RESEARCH ARTICLE

Characterizing developing executive functions in the first 1000 days in South Africa and Malawi: The Khula Study [version 1; peer review: 2 approved with reservations]

Michal R Zieff[1], Marlie Miles[1], Emmie Mbale[2], Emma Eastman[1], Lorna Ginnell[3], Steven C R Williams[4,5], Derek K Jones[6], Daniel C Alexander[7], Peter A. Wijeratne[7,8], Laurel J Gabard-Durnam[9], Vanja Klepac-Ceraj[10], Kevin S Bonham[10], Nicolò Pini[11,12], Ayesha Sania[11,12], Maristella Lucchini[11,12], Sean Deoni[13,14], William P Fifer[11,12,15], Melissa Gladstone[3], Dima Amso[16*], Kirsten A Donald[1,17*]

[1]Division of Developmental Paediatrics, Department of Paediatrics and Child Health, University of Cape Town, Rondebosch, Western Cape, South Africa
[2]Department of Paediatrics, Kamuzu University of Health Sciences, Blantyre, Malawi
[3]Department of Women and Children's Health, Institute of Life Course and Medical Science, Alder Hey Children's NHS Foundation Trust, University of Liverpool, Liverpool, England, UK
[4]Department of Neuroimaging, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London, England, UK
[5]NIHR Maudsley Biomedical Research Centre, South London and Maudsley NHS Foundation Trust, London, England, UK
[6]Cardiff University Brain Research Imaging Centre, Cardiff University, Cardiff, Wales, UK
[7]Centre for Medical Image Computing, Department of Computer Science, University College London, London, England, WC1E 6BT, UK
[8]Department of Informatics, University of Sussex, Brighton, England, UK
[9]Department of Psychology, Northeastern University, Boston, Massachusetts, USA
[10]Department of Biological Sciences, Wellesley College, Wellesley, Massachusetts, USA
[11]Department of Psychiatry, Irving Medical Center, Columbia University, New York, New York, USA
[12]Division of Developmental Neuroscience, New York State Psychiatric Institute, New York, New York, USA
[13]Maternal, Newborn, and Child Health: Discovery & Tools, Bill & Melinda Gates Foundation, Seattle, Washington, USA
[14]Advanced Baby Imaging Lab, Providence, Rhode Island, USA
[15]Department of Pediatrics, Irving Medical Center, Columbia University, New York, New York, USA
[16]Department of Psychology, Columbia University, New York, New York, USA
[17]Neuroscience Institute, University of Cape Town, Rondebosch, Western Cape, South Africa

* Equal contributors

v1 **First published:** 20 Mar 2024, **9**:157
https://doi.org/10.12688/wellcomeopenres.19638.1
Latest published: 20 Mar 2024, **9**:157
https://doi.org/10.12688/wellcomeopenres.19638.1

Abstract

The term 'executive functions' (EFs) refers to a set of skills that support flexible control over thought and action. Classic EFs (working memory, inhibitory control, and cognitive flexibility) do not show measurable

Open Peer Review

Approval Status ? ?

	1	2
version 1 20 Mar 2024	? view	? view

stable function until after the third year of life and continue to develop into early adulthood. However, even at the earliest ages, these EFs are shown to have value for predicting school readiness and academic achievement. They continue to have predictive value for success, mental health, and general well-being across the lifespan including in ageing populations. As such, understanding the developing brain and cognitive developmental dynamics that set the stage for the development of EFs, in the first three years of life, is crucial for developing programming that supports healthy EFs development. The goal of this manuscript is to describe the goals, hypotheses, participant populations, and methodology of the Khula Study. Khula is a multi-modal multi-site longitudinal birth cohort study designed to characterise emerging EFs in the first 1000 days of life in global majority settings. Most research to date has been conducted in highincome countries rather than low- and middle-income countries that comprise most of the world's child population. We assert that understanding and supporting EF development has global importance, but this must be done with the understanding that EFs are skills that develop within the context of adaptation to one's environment. As such, the Khula Study aims to understand which EF influences are common across cultures but also which are culture specific. We will address these questions by incorporating data from South Africa and Malawi to understand influences on EF development and outcomes for children living in these contexts. We enrolled 394 mothers (84% antenatally) from Gugulethu in Cape Town, South Africa and 507 mothers (42% antenatally) from Blantyre, Malawi.

Keywords
executive functions, longitudinal birth cohort, infant neurodevelopment, sub-Saharan Africa, cognitive development

1. **Barbara Barth**, McGill University, Montreal, Canada
2. **Trecia Wouldes**, The University of Auckland, Auckland, New Zealand

Any reports and responses or comments on the article can be found at the end of the article.

Corresponding author: Michal R Zieff (michal.zieff@uct.ac.za)

Author roles: Zieff MR: Data Curation, Formal Analysis, Investigation, Methodology, Project Administration, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Miles M**: Data Curation, Project Administration, Writing – Review & Editing; **Mbale E**: Conceptualization, Funding Acquisition, Methodology, Supervision, Writing – Review & Editing; **Eastman E**: Project Administration, Writing – Review & Editing; **Ginnell L**: Methodology, Project Administration, Writing – Review & Editing; **Williams SCR**: Conceptualization, Funding Acquisition, Methodology, Supervision, Writing – Review & Editing; **Jones DK**: Conceptualization, Funding Acquisition, Methodology, Supervision, Writing – Review & Editing; **Alexander DC**: Conceptualization, Funding Acquisition, Methodology, Supervision, Writing – Review & Editing; **Wijeratne PA**: Formal Analysis, Writing – Review & Editing; **Gabard-Durnam LJ**: Conceptualization, Funding Acquisition, Methodology, Writing – Review & Editing; **Klepac-Ceraj V**: Conceptualization, Funding Acquisition, Methodology, Writing – Review & Editing; **Bonham KS**: Conceptualization, Methodology, Writing – Review & Editing; **Pini N**: Conceptualization, Methodology, Project Administration, Writing – Review & Editing; **Sania A**: Conceptualization, Methodology, Writing – Review & Editing; **Lucchini M**: Methodology, Writing – Review & Editing; **Deoni S**: Conceptualization, Funding Acquisition, Methodology, Writing – Review & Editing; **Fifer WP**: Conceptualization, Funding Acquisition, Methodology, Supervision, Writing – Review & Editing; **Gladstone M**: Conceptualization, Funding Acquisition, Methodology, Supervision, Writing – Review & Editing; **Amso D**: Conceptualization, Funding Acquisition, Methodology, Writing – Original Draft Preparation, Writing – Review & Editing; **Donald KA**: Conceptualization, Funding Acquisition, Methodology, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by the Wellcome Leap 1kD programme (The First 1000 Days) [222076/Z/20/Z].
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Zieff MR, Miles M, Mbale E *et al.* **Characterizing developing executive functions in the first 1000 days in South Africa and Malawi: The Khula Study [version 1; peer review: 2 approved with reservations]** Wellcome Open Research 2024, **9**:157 https://doi.org/10.12688/wellcomeopenres.19638.1

First published: 20 Mar 2024, **9**:157 https://doi.org/10.12688/wellcomeopenres.19638.1

Introduction

'Executive functions' (EFs) is an umbrella term for a set of skills that enable us to effectively plan, make decisions, and regulate behaviour and emotions (Badre, 2008; Cohen *et al.,* 2002; Miller & Cohen, 2001; O'Reilly, 2006). EFs are central to successful outcomes throughout life. They are also widely known to be disrupted in neurodevelopmental disorders, such as Attention-Deficit/Hyperactivity Disorder, and in children with learning differences, such as dyslexia. Here, we describe the Khula Study, designed to characterise the development of sensory, cognitive, and neural systems, beginning in early infancy, that support stable EFs emergence by the preschool years.

EFs are divided into separate constructs, including working memory, inhibitory control, and cognitive flexibility (Davidson *et al.,* 2006; Diamond *et al.,* 2002; Friedman & Miyake, 2004). Working memory is defined as the capacity to hold and manipulate items in mind in the service of goal-relevant actions. Inhibitory control refers to the ability to refrain from goal- or task-inappropriate actions. Cognitive flexibility involves adaptively switching behaviours or thoughts as demands change. These particular EFs emerge when children are between 3–5 years of age. However, rudimentary versions of these constructs, for example, involving spatial- or object-based attentional skills, emerge as early as when infants are 6–12 months of age (see Amso & Scerif, 2015 for a review). Functional neuroimaging data have consistently shown that the prefrontal cortex (PFC) is engaged in EFs (Miller & Cohen, 2001), especially when the number of items that must be held in working memory increases, when demand for inhibitory control increases, and when one must shift from one rule or dimension to another.

