2: Demographic and health data for participants of this study

* HC = Head Circumference; ICV = Intra Cranial Volume; AADxp = Ounces of absolute alcohol per day averaged across pregnancy; Meth= Methamphetamine

3.2 Potential maternal and infant covariates

We summarize the maternal and infant variables that were considered as potential covariates for each volume in table 4. The results of the pearson correlation coefficient (r) and p-value were recorded (r (p-value)). For the regression models presented in later sections, the significant outcomes will be included as potential confounders. The majority of volumes relate to infant variables at the time of scan, with a few volumes showing relationships with maternal factors in pregnancy.

Table 3: Exploring associations between ROIs and potential covariates using Pearson correlations

ROI	Infant Indices at MRI scan				Maternal Indices during pregnancy					
	Sex	**Age**	**Weight**	**ICV**	**AAD**	**Tobacco**	**Cannabis**	**Meth**	**Edu**	**Weight change**
Left Cerebral Cortex	-3.04 **(0.003)**	4.30 **(3.03e-05)**	8.38 **(4.12e-14)**	68.04 **(<2.2e-16)**	-0.10 (0.92)	0.57 (0.57)	-0.14 (0.89)	0.16 (0.87)	-0.93 (0.36)	0.23 (0.82)
Right Cerebral Cortex	-3.05 **(0.003)**	4.35 **(2.55e-05)**	8.33 **(5.35e-14)**	65.13 **(<2.2e-16)**	-0.50 (0.62)	0.40 (0.69)	-0.38 (0.71)	0.07 (0.94)	-1.11 (0.23)	0.32 (0.75)
Left Cerebral WM	-3.54 **(5.43e-04)**	4.42 **(1.88e-05)**	7.74 **(1.52e-12)**	69.64 **(<2.2e-16)**	-0.58 (0.56)	0.15 (0.88)	-0.06 (0.95)	0.37 (0.72)	-0.30 (0.77)	0.22 (0.82)
Right Cerebral WM	-3.42 **(8.11e-04)**	4.52 **(1.25e-05)**	7.98 **(4.05e-13)**	73.53 **(<2.2e-16)**	-0.85 (0.40)	0.26 (0.79)	-0.15 (0.88)	0.36 (0.72)	-0.46 (0.64)	0.36 (0.72)
Left Hippocampus	-1.32 (0.19)	3.06 **(0.003)**	3.23 **(0.002)**	8.26 **(7.21e-14)**	-0.80 (0.42)	0.95 (0.34)	0.49 (0.62)	0.19 (0.85)	0.49 (0.62)	-0.43 (0.67)
Right Hippocampus	-1.08 (0.28)	-0.20 (0.84)	2.13 **(0.03)**	5.22 **(5.86e-07)**	-1.53 (0.13)	-0.96 (0.34)	-0.26 (0.79)	-0.31 (0.76)	0.84 (0.40)	-0.45 (0.65)
Left Amygdala	-2.93 **(0.004)**	2.47 **(0.01)**	4.04 **(8.55e-05)**	12.98 **(<2.2e-16)**	-1.05 (0.29)	1.31 (0.19)	0.49 (0.62)	0.72 (0.47)	-0.20 (0.85)	0.77 (0.44)
Right Amygdala	-2.79 **(0.006)**	1.12 (0.26)	3.33 **(0.001)**	9.28 **(<2.2e-16)**	-2.99 **(0.003)**	1.28 (0.20)	0.66 (0.51)	0.55 (0.58)	-0.44 (0.66)	1.05 (0.30)
Left Thalamus	-2.11 **(0.04)**	7.29 **(1.77e-11)**	6.94 **(1.20e-10)**	15.16 **(<2.2e-16)**	0.55 (0.58)	0.12 (0.90)	0.17 (0.86)	-0.35 (0.73)	-0.56 (0.57)	-0.69 (0.49)

Right Thalamus	-2.01 **(0.05)**	7.13 **(4.22e-11)**	6.71 **(3.99e-10)**	15.99 **(<2.2e-16)**	0.60 (0.55)	0.47 (0.64)	0.25 (0.80)	0.09 (0.93)	-0.26 (0.79)	-0.12 (0.91)
Left Caudate	-1.66 (0.10)	1.77 (0.08)	3.14 **(0.002)**	8.00 **(3.18e-13)**	-2.39 **(0.02)**	1.31 (0.19)	-0.51 (0.61)	0.94 (0.35)	0.09 (0.92)	2.24 **(0.03)**
Right Caudate	-1.77 (0.08)	2.90 **(0.004)**	4.16 **(5.42e-05)**	10.27 **(<2.2e-16)**	-2.77 **(0.006)**	1.17 (0.25)	-0.37 (0.71)	1.42 (0.16)	-0.54 (0.59)	2.36 **(0.02)**
Left Putamen	-0.92 (0.36)	-2.08 **(0.04)**	0.72 (0.47)	3.44 **(7.59e-04)**	-2.43 **(0.02)**	0.08 (0.94)	0.36 (0.72)	0.32 (0.75)	0.63 (0.53)	0.57 (0.57)
Right Putamen	-1.08 (0.28)	-1.13 (0.26)	-0.18 (0.85)	4.89 **(2.59e-06)**	-2.70 **(0.008)**	0.56 (0.58)	-0.13 (0.90)	0.70 (0.49)	0.26 (0.80)	0.51 (0.61)
Left Pallidus	-1.40 (0.16)	5.82 **(3.53e-08)**	9.09 **(6.54e-16)**	9.52 **(<2.2e-16)**	1.09 (0.28)	0.25 (0.80)	-1.42 (0.16)	0.37 (0.71)	-0.73 (0.47)	0.85 (0.39)
Right Pallidus	-1.61 (0.11)	5.59 **(1.07e-07)**	8.74 **(4.94e-15)**	9.52 **(<2.2e-16)**	1.15 (0.25)	-0.05 (0.96)	-1.58 (0.12)	0.21 (0.83)	-1.20 (0.23)	1.25 (0.21)
Left Cerebellum	-2.34 **(0.02)**	5.18 **(7.02e-07)**	8.41 **(3.33e-14)**	22.10 **(<2.2e-16)**	-1.96 **(0.05)**	-0.18 (0.86)	-1.42 (0.16)	-0.15 (0.88)	-0.21 (0.84)	1.07 (0.29)
Right Cerebellum	-2.82 **(0.005)**	4.93 **(2.23e-06)**	8.21 **(1.08e-13)**	24.27 **(<2.2e-16)**	-1.89 (0.06)	0.23 (0.82)	-0.91 (0.36)	-0.06 (0.96)	-0.41 (0.69)	1.01 (0.32)

Vermis	-3.01 **(0.003)**	5.09 **(1.08e-06)**	7.27 **(2.07e-11)**	15.99 **(<2.2e-16)**	-1.73 (0.09)	-0.32 (0.75)	-1.50 (0.14)	-0.29 (0.77)	-0.31 (0.76)	1.54 (0.12)
Left Lateral Ventricle	-1.39 (0.17)	1.54 (0.13)	2.89 **(0.004)**	6.93 **(1.17e-10)**	0.01 (0.99)	-0.51 (0.61)	-0.38 (0.70)	0.62 (0.53)	0.40 (0.69)	0.36 (0.72)
Right Lateral Ventricle	-0.73 (0.47)	2.36 **(0.02)**	3.21 **(0.002)**	6.26 **(3.84e-09)**	0.16 (0.87)	2.52 **(0.01)**	-0.49 (0.62)	0.66 (0.51)	-0.31 (0.76)	0.95 (0.35)
3rd Ventricle	-1.45 (0.15)	1.77 (0.08)	4.02 **(9.23e-05)**	7.30 **(1.62e-11)**	-0.26 (0.80)	-0.53 (0.60)	0.01 (0.99)	0.30 (0.76)	-0.49 (0.63)	-1.00 (0.32)
4th Ventricle	-1.53 (0.13)	2.41 **(0.02)**	3.62 **(4.02e-04)**	5.07 **(1.17e-06)**	-0.93 (0.35)	0.36 (0.72)	0.49 (0.63)	1.17 (0.24)	0.66 (0.51)	-0.44 (0.66)
Midbrain	-2.38 **(0.02)**	3.55 **(5.12e-04)**	8.58 **(1.31e-14)**	15.33 **(<2.2e-16)**	0.002 (0.99)	-0.32 (0.75)	-2.06 **(0.04)**	-0.45 (0.65)	0.27 (0.79)	0.93 (0.35)
Pons	-2.03 **(0.04)**	3.73 **(2.76e-04)**	6.94 **(1.19e-10)**	15.68 **(<2.2e-16)**	-1.74 (0.08)	0.12 (0.90)	-1.71 (0.09)	0.18 (0.85)	-0.31 (0.76)	1.34 (0.18)
Medulla	-1.54 (0.13)	2.34 **(0.02)**	7.66 **(2.36e-12)**	12.90 **(<2.2e-16)**	-1.27 (0.21)	-0.61 (0.54)	-1.81 (0.07)	0.05 (0.96)	-0.39 (0.70)	1.42 (0.16)

3.3 Associations between volumes and maternal health factors in pregnancy

Table 4 presents the result of a linear regression exploring the association between maternal trauma, as measured by the HTQ, and brain volumes across all infants. After adjusting for covariates, the left and right amygdalae are significantly associated with maternal HTQ scores ($p<0.01$).

