BMJ Open

The Prenatal Exposure And Child brain and mental Health (PEACH) Study: protocol for a cohort study of children and youth with prenatal alcohol exposure

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-051660
Article Type:	Protocol
Date Submitted by the Author:	24-Mar-2021
Complete List of Authors:	Lebel, Catherine; University of Calgary, Gibbard, Ben; University of Calgary, Department of Pediatrics Tortorelli, Chris; Social Work Mount Royal University Pei, Jacqueline; University of Alberta, Department of Biomedical Engineering Beaulieu, Christian; Univeristy of Alberta Bagshawe, Mercedes; University of Calgary, Department of Radiology McMorris, Carly A.; University of Calgary
Keywords:	MENTAL HEALTH, Child & adolescent psychiatry < PSYCHIATRY, Magnetic resonance imaging < RADIOLOGY & IMAGING, Depression & mood disorders < PSYCHIATRY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

The Prenatal Exposure And Child brain and mental Health (PEACH) Study: protocol for a cohort study of children and youth with prenatal alcohol exposure

Catherine Lebel[1,2,3], Ben Gibbard[2,4], Chris Tortorelli[5], Jacqueline Pei[6], Christian Beaulieu[7], Mercedes Bagshawe[1,2,3], Carly A. McMorris[2,8]

[1]Department of Radiology, University of Calgary

[2]Alberta Children's Hospital Research Institute, University of Calgary

[3]Hotchkiss Brain Institute, University of Calgary

[4]Department of Pediatrics, University of Calgary

[5]Social Work, Mount Royal University

[6]Department of Biomedical Engineering, University of Alberta

[7]Department of Educational Psychology, University of Alberta

[8]Werklund School of Education, University of Calgary

Corresponding Author:

Catherine Lebel
Alberta Children's Hospital
28 Oki Dr. NW
Calgary, AB
T3B 6A8
clebel@ucalgary.ca

Authors' contributions: All authors contributed to conceptualization of this study. CL wrote the first draft, and all authors edited the manuscript.

Competing interests. None.

Funding statement: This work is supported by the Canadian Institutes of Health Research (PJT-162217).

Word Count: 3776

Abstract

Introduction: Fetal alcohol spectrum disorder (FASD), which is caused by prenatal alcohol exposure (PAE), affects an estimated 4% of North Americans, and is the most common preventable cause of intellectual disability. Mental health issues, including anxiety and depression, are experienced by nearly all individuals with FASD. However, there is very limited knowledge about effect treatments for mental health problems in individuals with FASD; effective treatments are hindered in part due to a lack of understanding of the basic neurobiology underlying internalizing disorders in youth with FASD.

Methods and analysis: The Prenatal Exposure And Child brain and mental Health (PEACH) study includes children aged 7-18 years. We will use longitudinal neuroimaging (anatomical T1-weighted, diffusion, and passive viewing function magnetic resonance imaging) and mental health assessments (Behaviour Assessment Scale for Children [BASC-3], Multi-dimensional Anxiety Scale for Children [MASC-2], Children's Depression Inventory [CDI-2], Kiddie Scale of Affective Disorders [K-SADS]) to: (1) characterize trajectories of brain development in youth with FASD, (2) determine whether brain abnormalities mediate increases in anxiety and depression in youth with FASD, and (3) identify baseline brain features that predict changes of internalizing symptoms over the next 2 years in FASD. All of this will be done while considering sex and adverse postnatal experiences, which can significantly impact mental health and brain outcomes.

Ethics and dissemination: Ethics approval has been obtained in both testing sites (Edmonton and Calgary). The results of this study will be disseminated in peer-reviewed journals, at relevant conferences, and in conjunction with our knowledge mobilization partners.

Significance: This project will forge new understanding of FASD and mental health from a neurobiological perspective, highlighting key time periods (i.e., sensitive windows) and brain regions (i.e., that may be susceptible to neurostimulation), while identifying factors that predict individual trajectories of anxiety and depression symptoms.

Strengths and Limitations of this Study

- We will measure brain alterations in children and youth with prenatal alcohol exposure (PAE) using magnetic resonance imaging
- Alcohol-exposed participants will have confirmed prenatal alcohol exposure, though specific measures of timing, frequency, and dose of prenatal alcohol exposure are often difficult to obtain
- We use multiple mental health assessments to measure symptoms of depression and anxiety, and include a comprehensive neurocognitive battery
- This study uses a longitudinal, prospective design and will follow children over 2 years
- We incorporate comprehensive assessment and analysis of both prenatal and postnatal adverse exposures

Introduction

Fetal alcohol spectrum disorder (FASD) is a neurodevelopmental disorder caused by prenatal alcohol exposure (PAE). It is characterized by life-long cognitive, behavioural, and neurological deficits,[1] and its prevalence of FASD in North America is estimated to be 4%,[2 3] with lifetime costs over $1M per individual.[3-5] Beyond the primary cognitive and behavioural deficits, over 90% of individuals with FASD experience co-occurring mental health problems,[6-8] compared to 20% in the general population[9]. Depression and anxiety are among the most common, affecting 45-50% and 20-40% of individuals with FASD, respectively.[10-12] Developing early and appropriate interventions to minimize mental health problems and maximize adaptive outcomes in FASD is critical for improving quality of life and reducing the societal burden of FASD. Concerns have been raised that existing mental health treatments for individuals with FASD may be less effective than for the general population,[13 14] perhaps hindered by a lack of understanding of their neurobiological basis.

Magnetic resonance imaging (MRI) can be used to investigate neurological abnormalities in

FASD. The most common MRI finding in individuals with FASD is widespread reductions in brain volume, which have been observed with anatomical MRI from neonates to adults.[15-17] Diffusion tensor imaging (DTI) assesses microstructure of structural white matter connections via fractional anisotropy (FA) and mean diffusivity (MD), measures sensitive to myelination and axonal density.[18] Numerous studies have shown lower FA and/or higher MD in children, adolescents, and young adults with FASD.[19-22] Recent studies suggest that brain diffusion alterations are also present in infants and young children, though in the opposite direction (i.e., higher FA and lower diffusivity).[23 24] Resting state functional MRI (rs-fMRI) measures patterns of spontaneous brain connectivity by correlating functional signals across regions ("functional connectivity");[25] findings suggest atypical functional connectivity in children and youth with FASD.[26-29] Regional brain volume reductions, weaker white matter connectivity (lower FA/higher MD), and atypical functional connectivity have been reported throughout the brain, but alterations are most prominent in subcortical structures[17 30] and prefrontal areas[29-32]. Most studies to date have been cross-sectional, and thus the trajectories of brain maturation remain unclear. The few longitudinal MRI studies that do exist in FASD, show that children with FASD have faster changes of cortical thickness,[33] volume,[34] and white matter connectivity[35] than unexposed controls; these faster changes possibly reflect a "catch-up" in brain maturation. It is not known how key functional networks change with age in FASD. Longitudinal research is critical for revealing the developmental trajectories of brain connectivity in FASD.

Previous studies have related cognitive abilities and clinical features (e.g., dysmorphology) to brain structure in FASD;[29 34 36-39] however few studies have examined relationships between brain measures and mental health.[40] In individuals without FASD, internalizing symptoms are most commonly associated with brain alterations in the anterior cingulate cortex (ACC), medial prefrontal cortex (mPFC), dorsolateral prefrontal cortex (dlPFC), amygdala, and hippocampus, as well as connections between these structures.[41-43] Resting state functional connectivity is higher in the ACC and mPFC in adolescents with depression,[43 44] while weaker structural connectivity (lower FA/higher MD) in frontal

white matter (e.g., cingulum, uncinate) is associated with depression and anxiety in youth.[45-50] Areas identified by neuroimaging (e.g., PFC, cingulate) are used as brain targets for neurostimulation to treat adults with depression and anxiety,[51-53] highlighting the importance of understanding the neurological correlates of internalizing symptoms. Thus, brain alterations induced by PAE may increase the risk of internalizing disorders.

Little is known about the trajectories of mental health symptoms in youth with FASD, though difficulties tend to persist or worsen with age.[54 55] However, FASD is a heterogenous disorder, with heterogeneous outcomes, [56] so it is critical to consider differences at the individual level. In adolescents without FASD, functional connectivity between the amygdala and mPFC predicts the severity of future internalizing symptoms[57]. Brain volumes in the hippocampus[58] and ACC[59] also predict treatment response in adults with depression (but without FASD). However, it is unclear which baseline features predict future mental health outcomes in youth with FASD, though this could inform treatment decisions. Thus, longitudinal research is needed to understand associations between brain alterations and trajectories of depressive and anxiety symptoms in individuals with FASD, to help predict individual outcomes.

Individuals with FASD frequently have adverse postnatal experiences (~43% have abuse or neglect).[60-62] Such early adversity is commonly operationalized as adverse childhood experiences (ACEs),[63] which provides a cumulative risk score accounting for abuse, neglect, and other household dysfunction in childhood. In the general population, ACEs are associated with heightened risk of anxiety and depression[64] and alterations to frontal and limbic brain structure and function.[65-73] Animal studies show that PAE and postnatal adversity interact to increase depression risk[74]. Very few human studies of FASD have incorporated any measure of postnatal risk. Two recent human studies show differential associations between socio-economic status and brain volumes in children with and without PAE,[75 76] and one showed that postnatal adversity (neglect, abuse, etc.) moderates the association between PAE

and brain connectivity.[22] All of this evidence underscores the need for FASD studies to consider early adversity. ACEs treats all adverse experiences similarly, although different types of adversity may have different effects on individuals.[77] We recently developed a risk characterization framework that accounts for the duration, frequency, timing, and type of risks, which we believe is more appropriate for children with FASD, who may experience a wide range of adversities.[61]

Understanding brain development in FASD, its relation to internalizing symptoms, and predictors of positive outcomes is critical for targeting treatments at the right time (e.g., age-appropriate therapy),[78] for the right brain regions (e.g., for neurostimulation),[51] and for the right person (e.g., considering individual circumstances).[79] In this study, we will recruit 125 FASD and 125 control youth (7-18 years) and acquire longitudinal MRI and mental health assessments to study trajectories of brain and mental health with the following aims:

1. Characterize developmental trajectories of brain connectivity in youth with FASD. *Hypothesis 1:* Structural and functional connectivity to the prefrontal cortex, hippocampus, and amygdala will show faster increases in FASD compared to unexposed controls.
2. Determine whether brain structure and function mediate the relationship between FASD and internalizing symptoms. *Hypothesis 2:* Brain connectivity between the amygdala, hippocampus, and prefrontal cortex (specifically, lower FA and stronger functional connectivity) will mediate the association between FASD and symptoms of anxiety and depression.
3. Identify baseline factors that predict changes of internalizing symptoms over time in FASD. *Hypothesis 3:* Weaker structural connectivity, stronger functional connectivity, and smaller brain volumes at baseline will predict worsening anxiety and depressive symptoms over the subsequent two years.

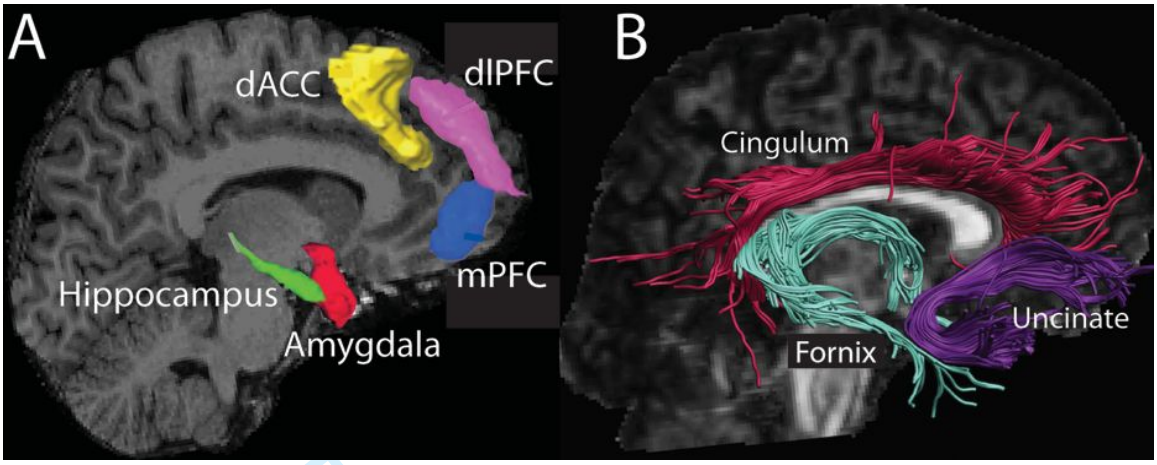

Figure 1: Key gray matter regions (A) and white matter connection (B) related to anxiety and/or depression symptoms. Volume of the regions in A, functional connectivity between pairs of regions in A, and structural connectivity (diffusion metrics) of tracts in B will be measured.

Methods and Analysis

Participants. We will recruit 125 children and youth with PAE and 125 unexposed controls aged 7-18 years. This age range was chosen because: (a) FASD diagnosis typically occurs at ~6-7 years in Alberta,[80] (b) 7-18 years includes the most common ages of onset for anxiety disorders,[81] (c) depression and anxiety symptoms are common in youth with PAE of this age range,[8] and (d) children this age are more likely to tolerate MRI scanning than younger children.[82] Repeat assessments and MRI scanning will occur 2 years after baseline, which allows for measurable brain development within individuals,[83] as well as meaningful changes in mental health symptoms. Approximately equal numbers of males and females will be recruited to be able to appropriately examine sex effects, and approximately half in Edmonton and half in Calgary. Informed written consent will be obtained from parents/guardians, as well as written assent from children/youth.

PAE Group. Participants will be recruited through diagnostic clinics throughout Alberta (including the Pediatric RASD Clinic at the Glenrose Hospital in Edmonton and the Cumulative Risk Diagnostic Clinic at the Alberta Children's Hospital in Calgary), Alberta Children's Services, parent/caregiver support groups for FASD, community groups (e.g., Calgary and Edmonton Fetal

Alcohol Networks), as well as online advertisements and word of mouth.

Participants in the PAE group must have a diagnosis of FASD or confirmed PAE at levels consistent with Canadian FASD diagnostic guidelines (≥7 drinks/week or ≥2 binge episodes at some point during pregnancy).[1] Alcohol exposure will be confirmed via biological mother's self-report, reliable observations by close family or friends, clinical observation, and/or medical, legal, or child services records. Participants with genetic disorders associated with significant intellectual or developmental impairments, diagnosed with a neurological disorder (e.g., epilepsy, cerebral palsy), or with contraindications to MRI (i.e., metal implants, dental devices, claustrophobia) will be excluded.

Control Group. Controls must have confirmed absence or minimal PAE (≤5 drinks total in pregnancy, with no binge episodes) via biological maternal report, no diagnosis of genetic, neurological, or developmental disorders, and no contraindications to MRI scanning. Controls will be recruited through online advertisements, parent groups in Edmonton and Calgary, and word of mouth.

MRI Scanning. MRI scanning at baseline and 2-year follow-up will take place at the Alberta Children's Hospital (ACH, Calgary) on a research-dedicated General Electric 3T MR750w system, or at the Peter S Allen MRI Centre (Edmonton) on a research-dedicated Siemens 3T Prisma. The imaging protocol is detailed in Table 1.

Table 1: Magnetic resonance imaging protocol. Parameters are given for GE MR750w before Siemens Prisma; if only one set of parameters is given, they were the same for both scanners.