The Khula Study is designed to comprehensively characterise foundational brain development that sets the stage for the emergence of executive functions (EFs) over the first 1000 days of life (longitudinally at 3, 6, 12, 18, and 24 months) in South Africa and Malawi. Khula uses a combined methods approach that incorporates magnetic resonance imaging (MRI), electroencephalography (EEG), and behavioural measures to capture trajectories of change. Our approach in designing this longitudinal investigation was to incorporate data collection of environmental variables known to impact early brain development and to examine their relative impact (protective versus risk factors) on the predicted structural and functional development of cortical caudal-to-rostral gradients of regions and pathways relevant to PFC and EFs development in two majority world low resource settings in Africa. Complementing our neuroimaging, our multi-modal multi-scale assessments are state-of-the-art deep phenotypic measurements, including comprehensive surveys, sleep and activity wearables, psychosocial and sociodemographic contextual factors, and biospecimen data to characterise relevant health and environmental factors. Figure 1 shows our predicted model where early infant and maternal health, nutrition, gut microbiota, and sleep health may impact, and be impacted by, early cortical functional and structural development. We expect that these early variations form the foundation upon which hierarchically developing cortical pathways to and from the PFC (Figure 1) functionally develop. In summary, our transformative work aims to characterise brain network trajectories of EFs development within each country and variables that are universally valuable for EFs development across countries beginning in early infancy. In this report, we describe the methods of the Khula Study and the participant sample with respect to demographics, maternal health, and contextual family factors at both sites.

Methods

Participants and recruitment

We recruited 394 participants from South Africa and 507 from Malawi. Including expected attrition (~30%), we expect to have a full sample of approximately 600 participants completing the longitudinal assessments from 3 to 24 months. Women who were (i) pregnant, (ii) in their third trimester of pregnancy (28–36 weeks) or up to three months postpartum, and

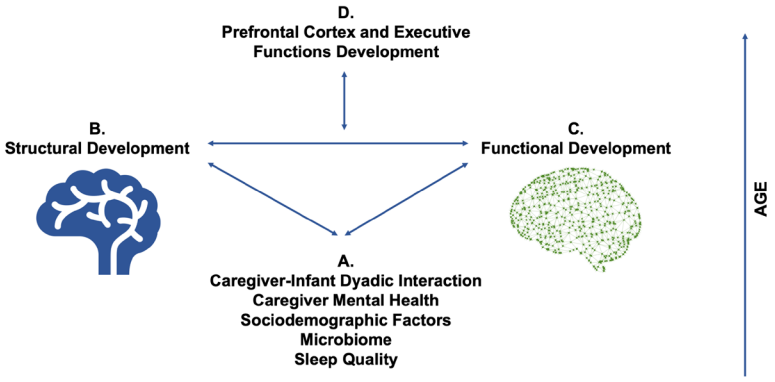

Figure 1. Khula developmental model. The overarching goal of this project is to test a developmental model of pathways to EF emergence, using a combination of exploratory machine learning analyses and confirmatory hypothesis-testing structural equation modelling (SEM) methods, as appropriate and depending on availability of all modality data at the project end. Our broad hypothesis is that (**A**) environmental variables early in life will reciprocally impact both structural (**B**) and functional (**C**) development (Timepoints 1–5, see Table 1) along a caudal (back) to rostral (front) dorsal and ventral pathway cortical gradient, (**D**) ultimately shaping PFC and EFs development (Timepoint 5, see Table 1).

(iii) over the age of 18 years at the time of recruitment were eligible to participate in the study. To limit potential confounders, modest exclusion criteria included multiple pregnancy, psychotropic drug use during pregnancy, infant congenital malformations and abnormalities (e.g., spina bifida, Down's syndrome), and significant delivery complications (e.g., uterine rupture, birth asphyxia).

In South Africa, we recruited 329 pregnant women from the Gugulethu Midwife Obstetrics Unit (MOU), an antenatal clinic in the Nyanga Health District of the Metro Region in Gugulethu, an informal settlement in the Western Cape, South Africa. We also recruited 65 mothers postpartum at various clinics in Gugulethu. We approached women in the clinics' waiting areas and invited them to participate in the study. All follow-up visits with the children were conducted at the Neuroscience Institute of the University of Cape Town. In Malawi, we recruited 507 women, both antenatally (42%) and postpartum (58%), from clinics in and around the Blantyre region. We selected these clinics because they have established MOUs and affiliated research infrastructure with the University of Cape Town and University of Malawi respectively, which makes them accessible for community recruitment. In South Africa, we recruited participants from 6 December 2021 to 29 November 2022, and in Malawi, between 22 January 2022 to 6 August 2023.

Ethical statement

All procedures were approved by the Human Research Ethics Committee at the University of Cape Town in South Africa or the College of Medicine Research Support Centre at the University of Malawi. Participation in the study is intensive, with five study visits over a 3-year period. For this reason, we obtain consent from participants on an annual basis, giving parents more agency to opt out if they want to stop participating at any point. Annual consent also provides participants with an opportunity to learn what to expect in each year of the study and to ask questions as new study elements are added. Trained study staff obtain consent from the participants prior to participation in any study-specific procedures. The consent forms are translated into local languages, and participants are informed that participation is voluntary and that they are free to withdraw at any point without any repercussions. Families are compensated with ZAR 300 (~ USD $15) in South Africa, or the MWK equivalent of USD $10 in Malawi after each visit they attend (according to ethics committee guidelines and regulations) for their time and to cover transport costs (in Malawi). In addition, both parents and children are provided with food and beverages during the visit. Transportation is made available to and from research sites in South Africa for participant convenience.

Participant location information

Gugulethu, South Africa. Gugulethu, South Africa, is a township located 18 km from Cape Town in the Western Cape Province of South Africa. The majority of its residents speak Xhosa as their home language. The majority of Gugulethu's inhabitants, aged 15 to 64 years, are employed (70%). Housing comprises formal dwellings (52%), informal/shack dwellings (47%), and other nonspecific dwelling types (1%). The majority of households have access to clean water (58%), flush toilets connected to a public sewage system (63%), weekly garbage removal (89%), and electric lighting (97%) (Strategic Development Information and GIS Department City of Cape Town, 2013).

Blantyre, Malawi. Blantyre is a district in Malawi composed of both urban and rural areas. Residents in these communities predominantly speak Chewa. There are, on average, four individuals per household in the Southern regions of Malawi. In Blantyre, most homes are non-permanent small dwellings with either grass thatched roofs or iron sheet roofs. Approximately 11% of households have electricity, and the majority (53%) use batteries as their main source of energy (National Statistical Office of Malawi, 2019). Approximately 85% of the population access drinking water via public standpipes, tubes, or protected wells and boreholes (Magombo & Kosamu, 2016; National Statistical Office of Malawi, 2019). Many Blantyre residents (47%) use a pit latrine with earth/sand slabs as toilet facilities, and 3% of homes use flush toilets (National Statistical Office of Malawi, 2019).

General study procedures

Parents and infants are tested for as many as five time points (pending availability) when infants are approximately between 3–24 months of age. Blood samples for general health information are taken by medical officers from infants at every visit and from mothers antenatally and at 3-months postpartum. Trained research assistants, research nurses, medical officers, and fieldworkers collect data at initial enrolment and as relevant throughout the study. Parents and infants are brought into the testing rooms housing our computer, EEG, and camera setups. Table 1 presents an overview of the data modalities and their collection in the Khula Study. Table 2 describes questionnaires administered at enrolment and reported in this article (Zieff, 2024b).

Methods and data processing specific to modality

EEG. High-density EEG recordings are collected using a fast-application 128-lead saline-based net system and NetAmps 400 amplifier (Electrical Geodesics, Inc.). EEG data are acquired at a sampling rate of 1000 Hz, with online referencing to the vertex (channel Cz; Ferree *et al.*, 2001). Data quality assessment is performed using the Harvard Automated Pre-processing Pipeline for EEG (HAPPE; Ferree *et al.*, 2001; Gabard-Durnam *et al.*, 2018; Monachino *et al.*, 2022). Recordings from a low-density gel-based Mentalab system supporting seven sensors with an online reference to Cz are used to test the generalisation of findings from high-density EEG signatures. Low-density EEG undergoes the same pre-processing steps as high-density EEG through HAPPE software's HAPPILEE pipeline with parameters optimised for low-density EEG (Lopez *et al.*, 2022).

Parent-child interaction. We capture naturalistic social interactions and infant visual attention using three video cameras: one facing the mother, one facing the infant, and a third

Table 1. Overview of data collection.