Table 4: Exploration of volumetric variation with maternal HTQ scores

ROI	Harvard Trauma Questionnaire Score (β (CI) p-value)	Confounders
Left Cerebral Cortex	-8.28e-04 (8.21e-04) 0.32	Sex, Age, Weight, ICV
Right Cerebral Cortex	-8.60e-04 (7.78e-04) 0.27	Sex, Age, Weight, ICV
Left Cerebral White Matter	7.78e-04 (9.38e-04) 0.41	Sex, Age, Weight, ICV
Right Cerebral White Matter	1.14e-03 (9.40e-04) 0.23	Sex, Age, Weight, ICV
Left Hippocampus	0.02 (0.01) 0.09	Age, Weight, ICV
Right Hippocampus	0.01 (8.79e-03) 0.15	Weight, ICV
Left Amygdala	0.092 (0.032) **0.005**	Sex, Age, Weight, ICV
Right Amygdala	0.078 (0.028) **0.006**	Sex, Weight, AADxp, ICV
Left Thalamus	3.39e-03 (3.54e-03) 0.34	Sex, Age, Weight, ICV
Right Thalamus	4.62e-03 (3.80e-03) 0.23	Sex, Age, Weight, ICV
Left Caudate	6.48e-03 (8.64e-03) 0.45	Weight, AADxp, Maternal Weight change, ICV
Right Caudate	0.01 (0.009) 0.12	Age, Weight, AADxp, Maternal Weight change, ICV
Left Putamen	6.73e-04 (3.96e-03) 0.87	Age, AADxp, ICV
Right Putamen	-1.10e-04 (4.29e-03) 0.98	AADxp, ICV
Left Pallidus	-0.006 (0.02) 0.75	Age, Weight, ICV
Right Pallidus	-0.01 (0.002) 0.52	Age, Weight, ICV
Left Cerebellum	9.37e-04 (2.07e-03) 0.65	Sex, Age, Weight, AADxp, ICV
Right Cerebellum	2.04e-03 (2.08e-03) 0.33	Sex, Age, Weight, ICV
Vermis	9.14e-04 (7.88e-03) 0.91	Sex, Age, Weight, ICV
Left Lateral Ventricle	-1.50e-03 (2.37e-03) 0.53	Weight, ICV
Right Lateral Ventricle	-2.69e-03 (2.33e-03) 0.25	Age, Weight, ICV
3rd Ventricle	2.15e-02 (2.17e-02) 0.32	Weight, ICV
4th Ventricle	0.014 (0.013) 0.37	Age, Weight, ICV
Midbrain	-8.69e-03 (9.51e-03) 0.36	Sex, Age, Weight, ICV
Pons	9.32e-03 (7.57e-03) 0.22	Sex, Age, Weight, ICV
Medulla	0.011 (0.012) 0.36	Age, Weight, ICV

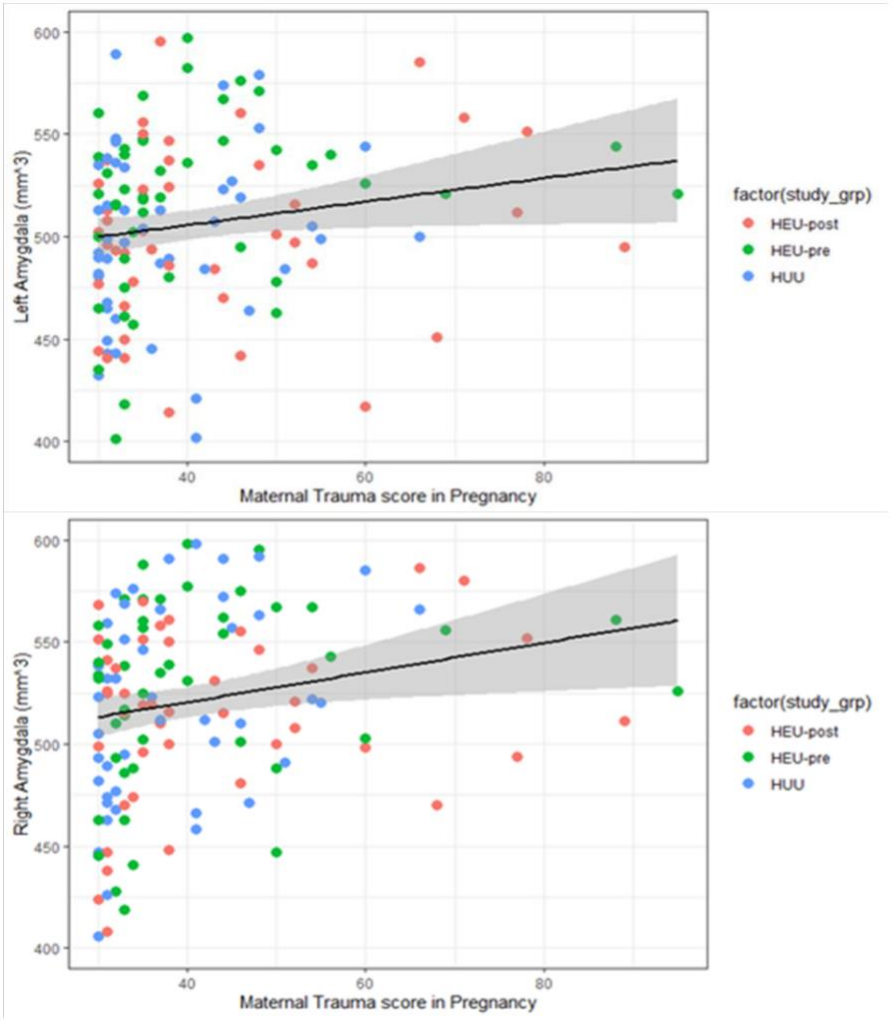


Figure 8: Correlation plot of amygdala volumes against HTQ score during pregnancy

Table 5 shows the result of the linear regression exploring the impact(s) of maternal CD4 count on the brain volumes of HIV-exposed infants. After adjusting for covariates, the right thalamus (*p=0.03*) and left caudate (*p=0.03*) were found to be significantly associated with maternal CD4 count while the right caudate presents at the less strict threshold (*p=0.08*).

ROI	Maternal CD4 count in pregnancy (β (CI) p-value)	Confounders
Left Cerebral Cortex	0.01 (0.01) 0.40	Sex, Age, Weight, ICV
Right Cerebral Cortex	0.01 (0.01) 0.55	Sex, Age, Weight, ICV
Left Cerebral White Matter	-1.89e-02 (1.72e-02) 0.28	Sex, Age, Weight, ICV
Right Cerebral White Matter	-0.02 (0.02) 0.33	Sex, Age, Weight, ICV
Left Hippocampus	-2.96e-01 (0.20) 0.14	Age, Weight, ICV
Right Hippocampus	-2.37 (0.15) 0.12	Weight, ICV
Left Amygdala	7.57e-03 (0.57) 0.99	Sex, Age, Weight, ICV
Right Amygdala	-0.18 (0.51) 0.73	Sex, Weight, AADxp, ICV
Left Thalamus	-9.39e-02 (0.07) 0.16	Sex, Age, Weight, ICV
Right Thalamus	-0.17 (0.07) **0.03**	Sex, Age, Weight, ICV
Left Caudate	0.32 (0.14) **0.03**	Weight, AADxp, Maternal Weight change, ICV
Right Caudate	0.25 (0.15) **0.08**	Age, Weight, AADxp, Maternal Weight change, ICV
Left Putamen	0.04 (0.07) 0.61	Age, AADxp, ICV
Right Putamen	-3.32e-02 (0.08) 0.68	AADxp, ICV
Left Pallidus	-0.14 (0.36) 0.69	Age, Weight, ICV
Right Pallidus	-0.23 (-0.43) 0.59	Age, Weight, ICV
Left Cerebellum	2.08e-02 (3.66e-02) 0.57	Sex, Age, Weight, AADxp, ICV
Right Cerebellum	1.96e-02 (3.58e-02) 0.59	Sex, Age, Weight, ICV
Vermis	3.08e-02 (0.14) 0.83	Sex, Age, Weight, ICV
Left Lateral Ventricle	1.24e-02 (3.99e-02) 0.76	Weight, ICV
Right Lateral Ventricle	5.37e-03 (3.85e-02) 0.89	Age, Weight, ICV
3rd Ventricle	-8.91e-02 (0.36) 0.81	Weight, ICV
4th Ventricle	0.28 (0.21) 0.19	Age, Weight, ICV

Midbrain	-5.63e-02 (0.18) 0.75	Sex, Age, Weight, ICV
Pons	-0.10 (0.13) 0.44	Sex, Age, Weight, ICV
Medulla	-0.19 (0.23) 0.40	Age, Weight, ICV

Table 5: Linear regression results of the relationship between brain volumes and maternal CD4 count in HIV exposed infants.