Sequence	Scan Time (min:sec)	Resolution (mm^3)	Slices	FOV (cm)	TR (ms)	TE (ms)	Other information
Passive viewing fMRI (ss-EPI)	8:10	3.6x3.6x3.6	36	23	2000	30	Acquired while watching a clip from Planet Earth
ASL (3D)	5:01	3.5x3.5x3.5 / 1.9x1.9x3.5	34	23/24	4600/4845	15.6/10.1	TI 1990/2025 ms
DTI	7:08/14:12	2.2x2.2x2.2	57	22/24.2	6300/12000	55/98	5/10 b0, 30 dir b900, 30 dir b2000
3D T1 (FSPGR BRAVO/ MP-RAGE)	4:57	0.8x0.8x0.8	192	25.6/24	1880/8.25	2.9/3.16	Flip angle 10, TI 948/600 ms,

QSM (3D SPGR/R2Star)	5:16	1.0x1.0x2.0 / 0.47x0.47x2.0	80	24	42/44.8	3.8-36.8/4.1-37.9	7/8 echoes, flip angle 17/15
ihMT	3:09	0.9x0.9x5.0	30	22	8500/15000	85/103	

fMRI = functional magnetic resonance imaging; EPI = echo planar imaging; SPGR = spoiled gradient; ASL = arterial spin labeling; DTI = diffusion tensor imaging; FSPGR = fast spoiled gradient; MP RAGE = magnetization prepared rapid acquisition gradient echo; QSM = quantitative susceptibility mapping; ihMT=inhomogeneous magnetization transfer

Image Analysis. T1-weighted images will be processed using FreeSurfer's[84] longitudinal processing stream.[85] Each subject's parcellation will be manually checked and receive minor corrections if necessary. Brain volumes of the left and right hippocampus, amygdala, and prefrontal cortical areas (dACC, dlPFC, mPFC) will be extracted.

Diffusion data will be quality checked, brain extracted, and corrected for eddy currents and head motion. Fractional anisotropy (FA) and mean diffusivity (MD) maps will be generated for each subject. Tractography (FA>0.2, angle<30°) will be used to reconstruct white matter fibers connecting frontal and limbic regions (uncinate fasciculus, cingulum, fornix). FA and MD will be assessed within each white matter fiber bundle as primary variables of interest. The dual b-value scan also allows for more advanced diffusion models and analysis,[86 87] which will be examined in follow-up analyses after primary aims are complete.

Assessment of rs-fMRI data will use AFNI and FSL tools.[88 89]. Each individual's fMRI data will be registered to their anatomical (T1-weighted) scan, then to a pediatric brain template for 5-18 year olds.[89 90] Volumes with high framewise displacement (>0.25 mm) will be identified and regressed out. Scans with <5 minutes of low-motion data will be eliminated. For each prefrontal region (dlPFC, mPFC, dACC) and the hippocampus and amygdala, averaged time courses will be generated. Correlations between time courses in each pair of regions will be analyzed to measure functional connectivity.

Mental Health Assessments. Mental health assessments will occur at baseline, a subset of tests will be administered online at 1-year follow-up, and the full set will be administered again at 2-year follow-up (see Table 2). Symptoms of depression and anxiety will be measured using both self- and caregiver-

reports on the Behavior Assessment System for Children (BASC-3)[91], Child Depression Inventory-2 (CDI-2),[92 93] and the Multidimensional Anxiety Scale for Children (MASC-2).[94] The MASC-2 is a self-report assessment of anxiety symptoms in children and adolescents (8-19 years of age). The CDI-2 is a brief questionnaire that measures cognitive, affective, and behavioural signs of depression in children and adolescent ages 7-17 years. For youth/young adults ≥18 years, we will use the Beck Depression Inventory (BDI)[95] and PROMIS Anxiety Short Form[96] to assess depression and anxiety symptoms, respectively. The BASC-3 provides a validated assessment of a range of mental health symptoms, including anxiety and depression, while the CDI-2 and MASC-2 provide more specific measures of depression and anxiety symptoms, respectively, that are consistent with diagnostic criteria. The BASC-3 caregiver report and self-report (only children ≥12) will be used to assess behaviour.

To determine whether an individual meets diagnostic criteria for anxiety or depression, youth ≥12 years of age and all caregivers will complete a diagnostic assessment of internalizing mental health disorders with a trained and reliable clinician using the mood and affective disorders subscales of Kiddie Schedule for Affective Disorders and Schizophrenia – Lifetime Version (K-SADS-PL).[97] The K-SADS-PL is a semi-structured diagnostic interview and gold standard for assessing a variety of mental health disorders in youth based on DSM-5 diagnostic criteria.[98] The Diagnostic Interview for Anxiety and Mood, and OCD and Related Neuropsychiatric Disorders (DIAMOND)[99] will be used for participants over 18 years.

We also examine the frequency, chronicity and location of pain of children and youth in the past 30 days.[100 101] The Adaptive Behavior Assessment System (ABAS-3) is a comprehensive parent report measure of the adaptive or daily functioning skills of children and youth across the lifespan. [102] The Sensory Profile (SP-2) measures a child's sensory processing patterns in various contexts (home, school, and community settings).[103]

Table 2: Questionnaires and Assessments

Questionnaire and assessments are listed below for each study time point. Caregiver refers to a parent or guardian who regularly cares for the child. Study personnel support younger children in completing the questionnaires if necessary.

	Time 1 (in person)	Time 2 (online)	Time 3 (in person)	Age limits
Mental Health				
Child Depression Index (CDI-2)	Caregiver report Self-report	Caregiver report Self-report	Caregiver report Self-report	Beck Depression Inventory (BDI-II) for youth >17 years
Multidimensional Anxiety Scale for Children (MASC-2)	Caregiver report Self-report	Caregiver report Self-report	Caregiver report Self-report	PROMIS Anxiety for young adults ≥18 years
Behavior Assessment System for Children (BASC-3)	Caregiver report Self-report	Caregiver report Self-report	Caregiver report Self-report	Self-report only for children ≥12 years
Kiddie Scale of Affective Disorders and Schizophrenia (K-SADS)	Caregiver interview Child interview		Caregiver interview Child interview	Child interview for children ≥12 years DIAMOND for young adults >18
Adaptive Behavior Assessment System (ABAS-3)	Caregiver report		Caregiver report	
Pain questionnaire		Caregiver report Child report		
Sensory Profile 2 (SP-2)	Caregiver report		Caregiver report	For children <15 years
Cognitive Function				
Weschler Abbreviated Scale of Intelligence (WASI-II) 2-subtest form	Matrix Reasoning, Vocabulary			
Rey-Osterrieth Complex Figure Test (Rey-O)	Child		Child	
Weschler Individual Achievement Test (WIAT-III)	Word Reading, Pseudo-Word Reading, Oral Reading Fluency, Reading Comprehension, Numerical Operations		Word Reading, Pseudo-Word Reading, Oral Reading Fluency, Reading Comprehension, Numerical Operations	
NEPSY-II	Inhibition, Word Generation		Inhibition, Word Generation	Children <17 years
California Verbal Learning Task -Child (CVLT-C)	Child		Child	CVLT-3 for youth ≥17 years

Wisconsin Card Sort Task (WCST)	Child		Child
Other information			
Demographic questionnaire	Caregiver	Caregiver	Caregiver
Prenatal and postnatal exposure assessment	Caregiver; medical, legal, Children's Services records		
Puberty questionnaire	Caregiver, child	Caregiver, child	Caregiver, child
Gender identity questionnaire	Caregiver, child		
Adverse childhood experiences (ACEs)	Caregiver on behalf of child		

Cognitive Assessments. IQ will be assessed using the Wechsler Abbreviated Scale of Intelligence – Second Edition (WASI-II)[104] 2-subtest form at baseline to obtain an estimate of Full Scale IQ (FSIQ). The Rey-Osterrieth Complex Figure Test (Rey-O) [105] examines visuospatial ability and visuospatial memory in individuals between 6-89 years of age. The Wisconsin Card Sorting Test (WCST)[106] is an executive functioning measure used to determine cognitive flexibility and set shifting in individuals 6 to 89 years of age. The California Verbal Learning Test (CVLT-C) measures learning and long-term recall and recognition of verbal information in 5-16.11 year olds.[107] The CVLT-3 will be used for youth ≥17 years. The NEPSY-II is a neuropsychological assessment tool for children aged 3-16 years of age that assesses functioning in 6 domains;[108] we will use the subscales of Inhibition (measuring inhibition) and Word Generation (verbal productivity). The Wechsler Individual Achievement Test (WIAT-III) measures academic abilities in children and adolescents aged 4-50.11 years[109].

Early Adversity. Adverse exposures are assessed using questions adapted from the National Crittenton Foundation ACEs survey,[63 110] a validated survey deemed acceptable by families and caregiving agencies.[111] For children in foster or adoptive care, adverse experiences will be ascertained through child services records and interviews with biological and/or adoptive parents. With this information, we will apply our own characterization tool, which accounts for the timing, amount, and

type of adverse exposure(s) experienced.[61]

Other Variables. Caregivers will complete a comprehensive demographic survey that includes information about other individuals in the house, household income, parent education, etc. Caregivers will be asked if youth have other diagnoses or if they are taking medications. Youth will be asked to self-report their sex and gender. Sex will be included as a covariate in all analyses, and sex-by-age or sex-by-anxiety/depression interaction terms will be included where appropriate. Gender and its interaction terms will be used as additional covariates if numbers permit.

Statistical Analysis. Statistical analysis will occur in SPSS (IBM), R (www.r-project.org), and Matlab. Aim 1 will begin with a cross-sectional analysis using a regression model including age, sex, group, and age-by-group interaction terms, run separately for each brain measure. Once longitudinal data is available, linear mixed effects models in R (using lme4 and lmerTest)[112 113] will be used to determine developmental patterns of structural and functional connectivity for the FASD and control groups. Subject will be modeled as a random factor, with sex, age, group, and age-by-group included in the model. For both cross-sectional and longitudinal analyses, postnatal adversity and IQ will be included as covariates. Mixed effects models will be run separately on volumes of each region, structural connectivity (FA) for each white matter tract, and functional connectivity (correlation) for connections between each pair of regions in the prefrontal-amygdala-hippocampus network (shown in Fig. 1). False discovery rate (FDR) will be used to correct for multiple comparisons.

For Aim 2, mediation will be carried out by testing the first pathway from the main predictor (PAE) to each brain measure (volume, structural or functional connectivity), controlling for age and sex of participants, and then testing the second pathway from each brain measure to each mental health measures (anxiety or depression symptoms), with age, sex, and postnatal adversity as covariates. The overall mediation effect will be tested using percentile-based bootstrap confidence intervals, computed from 5000 simulations.[114] Caregiver-report *T*-scores from the CDI-2 and MASC-2 will be used as

primary measures of depression and anxiety symptoms, respectively. BASC-3 *T*-scores on the anxiety and depression subscales of internalizing symptoms will be used as secondary information. Self-report scores will be used for supplementary analysis, as they sometimes provide different information.[115 116]

For Aim 3, change in anxiety and depression will be calculated by subtracting the *T*-scores at Time 2 from the *T*-score at Time 1. Primary variables will be caregiver-reported CDI-2 and MASC-2 scores for depression and anxiety, respectively. Brain measures, postnatal adversity, sex, IQ, age, and group will then be entered into a multiple regression model to determine which baseline factors predict anxiety and depression trajectories over time. Change in BASC-3 *T*-scores on the anxiety and depression subscales over the 2 years, as well as self-report scores on CDI-2 and MASC-2 will be used in a supplementary analysis. Initially, change in anxiety and depression outcomes will be used as a continuous measure. If enough youth meet criteria for a diagnosis of a depression or anxiety disorder (as measured by the K-SADS), a group analysis (those who symptoms changed in severity to meet criteria for a diagnosis vs those who do not) will also be conducted.

Power Calculations. We collected preliminary data, including MRI, mental health assessments, and postnatal adversity on 17 children with FASD and 19 controls without PAE aged 7-15 years. Control subjects (part of a different study)[86 87] had follow-up scans and assessments ~2 years later. This preliminary data showed age-by-group interactions in structural and functional connectivity with effect sizes of 0.067-0.192 (partial eta^2). To detect effects this size for Aim 1 with power ≥0.8 using an ANOVA with main effects and interactions, we require 112 individuals total (calculated in G*Power). Preliminary data shows small-medium effects for both FASD-brain and brain-anxiety pathways in the mediation. According to simulations,[117] these effects require a sample size of ≥162 total individuals to detect mediation using percentile bootstrap with power≥0.8 (Aim 2). Linear regression of relationships between brain measures and changes in anxiety and depression in controls showed effects of r=0.4-0.6. To detect these effects (Aim 3), we require at least 130 participants total (G*Power). Thus, we aim to

have ≥162 individuals total (81 per group) at time 1 (Aims 1, 2), and ≥130 (65 per group) with longitudinal data (Aims 1, 3).

Patient and Public Involvement. Ms. Tortorelli (co-investigator on the project) was an Associate Director of Alberta Children's Services, and was involved in study design. She has since moved on to a role as Assistant Professor at Mount Royal University, but we continue to involve staff from Children's Services in the design and execution of the study, and will involve them in interpretation and dissemination of findings. We also have active relationships with organizations serving individuals with FASD and their families, including the Calgary and Edmonton Fetal Alcohol Networks (CFAN, EFAN) and CanFASD. These organization have provided study feedback and support, and will assist with interpretation and dissemination of findings.

Ethics and Dissemination.

This study has been approved by ethics boards at both sites (Calgary: REB17-0663; Edmonton: Pro00093230). Data collection takes 4-6 hours for youth, and 2-3 hours for caregivers (Table 1) and occurs at the Alberta Children's Hospital (Calgary) or University of Alberta Hospital (Edmonton). Caregivers complete some questionnaires ahead of the visit, and additional questionnaires and interviews during the visit. Children complete the MRI scan, mental health and neuropsychological assessments, and questionnaires during the visit. Children ≥12 years complete the KSADS interview. Breaks are given and support is provided as necessary for children and youth. Snacks, parking, and an honorarium ($150 at baseline visit, $50 at one-year visit, $250 at two-year visit) are provided for each family. The honorarium reflects the commitment of the families, as this study requires substantial time commitments from both child and caregiver. If an MRI reveals any incidental findings, it will be referred to the site's medical director (a neuroradiologist) for review and follow-up. If the mental health assessments reveal any concerns, youth will be referred by a child clinical psychologist (Drs. McMorris

or Pei) for appropriate follow-up through the child's physician or other appropriate mental health services.

Communication of our findings to other researchers will occur via publications in peer-reviewed journals and presentations at pertinent conferences (e.g., Organization for Human Brain Mapping, Canadian Academy of Child & Adolescent Psychiatry). As we publish our research findings, we will produce lay summaries and infographics for distribution to stakeholders via our website, our twitter accounts, Kids Brain Health Network's website (researchimpact.ca), social media (including Kids Brain Health Network's YouTube, Facebook and twitter accounts), and email.

Knowledge translation to the wider community will include direct communication (via reports, presentations, meetings) with diagnostic clinics and Children's Services. Results will be presented at policy and practice meetings (e.g., International Conference on Child and Family Maltreatment, Canadian Association of Pediatric Health Centres, Canadian Pediatric Society, Alberta College of Social Workers).

Potential Limitations. Research on children and youth with FASD is complex due to confounding pre- and postnatal exposures, missing information, and behavioural difficulties. If recruitment or retention are lower than anticipated, we will recruit participants outside Calgary and Edmonton (e.g., through the other 10 FASD Networks across Alberta). The strict diagnostic criteria used here will ensure that all participants have a minimum level of PAE, though exact amounts may not be known. We will use all available sources to characterize other risks and diagnoses in our participants and will statistically control for these in our analyses.[61] In some cases, information will be missing, which is a challenging but unavoidable aspect of doing research in this population.