Assessment Domain	Measure	Respondent	Time Points					
			EN	T1	T2	T3	T4	T5
Maternal health and autonomy	Alcohol Exposure Questionnaire (AEQ)	Mother	■					
	The Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST)	Mother			■	■		■
	Child Trauma Questionnaire (CTQ)	Mother		■				
	Edinburgh Perinatal Depression Scale (EPDS)	Mother	■	■	■	■	■	■
	Intimate Partner Violence Questionnaire (IPVQ)	Mother	■	■	■	■	■	■
	Life Events Questionnaire (LEQ)	Mother	■				■	
	Mother Object Relation Scales (MORS-SF)	Mother		■	■			
	Multidimensional Scale of Perceived Social Support (MSPSS)	Mother	■	■	■	■	■	■
	Survey of Exposure to Community Violence (SECV)	Mother	■				■	
Contextual family factors	Chaos, Hubbub, and Order Scale – Modified Short Form (CHAOS)	Mother			■			
	Coronavirus Perinatal Experiences (COPE)	Mother		■				
	Infant Caregiver Movement Questionnaire (ICMQ)	Mother		■	■	■	■	■
	Screen Exposure Questionnaire	Mother			■	■	■	■
	Sociodemographic Questionnaire (South Africa)	Mother	■			■		■
	Demographic Health Survey (Malawi)	Mother	■			■		■
	Socialisation Goals Inventory (Modified)	Mother			■			
Child health and assessments	Anthropometry	Child		■	■	■	■	■
	Medical and developmental history	Mother		■	■	■	■	■
Sleep	Brief Infant Sleep Questionnaire - Revised Short Form (BISQ-R-SF)	Mother		■	■	■	■	■
	Phillips Respironics Actiwatch AW2	Child			■	■	■	■
	Gabi Smartcare Band	Child			■	■	■	■
	Sleep Diary	Mother			■	■	■	■
Neuroimaging	Low-Field MRI	Child		■	■	■	■	■
	High-Field MRI (*n* = 125 from South Africa only)	Child						
Biospecimens	Stool sample	Child		■	■	■	■	■
	Blood sample	Mother	■	■				
	Blood sample	Child		■	■	■	■	■
EEG and Behaviour	Resting State EEG	Child		■	■	■		■
	Visual Evoked Potentials (VEP)	Child		■	■	■		
	Face Individuation Task	Child			■	■		

Assessment Domain	Measure	Respondent	Time Points					
			EN	T1	T2	T3	T4	T5
	Statistical Learning Task	Child						
	Infant Visual Attention (parent-child interaction)	Child						
	Maternal Entropy (parent-child interaction)	Child						
	A-not-B	Child						
	Reverse Categorization Cognitive Flexibility Task	Child						
	Spin the Pots EF Working Memory Task	Child						
	Glitter Wand/Delay of Gratification	Child						
Child Development	Global Scales for Early Development (GSED)	Child						
	Bayley Scales of Infant Development (BSID-IV)	Child						

Note. Approximate age ranges for time points: T1 = 2–5 months, T2 = 6–11 months, T3 = 12–15 months, T4 = 16–19 months, T5 = 20–24 months.

Table 2. Self-report measures collected at enrolment.

Measure	Construct/Description
Khula Sociodemographic Questionnaire	Demographic (e.g., number of languages spoken in the child's home) and socioeconomic variables (e.g., maternal education). This tool was adapted from demographic questionnaires used in other South African birth cohorts (Donald *et al.*, 2018; Herman *et al.*, 2009).
Demographic Health Survey (DHS)	The Demographic Health Survey measures sociodemographic variables (National Statistical Office (NSO) [Malawi] & ICF, 2017). This questionnaire is administered in Malawi as an accompaniment to the Khula Sociodemographic Questionnaire.
Edinburgh Postnatal Depression Scale (EPDS)	The EPDS is a 10-item screening tool that screens for women who are at risk for peripartum depression (Cox *et al.*, 1987). Mothers responded to statements using a 4-point Likert scale (0-3). All scores are summed, with possible scores ranging from 0 (no depression) to 30 (highest level of depression).
Multidimensional Scale of Perceived Social Support (MPSS)	The MPSS is a validated, 12-item self-report questionnaire for measuring family and non-family social support (Zimet *et al.*, 1988). Mothers responded to statements such as "My family really tries to help me" with a Likert scale ranging from 0 ("Strongly disagree") to 4 ("Strongly agree"). Scores are averaged across three subscales: Significant Other, Family, and Friends. We adapted the MSPSS for our local contexts by reducing the number of response options from seven to five (Stewart *et al.*, 2014).
Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) V3.0	The ASSIST, developed for the World Health Organization, consists of eight questions or items, covering ten substances. ASSIST questionnaire is a reliable and valid screening test for problematic or risky substance use (Humeniuk *et al.*, 2010; WHO ASSIST Working Group, 2002).
Alcohol Exposure Questionnaire (AEQ)	The alcohol exposure questionnaire captures maternal use of alcohol during the three trimesters of pregnancy.
Intimate Partner Violence Questionnaire (IPVQ)	The IPVQ screens for women exposed to physical, sexual, and emotional abuse and controlling behaviours by an intimate partner (Garcia-Moreno *et al.*, 2006).
Survey of Exposure to Community Violence (SECV)	We administered an abbreviated version of the SECV (Richters & Saltzman, 1990) which measures how often people are exposed to different threats in their communities (e.g., being chased, drug activity, being arrested, physical assault, or witnessing a suicide or homicide). For this article, we present SECV results as frequencies (i.e., how many mothers have been direct victims of community violence, how many have witnessed acts of community violence, and how many mothers have heard about acts of violence in their community).
Life Events Questionnaire (LEQ)	Questions around exposure to traumatic and stress events. Items (51) were adapted from the Life Event Schedule of the World Mental Health Survey (Kessler & Üstün, 2004). In this article, we present scores as frequencies (i.e., how many mothers experienced each event in their lifetime and in the past year).

Note. Blank copies of these questionnaires, including the Xhosa and Chewa translations, may be found at ZivaHub Open Data UCT (Zieff, 2024b).

simultaneously capturing both. Mothers are instructed to play naturally with their babies as they might at home. Half of the play time (5 minutes) has three culture-appropriate objects. The other half is without objects. Their order is counterbalanced. We manually annotate each video for maternal behaviour and infant visual attention to the mother, using the Maternal Sensory Signal coding scheme (Davis *et al.*, 2017) and DataVyu software (Datavyu Team, 2014). Maternal entropy is a measure of predictability/unpredictability in maternal behaviour and is calculated as in Davis and colleagues (2017).

Biosamples

Blood. We measured hemoglobin levels in pregnant mothers at enrolment. In addition, we collect maternal (T1 visit) and child (all visits) blood samples to monitor specific proteins (ferritin, sTfR, RBP, CRP, and AGP), inflammatory markers (e.g., IL-6, TNF-alpha, IL-1 beta), folate, and thyroid function.

Stool. We perform shotgun metagenomic sequencing of stool samples at T1-T3, allowing for taxonomic and functional characterization of gut microbiota (bacteria, fungi, and viruses). We also store the T4-T5 samples for future analyses. To minimise the variance imposed by differences in procedure, we standardise sample collection kits, storage conditions, and extraction procedures across both sites. Microbial standards are used to enable post hoc normalisation (Gloor *et al.*, 2017; Mallick *et al.*, 2017). We also analyse faecal metabolomic profiles in richly characterised children (125 total from South Africa only) at T1-T3 to identify human and microbial metabolites. Metabolites from faecal samples are processed using a combination of LC-MS methods that measure polar metabolites, lipids, free fatty acids, bile acids, and metabolites of intermediate polarity.

Magnetic Resonance Imaging (MRI)

In South Africa, neuroimaging data are collected using both a standard high-field (3T) system and a newly introduced low-field (64 mT) MRI system. In Malawi, neuroimaging data are collected using an identical low-field (64 mT) MRI system. Multi-modal high-field MRI is performed using a 3T Siemens Skyra scanner with 32- or 48-channel head radiofrequency coil arrays. We assess the maturing brain macrostructure, tissue microstructure, and architecture using quantitative qT1, qT2, and qT2 relaxation time, myelin water fraction, and multi-directional multi-shell diffusion-weighted MRI. Imaging is performed during non-sedated sleep using strategies pioneered by our team and implemented at each site. Data are analysed using established quality control and post-processing routines, including registration to MNI space using custom age-appropriate templates. To complement our high-field 3T imaging, we acquire structural T1 and T2 weighted MRI on a portable low-field (Hyperfine Swoop) 64mT system (https://hyperfine.io). Using machine-learning techniques, specifically Image Quality Transfer, we will learn mappings between images collected at low field and corresponding high field images, so that the quality of subsequent images collected only at low field (such as at our Malawi site) can be enhanced, enabling advanced applications (e.g., tractography, tractometry, connectomics, and microstructure mapping) currently only achievable on higher-field MRI systems (Alexander *et al.*, 2017; Lin *et al.*, 2023; Tanno *et al.*, 2021).