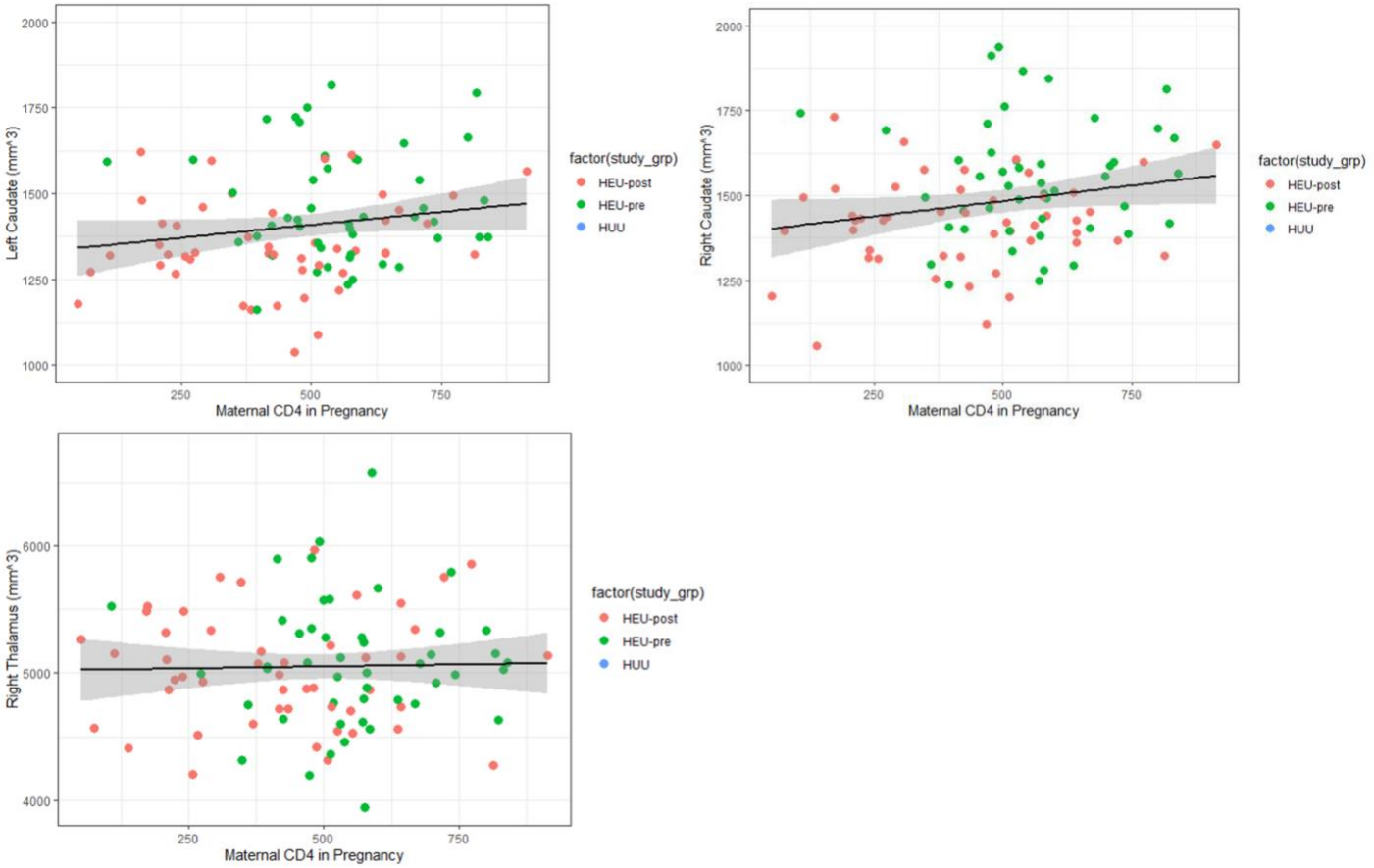
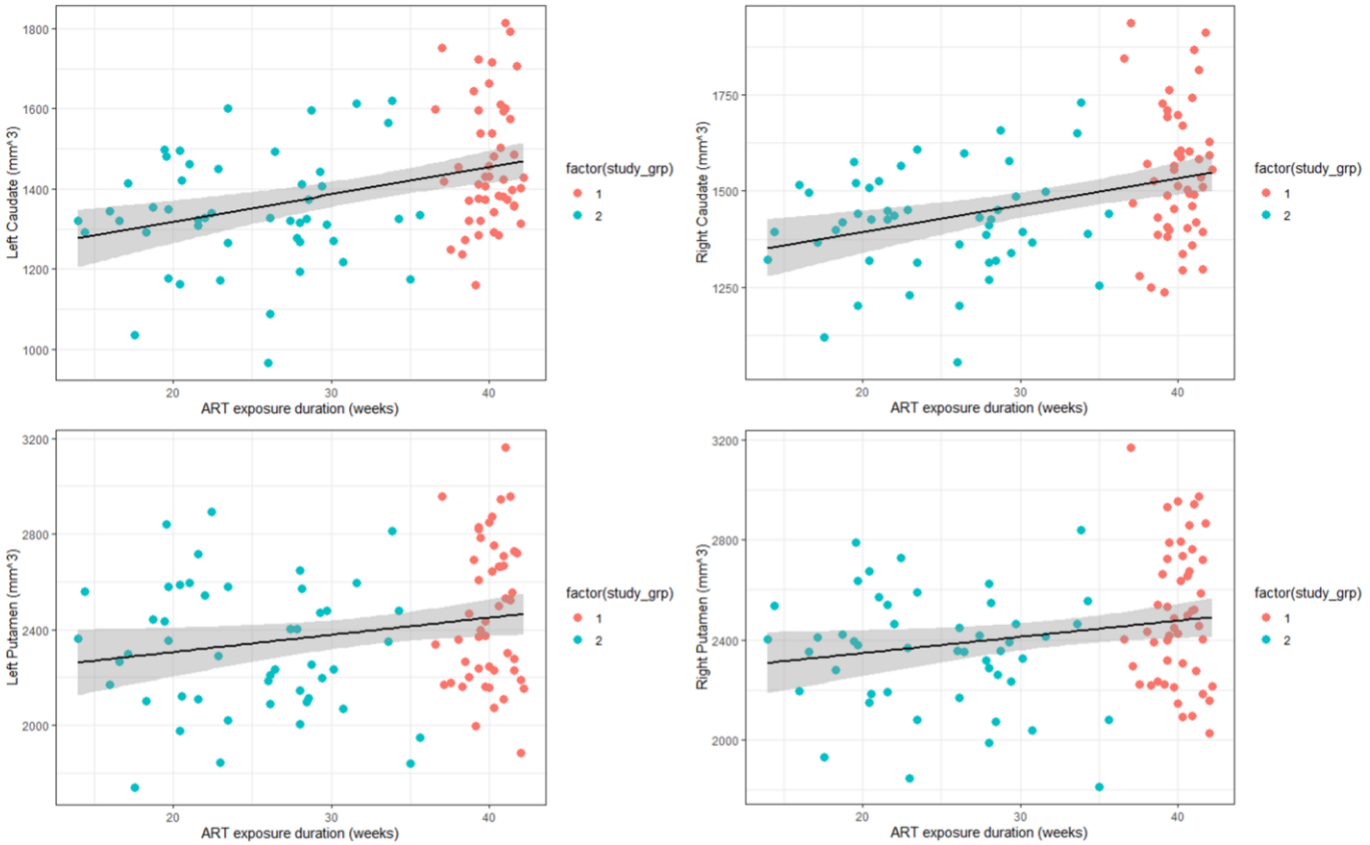


Figure 9: Association plot of the bilateral caudate and right thalamus with maternal CD4 count during pregnancy

The table below shows the result of the pearsons correlation between ART exposure duration (as a continuous variable) and brain volume in HEU infants. The bilateral caudate (p = <0.01) and putamen (p = 0.04) volumes were observed to be positively correlated to ART duration. The vermis and left lateral ventricle trends at p = 0.09.