Significance

FASD is a common disorder (~4% of Canadians) with a very high societal cost (>$17B annually). Most individuals with FASD experience co-occurring mental health issues throughout their

lifespan, but effective treatments are hindered by a lack of understanding of the neurobiological basis for these problems. The use of quantitative MRI to understand the brain abnormalities and atypical development patterns underlying mental health problems in youth with FASD is critical to early identification and appropriate intervention strategies to improve outcomes. This study will reveal developmental patterns of brain connectivity, identify the underlying neurological correlates of anxiety and depression symptoms in youth with FASD, and identify baseline brain features that can predict the worsening of anxiety and depression symptoms. This knowledge is crucial for advancing research and identifying prevention and early intervention strategies, which will have substantial benefits for these children and youth with FASD, their families, and the public health system and society. Thus, this innovative project will address significant gaps in the literature, inform prevention strategies, and promote early detection and intervention of internalizing issues in children and youth with FASD.

References

1. Cook JL, Green CR, Lilley CM, et al. Fetal alcohol spectrum disorder: a guideline for diagnosis across the lifespan. *CMAJ* 2016;188(3):191-7. doi: 10.1503/cmaj.141593
2. Flannigan K, Unsworth K, Harding K. The prevalence of fetal alcohol spectrum disorder, 2018.
3. Popova S, Lange S, Chudley A, et al. World Health Organization International Study on the Prevalence of Fetal Alcohol Spectrum Disorder (FASD) Canadian Component, 2018.
4. Thanh NX, Jonsson E. Costs of fetal alcohol spectrum disorder in Alberta, Canada. *Can J Clin Pharmacol* 2009;16(1):e80-90. [published Online First: 2009/01/20]
5. Stade B, Ungar WJ, Stevens B, et al. Cost of fetal alcohol spectrum disorder in Canada. *Canadian family physician Medecin de famille canadien* 2007;53(8):1303-4.
6. Kodituwakku PW. Defining the behavioral phenotype in children with fetal alcohol spectrum disorders: a review. *Neurosci Biobehav Rev* 2007;31(2):192-201.
7. May PA, Baete A, Russo J, et al. Prevalence and characteristics of fetal alcohol spectrum disorders. *Pediatrics* 2014;134(5):855-66. doi: 10.1542/peds.2013-3319
8. Pei J, Denys K, Hughes J, et al. Mental health issues in fetal alcohol spectrum disorder. *J Ment Health* 2011;20(5):438-48. doi: 10.3109/09638237.2011.577113
9. Smetanin P, Stiff D, Briante C, et al. The life and economic impact of major mental illnesses in Canada: 2011 to 2041, 2011.
10. Famy C, Streissguth AP, Unis AS. Mental illness in adults with fetal alcohol syndrome or fetal alcohol effects. *Am J Psychiatry* 1998;155(4):552-4. doi: 10.1176/ajp.155.4.552
11. Fagerlund A, Autti-Ramo I, Hoyme HE, et al. Risk factors for behavioural problems in foetal alcohol spectrum disorders. *Acta Paediatr* 2011;100(11):1481-8. doi: 10.1111/j.1651-2227.2011.02354.x
12. Denys K, Rasmussen C, Henneveld D. The effectiveness of a community-based intervention for parents with FASD. *Community Ment Health J* 2011;47(2):209-19. doi: 10.1007/s10597-009-9273-9
13. Anderson T, Mela M, Stewart M. The implementation of the 2012 mental health strategy for Canada through the lens of FASD. *Canadian Journal of Community Mental Health* 2017;36(4):1-13.
14. Flannigan K, Coons-Harding KD, Anderson T, et al. A Systematic Review of Interventions to Improve Mental Health and Substance Use Outcomes for Individuals with Prenatal Alcohol Exposure and Fetal Alcohol Spectrum Disorder. *Alcohol Clin Exp Res* 2020;44(12):2401-30. doi: 10.1111/acer.14490 [published Online First: 2020/10/30]
15. Donald KA, Eastman E, Howells FM, et al. Neuroimaging effects of prenatal alcohol exposure on the developing human brain: a magnetic resonance imaging review. *Acta Neuropsychiatr* 2015;27(5):251-69. doi: 10.1017/neu.2015.12
16. Lebel C, Roussotte F, Sowell ER. Imaging the impact of prenatal alcohol exposure on the structure of the developing human brain. *Neuropsychol Rev* 2011;21(2):102-18. doi: 10.1007/s11065-011-9163-0
17. Nardelli A, Lebel C, Rasmussen C, et al. Extensive deep gray matter volume reductions in children and adolescents with fetal alcohol spectrum disorders. *Alcoholism Clinical and Experimental Research* 2011;35(8):1404-17. doi: 10.1111/j.1530-0277.2011.01476.x [published Online First: 2011/05/18]
18. Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR Biomed* 2002;15(7-8):435-55.
19. Wozniak JR, Muetzel RL. What does diffusion tensor imaging reveal about the brain and cognition in fetal alcohol spectrum disorders? *Neuropsychology Review* 2011;21(2):133-47. doi: 10.1007/s11065-011-9162-1 [published Online First: 2011/02/25]

20. Lebel C, Rasmussen C, Wyper K, et al. Brain diffusion abnormalities in children with fetal alcohol spectrum disorder. *Alcohol Clin Exp Res* 2008;32(10):1732-40. doi: 10.1111/j.1530-0277.2008.00750.x
21. Wozniak JR, Mueller BA, Chang PN, et al. Diffusion tensor imaging in children with fetal alcohol spectrum disorders. *Alcohol Clinical and experimental research* 2006;30(10):1799-806.
22. Andre QR, Geeraert BL, Lebel C. Brain structure and internalizing and externalizing behavior in typically developing children and adolescents. *Brain Struct Funct* 2020;225(4):1369-78. doi: 10.1007/s00429-019-01973-y [published Online First: 2019/11/09]
23. Donald KA, Roos A, Fouche JP, et al. A study of the effects of prenatal alcohol exposure on white matter microstructural integrity at birth. *Acta Neuropsychiatr* 2015;27(4):197-205. doi: 10.1017/neu.2015.35
24. Kar P, Reynolds J, Grohs M, et al. White matter alterations in young children with prenatal alcohol exposure. *biorxiv* 2021 doi: https://doi.org/10.1101/2021.01.05.425489
25. van den Heuvel MP, Hulshoff Pol HE. Exploring the brain network: a review on resting-state fMRI functional connectivity. *Eur Neuropsychopharmacol* 2010;20(8):519-34. doi: 10.1016/j.euroneuro.2010.03.008 [published Online First: 2010/05/18]
26. Wozniak JR, Mueller BA, Bell CJ, et al. Global functional connectivity abnormalities in children with fetal alcohol spectrum disorders. *Alcohol Clin Exp Res* 2013;37(5):748-56. doi: 10.1111/acer.12024
27. Wozniak JR, Mueller BA, Mattson SN, et al. Functional connectivity abnormalities and associated cognitive deficits in fetal alcohol Spectrum disorders (FASD). *Brain Imaging Behav* 2017;11(5):1432-45. doi: 10.1007/s11682-016-9624-4 [published Online First: 2016/10/14]
28. Long X, Kar P, Gibbard B, et al. The brain's functional connectome in young children with prenatal alcohol exposure. *NeuroImage Clinical* 2019;24:102082. doi: 10.1016/j.nicl.2019.102082 [published Online First: 2019/12/05]
29. Long X, Little G, Beaulieu C, et al. Sensorimotor network alterations in children and youth with prenatal alcohol exposure. *Human Brain Mapping* 2018;in press
30. Zhou D, Rasmussen C, Pei J, et al. Preserved cortical asymmetry despite thinner cortex in children and adolescents with prenatal alcohol exposure and associated conditions. *Hum Brain Mapp* 2018;39(1):72-88. doi: 10.1002/hbm.23818 [published Online First: 2017/09/30]
31. Lebel C, Rasmussen C, Wyper K, et al. Brain diffusion abnormalities in children with fetal alcohol spectrum disorder. *Alcoholism Clinical and Experimental Research* 2008;32(10):1732-40. doi: ACER750 [pii]
10.1111/j.1530-0277.2008.00750.x [published Online First: 2008/08/02]
32. Paolozza A, Treit S, Beaulieu C, et al. Diffusion tensor imaging of white matter and correlates to eye movement control and psychometric testing in children with prenatal alcohol exposure. *Hum Brain Mapp* 2017;38(1):444-56. doi: 10.1002/hbm.23371
33. Treit S, Zhou D, Lebel C, et al. Longitudinal MRI reveals impaired cortical thinning in children and adolescents prenatally exposed to alcohol. *Hum Brain Mapp* 2014;35(9):4892-903. doi: 10.1002/hbm.22520
34. Lebel C, Mattson SN, Riley EP, et al. A longitudinal study of the long-term consequences of drinking during pregnancy: heavy in utero alcohol exposure disrupts the normal processes of brain development. *The Journal of neuroscience* 2012;32(44):15243-51. doi: 10.1523/JNEUROSCI.1161-12.2012 [published Online First: 2012/11/02]
35. Treit S, Lebel C, Baugh L, et al. Longitudinal MRI Reveals Altered Trajectory of Brain Development during Childhood and Adolescence in Fetal Alcohol Spectrum Disorders. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2013;33(24):10098-109. doi: 10.1523/JNEUROSCI.5004-12.2013 [published Online First: 2013/06/14]

36. Gautam P, Lebel C, Narr KL, et al. Volume changes and brain-behavior relationships in white matter and subcortical gray matter in children with prenatal alcohol exposure. *Hum Brain Mapp* 2015;36(6):2318-29. doi: 10.1002/hbm.22772
37. Green CR, Lebel C, Rasmussen C, et al. Diffusion Tensor Imaging Correlates of Saccadic Reaction Time in Children with Fetal Alcohol Spectrum Disorder. *Alcoholism, clinical and experimental research* 2013;37(9):1499-507. doi: 10.1111/acer.12132 [published Online First: 2013/04/05]
38. Lebel C, Rasmussen C, Wyper K, et al. Brain Microstructure Is Related to Math Ability in Children With Fetal Alcohol Spectrum Disorder. *Alcohol Clin Exp Res* 2010;34(2):356-63. doi: ACER1097 [pii]
10.1111/j.1530-0277.2009.01097.x [published Online First: 2009/11/26]
39. Treit S, Chen Z, Zhou D, et al. Sexual dimorphism of volume reduction but not cognitive deficit in fetal alcohol spectrum disorders: A combined diffusion tensor imaging, cortical thickness and brain volume study. *NeuroImage Clinical* 2017;15:284-97. doi: 10.1016/j.nicl.2017.05.006 [published Online First: 2017/06/01]
40. Andre QR, McMorris CA, Kar P, et al. Different brain profiles in children with prenatal alcohol exposure with or without early adverse exposures. *Hum Brain Mapp* 2020;41(15):4375-85. doi: 10.1002/hbm.25130 [published Online First: 2020/07/14]
41. Drevets WC, Price JL, Furey ML. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct Funct* 2008;213(1-2):93-118. doi: 10.1007/s00429-008-0189-x
42. Price JL, Drevets WC. Neurocircuitry of mood disorders. *Neuropsychopharmacology* 2010;35(1):192-216. doi: 10.1038/npp.2009.104
43. Kerestes R, Davey CG, Stephanou K, et al. Functional brain imaging studies of youth depression: a systematic review. *NeuroImage Clinical* 2014;4:209-31. doi: 10.1016/j.nicl.2013.11.009
44. Cullen KR, Gee DG, Klimes-Dougan B, et al. A preliminary study of functional connectivity in comorbid adolescent depression. *Neurosci Lett* 2009;460(3):227-31. doi: 10.1016/j.neulet.2009.05.022
45. Cullen KR, Klimes-Dougan B, Muetzel R, et al. Altered white matter microstructure in adolescents with major depression: a preliminary study. *J Am Acad Child Adolesc Psychiatry* 2010;49(2):173-83 e1.
46. Hulvershorn LA, Cullen K, Anand A. Toward dysfunctional connectivity: a review of neuroimaging findings in pediatric major depressive disorder. *Brain Imaging Behav* 2011;5(4):307-28. doi: 10.1007/s11682-011-9134-3
47. Adluru N, Luo Z, Van Hulle CA, et al. Anxiety-related experience-dependent white matter structural differences in adolescence: A monozygotic twin difference approach. *Sci Rep* 2017;7(1):8749. doi: 10.1038/s41598-017-08107-6
48. Liao M, Yang F, Zhang Y, et al. White matter abnormalities in adolescents with generalized anxiety disorder: a diffusion tensor imaging study. *BMC Psychiatry* 2014;14:41. doi: 10.1186/1471-244X-14-41
49. Ducharme S, Albaugh MD, Hudziak JJ, et al. Anxious/depressed symptoms are linked to right ventromedial prefrontal cortical thickness maturation in healthy children and young adults. *Cereb Cortex* 2014;24(11):2941-50. doi: 10.1093/cercor/bht151
50. Boes AD, McCormick LM, Coryell WH, et al. Rostral anterior cingulate cortex volume correlates with depressed mood in normal healthy children. *Biol Psychiatry* 2008;63(4):391-7. doi: 10.1016/j.biopsych.2007.07.018
51. Akhtar H, Bukhari F, Nazir M, et al. Therapeutic Efficacy of Neurostimulation for Depression: Techniques, Current Modalities, and Future Challenges. *Neurosci Bull* 2016;32(1):115-26. doi: 10.1007/s12264-015-0009-2 [published Online First: 2016/01/20]

52. Delaloye S, Holtzheimer PE. Deep brain stimulation in the treatment of depression. *Dialogues Clin Neurosci* 2014;16(1):83-91. [published Online First: 2014/04/16]
53. Milev RV, Giacobbe P, Kennedy SH, et al. Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 Clinical Guidelines for the Management of Adults with Major Depressive Disorder: Section 4. Neurostimulation Treatments. *Can J Psychiatry* 2016;61(9):561-75. doi: 10.1177/0706743716660033 [published Online First: 2016/08/04]
54. Steinhausen HC, Spohr HL. Long-term outcome of children with fetal alcohol syndrome: psychopathology, behavior, and intelligence. *Alcohol Clin Exp Res* 1998;22(2):334-8.
55. Spohr HL, Willms J, Steinhausen HC. Prenatal alcohol exposure and long-term developmental consequences. *Lancet* 1993;341(8850):907-10.
56. McLachlan K, Paolozza A, Kully-Martens K, et al. Unpacking the heterogeneity of cognitive functioning in children and adolescents with fetal alcohol spectrum disorder: determining the role of moderators and strengths. *Advances in Neurodevelopmental Disorders* 2017;1(4):271-82.
57. Burghy CA, Stodola DE, Ruttle PL, et al. Developmental pathways to amygdala-prefrontal function and internalizing symptoms in adolescence. *Nat Neurosci* 2012;15(12):1736-41. doi: 10.1038/nn.3257
58. MacQueen GM, Yucel K, Taylor VH, et al. Posterior hippocampal volumes are associated with remission rates in patients with major depressive disorder. *Biol Psychiatry* 2008;64(10):880-3. doi: 10.1016/j.biopsych.2008.06.027 [published Online First: 2008/08/30]
59. Chen CH, Ridler K, Suckling J, et al. Brain imaging correlates of depressive symptom severity and predictors of symptom improvement after antidepressant treatment. *Biol Psychiatry* 2007;62(5):407-14. doi: 10.1016/j.biopsych.2006.09.018 [published Online First: 2007/01/16]
60. Astley SJ. Profile of the first 1,400 patients receiving diagnostic evaluations for fetal alcohol spectrum disorder at the Washington State Fetal Alcohol Syndrome Diagnostic & Prevention Network. *Can J Clin Pharmacol* 2010;17(1):e132-64.
61. Lebel CA, McMorris CA, Kar P, et al. Characterizing adverse prenatal and postnatal experiences in children. *Birth Defects Res* 2019;111(12):848-58. doi: 10.1002/bdr2.1464 [published Online First: 2019/01/29]
62. Flannigan K, Kapasi A, Pei J, et al. Characterizing adverse childhood experiences among children and adolescents with prenatal alcohol exposure and Fetal Alcohol Spectrum Disorder. *Child Abuse Negl* 2021;112:104888. doi: 10.1016/j.chiabu.2020.104888 [published Online First: 2021/01/04]
63. Felitti VJ, Anda RF, Nordenberg D, et al. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. *Am J Prev Med* 1998;14(4):245-58.
64. Chapman DP, Whitfield CL, Felitti VJ, et al. Adverse childhood experiences and the risk of depressive disorders in adulthood. *J Affect Disord* 2004;82(2):217-25. doi: 10.1016/j.jad.2003.12.013
65. Tottenham N, Hare TA, Quinn BT, et al. Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. *Dev Sci* 2010;13(1):46-61. doi: 10.1111/j.1467-7687.2009.00852.x
66. Gee DG, Gabard-Durnam LJ, Flannery J, et al. Early developmental emergence of human amygdala-prefrontal connectivity after maternal deprivation. *Proc Natl Acad Sci U S A* 2013;110(39):15638-43. doi: 10.1073/pnas.1307893110
67. Mehta MA, Golembo NI, Nosarti C, et al. Amygdala, hippocampal and corpus callosum size following severe early institutional deprivation: the English and Romanian Adoptees study pilot. *J Child Psychol Psychiatry* 2009;50(8):943-51. doi: 10.1111/j.1469-7610.2009.02084.x
68. Hanson JL, Adluru N, Chung MK, et al. Early neglect is associated with alterations in white matter integrity and cognitive functioning. *Child Dev* 2013;84(5):1566-78. doi: 10.1111/cdev.12069