Sleep health

At both sites, we monitor sleep and activity for three nights using two wearable devices, Gabi Band (https://gabismartcare.com/) and Actiwatch 2 (AW2) (https://www.usa.philips.com/healthcare/e/respironics). Infants and toddlers wear the former on the upper arm and the latter on the ankle. These devices allow the collection of heart rate and heart rate variability (Gabi) and movement (AW2) signals. To assess sleep measurement scalability, we also collect a sleep diary and administer the Brief Infant Sleep Questionnaire, Short Form Revised (Sadeh, 2004).

Maternal health and autonomy/contextual factors data collection

Throughout the study, we collect self-reported data related to maternal health and autonomy, as well as contextual family factors (see Table 1 for more details). In this manuscript, we present the results from self-report measures collected at enrolment (Table 2), including measures of substance use, depression, social support, traumatic life events, and exposure to community violence. All questionnaires were forward-translated by two independent bilingual translators from an external translation agency and back-translated by two different bilingual translators from the same agency. The translations were then evaluated by local team members and consolidated to create semi-final versions. Finally, we piloted the translations with five participants and made final tweaks to create the final translated versions.

Results

Data collection for the postnatal time points is still ongoing at the time of publication. In this article, we present data collected at enrolment and post-birth (via telephone check-ins) prior to the first formal infant assessment (T1) at three months of age. In South Africa, 329 pregnant women were enrolled antenatally, and 65 postnatally. Two antenatally enrolled women delivered stillborn infants. Of the 392 women who had live births, we were able to reach 321 (82%) after the initial enrolment. The child sample comprises 158 biological female infants (49%) and 163 biological male infants (51%). The maternal age at enrolment ranged from 18–44 years (M = 28.77, SD = 5.70).

In Malawi, 507 women aged 18-43 years (M = 26.52, SD = 5.98) were enrolled (43% postnatally). Follow-up data were collected for 307 women (61% of the original sample). Approximately 182 women (36%) were confirmed to be lost to follow-up, as they could not be reached via the given contact details. A further 13 (0.03%) withdrew before we arranged a follow-up visit. Sex was recorded in 299 child participants (97% of the "active" sample), with 149 biological female infants and 150 biological male infants (Zieff, 2024a). Figure 2 illustrates the flow of participants from enrolment until the first follow-up visit when the child reached 3 months of age.

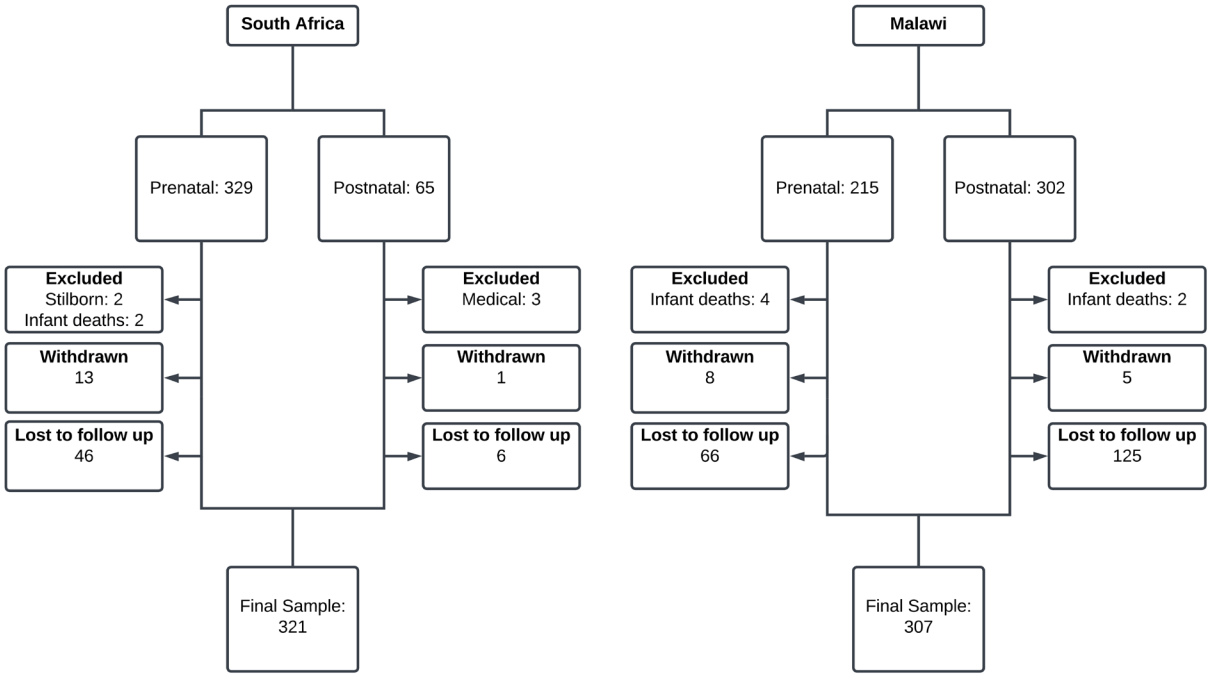

Figure 2. Flow of participants in South Africa and Malawi. Note. The "final" sample as depicted in this flow chart includes participants who were able to be contacted telephonically following initial enrolment to obtain the child's sex and date of birth, prior to scheduling the first in-person postnatal visit.

Sociodemographic data

Sociodemographic data from Malawi and South Africa are presented in Table 3 and Table 4, respectively. Both samples are representative of low- to low-middle-income households in their respective regions. The average number of individuals in participants' households was 3.57 (*SD* = 2.50) and 4.39 (*SD* = 2.36) in Malawi and South Africa, respectively.

Maternal mental health

Substance use

In South Africa, 103 women (26%) reported alcohol consumption during pregnancy. Reported alcohol consumption was highest in the first trimester (*n* = 89, 23%), followed by the second (*n* = 49, 12%) and third (*n* = 24, 6%) trimesters. In contrast, mothers from Malawi reported none to minimal drinking during pregnancy, with only six (1%), two (0.5%), and one (0.2%) mother(s) reporting drinking alcohol in the first, second, and third trimesters, respectively.

The ASSIST measured substance use in the previous three months. For mothers enrolled antenatally, these three months corresponded to the third trimester of pregnancy. In South Africa, 17 mothers (4%) used tobacco, whereas in Malawi, no mothers reported using tobacco in the preceding three months.

Depression

Scores on the EPDS in South Africa ranged from 0-26 (*M* = 6.96, *SD* = 5.99, *N* = 393), with 72 (18%) mothers scoring above the threshold (>12) for high risk of depression. In Malawi, the EPDS scores ranged from 0-27 (*M* = 3.82, *SD* = 4.12, *N* = 466), and 18 (4%) mothers were at risk for depression based on this assessment and threshold score. Figure 3 shows the distribution of the EPDS scores for both countries.

Contextual family and community factors

Intimate partner violence

Of the 406 participants who completed the IPVQ in Malawi, 86 (21%), 73 (18%), and 39 (10%) mothers had experienced emotional, physical, and sexual intimate partner violence respectively in their lifetimes. Thirty (7%), 20 (5%), and 12 (3%) mothers had experienced the three types of violence in the previous year. In South Africa, reports of lifetime intimate partner violence were higher for emotional violence (*n* = 145; 37%), physical violence (*n* = 124; 32%), and sexual violence (*n* = 59; 15%). Reported recent (past year) exposure to emotional (*n* = 66; 17%) and physical (*n* = 45; 11%) intimate partner violence respectively in the South African sample was higher in South Africa than in Malawi, although the proportion of mothers who experienced sexual violence in the past year was similar (*n* = 12; 3%).

Traumatic life experiences

The lifetime frequency of traumatic life events in South Africa ranged between 0.3% (military combat or military service in a war zone) and 70% (death of anyone close to you). Another

Table 3. Malawi participant sociodemographic information at enrolment (N = 415).

Variable	Frequency (%)
Maternal marital status	
Married	360 (86.75)
Single	39 (9.40)
Divorced/separated	14 (3.37)
Widowed	2 (0.48)
Maternal highest level of education	
None	13 (3.13)
Started primary school	74 (17.83)
Completed primary school	55 (13.25)
Started secondary school	137 (33.01)
Completed secondary school	104 (25.06)
Some post-school education	11 (2.65)
University	21 (5.06)
Home language	
Chewa	407 (97.83)
English	7 (1.69)
Yao	1 (0.24)
Water piped into dwelling	63 (15.18)
Flush toilet inside home	48 (11.57)
Electricity in the home	305 (73.49)

Note. Sociodemographic data at enrolment are missing for 92 participants (18%). Chi-square tests showed no significant differences in sociodemographic circumstances between participants who attended the first follow-up postnatal visit compared to those who were lost to follow-up after enrolment (see flow chart of participants in Figure 2).

Table 4. South African participant sociodemographic information at enrolment (N = 393).