Table 6: Correlation between ART duration and brain volumes

ROI	t (r) p-value
Left Cerebral Cortex	0.49(0.05) 0.62
Right Cerebral Cortex	0.51(0.05) 0.61
Left Cerebral White Matter	0.64(0.07) 0.53
Right Cerebral White Matter	0.85(0.09) 0.39
Left Hippocampus	-0.11(-0.01) 0.91
Right Hippocampus	0.31(0.03) 0.76
Left Amygdala	1.84(0.19) 0.07
Right Amygdala	1.13(0.11) 0.26
Left Thalamus	0.18(0.02) 0.85
Right Thalamus	0.26(0.03) 0.80
Left Caudate	3.90 (0.37) **0.00018**
Right Caudate	3.78 (0.36) **0.00027**
Left Putamen	2.13 (0.21) **0.036**
Right Putamen	2.13 (0.21) **0.035**
Left Pallidus	-0.23(-0.02) 0.82
Right Pallidus	-0.36(-0.04) 0.72
Left Cerebellum Cortex	1.24(0.13) 0.22
Right Cerebellum Cortex	1.44(0.15) 0.15
Vermis	1.70(0.17) 0.09
Left Lateral Ventricle	1.71(0.17) 0.09
Right Lateral Ventricle	0.49(0.05) 0.63
Third Ventricle	-0.09(-0.01) 0.93
Fourth Ventricle	0.43(0.04) 0.67
Midbrain	-0.15(-0.02) 0.88
Pons	1.38(0.14) 0.17
Medulla	1.23(0.13) 0.21



Study Group 1 = HEU-pre

2 = HEU-post

Figure 10: Association plot of the bilateral caudate and putamen with duration of ART exposure

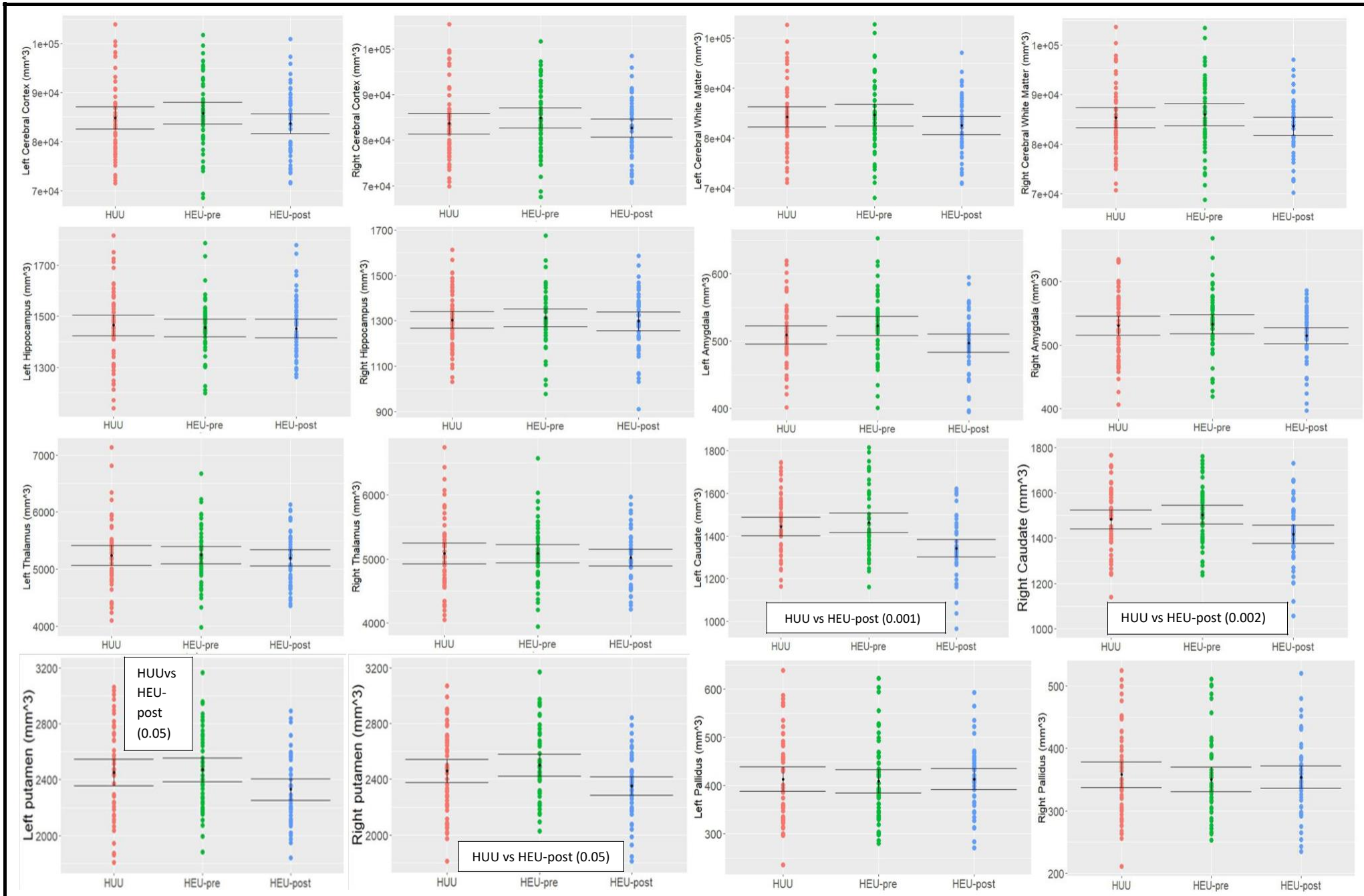
3.4 Volumetric differences across HIV and ART exposure groups

Table 7 shows the Student's t-test outcomes for volumetric differences between HIV unexposed (HUU), exposed uninfected (HEU) infants, infants whose mothers initiated ART pre-conception (HEU-pre) and infants whose mothers initiated ART post-conception (HEU-post). Results show no significant group difference between HIV exposed and unexposed infants, while there is a significant decrease in caudate volumes bilaterally ($p<0.01$), with a trending significance in the putamen bilaterally(*p=0.05*) among the post-conception group (HEU-post) compared to the unexposed group. The pre-conception group also shows a significant volume reduction in the left lateral ventricle (*p=0.04*).

A data point in the HEU pre-conception group was checked to see if it influenced the group difference in the left lateral ventricle. The outlier (4756 mm^3) was excluded and the Student's t-test was rerun. The result changed from -2.06 (0.04) to -1.77 (0.08).

Table 7: : Group differences of ROI in HIV exposed and unexposed infants, and in HIV unexposed and pre/post conception ART groups

ROI	HUU (54) vs HEU (97) t (p-value)	HUU vs HEU-Pre (49) t (p-value)	HUU vs HEU-Post (48) t (p-value)
Left Cerebral Cortex	0.07 (0.95)	-0.61 (0.54)	0.77 (0.44)
Right Cerebral Cortex	-0.10(0.92)	-0.78 (0.44)	0.66 (0.51)
Left Cerebral White Matter	0.53 (0.60)	-0.26 (0.79)	1.24 (0.22)
Right Cerebral White Matter	0.43 (0.67)	-0.40 (0.69)	1.22 (0.23)
Left Hippocampus	0.49 (0.62)	0.37 (0.71)	0.45 (0.66)
Right Hippocampus	-0.07(0.94)	-0.35 (0.73)	0.22 (0.82)
Left Amygdala	-0.16 (0.88)	-1.43 (0.16)	1.23 (0.22)
Right Amygdala	0.75 (0.45)	-0.23 (0.82)	1.59 (0.12)
Left Thalamus	0.18 (0.86)	-0.06 (0.95)	0.36 (0.72)
Right Thalamus	0.37 (0.71)	0.01 (0.99)	0.62 (0.54)
Left Caudate	1.50 (0.14)	-0.59 (0.56)	3.36 (**0.001**)
Right Caudate	1.27 (0.21)	-0.72 (0.47)	3.18 (**0.002**)
Left Putamen	0.84 (0.40)	-0.48 (0.63)	1.97 (**0.05**)
Right Putamen	0.70 (0.49)	-0.69 (0.49)	1.98 (**0.05**)
Left Pallidus	0.15 (0.88)	0.26 (0.80)	-0.01 (0.99)
Right Pallidus	0.50 (0.62)	0.53 (0.60)	0.29 (0.77)
Left Cerebellum	-0.66 (0.51)	-1.37 (0.17)	0.29 (0.77)
Right Cerebellum	-0.15 (0.88)	-1.01 (0.31)	0.85 (0.40)
Vermis	-0.70 (0.48)	-1.39 (0.17)	0.22 (0.83)
Left Lateral Ventricle	-1.10 (0.27)	-2.06 (**0.04**)	0.37 (0.71)
Right Lateral Ventricle	-0.31 (0.76)	-0.96 (0.34)	0.45 (0.65)
3rd Ventricle	-0.68 (0.50)	-0.57 (0.57)	-0.64 (0.52)
4th Ventricle	0.41 (0.68)	-0.07 (0.94)	0.76 (0.45)
Midbrain	0.89 (0.37)	0.60 (0.55)	0.88 (0.38)
Pons	-0.25 (0.80)	-1.11 (0.27)	0.72 (0.47)
Medulla	-0.48 (0.63)	-1.19 (0.24)	0.46 (0.65)