69. Behen ME, Muzik O, Saporta AS, et al. Abnormal fronto-striatal connectivity in children with histories of early deprivation: A diffusion tensor imaging study. *Brain Imaging Behav* 2009;3(3):292-97. doi: 10.1007/s11682-009-9071-6
70. Eluvathingal TJ, Chugani HT, Behen ME, et al. Abnormal brain connectivity in children after early severe socioemotional deprivation: a diffusion tensor imaging study. *Pediatrics* 2006;117(6):2093-100. doi: 10.1542/peds.2005-1727
71. Bick J, Zhu T, Stamoulis C, et al. Effect of early institutionalization and foster care on long-term white matter development: a randomized clinical trial. *JAMA Pediatr* 2015;169(3):211-9. doi: 10.1001/jamapediatrics.2014.3212
72. McLaughlin KA, Sheridan MA, Gold AL, et al. Maltreatment Exposure, Brain Structure, and Fear Conditioning in Children and Adolescents. *Neuropsychopharmacology* 2016;41(8):1956-64. doi: 10.1038/npp.2015.365
73. Gold AL, Sheridan MA, Peverill M, et al. Childhood abuse and reduced cortical thickness in brain regions involved in emotional processing. *J Child Psychol Psychiatry* 2016;57(10):1154-64. doi: 10.1111/jcpp.12630
74. Lam VYY, Raineki C, Takeuchi LE, et al. Chronic Stress Alters Behavior in the Forced Swim Test and Underlying Neural Activity in Animals Exposed to Alcohol Prenatally: Sex- and Time-Dependent Effects. *Front Behav Neurosci* 2018;12:42. doi: 10.3389/fnbeh.2018.00042
75. McLachlan K, Zhou D, Little G, et al. Current Socioeconomic Status Correlates With Brain Volumes in Healthy Children and Adolescents but Not in Children With Prenatal Alcohol Exposure. *Front Hum Neurosci* 2020;14:223. doi: 10.3389/fnhum.2020.00223 [published Online First: 2020/07/28]
76. Uban KA, Kan E, Wozniak JR, et al. The Relationship Between Socioeconomic Status and Brain Volume in Children and Adolescents With Prenatal Alcohol Exposure. *Front Hum Neurosci* 2020;14:85. doi: 10.3389/fnhum.2020.00085 [published Online First: 2020/04/24]
77. McLaughlin KA, Sheridan MA. Beyond Cumulative Risk: A Dimensional Approach to Childhood Adversity. *Curr Dir Psychol Sci* 2016;25(4):239-45. doi: 10.1177/0963721416655883
78. Kingery JN, Roblek TL, Suveg C, et al. They're not just "little adults": developmental considerations for implementing cognitive-behavioral therapy with anxious youth. *Journal of cognitive psychotherapy: an international quarterly* 2006;20(3):263-73.
79. Fernandes BS, Williams LM, Steiner J, et al. The new field of 'precision psychiatry'. *BMC Med* 2017;15(1):80. doi: 10.1186/s12916-017-0849-x [published Online First: 2017/04/14]
80. McLachlan K, Andrew G, Pei J, et al. Assessing FASD in young children: exploring clinical complexities and diagnostic challenges. *J Popul Ther Clin Pharmacol* 2015;22(1):e108-e24.
81. Kessler RC, Amminger GP, Aguilar-Gaxiola S, et al. Age of onset of mental disorders: a review of recent literature. *Curr Opin Psychiatry* 2007;20(4):359-64. doi: 10.1097/YCO.0b013e32816ebc8c [published Online First: 2007/06/07]
82. Greene DJ, Koller JM, Hampton JM, et al. Behavioral interventions for reducing head motion during MRI scans in children. *Neuroimage* 2018;171:234-45. doi: 10.1016/j.neuroimage.2018.01.023 [published Online First: 2018/01/18]
83. Lebel C, Beaulieu C. Longitudinal development of human brain wiring continues from childhood into adulthood. *J Neurosci* 2011;31(30):10937-47. doi: 10.1523/JNEUROSCI.5302-10.2011
84. Fischl B. FreeSurfer. *Neuroimage* 2012;62(2):774-81. doi: 10.1016/j.neuroimage.2012.01.021 [published Online First: 2012/01/18]
85. Reuter M, Schmansky NJ, Rosas HD, et al. Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage* 2012;61(4):1402-18. doi: 10.1016/j.neuroimage.2012.02.084 [published Online First: 2012/03/21]

86. Geeraert BL, Lebel RM, Mah AC, et al. A comparison of inhomogeneous magnetization transfer, myelin volume fraction, and diffusion tensor imaging measures in healthy children. *Neuroimage* 2017 doi: 10.1016/j.neuroimage.2017.09.019
87. Mah A, Geeraert BL, Lebel C. Detailing neuroanatomical development in late childhood and early adolescence using NODDI. *PLoS One* 2017;12(8):e0182340.
88. Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res* 1996;29(3):162-73.
89. Jenkinson M, Beckmann CF, Behrens TE, et al. Fsl. *Neuroimage* 2012;62(2):782-90. doi: 10.1016/j.neuroimage.2011.09.015 [published Online First: 2011/10/08]
90. Wilke M, Schmithorst VJ, Holland SK. Assessment of spatial normalization of whole-brain magnetic resonance images in children. *Hum Brain Mapp* 2002;17(1):48-60. doi: 10.1002/hbm.10053
91. Reynolds C, Kamphaus R. Behavioral assessment system for children, second edition. San Antonio, TX: Pearson 2004.
92. Kovacs M. Children's Depression Inventory 2: Pearson Clinical 2010.
93. March JS. Multidimensional anxiety scale for children 2nd edition: Pearson Clinical 2012.
94. March JS, Parker JD, Sullivan K, et al. The Multidimensional Anxiety Scale for Children (MASC): factor structure, reliability, and validity. *J Am Acad Child Adolesc Psychiatry* 1997;36(4):554-65. doi: 10.1097/00004583-199704000-00019 [published Online First: 1997/04/01]
95. Beck AT, Ward CH, Mendelson M, et al. An inventory for measuring depression. *Arch Gen Psychiatry* 1961;4:561-71. doi: 10.1001/archpsyc.1961.01710120031004 [published Online First: 1961/06/01]
96. Cella D, Choi SW, Condon DM, et al. PROMIS((R)) Adult Health Profiles: Efficient Short-Form Measures of Seven Health Domains. *Value Health* 2019;22(5):537-44. doi: 10.1016/j.jval.2019.02.004 [published Online First: 2019/05/21]
97. Kaufman J, Birmaher B, Brent D, et al. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. *J Am Acad Child Adolesc Psychiatry* 1997;36(7):980-8. doi: 10.1097/00004583-199707000-00021
98. American Psychological Association. Diagnostic and statistical manual of mental disorders, 5th edition. Arlington, VA: American Psychiatric Publishing 2013.
99. Tolin DF, Gilliam C, Wootton BM, et al. Psychometric Properties of a Structured Diagnostic Interview for DSM-5 Anxiety, Mood, and Obsessive-Compulsive and Related Disorders. *Assessment* 2018;25(1):3-13. doi: 10.1177/1073191116638410 [published Online First: 2016/03/19]
100. Noel M, Wilson AC, Holley AL, et al. Posttraumatic stress disorder symptoms in youth with vs without chronic pain. *Pain* 2016;157(10):2277-84. doi: 10.1097/j.pain.0000000000000642 [published Online First: 2016/06/09]
101. Palermo TM, Valenzuela D, Stork PP. A randomized trial of electronic versus paper pain diaries in children: impact on compliance, accuracy, and acceptability. *Pain* 2004;107(3):213-19. doi: 10.1016/j.pain.2003.10.005 [published Online First: 2004/01/23]
102. Harrison PL, Oakland T. ABAS-3: Adaptive behavior assessment system (3rd edition). Torrance, CA: Psychological Corporation 2015.
103. Dunn W. The Sensory Profile: User's Manual. San Antonio, TX: The Psychological Corporation 1999.
104. Wechsler D. Wechsler Abbreviated Scale of Intelligence (WASI). San Antonio, TX: The Psychological Corporation. 1999
105. Bernstein JH, Waber DP. Developmental scoring system for the Rey-Osterrieth complex figure. *Psychological Assessment Resources* 1996

106. Heaton RK, Chelune GJ, Talley JL, et al. Wisconsin Card Sorting Test manual: revised and expanded. Odessa, FL: Psychological Assessment Resources 1993.
107. Delis DC. CVLT-C: California verbal learning test. San Antonio, TX: Psychological Corporation 1994.
108. Korkman M, Kirk U, Kemp S. NEPSY II. Clinical and interpretative manual. San Antonio, TX: The Psychological Corporation 2007.
109. Wechsler D. Wechsler Individual Achievement Test - Third Edition. London, 2009.
110. National Crittenton Foundation. The adverse childhood experiences (ACEs) survey: Toolkit for providers, 2015.
111. National Crittenton Foundation. Adverse childhood experiences (ACE) questionnaire and Crittenton, 2013.
112. Bates D, Machler M, Bolker B, et al. Fitting linear mixed-effects models using lme4. *Journal of statistical software* 2015;67(1):48.
113. Kuznetsova A, Brockhoff PB, Chritensen RHB. lmerTest package: tests in linear mixed effects models. *Journal of statistical software* 2017;82(13):26.
114. Hayes AF. Introduction to mediation, moderation, and conditional process analysis. New York, USA: Guilford Press 2013.
115. Bennett DS, Pendley JS, Bates JE. Daughter and mother report of individual symptoms on the Children's Depression Inventory. *J Adolesc Health* 1997;20(1):51-7. doi: 10.1016/S1054-139X(96)00090-0 [published Online First: 1997/01/01]
116. Wei C, Hoff A, Villabo MA, et al. Assessing anxiety in youth with the multidimensional anxiety scale for children. *J Clin Child Adolesc Psychol* 2014;43(4):566-78. doi: 10.1080/15374416.2013.814541 [published Online First: 2013/07/13]
117. Fritz MS, Mackinnon DP. Required sample size to detect the mediated effect. *Psychol Sci* 2007;18(3):233-9. doi: 10.1111/j.1467-9280.2007.01882.x [published Online First: 2007/04/21]

Conjoint Health Research Ethics Board
Research Services Office
2500 University Drive, NW
Calgary AB T2N 1N4
Telephone: (403) 220-2297
chreb@ucalgary.ca

CERTIFICATION OF INSTITUTIONAL ETHICS APPROVAL

Ethics approval for the following research has been renewed by the Conjoint Health Research Ethics Board (CHREB) at the University of Calgary. The CHREB is constituted and operates in compliance with the *Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans* (TCPS 2); Health Canada Food and Drug Regulations Division 5; Part C; ICH Guidance E6: Good Clinical Practice and the provisions and regulations of the Health Information Act, RSA 2000 c H-5.

Ethics ID:	REB17-0663_REN4
Principal Investigator:	Catherine Lebel
Co-Investigator(s):	William (Ben) Gibbard Carly McMorris
Student Co-Investigator(s):	Sarah MacEachern Quinn Andre Kelsey Friesen Natalia Kruger Chantel Ritter Preeti Kar Daphne Nakhid
Study Title:	Mental health and brain alterations in children with multiple early risks
Sponsor:	Addiction & Mental Health Strategic Clinical Network Canadian Institutes of Health Research

Effective: 8-Jun-2020 **Expires:** 7-Jun-2021

Restrictions:

This Certification is subject to the following conditions:

1. Approval is granted only for the research and purposes described in the application.
2. Any modification to the approved research must be submitted to the CHREB for approval.
3. An annual application for renewal of ethics certification must be submitted and approved by the above expiry date.

4. A closure request must be sent to the CHREB when the research is complete or terminated.

Approved By:	**Date:**
Kathleen Oberle, PhD, Vice-Chair , CHREB	14-May-2020

Note: This correspondence includes an electronic signature (validation and approval via an online system).

Health Research Ethics Board

308 Campus Towne
University of Alberta, Edmonton, AB T6G 1R8
p. 780.492.9724 (Biomedical Panel)
p. 780.492.0302 (Health Panel)
p. 780.492.0459

Approval Form

Date:	August 12, 2019
Principal Investigator:	Christian Beaulieu
Study ID:	Pro00093230
Study Title:	Calgary-Led MRI Study of Children with Prental Alcohol Exposure - Mental Health & Cumulative Risks
Approval Expiry Date:	August 11, 2020

Date of Informed Consent:	Approval Date	Approved Document
	8/12/2019	Youth Consent Aug 2019
	8/12/2019	Guardian_Consent_Form_UofA - Aug 2019

Funding/Sponsor: Canadian Institutes of Health Research

Thank you for submitting the above study to the Health Research Ethics Board - Biomedical Panel. Your application has received a delegated review in light of the prior approval by the University of Calgary CHREB. The study is now approved. The following documentation forms part of this approval: Protocol (uploaded 8/8/2019), guardian consent (August 2019), youth consent (August 2019), child assent (August 2019), consent to contact, ADvertisements (Control and PAE) (each dated August 2019), CRDC Alcohol Exposure Assessment Form, Demographic Survey Form - Birth Parent, Demographic Brain Mental Health (2019-06-03), Gender Puberty (2019-06-03), Household Information (2019-06-03), AHS MRI Screening Form.

The Health Research Ethics Board assessed all matters required by section 50(1)(a) of the Health Information Act. Subject consent for access to identifiable health information is required for the research described in the ethics application, and appropriate procedures for such consent have been approved by the HREB - Biomedical Panel. In order to comply with the Health Information Act, a copy of the approval form is being sent to the Office of the Information and Privacy Commissioner.

Any proposed changes to the study must be submitted to the REB for approval prior to implementation. A renewal report must be submitted next year prior to the expiry of this approval if your study still requires ethics approval. If you do not renew on or before the renewal expiry date (August 11, 2020), you will have to re-submit an ethics application.