Variable	Frequency (%)
Maternal marital status	
Single (never married)	246 (62.60)
Married or in a marriage-like relationship	146 (37.15)
Divorced/separated	1 (0.25)
Maternal highest level of education	
Completed primary school	9 (2.29)
Started secondary school	183 (46.56)
Completed secondary school	150 (38.17)
Started post-school education	33 (8.40)
Completed post-school education	18 (4.58)
Home language	
Xhosa	383 (97.46)
English	4 (1.72)
Other	6 (1.53)
Water piped into dwelling	290 (73.60)
Flush toilet inside home	153 (38.93)

Note. Enrolment data are missing for 1 participant (0.3%). Chi-square tests showed no significant differences in sociodemographic circumstances between participants who attended the first follow-up postnatal visit compared to those who were lost to follow-up after enrolment (see Figure 2).

life event that occurred in more than half of the South African sample was the unsuccessful search for a new job for more than one month (56%). In Malawi, the least frequently occurring traumatic events were (i) military combat, (ii) sexual assault or rape, and (ii) injured or killed another person (all 0.3%), while the death of anyone close was also the most frequently reported traumatic life event (47%). A relatively large proportion of participants from both sites reported either having inadequate financial resources to support or feed their families (South Africa 30%; Malawi 24%) and/or being in a major financial crisis (South Africa 16%; Malawi 36%) in the past year. Appendix 1 displays the lifetime and recent (past year) frequencies of each traumatic event at both sites.

Community violence. In South Africa, 316 participants (80%) reported having been victims of some form of violence, 370 (94%) reported having seen someone else being victimised by some form of violence, and 375 (95%) reported having heard about someone else in their community being victimised by some form of violence described in the SECV. Overall, reported exposure to community violence was lower in Malawi. Of the 412 participants who completed the SECV, 224 (54%) reported having been a victim of violence, 360 (87%) had seen someone else being a victim of violence, and 379 (92%) had heard about someone in their community being a victim of violence.

Social support. In both South Africa and Malawi, participants reported the highest levels of social support from a significant other, followed by family members (Figure 4). Perceived social support from friends was relatively low in both countries. The distribution of responses was highest for social support from friends compared to social support from a significant other and family.

Discussion

Understanding and supporting EF development is of global importance but must be done via culturally appropriate methodologies. EFs develop in response to the challenges posed by the environment of each child. That is, how a child acts, plans, decides, and regulates their behaviour can only be

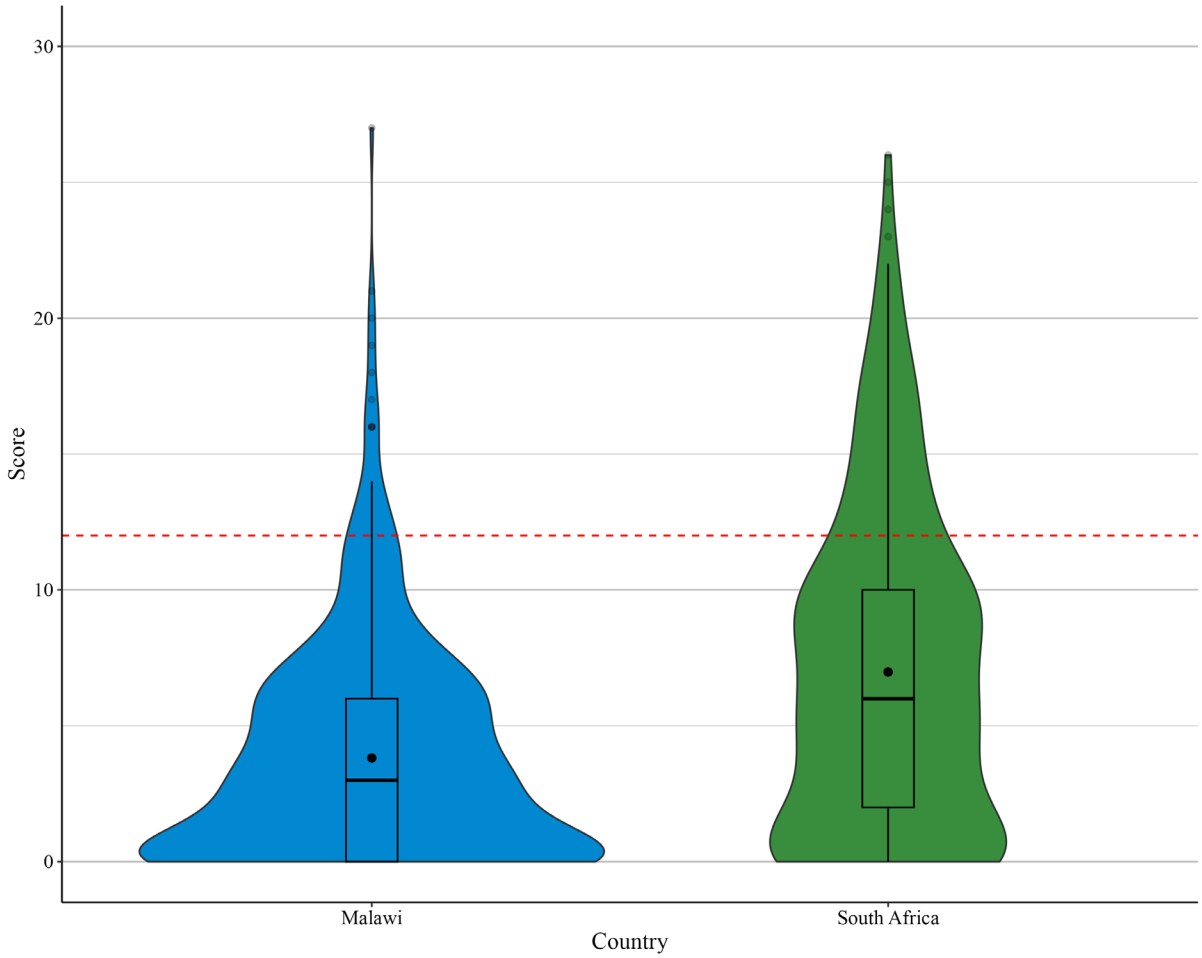

Figure 3. EPDS scores at enrolment of South African (N = 393) and Malawian (N = 466) participants. Note. EPDS = Edinburgh Postnatal Depression Scale. Black dots in the box represent the mean scores. Horizontal red dotted line indicates threshold score for depression risk (y >= 12).

considered successful when it is appropriate for wellness in their particular environment and cultural context.

Baseline data revealed the presence of different risk and protective factors that may affect infant and child development in South Africa and Malawi. While the average maternal depression scores were higher in South Africa, social support from significant others and families was similar across contexts. This is even as most (87%) of our Malawian sample were married compared to 37% of the South African participants. Exposure to intimate partner violence was reported in South Africa and Malawi. However, women in South Africa reported higher rates of both physical and sexual violence. Participants in South Africa also reported substantially higher rates of alcohol use during pregnancy than those in Malawi who reported little to no alcohol consumption. These data show both similarities and differences in the sociodemographic and familial contexts in which children grow and develop across these two cultural and environmental contexts in sub-Saharan Africa. This study aims to uncover the relationships between these environmental and contextual variables and emerging EFs. While previous studies from both high-income countries and low- and middle-income countries have demonstrated relationships between similar risk and protective factors and child cognition or development, (Barnett *et al.*, 2021; Naudé *et al.*, 2022; Urizar & Muñoz, 2022) few have examined the possible direct and indirect neural pathways to specific cognitive outcomes (e.g., inhibitory control).