Left Cerebral Cortex (mm^3)
Right Cerebral Cortex (mm^3)
Left Cerebral White Matter (mm^3)
Right Cerebral White Matter (mm^3)
Left Hippocampus (mm^3)
Right Hippocampus (mm^3)
Left Amygdala (mm^3)
Right Amygdala (mm^3)
Left Thalamus (mm^3)
Right Thalamus (mm^3)
Left Caudate (mm^3)
Right Caudate (mm^3)
Left putamen (mm^3)
Right putamen (mm^3)
Left Pallidus (mm^3)
Right Pallidus (mm^3)
HUU
HEU-pre
HEU-post
HUU vs HEU-post (0.001)
HUU vs HEU-post (0.002)
HUUvs HEU-post (0.05)
HUU vs HEU-post (0.05)

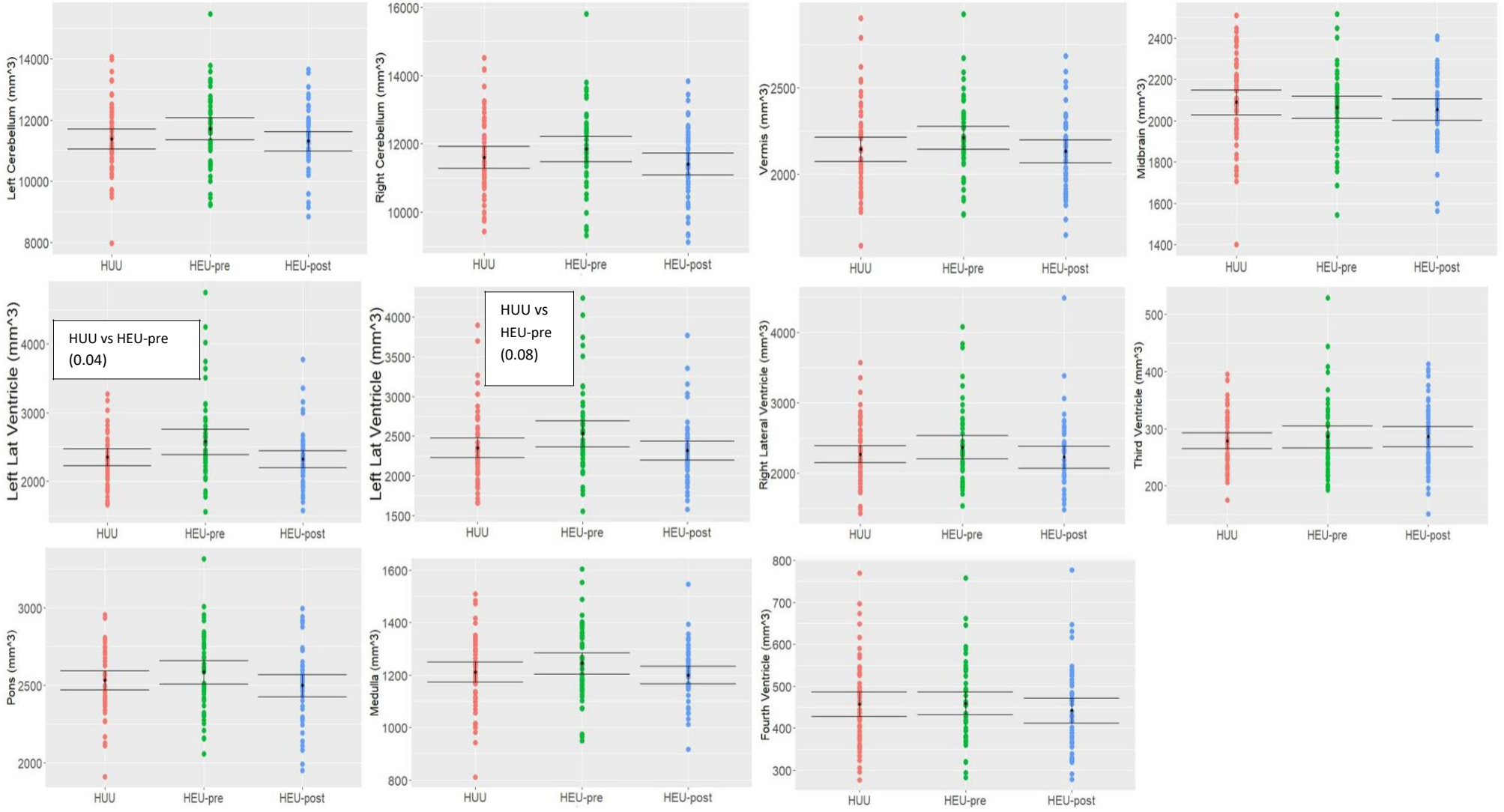


Figure 11: Mean plots showing the group differences in ROIs across groups using t-test

Table 8 shows the linear regression analysis for the volumetric differences between HUU, HEU-pre and HEU-post as shown in table 3 above. After adjusting for confounders, we report a significant difference in the bilateral caudate of HEU-post infants (*p<0.01*). The right amygdala was also found to be significantly different in the HEU-post group (*p=0.03*). In the HEU-pre group, a significant group difference was observed in the left lateral ventricle (*p=0.04*) and the left cerebral white matter (*p=0.03*).

Table 8: Linear regression analyses after adjustments for significant confounders

ROI	Unexposed (54) vs Preconception ART HEU(49) β (CI) p-value	Unexposed (54) vs Postconception ART HEU(48) β (CI) p-value	Confounders
Left Cerebral Cortex	-33.11 (277.3) 0.91	227.3 (278.6) 0.42	Sex, Age, Weight, ICV
Right Cerebral Cortex	271.7 (289.6) 0.35	358.2 (290.9) 0.22	Sex, Age, Weight, ICV
Left Cerebral White Matter	-511.7 (239.2) **0.03**	-312.2 (240.3) 0.20	Sex, Age, Weight, ICV
Right Cerebral White Matter	-296.0 (237.6) 0.22	-356.4 (238.7) 0.14	Sex, Age, Weight, ICV
Left Hippocampus	-237.3 (22.47) 0.29	-3.69 (22.55) 0.87	Age, Weight, ICV
Right Hippocampus	-0.86 (25.28) 0.97	-0.54 (25.53) 0.98	Weight, ICV
Left Amygdala	7.20 (0.68) 0.29	-1.01 (0.68) 0.15	Sex, Age, Weight, ICV, HTQ
Right Amygdala	-95.0 (0.79) 0.23	-18.0 (0.79) **0.03**	Sex, Weight, AADxp, ICV, HTQ
Left Thalamus	-34.37 (64.62) 0.60	13.68 (64.91) 0.83	Sex, Age, Weight, ICV
Right Thalamus	-42.49 (59.62) 0.48	-11.57 (59.89) 0.85	Sex, Age, Weight, ICV
Left Caudate	5.07 (26.16) 0.85	-73.28 (26.43) **0.006**	Weight, AADxp, Maternal Weight change, ICV
Right Caudate	6.21 (25.21) 0.84	-66.42 (25.27) **0.009**	Age, Weight, AADxp, Maternal Weight change, ICV
Left Putamen	-21.68 (56.97) 0.70	-90.75 (56.34) 0.11	Age, AADxp, ICV
Right Putamen	8.72 (50.66) 0.86	-84.83 (50.43) 0.09	AADxp, ICV
Left Pallidus	-5.14 (12.05) 0.67	7.17 (12.10) 0.55	Age, Weight, ICV
Right Pallidus	-8.71 (10.06) 0.39	2.06 (10.09) 0.84	Age, Weight, ICV
Left Cerebellum	186.8 (111.7) 0.097	149.5 (111.0) 0.18	Sex, Age, Weight, AADxp, ICV
Right Cerebellum	155.79 (108.39) 0.15	13.14 (108.87) 0.90	Sex, Age, Weight, ICV
Vermis	42.61 (28.97) 0.14	17.91 (29.10) 0.54	Sex, Age, Weight, ICV
Left Lateral Ventricle	185.5 (91.12) **0.04**	-0.02 (92.03) 0.99	Weight, ICV
Right Lateral Ventricle	69.59 (95.80) 0.47	-1.17 (96.15) 0.99	Age, Weight, ICV
3rd Ventricle	1.80 (10.20) 0.86	9.84 (10.30) 0.34	Weight, ICV
4th Ventricle	6.56 (18.47) 0.72	-1.88 (18.54) 0.92	Age, Weight, ICV
Midbrain	-43.06 (23.76) 0.07	-13.67 (23.86) 0.57	Sex, Age, Weight, ICV
Pons	29.17 (29.90) 0.33	-4.08 (30.03) 0.89	Sex, Age, Weight, ICV
Medulla	21.38 (18.35) 0.25	6.63 (18.41) 0.72	Age, Weight, ICV

3.5 Multiple comparison analyses

After applying corrections for multiple comparisons, we report the positive relationship between bilateral amygdala in infants and maternal HTQ scores during pregnancy, as well as between the bilateral caudate and duration of ART exposure at the less strict level of p≤0.10.