The membership of the Health Research Ethics Board - Biomedical Panel complies with the membership requirements for research ethics boards as defined in Division 5 of the Food and Drug Regulations and the Tri Council Policy Statement. The HREB - Biomedical Panel carries out its functions in a manner consistent with Good Clinical Practices.

Approval by the Health Research Ethics Board does not encompass authorization to access the patients, staff or resources of Alberta Health Services or other local health care institutions for the purposes of the research. Inquiries regarding administrative approval, and operational approval for areas impacted by the research should be directed to the Alberta Health Services Research Administration office (Edmonton Zone) at nactrc.contracts@albertahealthservices.ca or Covenant Health Research Administration (research@covenanthealth.ca) as applicable.

Sincerely,

Donald W. Morrish, MD, PhD, FRCPC
Associate Chair, Health Research Ethics Board – Biomedical Panel

Note: This correspondence includes an electronic signature (validation and approval via an online system).

UNIVERSITY OF ALBERTA Alberta Health Services Covenant Health

BMJ Open

The Prenatal Exposure And Child brain and mental Health (PEACH) Study: protocol for a cohort study of children and youth with prenatal alcohol exposure

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2021-051660.R1
Article Type:	Protocol
Date Submitted by the Author:	14-Apr-2021
Complete List of Authors:	Lebel, Catherine; University of Calgary, Gibbard, Ben; University of Calgary, Department of Pediatrics Tortorelli, Chris; Social Work Mount Royal University Pei, Jacqueline; University of Alberta, Department of Biomedical Engineering Beaulieu, Christian; Univeristy of Alberta Bagshawe, Mercedes; University of Calgary, Department of Radiology McMorris, Carly A.; University of Calgary
Primary Subject Heading:	Paediatrics
Secondary Subject Heading:	Mental health, Radiology and imaging
Keywords:	MENTAL HEALTH, Child & adolescent psychiatry < PSYCHIATRY, Magnetic resonance imaging < RADIOLOGY & IMAGING, Depression & mood disorders < PSYCHIATRY

I, the Submitting Author has the right to grant and does grant on behalf of all authors of the Work (as defined in the below author licence), an exclusive licence and/or a non-exclusive licence for contributions from authors who are: i) UK Crown employees; ii) where BMJ has agreed a CC-BY licence shall apply, and/or iii) in accordance with the terms applicable for US Federal Government officers or employees acting as part of their official duties; on a worldwide, perpetual, irrevocable, royalty-free basis to BMJ Publishing Group Ltd ("BMJ") its licensees and where the relevant Journal is co-owned by BMJ to the co-owners of the Journal, to publish the Work in this journal and any other BMJ products and to exploit all rights, as set out in our licence.

The Submitting Author accepts and understands that any supply made under these terms is made by BMJ to the Submitting Author unless you are acting as an employee on behalf of your employer or a postgraduate student of an affiliated institution which is paying any applicable article publishing charge ("APC") for Open Access articles. Where the Submitting Author wishes to make the Work available on an Open Access basis (and intends to pay the relevant APC), the terms of reuse of such Open Access shall be governed by a Creative Commons licence – details of these licences and which Creative Commons licence will apply to this Work are set out in our licence referred to above.

Other than as permitted in any relevant BMJ Author's Self Archiving Policies, I confirm this Work has not been accepted for publication elsewhere, is not being considered for publication elsewhere and does not duplicate material already published. I confirm all authors consent to publication of this Work and authorise the granting of this licence.

The Prenatal Exposure And Child brain and mental Health (PEACH) Study: protocol for a cohort study of children and youth with prenatal alcohol exposure

Catherine Lebel[1,2,3], Ben Gibbard[2,4], Chris Tortorelli[5], Jacqueline Pei[6], Christian Beaulieu[7], Mercedes Bagshawe[1,2,3], Carly A. McMorris[2,8]

[1]Department of Radiology, University of Calgary

[2]Alberta Children's Hospital Research Institute, University of Calgary

[3]Hotchkiss Brain Institute, University of Calgary

[4]Department of Pediatrics, University of Calgary

[5]Social Work, Mount Royal University

[6]Department of Biomedical Engineering, University of Alberta

[7]Department of Educational Psychology, University of Alberta

[8]Werklund School of Education, University of Calgary

Corresponding Author:

Catherine Lebel
Alberta Children's Hospital
28 Oki Dr. NW
Calgary, AB
T3B 6A8
clebel@ucalgary.ca

Authors' contributions: CL, BG, CT, JP, CB, MB, and CM contributed to conceptualization of this study. CL wrote the first draft of this manuscript; all authors (CL, BG, CT, JP, CB, MB, and CM) edited the manuscript and approved the final version.

Competing interests. None.

Funding statement: This work is supported by the Canadian Institutes of Health Research (PJT-162217).

Word Count: 3861

Abstract

Introduction: Fetal alcohol spectrum disorder (FASD), which is caused by prenatal alcohol exposure (PAE), affects an estimated 4% of North Americans, and is the most common preventable cause of intellectual disability. Mental health problems, including anxiety and depression, are experienced by nearly all individuals with FASD. However, there is very limited knowledge about effect mental health treatments for individuals with FASD; effective treatments are hindered in part due to a lack of understanding of the basic neurobiology underlying internalizing disorders in youth with FASD.

Methods and analysis: The Prenatal Exposure And Child brain and mental Health (PEACH) study includes children aged 7-18 years. We will use longitudinal neuroimaging (anatomical T1-weighted, diffusion, and passive viewing function magnetic resonance imaging) and mental health assessments (Behaviour Assessment Scale for Children [BASC-3], Multi-dimensional Anxiety Scale for Children [MASC-2], Children's Depression Inventory [CDI-2], Kiddie Scale of Affective Disorders [K-SADS]) to: (1) characterize brain development trajectories in youth with FASD, (2) determine whether brain alterations mediate increased anxiety and depression in youth with FASD, and (3) identify baseline brain features that predict changes of anxiety and depression symptoms over the next 2 years. All of this will be done while considering sex and adverse postnatal experiences, which can significantly impact mental health and brain outcomes. This project will forge new understanding of FASD and mental health from a neurobiological perspective, highlighting key time periods (i.e., sensitive windows) and brain regions (i.e., that may be susceptible to neurostimulation), while identifying factors that predict individual trajectories of anxiety and depression symptoms.

Ethics and dissemination: This study was approved by the University of Calgary Conjoint Health Research Ethics Board and the University of Alberta Health Research Ethics Board. Study results will be disseminated in peer-reviewed journals, at relevant conferences, and in conjunction with our knowledge mobilization partners.

Strengths and Limitations of this Study

- We use longitudinal neuroimaging to assess brain structure and brain growth
- Alcohol-exposed participants will have confirmed prenatal alcohol exposure, though specific measures of timing, frequency, and dose of prenatal alcohol exposure may be difficult to obtain
- We use multiple mental health assessments to measure symptoms of depression and anxiety, and include a comprehensive neurocognitive battery
- This study uses a longitudinal, prospective design and will follow children over 2 years
- We incorporate comprehensive assessment and analysis of both prenatal and postnatal adverse exposures

Introduction

Fetal alcohol spectrum disorder (FASD) is a neurodevelopmental disorder caused by prenatal alcohol exposure (PAE). It is characterized by life-long cognitive, behavioural, and neurological deficits.[1] The prevalence of FASD in North America is estimated to be 4%,[2 3] with lifetime costs over $1M per individual.[3-5] Beyond the primary cognitive and behavioural deficits, over 90% of individuals with FASD experience co-occurring mental health problems,[6-8] compared to 20% in the general population[9]. Depression and anxiety are among the most common, affecting 45-50% and 20-40% of individuals with FASD, respectively.[10-12] Developing early and appropriate interventions to minimize mental health problems and maximize adaptive outcomes in FASD is critical for improving quality of life and reducing the societal burden of FASD. Concerns have been raised that existing mental health treatments for individuals with FASD may be less effective than for the general population,[13 14] perhaps hindered by a lack of understanding of their neurobiological basis.

Magnetic resonance imaging (MRI) can be used to investigate neurological abnormalities in FASD. The most common MRI finding in individuals with FASD is widespread reductions in brain

volume, which have been observed with anatomical MRI from neonates to adults.[15-17] Diffusion tensor imaging (DTI) assesses microstructure of structural white matter connections via fractional anisotropy (FA) and mean diffusivity (MD), measures sensitive to myelination and axonal density.[18] Numerous studies have shown lower FA and/or higher MD in children, adolescents, and young adults with FASD.[19-22] Recent studies suggest that brain diffusion alterations are also present in infants and young children, though in the opposite direction (i.e., higher FA and lower diffusivity).[23 24] Resting state functional MRI (rs-fMRI) measures patterns of spontaneous brain connectivity by correlating functional signals across regions ("functional connectivity");[25] findings suggest atypical functional connectivity in children and youth with FASD.[26-29] Regional brain volume reductions, weaker white matter connectivity (lower FA/higher MD), and atypical functional connectivity have been reported throughout the brain, but alterations are most prominent in subcortical structures[17 30] and prefrontal areas[29-32]. Most studies to date have been cross-sectional, and thus the trajectories of brain maturation remain unclear. The few longitudinal MRI studies that do exist in FASD, show that children with FASD have faster changes of cortical thickness,[33] volume,[34] and white matter connectivity[35] than unexposed controls; these faster changes possibly reflect a "catch-up" in brain maturation. It is not known how key functional networks change with age in FASD. Longitudinal research is critical for revealing the developmental trajectories of brain connectivity in FASD.

Previous studies have related cognitive abilities and clinical features (e.g., dysmorphology) to brain structure in FASD;[29 34 36-39] however few studies have examined relationships between brain measures and mental health.[40] In individuals without FASD, internalizing symptoms are most commonly associated with brain alterations in the anterior cingulate cortex (ACC), medial prefrontal cortex (mPFC), dorsolateral prefrontal cortex (dlPFC), amygdala, and hippocampus, as well as connections between these structures.[41-43] Resting state functional connectivity is higher in the ACC and mPFC in adolescents with depression,[43 44] while weaker structural connectivity (lower FA/higher MD) in frontal white matter (e.g., cingulum, uncinate) is associated with depression and anxiety in youth.[45-50] Areas

identified by neuroimaging (e.g., PFC, cingulate) can be used as brain targets for neurostimulation to treat adults with depression and anxiety,[51-53] highlighting the importance of understanding the neurological correlates of internalizing symptoms. Given the overlap between structures identified as atypical in children and youth with FASD, and brain areas associated with anxiety and depression, brain alterations induced by PAE may underlie, at least in part, the increased the risk of internalizing disorders.

Little is known about the trajectories of mental health symptoms in youth with FASD, though difficulties tend to persist or worsen with age.[54 55] However, FASD is a heterogenous disorder, with heterogeneous outcomes, [56] so it is critical to consider differences at the individual level. In adolescents without FASD, functional connectivity between the amygdala and mPFC predicts the severity of future internalizing symptoms[57]. Brain volumes in the hippocampus[58] and ACC[59] also predict treatment response in adults with depression (but without FASD). However, it is unclear which baseline features predict future mental health outcomes in youth with FASD, though this could inform treatment decisions. Thus, longitudinal research is needed to understand associations between brain alterations and trajectories of depressive and anxiety symptoms in individuals with FASD, to help predict individual outcomes.

Individuals with PAE/FASD frequently have adverse postnatal experiences (~43% have abuse or neglect).[60-62] Such early adversity is commonly operationalized as adverse childhood experiences (ACEs),[63] which provides a cumulative risk score accounting for abuse, neglect, and other household dysfunction in childhood. In the general population, ACEs are associated with heightened risk of anxiety and depression[64] and alterations to frontal and limbic brain structure and function.[65-73] Animal studies show that PAE and postnatal adversity interact to increase depression risk.[74] However, few human studies of FASD have incorporated any measure of postnatal risk. Two recent human studies show differential associations between socio-economic status and brain volumes in children with and without

PAE,[75 76] and one showed that postnatal adversity (neglect, abuse, etc.) moderates the association between PAE and brain connectivity.[22] All of this evidence underscores the need for FASD studies to consider postnatal adversity. ACEs treats all adverse experiences similarly, although different types of adversity may have different effects on individuals.[77] We recently developed a risk characterization framework that accounts for the duration, frequency, timing, and type of risks, which we believe is more appropriate for children with PAE who may experience a wide range of adversities.[61]

Understanding brain development in individuals with FASD, its relation to internalizing symptoms, and predictors of positive outcomes is critical for targeting treatments at the right time (e.g., age-appropriate therapy),[78] for the right brain regions (e.g., for neurostimulation),[51] and for the right person (e.g., considering individual circumstances).[79] In this study, we will recruit 125 youth with heavy PAE or FASD and 125 control youth (7-18 years) and acquire longitudinal MRI and mental health assessments to study trajectories of brain and mental health with the following aims:

1. Characterize developmental trajectories of brain connectivity in youth with FASD. *Hypothesis 1:* Structural and functional connectivity to the prefrontal cortex, hippocampus, and amygdala will show faster increases in FASD compared to unexposed controls.
2. Determine whether brain structure and function mediate the relationship between FASD and internalizing symptoms. *Hypothesis 2:* Brain connectivity between the amygdala, hippocampus, and prefrontal cortex (specifically, lower FA and stronger functional connectivity) will mediate the association between FASD and symptoms of anxiety and depression.
3. Identify baseline factors that predict changes of internalizing symptoms over time in FASD. *Hypothesis 3:* Weaker structural connectivity, stronger functional connectivity, and smaller brain volumes at baseline will predict worsening anxiety and depressive symptoms over the subsequent two years.

Methods and Analysis

Participants. We will recruit 125 children and youth with heavy PAE or FASD and 125 unexposed controls aged 7-18 years. This age range was chosen because: (a) FASD diagnosis typically occurs at ~6-7 years in Alberta,[80] (b) 7-18 years includes the most common ages of onset for anxiety disorders,[81] (c) depression and anxiety symptoms are common in youth with PAE of this age range,[8] and (d) children this age are more likely to tolerate MRI scanning than younger children.[82] Repeat assessments and MRI scanning will occur 2 years after baseline, which allows for measurable brain development within individuals,[83] as well as meaningful changes in mental health symptoms. Approximately equal numbers of males and females will be recruited to be able to appropriately examine sex effects, and approximately half in Edmonton and half in Calgary. Informed written consent will be obtained from parents/guardians, as well as written assent from children/youth.

FASD/PAE Group. Participants will be recruited through diagnostic clinics throughout Alberta (including the Pediatric RASD Clinic at the Glenrose Hospital in Edmonton and the Cumulative Risk Diagnostic Clinic at the Alberta Children's Hospital in Calgary), Alberta Children's Services, parent/caregiver support groups for FASD, community groups (e.g., Calgary and Edmonton Fetal Alcohol Networks), as well as online advertisements and word of mouth.

Participants in the PAE group must have a diagnosis of FASD or confirmed heavy PAE at levels consistent with Canadian FASD diagnostic guidelines (≥7 drinks/week or ≥2 binge episodes at some point during pregnancy).[1] Alcohol exposure will be confirmed via biological mother's self-report, reliable observations by close family or friends, clinical observation, and/or medical, legal, or child services records. Additional prenatal exposures (e.g., tobacco, cannabis, illicit drugs) and adverse experiences (e.g., lack of prenatal care, maternal mental health problems) will be documented where information is available.[61] Participants with genetic disorders associated with significant intellectual or

developmental impairments, diagnosed with a neurological disorder (e.g., epilepsy, cerebral palsy), or with contraindications to MRI (i.e., metal implants, dental devices, claustrophobia) will be excluded. Participants will not be excluded for common comorbid developmental disorders such as attention deficit hyperactivity disorder (ADHD) or learning disabilities.