The longitudinal, multidimensional design of the study will allow us to richly characterise this sample over time using a wide variety of relevant and complementary modalities. This study investigates factors that interface between genetic and environmental variation, including factors that are novel both in this specific geographical region and globally. For example, Khula is one of the first studies to longitudinally investigate

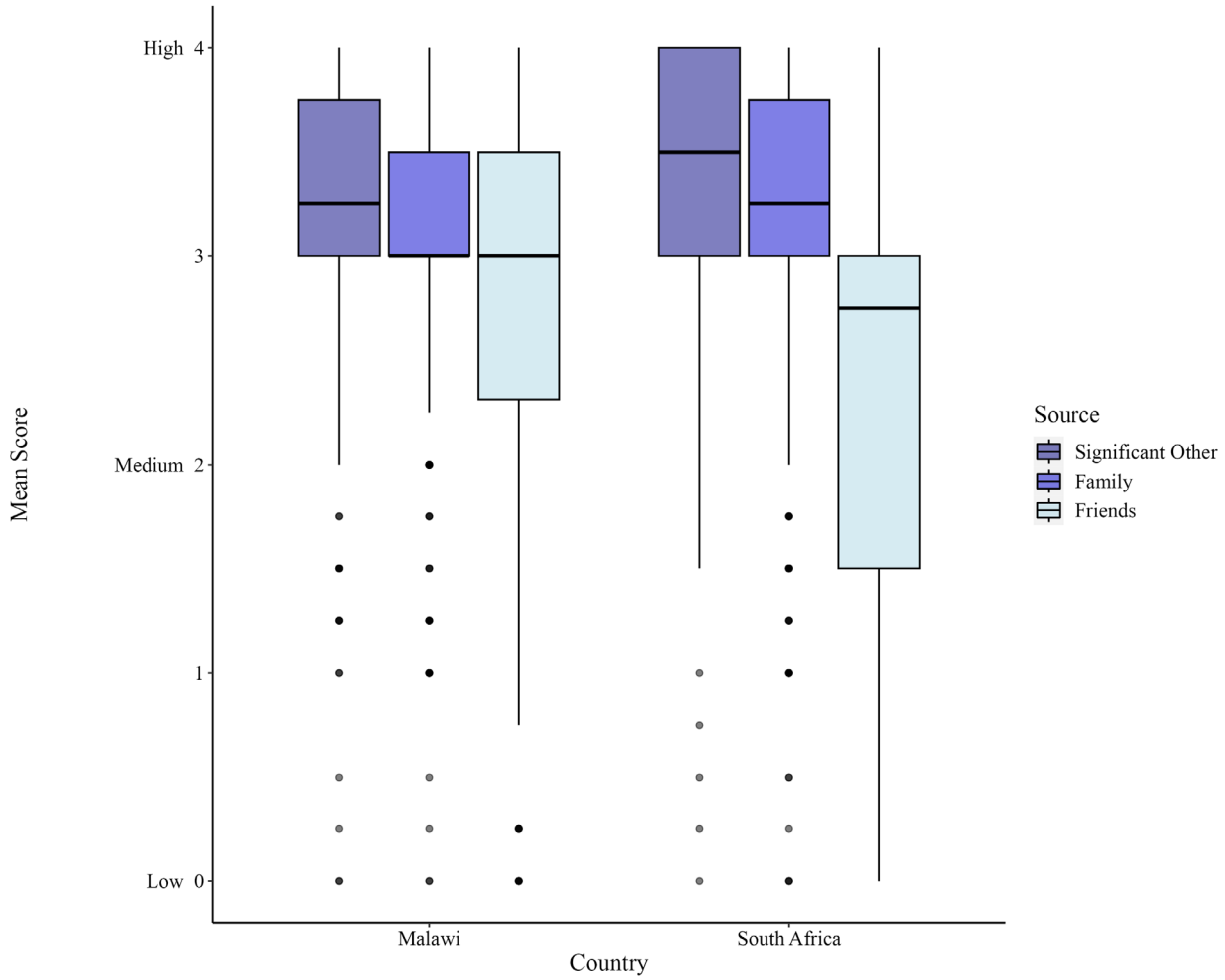

Figure 4. Distribution of MSPSS scores at enrolment for South Africa and Malawi sites. Note. MSPSS = Multidimensional Scale of Perceived Support. An average score of 0 indicates low levels of perceived support, 2 indicates medium levels of perceived support, and 4 indicates high levels of perceived support.

the early microbiome together with direct brain measures (i.e., MRI and EEG). Previous research has demonstrated a link between gut microbiota and neurodevelopment (Carlson *et al.*, 2018; Christian *et al.*, 2015; Gao *et al.*, 2019). However, these studies were mostly cross-sectional and based on samples from high-income settings. In addition, the impact of sleep during infancy is relatively understudied. Sleep is a universal human need, and perhaps the most preserved need from an evolutionary perspective, especially in infancy. However, little is known about infant and child sleep patterns and circumstances in different environments (e.g., living in informal housing, living without electricity, family co-sleeping) and their possible protective value in relation to emerging cognitive functions and development. The Khula study's multidimensional measurement of sleep using two different wearable devices, self-report measures (BISQ-R-SF), and sleep diaries across the first three years of life is novel in our contexts and serves to enhance our understanding of infant sleep and its role in development in these regions.

Another unique feature of the Khula Study is the emphasis on scalable methods aimed at obtaining high-quality data at a more accessible cost. Scanning infants using low- and high-field MRI systems across multiple time points in South Africa will allow us to develop techniques that enhance the quality of the subsequently collected low-field MRI scans. This will have a great impact on different low- and middle-income regions, where access to high-field MRI systems (for clinical or research purposes) may be limited or entirely lacking. The concurrent use of high- and low-density EEG at both sites will also enable us to evaluate the reliability of these newer, more affordable, and more user-friendly systems against "gold standard" high-density EEG systems, which involve significant equipment costs. In addition, we will be able to

evaluate the feasibility of using such systems in children with different hair textures and styles (e.g., thick, high-porosity, curly, coily, braids, etc; see Choy *et al.*, 2022) and recommend techniques to optimise data quality in diverse populations.

The ethical issues in this study require careful attention. Conducting research with pregnant or postpartum women and their infants requires special care as they constitute a vulnerable research population. A key ethical issue is the screening for perinatal depression and intimate-partner violence and ensuring that participants are referred to the correct provincial health services or appropriate non-governmental organisations, as indicated. In addition, infants undergo general medical examinations by trained medical officers during all study visits in South Africa. This is significant because children are not typically seen by medical doctors within the governmental primary healthcare system on a regular basis in infancy. We refer participants to the appropriate standard paediatric services if any issues are identified. Other challenges of conducting this research in our contexts include language and cultural barriers, trust in researchers and the research process, and misconceptions about exploratory research versus therapeutic interventions. While interactions with clinically trained research officers are an important part of the study, it is important that participants are aware that they are taking part in the research and that the study does not offer direct treatment or intervention. To prevent this potential misconception, we consent participants thoroughly and give them opportunities to ask questions in their home language and receive answers in their home language. Participation in this study comprises six intensive study visits over three years and may be experienced as a considerable burden. Rather than asking participants to commit to three years into the future at the outset, we re-consent participants on an annual basis to give them an opportunity to "opt-in" to each year of the study. This strategy aims to ensure that participants are well-informed about what to expect with regard to study activities over the next year. We also ensure that participants are compensated appropriately for their time, though in strict accordance with local human research ethics committee guidelines to avoid the risk of unintended "coercion" given that many participants live in poorly resourced areas and most come from low to low-middle income households.

Another challenge of such studies is the retention of participants over time. As demonstrated in Figure 2, we lost contact with approximately 180 mothers across both sites since the initial enrolment. At both sites, the primary reason for loss of contact is that mothers frequently change their mobile phone numbers due to mobile phones being lost, stolen, or broken. At enrolment, we recorded participants' physical addresses as well as alternative contact numbers (i.e., of partners, family members, neighbours), which helped us track down a portion of our participants successfully. Some participants moved outside the study area. In Malawi, the devastating effects of Cyclone Freddy forced participants out of their homes. In South Africa, some mothers relocated their infants to other provinces to receive care from family members while continuing to work in Cape Town. Relatively fewer participants withdrew from the study after their initial enrolment. One common reason for withdrawal was the child's father disapproving of the child's involvement in the study. In our context, it is unusual for fathers to accompany mothers to antenatal or postnatal clinic visits, where recruitment took place. It is possible that fathers may have been mistrustful of the study, especially when there was no opportunity to explain to them directly. To help familiarize other caregivers (i.e., not present at enrolment) with the study, we always provided copies of the information and informed consent documents. We also leave our study contact details in the information document so that any concerned parties may contact us for further information. In Malawi, we have also engaged with local community leaders and other trusted stakeholders, who are able to provide a space for community members to ask questions and address any misconceptions or concerns about their participation.

This study has two important limitations. The first limitation is the modest size of the cohort. Our target sample size at the end of the three years is 300 dyads at each site (N = 600 in total). To account for this modest sample size, we may use various statistical techniques to ensure adequate statistical power for our predictive models. While we have over-recruited by at least 30% at both sites to account for the loss of participants over time, it is likely that we will lose more along the way. There are, however, important non-measurable factors that may help prevent further attrition, including building relationships with families during follow-up in-person visits. The second limitation concerns the suitability of assessments and tools from the global North in our study contexts. To our knowledge, most executive function tasks (e.g., Spin the Pots) have not been validated in South African or Malawian populations. While we adapted the stimuli in these tasks to be familiar, we cannot know how they will perform. In addition, there are limited data to support the use of certain behavioural measures in these contexts, especially translated versions. To account for this, we will be piloting each questionnaire and task with at least 10% of our sample before formally commencing administration. We will make every effort to ensure that our tools are translated, back-translated, and reviewed to the highest possible standard.