Table 9:Uncorrected and corrected (FDR) p-values for the volumetric differences in HEU-pre and HEU-post infants

ROI	HEU-pre (p-value)	HEU-pre (p-adjusted)	HEU-post (p-value)	HEU-post (p-adjusted)
Left Cerebral Cortex	0.91	0.95	0.42	0.98
Right Cerebral Cortex	0.35	0.76	0.22	0.66
Left Cerebral White Matter	**0.03**	0.52	0.2	0.66
Right Cerebral White Matter	0.22	0.715	0.14	0.66
Left Hippocampus	0.29	0.75	0.87	0.99
Right Hippocampus	0.97	0.97	0.98	0.99
Left Amygdala	0.29	0.72	0.15	0.98
Right Amygdala	0.23	0.85	**0.03**	0.52
Left Thalamus	0.6	0.92	0.83	0.99
Right Thalamus	0.48	0.80	0.85	0.99
Left Caudate	0.85	0.93	**0.006**	0.12
Right Caudate	0.84	0.93	**0.009**	0.12
Left Putamen	0.7	0.93	0.11	0.66
Right Putamen	0.86	0.93	0.09	0.66
Left Pallidus	0.67	0.93	0.55	0.99
Right Pallidus	0.39	0.78	0.84	0.99
Left Cerebellum	0.097	0.63	0.18	0.66
Right Cerebellum	0.15	0.65	0.9	0.99
Vermis	0.14	0.65	0.54	0.99
Left Lateral Ventricle	**0.04**	0.52	0.99	0.99
Right Lateral Ventricle	0.47	0.80	0.99	0.99
3rd Ventricle	0.86	0.93	0.34	0.89
4th Ventricle	0.72	0.93	0.92	0.99
Midbrain	**0.07**	0.61	0.57	0.99
Pons	0.33	0.76	0.89	0.99
Medulla	0.25	0.72	0.72	0.99

Table 10: Summary of uncorrected and corrected (FDR) p-values for the associations with maternal HTQ score in pregnancy

ROI	p_value	p_adjusted
Left Cerebral Cortex	0.32	0.60
Right Cerebral Cortex	0.27	0.60
Left Cerebral White Matter	0.41	0.63
Right Cerebral White Matter	0.23	0.60
Left Hippocampus	0.09	0.60
Right Hippocampus	0.15	0.60
Left Amygdala	**0.005**	**0.078**
Right Amygdala	**0.006**	**0.078**
Left Thalamus	0.34	0.60
Right Thalamus	0.23	0.60
Left Caudate	0.45	0.63
Right Caudate	0.12	0.60
Left Putamen	0.87	0.94
Right Putamen	0.98	0.98
Left Pallidus	0.75	0.85
Right Pallidus	0.52	0.66
Left Cerebellum	0.65	0.77
Right Cerebellum	0.33	0.60
Vermis	0.91	0.95
Left Lateral Ventricle	0.53	0.66
Right Lateral Ventricle	0.25	0.60
3rd Ventricle	0.32	0.60
4th Ventricle	0.37	0.60
Midbrain	0.46	0.63
Pons	0.22	0.60
Medulla	0.36	0.60

Table 11: Uncorrected and corrected (FDR) p-values for the volumetric associations with maternal CD4 count

ROI	p_value	p_adjusted
Left Cerebral Cortex	0.4	0.90
Right Cerebral Cortex	0.55	0.90
Left Cerebral White Matter	0.28	0.90
Right Cerebral White Matter	0.33	0.90
Left Hippocampus	0.14	0.70
Right Hippocampus	0.12	0.70
Left Amygdala	0.99	0.99
Right Amygdala	0.73	0.90
Left Thalamus	0.16	0.70
Right Thalamus	**0.03**	0.39

Left Caudate	**0.03**	0.39
Right Caudate	**0.08**	0.70
Left Putamen	0.61	0.90
Right Putamen	0.68	0.90
Left Pallidus	0.69	0.90
Right Pallidus	0.59	0.90
Left Cerebellum	0.57	0.90
Right Cerebellum	0.59	0.90
Vermis	0.83	0.90
Left Lateral Ventricle	0.76	0.90
Right Lateral Ventricle	0.89	0.93
3rd Ventricle	0.81	0.90
4th Ventricle	0.19	0.71
Midbrain	0.75	0.90
Pons	0.44	0.90
Medulla	0.4	0.90

Table 12: Summary of uncorrected and corrected (FDR) p-values for the association of volumes with ART exposure duration

ROI	p_value	p_adjusted
Left Cerebral Cortex	0.62	0.93
Right Cerebral Cortex	0.61	0.93
Left Cerebral White Matter	0.53	0.93
Right Cerebral White Matter	0.39	0.78
Left Hippocampus	0.91	0.93
Right Hippocampus	0.76	0.93
Left Amygdala	**0.07**	0.33
Right Amygdala	0.26	0.56
Left Thalamus	0.85	0.93
Right Thalamus	0.8	0.93
Left Caudate	**0.00018**	**0.00351**
Right Caudate	**0.00027**	**0.00351**
Left Putamen	**0.036**	0.234
Right Putamen	**0.035**	0.234
Left Pallidus	0.82	0.93
Right Pallidus	0.72	0.93
Left Cerebellum Cortex	0.22	0.52
Right Cerebellum Cortex	0.15	0.49
Vermis	**0.09**	0.33
Left Lateral Ventricle	**0.09**	0.33
Right Lateral Ventricle	0.63	0.93
3rd Ventricle	0.93	0.93
4th Ventricle	0.67	0.93
Midbrain	0.88	0.93
Pons	0.17	0.49
Medulla	0.21	0.52

4. DISCUSSION

This study examined the influence of maternal factors during pregnancy, including trauma assessments, maternal immune health and in utero exposure to HIV/ART on uninfected neonates' brain volumes using an automated segmentation tool.

Our finding of maternal HTQ scores associated with infant amygdala volumes bilaterally supports our first hypothesis that maternal trauma in pregnancy relates to amygdala volumes across all newborns. This result is particularly worth noting as it points to intergenerational consequences of maternal trauma. And, it is highly relevant due to the high prevalence globally, and locally, of gendered based violence.

And among HEU newborns, we report positive associations between immune system related measures (CD4 count and maternal ART duration) and caudate volumes. When examining the potential influence of HIV and ART exposure, we noted a decrease in the mean caudate volume bilaterally in the ART post-conception group. These findings support our hypothesis that longer ART exposure provides neuroprotection, particularly in the caudate. Further, we reported lower right amygdala volumes in the ART post-conception group after controlling for HTQ scores, suggesting an intersection between HIV/ART exposure and maternal trauma in pregnancy on the developing brain.

In addition, we reported a decrease in left cerebral white matter volume as well as an increase in the left lateral ventricle in the ART pre-conception group. While we hypothesized white matter volumes would be affected, we expected longer ART exposure to provide protection.

We find it noteworthy that the HIV/ART exposure regression results reported may be viewed as complementary. In the HEU-post group, we find reduced volumes in the bilateral caudate compared to HUU infants. And, in the HEU-pre group, we find reductions in cerebral white matter volume in combination with increased lateral ventricle volume in the left hemisphere. Given the proximity of the lateral ventricle to cerebral white matter, these two results may be related. Due to these observations, we present a discussion of these results with the caveat that these findings do not remain after multiple comparison corrections.

4.1 Associations between volumes and maternal health in pregnancy

While not our focus, it is worth noting the correlations observed among the maternal factors included in our regression models. The maternal indices explored as possible confounders included weekly weight change, education, and drug consumption (methamphetamine, cannabis, tobacco and alcohol).

We reported that the bilateral caudate was positively correlated with maternal weight change. Cannabis use was negatively correlated with midbrain volumes. Tobacco use was positively correlated with right lateral ventricle volume. However, the number of reported maternal users of tobacco (1 mother) and cannabis (4 mothers) was very low, meaning we cannot make any interpretations regarding drug use. And, alcohol use – although in very low quantities – was negatively associated with right amygdala, left cerebellum as well as bilateral caudate and putamen.

While there are no previous studies that explored the direct relationship between maternal weight change during pregnancy and infant caudate volume, there are studies that suggest that maternal factors such as diet and weight gain during pregnancy can influence fetal brain development, including the development of the caudate (Fitzgerald et al., 2020, Cortés-Albornoz et al., 2021). One study also suggests that inadequate gestational weight gain in mothers may have a negative impact on the neurodevelopment of infants at 12 months (Motoki et al., 2022).

Our findings are in line with previous literature suggesting a relationship between exposure to cannabis and tobacco with infant neurodevelopment. Studies have found that prenatal exposure to cannabis and tobacco use is related to decreased general cognitive functioning, and deficits in learning and memory tasks, indicating that maternal cannabis use may have disrupted the normal brain maturation process (Huizink & Mulder, 2006, Wu et al., 2011, Corsi et al., 2020).Though there have been no previous studies that have examined the link between maternal tobacco use during pregnancy and the volumes of the brains of infants to the best of our knowledge. Previous studies however observed reduced brain volumes in children and

adolescents exposed to tobacco in-utero (Elmarroun et al., 2014, Zou et al., 2022, Ekblad et al., 2023).