Control Group. Controls must have confirmed absence or minimal PAE (≤5 drinks total in pregnancy, with no binge episodes) via biological maternal report, no diagnosis of genetic or neurological disorders, and no contraindications to MRI scanning. Controls will be recruited through online advertisements, parent groups in Edmonton and Calgary, and word of mouth. Controls must not have significant intellectual or developmental impairments, but will not be excluded for neurodevelopmental disorders such as ADHD or learning disabilities.

MRI Scanning. MRI scanning at baseline and 2-year follow-up will take place at the Alberta Children's Hospital (ACH, Calgary) on a research-dedicated General Electric 3T MR750w system, or at the Peter S Allen MRI Centre (Edmonton) on a research-dedicated Siemens 3T Prisma. The imaging protocol is detailed in Table 1.

Table 1: Magnetic resonance imaging protocol. Parameters are given for GE MR750w before Siemens Prisma; if only one set of parameters is given, they were the same for both scanners.

Sequence	Scan Time (min:sec)	Resolution (mm^3)	Slices	FOV (cm)	TR (ms)	TE (ms)	Other information
Passive viewing fMRI (ss-EPI)	8:10	3.6x3.6x3.6	36	23	2000	30	Acquired while watching a clip from Planet Earth
ASL (3D)	5:01	3.5x3.5x3.5 / 1.9x1.9x3.5	34	23/24	4600/4845	15.6/10.1	TI 1990/2025 ms
DTI	7:08/14:12	2.2x2.2x2.2	57	22/24.2	6300/12000	55/98	5/10 b0, 30 dir b900, 30 dir b2000
3D T1 (FSPGR BRAVO/ MP-RAGE)	4:57	0.8x0.8x0.8	192	25.6/24	1880/8.25	2.9/3.16	Flip angle 10, TI 948/600 ms,
QSM (3D SPGR/R2Star)	5:16	1.0x1.0x2.0 / 0.47x0.47x2.0	80	24	42/44.8	3.8-36.8/4.1-37.9	7/8 echoes, flip angle 17/15
ihMT	3:09	0.9x0.9x5.0	30	22	8500/15000	85/103	

fMRI = functional magnetic resonance imaging; EPI = echo planar imaging; SPGR = spoiled gradient; ASL = arterial spin labeling; DTI = diffusion tensor imaging; FSPGR = fast spoiled gradient; MP RAGE = magnetization prepared rapid acquisition gradient echo; QSM = quantitative susceptibility mapping; ihMT=inhomogeneous magnetization transfer

Image Analysis. T1-weighted images will be processed using FreeSurfer's[84] longitudinal processing stream.[85] Each subject's parcellation will be manually checked and receive minor corrections if necessary. Brain volumes of the left and right hippocampus, amygdala, and prefrontal cortical areas (dACC, dlPFC, mPFC) will be extracted.

Diffusion data will be quality checked, brain extracted, and corrected for eddy currents and head motion. Fractional anisotropy (FA) and mean diffusivity (MD) maps will be generated for each subject. Tractography (FA>0.2, angle<30°) will be used to reconstruct white matter fibers connecting frontal and limbic regions (uncinate fasciculus, cingulum, fornix). FA and MD will be assessed within each white matter fiber bundle as primary variables of interest. The dual b-value scan also allows for more advanced diffusion models and analysis,[86 87] which will be examined in follow-up analyses after primary aims are complete.

Assessment of rs-fMRI data will use AFNI and FSL tools.[88 89]. Each individual's fMRI data will be registered to their anatomical (T1-weighted) scan, then to a pediatric brain template for 5-18 year olds.[89 90] Volumes with high framewise displacement (>0.25 mm) will be identified and regressed out. Scans with <5 minutes of low-motion data will be eliminated. For each prefrontal region (dlPFC, mPFC, dACC) and the hippocampus and amygdala, averaged time courses will be generated. Correlations between time courses in each pair of regions will be analyzed to measure functional connectivity.

Mental Health Assessments. Mental health assessments will occur at baseline, a subset of tests will be administered online at 1-year follow-up, and the full set will be administered again at 2-year follow-up (see Table 2). Symptoms of depression and anxiety will be measured using both self- and caregiver-reports on the Behavior Assessment System for Children (BASC-3)[91], Child Depression Inventory-2

(CDI-2),[92 93] and the Multidimensional Anxiety Scale for Children (MASC-2).[94] The MASC-2 is a self-report assessment of anxiety symptoms in children and adolescents (8-19 years of age). The CDI-2 is a brief questionnaire that measures cognitive, affective, and behavioural signs of depression in children and adolescent ages 7-17 years. For youth/young adults ≥18 years, we will use the Beck Depression Inventory (BDI)[95] and PROMIS Anxiety Short Form[96] to assess depression and anxiety symptoms, respectively. The BASC-3 provides a validated assessment of a range of mental health symptoms, including anxiety and depression, while the CDI-2 and MASC-2 provide more specific measures of depression and anxiety symptoms, respectively, that are consistent with diagnostic criteria. The BASC-3 caregiver report and self-report (only children ≥12) will be used to assess behaviour.

To determine whether an individual meets diagnostic criteria for anxiety or depression, youth ≥12 years of age and all caregivers will complete a diagnostic assessment of internalizing mental health disorders with a trained and reliable clinician using the mood and affective disorders subscales of Kiddie Schedule for Affective Disorders and Schizophrenia – Lifetime Version (K-SADS-PL).[97] The K-SADS-PL is a semi-structured diagnostic interview and gold standard for assessing a variety of mental health disorders in youth based on DSM-5 diagnostic criteria.[98] The Diagnostic Interview for Anxiety and Mood, and OCD and Related Neuropsychiatric Disorders (DIAMOND)[99] will be used for participants over 18 years.

We also examine the frequency, chronicity and location of pain of children and youth in the past 30 days.[100 101] The Adaptive Behavior Assessment System (ABAS-3) is a comprehensive parent report measure of the adaptive or daily functioning skills of children and youth across the lifespan. [102] The Sensory Profile (SP-2) measures a child's sensory processing patterns in various contexts (home, school, and community settings).[103]

Table 2: Questionnaires and Assessments

Questionnaire and assessments are listed below for each study time point. Caregiver refers to a parent or guardian who regularly cares for the child. Study personnel support younger children in completing the questionnaires if necessary.

	Time 1 (in person)	Time 2 (online)	Time 3 (in person)	Age limits
Mental Health				
Child Depression Index (CDI-2)	Caregiver report Self-report	Caregiver report Self-report	Caregiver report Self-report	Beck Depression Inventory (BDI-II) for youth >17 years
Multidimensional Anxiety Scale for Children (MASC-2)	Caregiver report Self-report	Caregiver report Self-report	Caregiver report Self-report	PROMIS Anxiety for young adults ≥18 years
Behavior Assessment System for Children (BASC-3)	Caregiver report Self-report	Caregiver report Self-report	Caregiver report Self-report	Self-report only for children ≥12 years
Kiddie Scale of Affective Disorders and Schizophrenia (K-SADS)	Caregiver interview Child interview		Caregiver interview Child interview	Child interview for children ≥12 years DIAMOND for young adults >18
Adaptive Behavior Assessment System (ABAS-3)	Caregiver report		Caregiver report	
Pain questionnaire		Caregiver report Child report		
Sensory Profile 2 (SP-2)	Caregiver report		Caregiver report	For children <15 years
Cognitive Function				
Weschler Abbreviated Scale of Intelligence (WASI-II) 2-subtest form	Matrix Reasoning, Vocabulary			
Rey-Osterrieth Complex Figure Test (Rey-O)	Child		Child	
Weschler Individual Achievement Test (WIAT-III)	Word Reading, Pseudo-Word Reading, Oral Reading Fluency, Reading Comprehension, Numerical Operations		Word Reading, Pseudo-Word Reading, Oral Reading Fluency, Reading Comprehension, Numerical Operations	
NEPSY-II	Inhibition, Word Generation		Inhibition, Word Generation	Children <17 years
California Verbal Learning Task -Child (CVLT-C)	Child		Child	CVLT-3 for youth ≥17 years
Wisconsin Card Sort Task (WCST)	Child		Child	
Other information				

Demographic questionnaire	Caregiver	Caregiver	Caregiver
Prenatal and postnatal exposure assessment	Caregiver; medical, legal, Children's Services records		
Puberty questionnaire	Caregiver, child	Caregiver, child	Caregiver, child
Gender identity questionnaire	Caregiver, child		
Adverse childhood experiences (ACEs)	Caregiver on behalf of child		

Cognitive Assessments. IQ will be assessed using the Wechsler Abbreviated Scale of Intelligence – Second Edition (WASI-II)[104] 2-subtest form at baseline to obtain an estimate of Full Scale IQ (FSIQ). The Rey-Osterrieth Complex Figure Test (Rey-O) [105] examines visuospatial ability and visuospatial memory in individuals between 6-89 years of age. The Wisconsin Card Sorting Test (WCST)[106] is an executive functioning measure used to determine cognitive flexibility and set shifting in individuals 6 to 89 years of age. The California Verbal Learning Test (CVLT-C) measures learning and long-term recall and recognition of verbal information in 5-16.11 year olds.[107] The CVLT-3 will be used for youth ≥17 years. The NEPSY-II is a neuropsychological assessment tool for children aged 3-16 years of age that assesses functioning in 6 domains;[108] we will use the subscales of Inhibition (measuring inhibition) and Word Generation (verbal productivity). The Wechsler Individual Achievement Test (WIAT-III) measures academic abilities in children and adolescents aged 4-50.11 years[109].

Early Adversity. Adverse postnatal exposures are assessed using questions adapted from the National Crittenton Foundation ACEs survey,[63 110] a validated survey deemed acceptable by families and caregiving agencies.[111] For children in foster or adoptive care, adverse experiences will be ascertained through child services records and interviews with biological and/or adoptive parents. With this information and prenatal exposure (see above), we will apply our own characterization tool, which accounts for the timing, amount, and type of adverse exposure(s) experienced both prenatally and

postnatally.[61]

Other Variables. Caregivers will complete a comprehensive demographic survey that includes information about other individuals in the house, household income, parent education, etc. Caregivers will be asked if youth have other diagnoses or if they are taking medications. Youth will be asked to self-report their sex and gender. Sex will be included as a covariate in all analyses, and sex-by-age or sex-by-anxiety/depression interaction terms will be included where appropriate. Gender and its interaction terms will be used as additional covariates if numbers permit.

Statistical Analysis. Statistical analysis will occur in SPSS (IBM), R (www.r-project.org), and Matlab. Aim 1 will begin with a cross-sectional analysis using a regression model including age, sex, group, and age-by-group interaction terms, run separately for each brain measure. Once longitudinal data is available, linear mixed effects models in R (using lme4 and lmerTest)[112 113] will be used to determine developmental patterns of structural and functional connectivity for the FASD and control groups. Subject will be modeled as a random factor, with sex, age, group, and age-by-group included in the model. For both cross-sectional and longitudinal analyses, postnatal adversity and IQ will be included as covariates. Mixed effects models will be run separately on volumes of each region, structural connectivity (FA) for each white matter tract, and functional connectivity (correlation) for connections between each pair of regions in the prefrontal-amygdala-hippocampus network (shown in Fig. 1). False discovery rate (FDR) will be used to correct for multiple comparisons.

For Aim 2, mediation will be carried out by testing the first pathway from the main predictor (PAE) to each brain measure (volume, structural or functional connectivity), controlling for age and sex of participants, and then testing the second pathway from each brain measure to each mental health measures (anxiety or depression symptoms), with age, sex, and postnatal adversity as covariates. The overall mediation effect will be tested using percentile-based bootstrap confidence intervals, computed from 5000 simulations.[114] Caregiver-report *T*-scores from the CDI-2 and MASC-2 will be used as

primary measures of depression and anxiety symptoms, respectively. BASC-3 *T*-scores on the anxiety and depression subscales of internalizing symptoms will be used as secondary information. Self-report scores will be used for supplementary analysis, as they sometimes provide different information.[115 116]

For Aim 3, change in anxiety and depression symptoms will be calculated by subtracting the *T*-scores at Time 2 from the *T*-score at Time 1. Primary variables will be caregiver-reported CDI-2 and MASC-2 scores for depression and anxiety, respectively. Brain measures, postnatal adversity, sex, IQ, age, and group will then be entered into a multiple regression model to determine which baseline factors predict anxiety and depression trajectories over time. Change in BASC-3 *T*-scores on the anxiety and depression subscales over the 2 years, as well as self-report scores on CDI-2 and MASC-2 will be used in a supplementary analysis. Initially, change in anxiety and depression outcomes will be used as a continuous measure. If enough youth meet criteria for a diagnosis of a depression or anxiety disorder (as measured by the K-SADS), a group analysis (those who symptoms changed in severity to meet criteria for a diagnosis vs those who do not) will also be conducted.

Power Calculations. We collected preliminary data, including MRI, mental health assessments, and postnatal adversity on 17 children with FASD and 19 controls without PAE aged 7-15 years. Control subjects (part of a different study)[86 87] had follow-up scans and assessments ~2 years later. This preliminary data showed age-by-group interactions in structural and functional connectivity with effect sizes of 0.067-0.192 (partial eta^2). To detect effects this size for Aim 1 with power ≥0.8 using an ANOVA with main effects and interactions, we require 112 individuals total (calculated in G*Power). Preliminary data shows small-medium effects for both FASD-brain and brain-anxiety pathways in the mediation. According to simulations,[117] these effects require a sample size of ≥162 total individuals to detect mediation using percentile bootstrap with power≥0.8 (Aim 2). Linear regression of relationships between brain measures and changes in anxiety and depression in controls showed effects of r=0.4-0.6. To detect these effects (Aim 3), we require at least 130 participants total (G*Power). Thus, we aim to

have ≥162 individuals total (81 per group) at time 1 (Aims 1, 2), and ≥130 (65 per group) with longitudinal data (Aims 1, 3).

Patient and Public Involvement. Ms. Tortorelli (co-investigator on the project) was an Associate Director of Alberta Children's Services and was involved in study design. She has since moved on to a role as Assistant Professor at Mount Royal University and remains involved with the project. We continue to involve staff from Children's Services in the design and execution of the study, and will involve them in interpretation and dissemination of findings. We also have active relationships with organizations serving individuals with FASD and their families, including the Calgary and Edmonton Fetal Alcohol Networks (CFAN, EFAN) and CanFASD. These organizations have provided study feedback and support and will assist with interpretation and dissemination of findings.

Potential Limitations. Research on children and youth with FASD is complex due to confounding pre- and postnatal exposures, missing information, and behavioural difficulties. If recruitment or retention are lower than anticipated, we will recruit participants outside Calgary and Edmonton (e.g., through the other 10 FASD Networks across Alberta). The strict diagnostic criteria used here will ensure that all participants have a minimum level of PAE, though exact amounts may not be known. We will use all available sources to characterize other risks and diagnoses in our participants and will statistically control for these in our analyses.[61] In some cases, information will be missing, which is a challenging but unavoidable aspect of doing research in this population.

Significance. FASD is a common disorder (~4% of Canadians) with a very high societal cost (>$17B annually). Most individuals with FASD experience co-occurring mental health issues throughout their lifespan, but effective treatments are hindered by a lack of understanding of the neurobiological basis for these problems. The use of quantitative MRI to understand the brain abnormalities and atypical development patterns underlying mental health problems in youth with FASD is critical to early identification and appropriate intervention strategies to improve outcomes. This study will reveal

developmental patterns of brain connectivity, identify the underlying neurological correlates of anxiety and depression symptoms in youth with FASD, and identify baseline brain features that can predict the worsening of anxiety and depression symptoms. This knowledge is crucial for advancing research and identifying prevention and early intervention strategies, which will have substantial benefits for these children and youth with FASD, their families, and the public health system and society. Thus, this innovative project will address significant gaps in the literature, inform prevention strategies, and promote early detection and intervention of internalizing issues in children and youth with FASD.