Conclusions

To date, few birth cohorts have explored the development of EFs in the early years of life. The Khula Study will richly characterise samples from two African countries using multimodal measurements of brain development, cognition, and behaviour. Collecting data at three different time points in the first year alone presents a valuable opportunity to gain insights into the early sensitive periods for certain risk and protective factors. This will help us to understand how these factors contribute to the early formation of pathways for the development of executive functions. Furthermore, understanding the timing

of these relationships will allow us to develop effective and culture-specific interventions aimed at promoting resilient development in children living in high-risk environments.

Data availability

Underlying data

Figshare (ZivaHub Open Data UCT): Khula Methods Paper – Data, https://www.doi.org/10.25375/uct.24610932 (Zieff, 2024a)

The project contains the following underlying data:

- KhulaSouthAfrica_MethodsPaper_28.10.2023.xlsx (anonymised data presented in the main body of the article)

Extended data

Figshare (ZivaHub Open Data UCT): Khula Methods Paper – Extended Data, https://www.doi.org/10.25375/uct.24649893 (Zieff, 2024b)

This project contains the following extended data:

- KhulaMethodsPaper_Appendix1.pdf (Appendix 1 referenced in the main body of the article, Life Events Questionnaire)
- Questionnaires (blank copies of questionnaires in English and Xhosa/Chewa respectively)

Acknowledgements

We thank the mothers and infants for generously providing their time to participate in this study.

We are also grateful to our recruitment and data collection teams for their tireless efforts in ensuring the success of the study.

References

Alexander DC, Zikic D, Ghosh A, *et al.*: **Image quality transfer and applications in diffusion MRI.** *Neuroimage.* 2017; **152**: 283–298.
PubMed Abstract | Publisher Full Text

Amso D, Scerif G: **The attentive brain: Insights from developmental cognitive neuroscience.** *Nat Rev Neurosci.* 2015; **16**(10): 606–619.
PubMed Abstract | Publisher Full Text | Free Full Text

Badre D: **Cognitive control, hierarchy, and the rostro-caudal organization of the frontal lobes.** *Trends Cogn Sci.* 2008; **12**(5): 193–200.
PubMed Abstract | Publisher Full Text

Barnett W, Halligan SL, Wedderburn C, *et al.*: **Maternal emotional and physical intimate partner violence and early child development: Investigating mediators in a cross-sectional study in a South African birth cohort.** *BMJ Open.* 2021; **11**(10): e046829.
PubMed Abstract | Publisher Full Text | Free Full Text

Carlson AL, Xia K, Azcarate-Peril MA, *et al.*: **Infant gut microbiome associated with cognitive development.** *Biol Psychiatry.* 2018; **83**(2): 148–159.
PubMed Abstract | Publisher Full Text | Free Full Text

Choy T, Baker E, Stavropoulos K: **Systemic racism in EEG research: Considerations and potential solutions.** *Affect Sci.* 2022; **3**(1): 14–20.
PubMed Abstract | Publisher Full Text | Free Full Text

Christian LM, Galley JD, Hade EM, *et al.*: **Gut microbiome composition is associated with temperament during early childhood.** *Brain Behav Immun.* 2015; **45**: 118–127.
PubMed Abstract | Publisher Full Text | Free Full Text

Cohen JD, Braver TS, Brown JW: **Computational perspectives on dopamine function in prefrontal cortex.** *Curr Opin Neurobiol.* 2002; **12**(2): 223–229.
PubMed Abstract | Publisher Full Text

Cox JL, Holden JM, Sagovsky R: **Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale.** *Br J Psychiatry.* 1987; **150**: 782–786.
PubMed Abstract | Publisher Full Text

Datavyu Team: **Datavyu: A video coding tool.** New York University, 2014.
Reference Source

Davidson MC, Amso D, Anderson LC, *et al.*: **Development of cognitive control and executive functions from 4 to 13 years: Evidence from manipulations of memory, inhibition, and task switching.** *Neuropsychologia.* 2006; **44**(11): 2037–2078.
PubMed Abstract | Publisher Full Text | Free Full Text

Davis EP, Stout SA, Molet J, *et al.*: **Exposure to unpredictable maternal sensory signals influences cognitive development across species.** *Proc Natl Acad Sci U S A.* 2017; **114**(39): 10390–10395.
PubMed Abstract | Publisher Full Text | Free Full Text

Diamond A, Kirkham N, Amso D: **Conditions under which young children can hold two rules in mind and inhibit a prepotent response.** *Dev Psychol.* 2002; **38**(3): 352–362.
PubMed Abstract | Publisher Full Text

Donald KA, Hoogenhout M, du Plooy CP, *et al.*: **Drakenstein Child Health Study (DCHS): Investigating determinants of early child development and cognition.** *BMJ Paediatr Open.* 2018; **2**(1): e000282.
PubMed Abstract | Publisher Full Text | Free Full Text

Ferree TC, Luu P, Russell GS, *et al.*: **Scalp electrode impedance, infection risk, and EEG data quality.** *Clin Neurophysiol.* 2001; **112**(3): 536–544.
PubMed Abstract | Publisher Full Text

Friedman NP, Miyake A: **The relations among inhibition and interference control functions: A latent-variable analysis.** *J Exp Psychol Gen.* 2004; **133**(1): 101–135.
PubMed Abstract | Publisher Full Text

Gabard-Durnam LJ, Leal ASM, Wilkinson CL, *et al.*: **The Harvard Automated Processing Pipeline for Electroencephalography (HAPPE): Standardized processing software for developmental and high-artifact data.** *Front Neurosci.* 2018; **12**: 97.
PubMed Abstract | Publisher Full Text | Free Full Text

Gao W, Salzwedel AP, Carlson AL, *et al.*: **Gut microbiome and brain functional connectivity in infants-a preliminary study focusing on the amygdala.** *Psychopharmacology (Berl).* 2019; **236**(5): 1641–1651.
PubMed Abstract | Publisher Full Text | Free Full Text

Garcia-Moreno C, Jansen HA, Ellsberg M, *et al.*: **Prevalence of intimate partner violence: Findings from the WHO multi-country study on women's health and domestic violence.** *Lancet.* 2006; **368**(9543): 1260–1269.
PubMed Abstract | Publisher Full Text

Gloor GB, Macklaim JM, Pawlowsky-Glahn V, *et al.*: **Microbiome datasets are compositional: And this is not optional.** *Front Microbiol.* 2017; **8**: 294209.
PubMed Abstract | Publisher Full Text | Free Full Text

Herman AA, Stein DJ, Seedat S, *et al.*: **The South African Stress and Health (SASH) study: 12-month and lifetime prevalence of common mental disorders.** *S Afr Med J.* 2009; **99**(5 Pt 2): 339–44.
PubMed Abstract | Free Full Text

Humeniuk R, Henry-Edwards S, Ali R, *et al.*: **The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST): Manual for use in primary care**. In: *World Health Organization*. 2010.
Reference Source

Kessler RC, Üstün TB: **The World Mental Health (WMH) Survey Initiative Version of the World Health Organization (WHO) Composite International Diagnostic Interview (CIDI).** *Int J Methods Psychiatr Res.* 2004; **13**(2): 93–121.
PubMed Abstract | Publisher Full Text | Free Full Text

Lin H, Figini M, D'Arco F, *et al.*: **Low-field magnetic resonance image enhancement via stochastic image quality transfer.** *Med Image Anal.* 2023; **87**: 102807.
PubMed Abstract | Publisher Full Text

Lopez KL, Monachino AD, Morales S, *et al.*: **HAPPILEE: HAPPE In Low Electrode Electroencephalography, a standardized pre-processing software for lower density recordings.** *Neuroimage.* 2022; **260**: 119390.
PubMed Abstract | Publisher Full Text | Free Full Text

Magombo PU, Kosamu IBM: **Challenges of water accessibility in the urban centres of Malawi: A case study of Blantyre City.** *Afr J Eviron Sci Tech.* 2016; **10**(10): 380–385.
Reference Source

Mallick H, Ma S, Franzosa EA, *et al.*: **Experimental design and quantitative analysis of microbial community multiomics.** *Genome Biol.* 2017; **18**(1): 228.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Miller EK, Cohen JD: **An integrative theory of prefrontal cortex function.** *Annu Rev Neurosci.* 2001; **24**: 167–202.
PubMed Abstract | **Publisher Full Text**

Monachino AD, Lopez KL, Pierce LJ, *et al.*: **The HAPPE plus Event-Related (HAPPE+ER) software: A standardized preprocessing pipeline for event-related potential analyses.** *Dev Cogn Neurosci.* 2022; **57**: 101140.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

National Statistical Office (NSO) [Malawi], & ICF: **Malawi Demographic and Health Survey 2015-16**. NSO and ICF, 2017.
Reference Source

National Statistical Office of Malawi: **Malawi Population and Housing Census 2018**. 2019.
Reference Source