Lastly, the negative association between prenatal exposure to alcohol and infant neurodevelopment observed in this study corroborates those reported in previous studies. These studies report reduced total brain volume (Lebel et al., 2011, Treit et al., 2016). Specifically, reductions were noted in the corpus callosum (Lebel et al, 2011, Jacobson et al., 2017), bilateral amygdala, left hippocampus, left thalamus (Donald et al., 2016) and the basal ganglia especially the caudate (Mattson et al., 1996, Astley et al., 2009) and putamen (Subramoney et al., 2022).

Taken together, these associations highlight the importance of including maternal variables in infant neurodevelopmental studies. These results also point to the need for a better understanding of the role of maternal health in neonate brain development, in typical and atypical development.

Maternal trauma scores and brain volumes

In this study, we found a positive relationship between HTQ score and amygdala volume. Infants whose mothers had higher scores on the HTQ during pregnancy were found to have increased amygdala volumes compared to their counterparts (who were born to mothers with lower HTQ scores). Trauma is a psychological and physiological response to a distressing or disturbing experience (single incidence or repeated exposure). Maternal trauma can lead to poor pregnancy outcomes, including preterm birth, low birth weight, and developmental delays in the infant (Tenami et al., 2023). The fetus/infant can also experience trauma if the mother experiences high levels of stress, which can affect the fetal brain development and increase the risk of developmental and behavioral problems (Guintivanoet al., 2016). Research has shown that exposure to trauma during pregnancy can affect fetal development and increase the risk of behavioral, emotional, and cognitive problems in children (Kinsella and Monk, 2009; Glover, 2019).

The prevalence of Intimate Partner Violence (IPV), the most common form of violence against women, in South Africa is 20 – 24% among women 15 to 49 years old (Sardinha et al., 2022).

Antenatal exposure to maternal IPV, which is a form of trauma, has been found to have effects on early brain development in a South African study. They observed that IPV was associated with smaller gray matter volumes in the left insula and left parietal lobe in male infants and larger gray matter volumes in the right insula and right parietal lobe in female infants (Hiscox et al., 2023). Another study in a South African cohort also reported a significant increase in the right amygdala volume of infants exposed to antenatal maternal depression (Groenewold et al., 2022).

Infants who are exposed to trauma in utero may have an altered stress response and be more susceptible to stress-related disorders later in life (Glover et al., 2015). Trauma can lead to structural changes and altered connectivity in the amygdala (Hay et al., 2020; Mareckova et al., 2022). The amygdala is a brain region that plays a key role in the processing of emotions, including fear and anxiety. In individuals who have experienced trauma, the amygdala can become hyperactive and more sensitive to potential threats, which can lead to a heightened stress response (Buss et al., 2010).

Research has shown that antenatal maternal depression and anxiety are associated with altered amygdala development in infants. Children who were exposed to high levels of maternal depression during pregnancy were noted to be more reactive to stress at ages 5 and 9 (Gunnar & Donzella, 2002, Gunnar et al., 2009). One MRI study found significantly lower FA, which suggests reduced white matter integrity, in the right amygdala of neonates born to prenatally depressed mothers (Rifkin-Graboi et al., 2013). Hay and colleagues also reported links between antenatal maternal depression and the amygdala-prefrontal cortex functional connectivity (Hay et al., 2020). Several studies have also reported an association between maternal anxiety and infant neurodevelopment, including an increase in the amygdala volume (Adamson et al., 2018, Acosta et al., 2019).

One potential explanation for these findings is the activation of the hypothalamic-pituitary-adrenal (HPA) axis which helps to regulate stress response. Activation of the HPA axis increases stress hormones such as cortisol which can then cross the placenta to affect the developing

brain of the fetus, specifically the amygdala which is a key structure in emotion regulation (Buss et al., 2010).

We also observed a significant increase in the HTQ score of mothers living with HIV as compared to mothers living without HIV. For mothers living with HIV, receiving an HIV diagnosis may have been experienced as traumatic. A study found that women living with HIV have significantly higher rates of both depression and trauma (Spies et al., 2018). Women living with HIV have been noted to be at a higher risk for mental health issues (Orza et al., 2015). It is postulated that stigma and discrimination (Fekete et al., 2018), as well as the release of inflammatory cytokines triggered by HIV infection that may contribute to behaviors that resemble symptoms of depression could be responsible for the observed differences (Readler, 2011). Another study in China also found a link between ART and mental health status, specifically depression (Xie et al., 2021). This finding may be as a result of more than one factor, such as direct exposure to HIV and ART, as well as stigma.

To the best of our knowledge, this is the first study to demonstrate a relationship between prenatal maternal HTQ scores and newborn amygdala volumes. Our findings add to the growing body of evidence across imaging modalities linking prenatal maternal stressors to the development of the infant amygdala.

CD4 count and brain volumes in HEU neonates

Among the infants born to mothers living with HEU we observe positive associations between maternal CD4 count in pregnancy and bilateral caudate and left thalamus volumes. These associations do not survive multiple comparison corrections. It is worth noting that both the left and right caudate are significant before correction, suggesting these may not be spurious results. We also report significant correlations between duration of maternal ART during pregnancy and caudate and putamen volumes. These results are unsurprising as maternal CD4 count and ART duration are likely highly associated as treatment strengthens the immune system, thereby increasing CD4 count. Further, within our cohort, we reported lower mean CD4 count in mothers who initiated ART post conception as compared to mothers who were on treatment at conception.

A recent study, as well as a manually segmented study of a smaller sample of our cohort, has shown an association between the volume of the caudate in infants and maternal immune status during pregnancy (Wedderburn et al., 2022, Ibrahim et al., 2023). The caudate is connected to various brain regions, including the prefrontal cortex, the thalamus, and the globus pallidus. The caudate plays a critical role in the cortico-striato-thalamo-cortical circuit, which is involved in the regulation of motor function, motivation and reward. Damage to the caudate may lead to problems with motor coordination and balance, as well as difficulties in initiating movement. It can also affect the ability to learn new motor skills, and to make decisions based on rewards and punishments. The thalamus on the other hand, is a key relay station for sensory information going to the cerebral cortex, and its reduction can lead to deficits in attention and consciousness. This observation could be due to the production of pro-inflammatory cytokines and the inflammatory effects of MIA which occurs when a pregnant woman's immune system is stimulated. MIA can cross the placenta and affect the developing brain. The caudate and thalamus have been noted to be especially sensitive to the effects of MIA (McAllister et al., 2015). Further, these effects may be due to proximity of these structures to the ventricles of the brain which produces and contains CSF making them more prone to HIV-induced inflammation and neurotoxicity.

Our data suggest that ART timing offers neuroprotection and maternal immune health plays a role in subcortical development in HEU newborns. Overall, the findings presented add to the growing body of evidence linking maternal health in pregnancy to early brain development. These results suggest that interventions aimed at reducing prenatal maternal stress and boosting immune health may be an important way to promote healthy brain development in infants.

4.2 Neonate volumes: HUU vs HEU-pre-conception

The first month after birth is a crucial period for white matter development, with rapid maturation and increased myelination of axons (Dubois et al., 2014). In this study, we observed a decrease in the cerebral white matter volume of HEU neonates exposed to ART since conception as compared to HUU infants. Even though there are no prior studies on cerebral

white matter volume in infants who are HEU, a DTI study in infants uninfected but exposed to HIV reported higher mean localized FA, a measure of white matter integrity, in the cerebral peduncles (Tran et al., 2016). Studies in older children have reported HIV-exposure related increases in FA in cerebral white matter regions (Jankiewicz et al, 2017, Madzime et al., 2021), as well as reduced NAA, a metabolite related to axonal integrity, in the peritrigonal white matter region (Graham et al., 2020).

In animal models, ART has been associated with white matter damage and cognitive impairments. One study demonstrated that exposure to the ART drugs AZT and EFV caused oxidative stress, inflammation, and demyelination in the white matter of rats, which led to a reduction in the number of myelinated axons and impaired cognitive function (Sharma et al., 2017). Other studies showed that ART exposure in macaque monkeys resulted in decreased white matter volume, altered myelin structure, and increased microglial activation in the brain (Anderson et al., 2014, Thompson et al., 2020).

Even though ART may help prevent HIV-related brain abnormalities, it may also have neurotoxic effects on the brain of exposed infants (Hameed et al., 2017). Further, exposure to ART medications could have toxic effects on the developing brain, or interact with other prenatal medication to induce toxicity. Although myelination does not begin until the fifth fetal month, a premyelinating phase is an essential step for myelination. This phase is characterized by the presence of myelination glia (made up of precursor and immature oligodendrocytes) in the first fetal month (Zanin et al., 2011). It is possible that the neurotoxic effect of ART impacted the premyelinating phase because oligodendrocytes are highly susceptible to toxins (Barateiro et al., 2016). Although there are no previous studies linking the ART combination used by mothers in this cohort (Tenofovir/Efavirenz/Emtricitabine) to infant brain structures, a study on HEU children in Botswana suggests that infants who were exposed to the antiretroviral drug Efavirenz in utero may have an increased risk of developing deficits in expressive language, fine motor and social-emotional skills compared to infants exposed to other types of antiretroviral drugs (Cassidy et al., 2019). While other studies found no link between Tenofovir exposure and infant outcomes (Gibb et al., 2012, Siberry et al., 2012).