Ethics and Dissemination.

This study has been approved by the University of Calgary Conjoint Health Research Ethics Board (REB17-0663) and the University of Alberta Health Research Ethics Board (Pro00093230). Data collection takes 4-6 hours for youth, and 2-3 hours for caregivers (Table 1) and occurs at the Alberta Children's Hospital (Calgary) or University of Alberta Hospital (Edmonton). Caregivers complete some questionnaires ahead of the visit, and additional questionnaires and interviews during the visit. Children complete the MRI scan, mental health and neuropsychological assessments, and questionnaires during the visit. Children ≥12 years complete the KSADS interview. Breaks are given and support is provided as necessary for children and youth. Snacks, parking, and an honorarium ($150 at baseline visit, $50 at one-year visit, $250 at two-year visit) are provided for each family. The honorarium reflects the commitment of the families, as this study requires substantial time commitments from both child and caregiver. If an MRI reveals any incidental findings, it will be referred to the site's medical director (a neuroradiologist) for review and follow-up. If the mental health assessments reveal any concerns, youth will be referred by a child clinical psychologist (Drs. McMorris or Pei) for appropriate follow-up through the child's physician or other appropriate mental health services.

Communication of our findings to other researchers will occur via publications in peer-reviewed journals and presentations at pertinent conferences (e.g., Organization for Human Brain Mapping,

Canadian Academy of Child & Adolescent Psychiatry). As we publish our research findings, we will produce lay summaries and infographics for distribution to stakeholders via our website, our twitter accounts, Kids Brain Health Network's website (researchimpact.ca), social media (including Kids Brain Health Network's YouTube, Facebook and twitter accounts), and email.

Knowledge translation to the wider community will include direct communication (via reports, presentations, meetings) with diagnostic clinics and Children's Services. Results will be presented at policy and practice meetings (e.g., International Conference on Child and Family Maltreatment, Canadian Association of Pediatric Health Centres, Canadian Pediatric Society, Alberta College of Social Workers).

References

1. Cook JL, Green CR, Lilley CM, et al. Fetal alcohol spectrum disorder: a guideline for diagnosis across the lifespan. *CMAJ* 2016;188(3):191-7. doi: 10.1503/cmaj.141593
2. Flannigan K, Unsworth K, Harding K. The prevalence of fetal alcohol spectrum disorder, 2018.
3. Popova S, Lange S, Chudley A, et al. World Health Organization International Study on the Prevalence of Fetal Alcohol Spectrum Disorder (FASD) Canadian Component, 2018.
4. Thanh NX, Jonsson E. Costs of fetal alcohol spectrum disorder in Alberta, Canada. *Can J Clin Pharmacol* 2009;16(1):e80-90. [published Online First: 2009/01/20]
5. Stade B, Ungar WJ, Stevens B, et al. Cost of fetal alcohol spectrum disorder in Canada. *Canadian family physician Medecin de famille canadien* 2007;53(8):1303-4.
6. Kodituwakku PW. Defining the behavioral phenotype in children with fetal alcohol spectrum disorders: a review. *Neurosci Biobehav Rev* 2007;31(2):192-201.
7. May PA, Baete A, Russo J, et al. Prevalence and characteristics of fetal alcohol spectrum disorders. *Pediatrics* 2014;134(5):855-66. doi: 10.1542/peds.2013-3319
8. Pei J, Denys K, Hughes J, et al. Mental health issues in fetal alcohol spectrum disorder. *J Ment Health* 2011;20(5):438-48. doi: 10.3109/09638237.2011.577113
9. Smetanin P, Stiff D, Briante C, et al. The life and economic impact of major mental illnesses in Canada: 2011 to 2041, 2011.
10. Famy C, Streissguth AP, Unis AS. Mental illness in adults with fetal alcohol syndrome or fetal alcohol effects. *Am J Psychiatry* 1998;155(4):552-4. doi: 10.1176/ajp.155.4.552
11. Fagerlund A, Autti-Ramo I, Hoyme HE, et al. Risk factors for behavioural problems in foetal alcohol spectrum disorders. *Acta Paediatr* 2011;100(11):1481-8. doi: 10.1111/j.1651-2227.2011.02354.x
12. Denys K, Rasmussen C, Henneveld D. The effectiveness of a community-based intervention for parents with FASD. *Community Ment Health J* 2011;47(2):209-19. doi: 10.1007/s10597-009-9273-9
13. Anderson T, Mela M, Stewart M. The implementation of the 2012 mental health strategy for Canada through the lens of FASD. *Canadian Journal of Community Mental Health* 2017;36(4):1-13.
14. Flannigan K, Coons-Harding KD, Anderson T, et al. A Systematic Review of Interventions to Improve Mental Health and Substance Use Outcomes for Individuals with Prenatal Alcohol Exposure and Fetal Alcohol Spectrum Disorder. *Alcohol Clin Exp Res* 2020;44(12):2401-30. doi: 10.1111/acer.14490 [published Online First: 2020/10/30]
15. Donald KA, Eastman E, Howells FM, et al. Neuroimaging effects of prenatal alcohol exposure on the developing human brain: a magnetic resonance imaging review. *Acta Neuropsychiatr* 2015;27(5):251-69. doi: 10.1017/neu.2015.12
16. Lebel C, Roussotte F, Sowell ER. Imaging the impact of prenatal alcohol exposure on the structure of the developing human brain. *Neuropsychol Rev* 2011;21(2):102-18. doi: 10.1007/s11065-011-9163-0
17. Nardelli A, Lebel C, Rasmussen C, et al. Extensive deep gray matter volume reductions in children and adolescents with fetal alcohol spectrum disorders. *Alcoholism Clinical and Experimental Research* 2011;35(8):1404-17. doi: 10.1111/j.1530-0277.2011.01476.x [published Online First: 2011/05/18]
18. Beaulieu C. The basis of anisotropic water diffusion in the nervous system - a technical review. *NMR Biomed* 2002;15(7-8):435-55.

19. Wozniak JR, Muetzel RL. What does diffusion tensor imaging reveal about the brain and cognition in fetal alcohol spectrum disorders? *Neuropsychology Review* 2011;21(2):133-47. doi: 10.1007/s11065-011-9162-1 [published Online First: 2011/02/25]
20. Lebel C, Rasmussen C, Wyper K, et al. Brain diffusion abnormalities in children with fetal alcohol spectrum disorder. *Alcohol Clin Exp Res* 2008;32(10):1732-40. doi: 10.1111/j.1530-0277.2008.00750.x
21. Wozniak JR, Mueller BA, Chang PN, et al. Diffusion tensor imaging in children with fetal alcohol spectrum disorders. *Alcohol Clinical and experimental research* 2006;30(10):1799-806.
22. Andre QR, Geeraert BL, Lebel C. Brain structure and internalizing and externalizing behavior in typically developing children and adolescents. *Brain Struct Funct* 2020;225(4):1369-78. doi: 10.1007/s00429-019-01973-y [published Online First: 2019/11/09]
23. Donald KA, Roos A, Fouche JP, et al. A study of the effects of prenatal alcohol exposure on white matter microstructural integrity at birth. *Acta Neuropsychiatr* 2015;27(4):197-205. doi: 10.1017/neu.2015.35
24. Kar P, Reynolds J, Grohs M, et al. White matter alterations in young children with prenatal alcohol exposure. *Developmental Neurobiology* 2021;in press doi: https://doi.org/10.1101/2021.01.05.425489
25. van den Heuvel MP, Hulshoff Pol HE. Exploring the brain network: a review on resting-state fMRI functional connectivity. *Eur Neuropsychopharmacol* 2010;20(8):519-34. doi: 10.1016/j.euroneuro.2010.03.008 [published Online First: 2010/05/18]
26. Wozniak JR, Mueller BA, Bell CJ, et al. Global functional connectivity abnormalities in children with fetal alcohol spectrum disorders. *Alcohol Clin Exp Res* 2013;37(5):748-56. doi: 10.1111/acer.12024
27. Wozniak JR, Mueller BA, Mattson SN, et al. Functional connectivity abnormalities and associated cognitive deficits in fetal alcohol Spectrum disorders (FASD). *Brain Imaging Behav* 2017;11(5):1432-45. doi: 10.1007/s11682-016-9624-4 [published Online First: 2016/10/14]
28. Long X, Kar P, Gibbard B, et al. The brain's functional connectome in young children with prenatal alcohol exposure. *NeuroImage Clinical* 2019;24:102082. doi: 10.1016/j.nicl.2019.102082 [published Online First: 2019/12/05]
29. Long X, Little G, Beaulieu C, et al. Sensorimotor network alterations in children and youth with prenatal alcohol exposure. *Human Brain Mapping* 2018;in press
30. Zhou D, Rasmussen C, Pei J, et al. Preserved cortical asymmetry despite thinner cortex in children and adolescents with prenatal alcohol exposure and associated conditions. *Hum Brain Mapp* 2018;39(1):72-88. doi: 10.1002/hbm.23818 [published Online First: 2017/09/30]
31. Lebel C, Rasmussen C, Wyper K, et al. Brain diffusion abnormalities in children with fetal alcohol spectrum disorder. *Alcoholism Clinical and Experimental Research* 2008;32(10):1732-40. doi: ACER750 [pii]
10.1111/j.1530-0277.2008.00750.x [published Online First: 2008/08/02]
32. Paolozza A, Treit S, Beaulieu C, et al. Diffusion tensor imaging of white matter and correlates to eye movement control and psychometric testing in children with prenatal alcohol exposure. *Hum Brain Mapp* 2017;38(1):444-56. doi: 10.1002/hbm.23371
33. Treit S, Zhou D, Lebel C, et al. Longitudinal MRI reveals impaired cortical thinning in children and adolescents prenatally exposed to alcohol. *Hum Brain Mapp* 2014;35(9):4892-903. doi: 10.1002/hbm.22520
34. Lebel C, Mattson SN, Riley EP, et al. A longitudinal study of the long-term consequences of drinking during pregnancy: heavy in utero alcohol exposure disrupts the normal processes of brain

development. *The Journal of neuroscience* 2012;32(44):15243-51. doi: 10.1523/JNEUROSCI.1161-12.2012 [published Online First: 2012/11/02]
35. Treit S, Lebel C, Baugh L, et al. Longitudinal MRI Reveals Altered Trajectory of Brain Development during Childhood and Adolescence in Fetal Alcohol Spectrum Disorders. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2013;33(24):10098-109. doi: 10.1523/JNEUROSCI.5004-12.2013 [published Online First: 2013/06/14]
36. Gautam P, Lebel C, Narr KL, et al. Volume changes and brain-behavior relationships in white matter and subcortical gray matter in children with prenatal alcohol exposure. *Hum Brain Mapp* 2015;36(6):2318-29. doi: 10.1002/hbm.22772
37. Green CR, Lebel C, Rasmussen C, et al. Diffusion Tensor Imaging Correlates of Saccadic Reaction Time in Children with Fetal Alcohol Spectrum Disorder. *Alcoholism, clinical and experimental research* 2013;37(9):1499-507. doi: 10.1111/acer.12132 [published Online First: 2013/04/05]
38. Lebel C, Rasmussen C, Wyper K, et al. Brain Microstructure Is Related to Math Ability in Children With Fetal Alcohol Spectrum Disorder. *Alcohol Clin Exp Res* 2010;34(2):356-63. doi: ACER1097 [pii]
10.1111/j.1530-0277.2009.01097.x [published Online First: 2009/11/26]
39. Treit S, Chen Z, Zhou D, et al. Sexual dimorphism of volume reduction but not cognitive deficit in fetal alcohol spectrum disorders: A combined diffusion tensor imaging, cortical thickness and brain volume study. *NeuroImage Clinical* 2017;15:284-97. doi: 10.1016/j.nicl.2017.05.006 [published Online First: 2017/06/01]
40. Andre QR, McMorris CA, Kar P, et al. Different brain profiles in children with prenatal alcohol exposure with or without early adverse exposures. *Hum Brain Mapp* 2020;41(15):4375-85. doi: 10.1002/hbm.25130 [published Online First: 2020/07/14]
41. Drevets WC, Price JL, Furey ML. Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct Funct* 2008;213(1-2):93-118. doi: 10.1007/s00429-008-0189-x
42. Price JL, Drevets WC. Neurocircuitry of mood disorders. *Neuropsychopharmacology* 2010;35(1):192-216. doi: 10.1038/npp.2009.104
43. Kerestes R, Davey CG, Stephanou K, et al. Functional brain imaging studies of youth depression: a systematic review. *NeuroImage Clinical* 2014;4:209-31. doi: 10.1016/j.nicl.2013.11.009
44. Cullen KR, Gee DG, Klimes-Dougan B, et al. A preliminary study of functional connectivity in comorbid adolescent depression. *Neurosci Lett* 2009;460(3):227-31. doi: 10.1016/j.neulet.2009.05.022
45. Cullen KR, Klimes-Dougan B, Muetzel R, et al. Altered white matter microstructure in adolescents with major depression: a preliminary study. *J Am Acad Child Adolesc Psychiatry* 2010;49(2):173-83 e1.
46. Hulvershorn LA, Cullen K, Anand A. Toward dysfunctional connectivity: a review of neuroimaging findings in pediatric major depressive disorder. *Brain Imaging Behav* 2011;5(4):307-28. doi: 10.1007/s11682-011-9134-3
47. Adluru N, Luo Z, Van Hulle CA, et al. Anxiety-related experience-dependent white matter structural differences in adolescence: A monozygotic twin difference approach. *Sci Rep* 2017;7(1):8749. doi: 10.1038/s41598-017-08107-6
48. Liao M, Yang F, Zhang Y, et al. White matter abnormalities in adolescents with generalized anxiety disorder: a diffusion tensor imaging study. *BMC Psychiatry* 2014;14:41. doi: 10.1186/1471-244X-14-41

49. Ducharme S, Albaugh MD, Hudziak JJ, et al. Anxious/depressed symptoms are linked to right ventromedial prefrontal cortical thickness maturation in healthy children and young adults. *Cereb Cortex* 2014;24(11):2941-50. doi: 10.1093/cercor/bht151
50. Boes AD, McCormick LM, Coryell WH, et al. Rostral anterior cingulate cortex volume correlates with depressed mood in normal healthy children. *Biol Psychiatry* 2008;63(4):391-7. doi: 10.1016/j.biopsych.2007.07.018
51. Akhtar H, Bukhari F, Nazir M, et al. Therapeutic Efficacy of Neurostimulation for Depression: Techniques, Current Modalities, and Future Challenges. *Neurosci Bull* 2016;32(1):115-26. doi: 10.1007/s12264-015-0009-2 [published Online First: 2016/01/20]
52. Delaloye S, Holtzheimer PE. Deep brain stimulation in the treatment of depression. *Dialogues Clin Neurosci* 2014;16(1):83-91. [published Online First: 2014/04/16]
53. Milev RV, Giacobbe P, Kennedy SH, et al. Canadian Network for Mood and Anxiety Treatments (CANMAT) 2016 Clinical Guidelines for the Management of Adults with Major Depressive Disorder: Section 4. Neurostimulation Treatments. *Can J Psychiatry* 2016;61(9):561-75. doi: 10.1177/0706743716660033 [published Online First: 2016/08/04]
54. Steinhausen HC, Spohr HL. Long-term outcome of children with fetal alcohol syndrome: psychopathology, behavior, and intelligence. *Alcohol Clin Exp Res* 1998;22(2):334-8.
55. Spohr HL, Willms J, Steinhausen HC. Prenatal alcohol exposure and long-term developmental consequences. *Lancet* 1993;341(8850):907-10.
56. McLachlan K, Paolozza A, Kully-Martens K, et al. Unpacking the heterogeneity of cognitive functioning in children and adolescents with fetal alcohol spectrum disorder: determining the role of moderators and strengths. *Advances in Neurodevelopmental Disorders* 2017;1(4):271-82.
57. Burghy CA, Stodola DE, Ruttle PL, et al. Developmental pathways to amygdala-prefrontal function and internalizing symptoms in adolescence. *Nat Neurosci* 2012;15(12):1736-41. doi: 10.1038/nn.3257
58. MacQueen GM, Yucel K, Taylor VH, et al. Posterior hippocampal volumes are associated with remission rates in patients with major depressive disorder. *Biol Psychiatry* 2008;64(10):880-3. doi: 10.1016/j.biopsych.2008.06.027 [published Online First: 2008/08/30]
59. Chen CH, Ridler K, Suckling J, et al. Brain imaging correlates of depressive symptom severity and predictors of symptom improvement after antidepressant treatment. *Biol Psychiatry* 2007;62(5):407-14. doi: 10.1016/j.biopsych.2006.09.018 [published Online First: 2007/01/16]
60. Astley SJ. Profile of the first 1,400 patients receiving diagnostic evaluations for fetal alcohol spectrum disorder at the Washington State Fetal Alcohol Syndrome Diagnostic & Prevention Network. *Can J Clin Pharmacol* 2010;17(1):e132-64.
61. Lebel CA, McMorris CA, Kar P, et al. Characterizing adverse prenatal and postnatal experiences in children. *Birth Defects Res* 2019;111(12):848-58. doi: 10.1002/bdr2.1464 [published Online First: 2019/01/29]
62. Flannigan K, Kapasi A, Pei J, et al. Characterizing adverse childhood experiences among children and adolescents with prenatal alcohol exposure and Fetal Alcohol Spectrum Disorder. *Child Abuse Negl* 2021;112:104888. doi: 10.1016/j.chiabu.2020.104888 [published Online First: 2021/01/04]
63. Felitti VJ, Anda RF, Nordenberg D, et al. Relationship of childhood abuse and household dysfunction to many of the leading causes of death in adults. The Adverse Childhood Experiences (ACE) Study. *Am J Prev Med* 1998;14(4):245-58.

64. Chapman DP, Whitfield CL, Felitti VJ, et al. Adverse childhood experiences and the risk of depressive disorders in adulthood. *J Affect Disord* 2004;82(2):217-25. doi: 10.1016/j.jad.2003.12.013
65. Tottenham N, Hare TA, Quinn BT, et al. Prolonged institutional rearing is associated with atypically large amygdala volume and difficulties in emotion regulation. *Dev Sci* 2010;13(1):46-61. doi: 10.1111/j.1467-7687.2009.00852.x
66. Gee DG, Gabard-Durnam LJ, Flannery J, et al. Early developmental emergence of human amygdala-prefrontal connectivity after maternal deprivation. *Proc Natl Acad Sci U S A* 2013;110(39):15638-43. doi: 10.1073/pnas.1307893110
67. Mehta MA, Golembo NI, Nosarti C, et al. Amygdala, hippocampal and corpus callosum size following severe early institutional deprivation: the English and Romanian Adoptees study pilot. *J Child Psychol Psychiatry* 2009;50(8):943-51. doi: 10.1111/j.1469-7610.2009.02084.x
68. Hanson JL, Adluru N, Chung MK, et al. Early neglect is associated with alterations in white matter integrity and cognitive functioning. *Child Dev* 2013;84(5):1566-78. doi: 10.1111/cdev.12069
69. Behen ME, Muzik O, Saporta AS, et al. Abnormal fronto-striatal connectivity in children with histories of early deprivation: A diffusion tensor imaging study. *Brain Imaging Behav* 2009;3(3):292-97. doi: 10.1007/s11682-009-9071-6
70. Eluvathingal TJ, Chugani HT, Behen ME, et al. Abnormal brain connectivity in children after early severe socioemotional deprivation: a diffusion tensor imaging study. *Pediatrics* 2006;117(6):2093-100. doi: 10.1542/peds.2005-1727
71. Bick J, Zhu T, Stamoulis C, et al. Effect of early institutionalization and foster care on long-term white matter development: a randomized clinical trial. *JAMA Pediatr* 2015;169(3):211-9. doi: 10.1001/jamapediatrics.2014.3212
72. McLaughlin KA, Sheridan MA, Gold AL, et al. Maltreatment Exposure, Brain Structure, and Fear Conditioning in Children and Adolescents. *Neuropsychopharmacology* 2016;41(8):1956-64. doi: 10.1038/npp.2015.365
73. Gold AL, Sheridan MA, Peverill M, et al. Childhood abuse and reduced cortical thickness in brain regions involved in emotional processing. *J Child Psychol Psychiatry* 2016;57(10):1154-64. doi: 10.1111/jcpp.12630
74. Lam VYY, Raineki C, Takeuchi LE, et al. Chronic Stress Alters Behavior in the Forced Swim Test and Underlying Neural Activity in Animals Exposed to Alcohol Prenatally: Sex- and Time-Dependent Effects. *Front Behav Neurosci* 2018;12:42. doi: 10.3389/fnbeh.2018.00042
75. McLachlan K, Zhou D, Little G, et al. Current Socioeconomic Status Correlates With Brain Volumes in Healthy Children and Adolescents but Not in Children With Prenatal Alcohol Exposure. *Front Hum Neurosci* 2020;14:223. doi: 10.3389/fnhum.2020.00223 [published Online First: 2020/07/28]
76. Uban KA, Kan E, Wozniak JR, et al. The Relationship Between Socioeconomic Status and Brain Volume in Children and Adolescents With Prenatal Alcohol Exposure. *Front Hum Neurosci* 2020;14:85. doi: 10.3389/fnhum.2020.00085 [published Online First: 2020/04/24]
77. McLaughlin KA, Sheridan MA. Beyond Cumulative Risk: A Dimensional Approach to Childhood Adversity. *Curr Dir Psychol Sci* 2016;25(4):239-45. doi: 10.1177/0963721416655883
78. Kingery JN, Roblek TL, Suveg C, et al. They're not just "little adults": developmental considerations for implementing cognitive-behavioral therapy with anxious youth. *Journal of cognitive psychotherapy: an international quarterly* 2006;20(3):263-73.
79. Fernandes BS, Williams LM, Steiner J, et al. The new field of 'precision psychiatry'. *BMC Med* 2017;15(1):80. doi: 10.1186/s12916-017-0849-x [published Online First: 2017/04/14]

80. McLachlan K, Andrew G, Pei J, et al. Assessing FASD in young children: exploring clinical complexities and diagnostic challenges. *J Popul Ther Clin Pharmacol* 2015;22(1):e108-e24.
81. Kessler RC, Amminger GP, Aguilar-Gaxiola S, et al. Age of onset of mental disorders: a review of recent literature. *Curr Opin Psychiatry* 2007;20(4):359-64. doi: 10.1097/YCO.0b013e32816ebc8c [published Online First: 2007/06/07]
82. Greene DJ, Koller JM, Hampton JM, et al. Behavioral interventions for reducing head motion during MRI scans in children. *Neuroimage* 2018;171:234-45. doi: 10.1016/j.neuroimage.2018.01.023 [published Online First: 2018/01/18]
83. Lebel C, Beaulieu C. Longitudinal development of human brain wiring continues from childhood into adulthood. *J Neurosci* 2011;31(30):10937-47. doi: 10.1523/JNEUROSCI.5302-10.2011
84. Fischl B. FreeSurfer. *Neuroimage* 2012;62(2):774-81. doi: 10.1016/j.neuroimage.2012.01.021 [published Online First: 2012/01/18]
85. Reuter M, Schmansky NJ, Rosas HD, et al. Within-subject template estimation for unbiased longitudinal image analysis. *Neuroimage* 2012;61(4):1402-18. doi: 10.1016/j.neuroimage.2012.02.084 [published Online First: 2012/03/21]
86. Geeraert BL, Lebel RM, Mah AC, et al. A comparison of inhomogeneous magnetization transfer, myelin volume fraction, and diffusion tensor imaging measures in healthy children. *Neuroimage* 2017 doi: 10.1016/j.neuroimage.2017.09.019
87. Mah A, Geeraert BL, Lebel C. Detailing neuroanatomical development in late childhood and early adolescence using NODDI. *PLoS One* 2017;12(8):e0182340.
88. Cox RW. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. *Comput Biomed Res* 1996;29(3):162-73.
89. Jenkinson M, Beckmann CF, Behrens TE, et al. Fsl. *Neuroimage* 2012;62(2):782-90. doi: 10.1016/j.neuroimage.2011.09.015 [published Online First: 2011/10/08]
90. Wilke M, Schmithorst VJ, Holland SK. Assessment of spatial normalization of whole-brain magnetic resonance images in children. *Hum Brain Mapp* 2002;17(1):48-60. doi: 10.1002/hbm.10053
91. Reynolds C, Kamphaus R. Behavioral assessment system for children, second edition. San Antonio, TX: Pearson 2004.
92. Kovacs M. Children's Depression Inventory 2: Pearson Clinical 2010.
93. March JS. Multidimensional anxiety scale for children 2nd edition: Pearson Clinical 2012.
94. March JS, Parker JD, Sullivan K, et al. The Multidimensional Anxiety Scale for Children (MASC): factor structure, reliability, and validity. *J Am Acad Child Adolesc Psychiatry* 1997;36(4):554-65. doi: 10.1097/00004583-199704000-00019 [published Online First: 1997/04/01]
95. Beck AT, Ward CH, Mendelson M, et al. An inventory for measuring depression. *Arch Gen Psychiatry* 1961;4:561-71. doi: 10.1001/archpsyc.1961.01710120031004 [published Online First: 1961/06/01]
96. Cella D, Choi SW, Condon DM, et al. PROMIS((R)) Adult Health Profiles: Efficient Short-Form Measures of Seven Health Domains. *Value Health* 2019;22(5):537-44. doi: 10.1016/j.jval.2019.02.004 [published Online First: 2019/05/21]
97. Kaufman J, Birmaher B, Brent D, et al. Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL): initial reliability and validity data. *J Am Acad Child Adolesc Psychiatry* 1997;36(7):980-8. doi: 10.1097/00004583-199707000-00021
98. American Psychological Association. Diagnostic and statistical manual of mental disorders, 5th edition. Arlington, VA: American Psychiatric Publishing 2013.

99. Tolin DF, Gilliam C, Wootton BM, et al. Psychometric Properties of a Structured Diagnostic Interview for DSM-5 Anxiety, Mood, and Obsessive-Compulsive and Related Disorders. *Assessment* 2018;25(1):3-13. doi: 10.1177/1073191116638410 [published Online First: 2016/03/19]
100. Noel M, Wilson AC, Holley AL, et al. Posttraumatic stress disorder symptoms in youth with vs without chronic pain. *Pain* 2016;157(10):2277-84. doi: 10.1097/j.pain.0000000000000642 [published Online First: 2016/06/09]
101. Palermo TM, Valenzuela D, Stork PP. A randomized trial of electronic versus paper pain diaries in children: impact on compliance, accuracy, and acceptability. *Pain* 2004;107(3):213-19. doi: 10.1016/j.pain.2003.10.005 [published Online First: 2004/01/23]
102. Harrison PL, Oakland T. ABAS-3: Adaptive behavior assessment system (3rd edition). Torrance, CA: Psychological Corporation 2015.
103. Dunn W. The Sensory Profile: User's Manual. San Antonio, TX: The Psychological Corporation 1999.
104. Wechsler D. Wechsler Abbreviated Scale of Intelligence (WASI). San Antonio, TX: The Psychological Corporation. 1999
105. Bernstein JH, Waber DP. Developmental scoring system for the Rey-Osterrieth complex figure. *Psychological Assessment Resources* 1996
106. Heaton RK, Chelune GJ, Talley JL, et al. Wisconsin Card Sorting Test manual: revised and expanded. Odessa, FL: Psychological Assessment Resources 1993.
107. Delis DC. CVLT-C: California verbal learning test. San Antonio, TX: Psychological Corporation 1994.
108. Korkman M, Kirk U, Kemp S. NEPSY II. Clinical and interpretative manual. San Antonio, TX: The Psychological Corporation 2007.
109. Wechsler D. Wechsler Individual Achievement Test - Third Edition. London, 2009.
110. National Crittenton Foundation. The adverse childhood experiences (ACEs) survey: Toolkit for providers, 2015.
111. National Crittenton Foundation. Adverse childhood experiences (ACE) questionnaire and Crittenton, 2013.
112. Bates D, Machler M, Bolker B, et al. Fitting linear mixed-effects models using lme4. *Journal of statistical software* 2015;67(1):48.
113. Kuznetsova A, Brockhoff PB, Chritensen RHB. lmerTest package: tests in linear mixed effects models. *Journal of statistical software* 2017;82(13):26.
114. Hayes AF. Introduction to mediation, moderation, and conditional process analysis. New York, USA: Guilford Press 2013.
115. Bennett DS, Pendley JS, Bates JE. Daughter and mother report of individual symptoms on the Children's Depression Inventory. *J Adolesc Health* 1997;20(1):51-7. doi: 10.1016/S1054-139X(96)00090-0 [published Online First: 1997/01/01]
116. Wei C, Hoff A, Villabo MA, et al. Assessing anxiety in youth with the multidimensional anxiety scale for children. *J Clin Child Adolesc Psychol* 2014;43(4):566-78. doi: 10.1080/15374416.2013.814541 [published Online First: 2013/07/13]
117. Fritz MS, Mackinnon DP. Required sample size to detect the mediated effect. *Psychol Sci* 2007;18(3):233-9. doi: 10.1111/j.1467-9280.2007.01882.x [published Online First: 2007/04/21]

Figure Caption

Figure 1: Key gray matter regions (A) and white matter connection (B) related to anxiety and/or depression symptoms. Volume of the regions in A, functional connectivity between pairs of regions in A, and structural connectivity (diffusion metrics) of tracts in B will be measured.

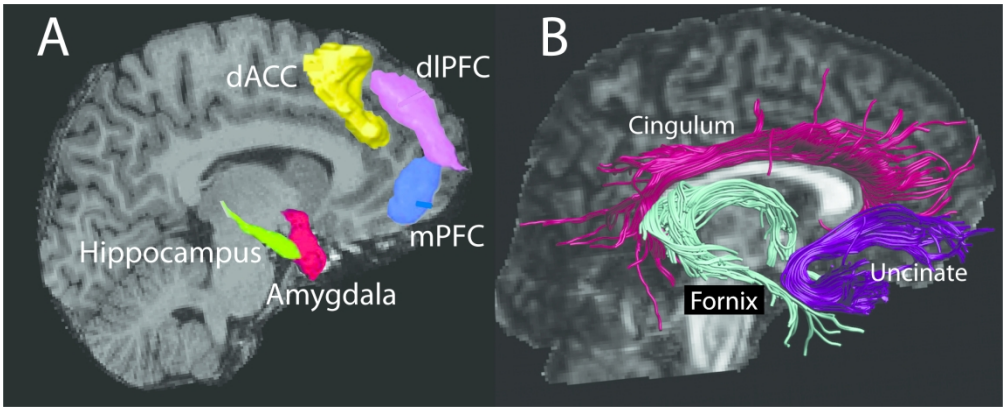

Figure 1
Key gray matter regions (A) and white matter connections (B) related to anxiety and/or depression symptoms. Volume of the regions in A, functional connectivity between pairs of regions in A, and structural connectivity (diffusion metrics) of tracts in B will be measured.