We found that infants whose mothers initiated ART before pregnancy also had increased left lateral ventricle volumes compared to HUU infants. This finding was unexpected and we suggest it may be due to the reduction in white matter. As white matter volume in the left hemisphere reduces, the left lateral ventricle volume expands to compensate. However, the lateral ventricle enlargement may also be due to the neurotoxic effect of ART exposure during the first trimester.

4.3 Neonate volumes: HUU vs HEU-post-conception

The caudate is a brain structure located in the basal ganglia and is involved in the regulation of voluntary movements, learning, and memory. During fetal development, the caudate undergoes a complex process of maturation, which involves the migration of neurons, the formation of synaptic connections, and the establishment of functional networks (Driscoll et al., 2022). Studies suggest that exposure to ART initiated post-conception may affect neurogenesis and neuronal migration and disrupt normal brain development - including the caudate (Sylor et al., 2019). Reduced caudate volume in infancy has been associated with a range of cognitive and behavioral impairments, such as deficits in attention, impulsivity, and executive function, later in life (Girard et al., 2011).

Our study found the caudate to be smaller in volume bilaterally in infants that are exposed to HIV and whose mothers initiated ART post-conception. Our finding is consistent with another study from the same cohort which employed manual segmentation in select structures (basal ganglia, thalamus and cerebellum) in a subset of infants (Ibrahim et al., 2023). They observed reduced caudate volumes bilaterally in infants exposed to ART post-conception (Ibrahim et al., 2023). This also builds on a recent study that reported a volume reduction in the caudate of HEU infants, although they found no difference in caudate volume reduction based on time of ART initiation (Wedderburn et al., 2022). Previous MRS studies found no difference in the basal ganglia of HEU and HUU children at ages 5, 7 &10 (Holmes et al., 2017, Robertson et al., 2018), while Robertson and colleagues found altered basal ganglia metabolite in HEU children at 9 years. These suggest that the basal ganglia may be especially vulnerable to HIV exposure in

utero at specific points in neurodevelopment, particularly the caudate which is in close proximity to the lateral ventricles (and cerebrospinal fluid).

Apart from HIV, there are other maternal infections that have been associated with changes in the infant caudate/basal ganglia. Studies have found that infants born to mothers with CMV infection during pregnancy are at increased risk for abnormalities in the basal ganglia and other brain regions (Grosse et al., 2018; Lim et al., 2019). Another example is maternal exposure to influenza virus. Some studies have also suggested that maternal influenza infection during pregnancy may be associated with increased risk of neurological and psychiatric disorders in offspring, which may involve alterations in the basal ganglia (Brown et al., 2006; Canetta et al., 2014). The effects of HIV and other maternal infections on cognitive development in infants and children are likely multifactorial, involving both direct and indirect effects of the virus on the developing brain. Maternal infections such as HIV can cross the placenta and infect the fetal brain, leading to inflammation and damage to brain cells. The infection can also indirectly affect the mother and the environment in which the child is raised. HIV infection can cause immune system dysfunction, which can lead to chronic inflammation and oxidative stress in the mother and the developing fetus (Dirajlal-Fargo et al., 2019).

In addition to the caudate, we also reported reduced right amygdala volume in the HEU-post conception as compared to the HUU infants. This result was present after adding HTQ scores as a confounder, pointing to the contribution of both trauma and HIV/ART trauma to the development of the right amygdala. This result points to the potential multifactorial nature of HIV exposure on the developing brain.

Future Work

Studies have reported neurodevelopmental delays in HEU infants, including deficits in cognitive, language, and motor domains compared to their HIV-unexposed uninfected peers (HUU) (Laughton et al., 2013; Le Doare et al., 2017; McHenry et al., 2018; Wedderburn et al., 2019;). The volumetric differences reported in this study may provide some insights into the underlying mechanisms of these deficits in HEU infants. The decrease in caudate volume observed in HEU-

post infants in this study is particularly interesting, as the caudate is known to play a crucial role in motor, language and cognitive processes (Arsalidou et al., 2012; Haber, 2016; Driscoll et al., 2022). Therefore, the decrease in caudate volume observed in HEU-post infants may contribute to an increased future risk of motor and cognitive deficits.

Similarly, the decrease in cerebral white matter observed in HEU-pre infants in this study may also be contribute to cognitive deficits observed in HEU children. White matter plays a crucial role in the communication between different regions of the brain, and abnormalities in white matter development have been associated with cognitive deficits in various neurological disorders (Kanaan et al., 2015). White matter integrity and volume during infancy has been linked with subsequent language (Zuk et al., 2021), motor (Gryga et al., 2012) and cognitive learning and abilities (Sánchez et al., 2023).

Overall, the volumetric differences reported in this study may provide some insights into the neural mechanisms underlying the cognitive deficits observed in HEU infants and children. Future work in this cohort is needed to examine the possible contributions of volume reductions in cognitive outcomes.

And, as this is a cross sectional analysis, future work could include longitudinal follow-up to identify if the changes and associations reported persist or resolve at later ages.

The study investigated the potential impact of ART initiation with respect to one specific ART regimen on neonatal brain volumes. It is possible that different ART drugs may have varying effects on the developing brain of the infant. Future research could explore this further by examining the impact of different ART regimens on neonatal brain volumes and long-term developmental outcomes. There's also a need to understand the extent to which ART is neuroprotective, and separating the neurotoxic effect(s) of ART from that of HIV. Future studies could include newborns living with HIV and compare their neurodevelopmental outcomes to those of HEU infants. This will help to determine whether any observed neurodevelopmental deficits in HEU infants are solely due to HIV exposure or are also influenced by ART. Future studies could utilize more objective measures of trauma exposure, such as cortisol levels, as well as other maternal health factors to improve the validity of the findings.

Methodological limitations

While structural MRI is a powerful tool for investigating aspects of brain structure, it is important to note that it does not provide information about brain function. Future studies may need to incorporate fMRI or other measures of function to better understand the functional consequences of these alterations.

A limitation of the study is that the timing of CD4 count testing during pregnancy may not have considered the possibility of immune health variations throughout pregnancy. Additionally, the HTQ measures were self-reported (which may be subject to bias and variability in reporting) and only assessed measures at a particular point in pregnancy and do not account for any trauma events that may have occurred after the test was conducted.

The use of a cross-sectional design for this study limits the ability to establish causal relationships between HIV/ART exposure and brain volumes since it only captures a single moment in time and does not follow participants over an extended period. It is difficult to determine whether the observed differences in brain volumes are due to HIV/ART exposure or other factors, such as genetic predisposition. A longitudinal design would be better suited to investigate the causal relationship between HIV/ART exposure and brain volumes over time, allowing for the identification of changes in brain volumes and the assessment of their relationship to HIV/ART exposure.

Strengths

The strength of this study includes a relatively large sample size (~150 participants), inclusion of maternal health data and duration of ART exposure. Because this study was carried out a few weeks after birth, the impact of other environmental factors (such as parenting, feeding practice) that could impact brain development have been limited.

This work follows a recently published study of a smaller group of infants from this cohort that examined manually segmented volumes (basal ganglia, thalamus and cerebellum) (Ibrahim et al., 2023). While this thesis examined more structures and a larger population, the overlap of results strengthens our confidence in the relatively new infant FreeSurfer tool. Both studies

reported reduced caudate volumes in neonates born to mothers who initiated ART post-conception. Further, both studies found associations between caudate and maternal CD4 count.

5. CONCLUSION

Taken together, the results presented in this thesis provide evidence that several maternal health factors – timing of maternal ART initiation, maternal immune health and maternal trauma assessed during pregnancy – influence HEU newborn neurodevelopment. Across newborns, higher evidence of maternal trauma in pregnancy relates to larger amygdala volumes. And, among uninfected neonates born to mothers living with HIV, higher maternal CD4 correlates with larger caudate and thalamus volumes. We find evidence of the neuroprotective properties of ART in pregnancy for the newborn, with longer exposure to ART in utero related to larger caudate and putamen volumes. Further, only HEU-post conception infants demonstrated reduced caudate volumes. Whereas cerebral white matter may be sensitive to the potential neurotoxic properties of early ART initiation. Lastly, we report reduced right amygdala volumes, influenced by maternal trauma as well as HIV/ART exposure. Our findings demonstrate the multifactorial approach needed in early infancy to understand the factors contributing to neurodevelopmental delays in HEU infants and children.

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