Naudé PJW, Pariante C, Hoffman N, *et al.*: **Antenatal maternal depression, early life inflammation and neurodevelopment in a South African birth cohort.** *Brain Behav Immun.* 2022; **105**: 160–168.
PubMed Abstract | **Publisher Full Text**

O'Reilly RC: **Modeling integration and dissociation in brain and cognitive development**. In: Y. Munakata & M. Johnson (Eds.), *Processes of Change in Brain and Cognitive Development: Attention and Performance.* Oxford University Press, 1st ed., 2006; 375–402.
Publisher Full Text

Richters J, Saltzman W: **Survey of Exposure to Community Violence: Self report version**. National Institute of Mental Health, 1990.
Publisher Full Text

Sadeh A: **A brief screening questionnaire for infant sleep problems: validation and findings for an Internet sample.** *Pediatrics.* 2004; **113**(6): e570–e577.
PubMed Abstract | **Publisher Full Text**

Stewart RC, Umar E, Tomenson B, *et al.*: **Validation of the multi-dimensional scale of perceived social support (MSPSS) and the relationship between social support, intimate partner violence and antenatal depression in Malawi.** *BMC Psychiatry.* 2014; **14**: 180.
PubMed Abstract | **Publisher Full Text** | **Free Full Text**

Strategic Development Information and GIS Department City of Cape Town: **City of Cape Town – 2011 Census Suburb Gugulethu**. 2013.
Reference Source

Tanno R, Worrall DE, Kaden E, *et al.*: **Uncertainty modelling in deep learning for safer neuroimage enhancement: Demonstration in diffusion MRI.** *Neuroimage.* 2021; **225**: 117366.
PubMed Abstract | **Publisher Full Text**

Urizar GG Jr, Muñoz RF: **Role of Maternal Depression on Child Development: A Prospective Analysis from Pregnancy to Early Childhood.** *Child Psychiatry Hum Dev.* 2022; **53**(3): 502–514.
PubMed Abstract | **Publisher Full Text**

WHO ASSIST Working Group: **The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST): development, reliability and feasibility.** *Addiction.* 2002; **97**(9): 1183–1194.
PubMed Abstract | **Publisher Full Text**

Zieff MR: **Khula Methods Paper - Data.** University of Cape Town. Dataset, 2024a.
http://www.doi.org/10.25375/uct.24610932.v1

Zieff MR: **Khula Methods Paper - Extended Data.** University of Cape Town, Journal contribution. 2024b.
http://www.doi.org/10.25375/uct.24649893.v1

Zimet GD, Dahlem NW, Zimet SG, *et al.*: **The Multidimensional Scale of Perceived Social Support.** *J Pers Assess.* 1988; **52**(1): 30–41.
Publisher Full Text

Open Peer Review

Current Peer Review Status: ? ?

Version 1

Reviewer Report 12 June 2024

https://doi.org/10.21956/wellcomeopenres.21754.r79035

?

Trecia Wouldes
The University of Auckland, Auckland, Auckland, New Zealand

This study aims to demonstrate the development of executive function (EF) in children over the first 1000 days postnatally in two settings, one in South Africa (SA) and one in Malawi. In SA, recruitment included 329 pregnant women and 65 mothers postpartum from an informal settlement in the Western Cape, Gugulethu. Of the 507 women recruited in Malawi, 42% were recruited during their pregnancy and 58% postpartum. The aim of this manuscript was to describe the study goals, hypotheses, and methodology of the study (Khula Study). The demographics, maternal health and environmental factors of the sample are reported. Data included a description of the sample that was collected at enrolment and post-birth (via telephone check-ins) prior to the first formal infant assessment at 3 months of age (T1). This is an important study, as the authors noted, most studies of EF have been carried out in high-income countries, therefore, the Khula Study has the potential to contribute important knowledge to our understanding of EF during a developmental stage of significant brain development in a more diverse sample in a moderate- to low-income country. This is an important study that has the ability to contribute significant knowledge around the development of EF.

Comments and questions:

1. This study will use multiple methods to examine EF including MRI, EEGs and behavioural measures and state of the art wearable sleep and activity monitors. The team of researchers provide expertise in all of these measures, but the preponderance of researchers appear to be from high-income countries (USA and UK). Could you provide some explanation around what input SA and Malawi parents/researchers had in the development of this study.
2. Attrition was quite high prior to T1, 82% in SA and 61% in Malawi. As telephone check-ins were used to collect these data, could you provide data on the number of families who had a phone?
3. Although it was reported that there was no difference in sociodemographic circumstances between participants who attended the first follow-up postnatal visit compared to those lost to follow-up it would be good to have a table that compares the two groups for both the SA and Malawi sample.
4. In addition, it appears the environments of this sample are reliant on questionnaire data,

but the home environment may be better characterised with a Home Observation for Measurement of the Environment (HOME) or a similar measure during a home visit. Is this part of the follow-up plan? From the description of the sample, the home environment and the laboratory environment where the testing will be done is likely to be very different. This could have positive or negative effects on outcomes. Also, do you plan to ask the mother or primary caregiver about their impression of the child's skills related to EF that they may see on a daily basis?

Thank you for the opportunity to review this paper and I look forward to seeing further results.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: My area of expertise is child development in populations where there may have been a perinatal insult due to exposure to psychoactive drugs such as methamphetamine, alcohol, or opioids. I am particularly interested in EF development, especially before formal school entry

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Reviewer Report 22 May 2024

https://doi.org/10.21956/wellcomeopenres.21754.r79037

Barbara Barth
McGill University, Montreal, Québec, Canada

The article describes the Khula study that aims to investigate the environmental factors influencing the development of executive functions in children from South Africa and Malawi. It also describes results on sample characteristics from mothers collected at the first phase of the study. The Khula study will follow children from birth to 24 months old, by collecting data in multiple modalities (MRI, EEG, behavioural and biomarkers). Data about mothers and child environment are also collected. The study is of utmost importance, as knowing the risk and protective factors involved in the development of executive functions in children has an impact on providing information for future intervention strategies and contributes to the body of knowledge on the impact of the early environment in children's health. As the authors state, culture and context-specific knowledge of the overall objective of the study are necessary. The manuscript will benefit from revisions. Specific impressions and suggestions are stated below:

Abstract
Consider replacing the "participant populations" with "participant sample". Population entails the entire group of individuals who meet the criteria for participating in a study, this would be all mothers from Gugulethu in Cape Town, South Africa and from Blantyre, Malawi.

I suggest reviewing the text for some word repetitions, for better reading flow, such as "the goal of this manuscript is to describe the goals…"

Not clear what global majority settings mean if the following phrase: "Khula is a multi-modal multi-site longitudinal birth cohort study designed to characterise emerging EFs in the first 1000 days of life in global majority settings." Is it an objective of the study for the results to be applicable to global majority settings?

I suggest including in the abstract that results from enrolment and post-birth check-ins via telephone prior to infant assessment T1 will be reported as it is a large proportion of the manuscript.

Introduction
I suggest including a section bout the impact of the early life environment on EF development. This would be appropriate as the study aims to investigate environmental factors that shape ED development. It would also give context to the measures being used in the study.
Add a description of the time of data collection of the data being presented in the manuscript. The text says "In this report, we describe the methods of the Khula Study and the participant sample with respect to demographics, maternal health, and contextual family factors at both sites." State that the results being presented are from enrolment and post-birth check-ins via telephone prior to infant assessment T1.

Methods
I suggest reviewing the text for some word repetitions, for better reading flow, such as "Including expected attrition (~30%), we expect to…"

I suggest clarifying what "modest" exclusion criteria mean.
In "general study procedures" it is stated that "Table 2 describes questionnaires administered at

enrolment and reported in this article (Zieff, 2024b).” I suggest replacing “reported in this article” with reported in this data repository.

Overall the description of all study measures would benefit from stating what is being measured and what is the objective for the data being collected. Addressing these measures in the introduction would also benefit the readers. For example, the relation between early environment, EF development and specific biomarkers (the ones being measured in the study), maternal-infant interaction, microbiome, and so on.
A Few questions emerged while reading the methods that led me to the suggestion made above. What are the objectives of the data being collected with EEG? What specific measure is being assessed with the parent-child interaction? What is maternal entropy and why is it relevant to the study? Why it is important to assess infant visual attention to the mother? Why those specific measures from blood samples? What are the objectives of the data being collected with MRI? Why does sleep health matter to EF development?
Section Magnetic Resonance Imaging (MRI), I suggest clarifying the strategies mentioned in this phrase for replication purposes: “Imaging is performed during non-sedated sleep using strategies pioneered by our team and implemented at each site”.
The objective of the manuscript is to present the Khula study, its study methods and some preliminary results from enrolment and post-birth check-ins via telephone prior to infant assessment T1. I suggest including the description of all measures used, in all time points, as the objective of the manuscript was to present the Khula study methodology in its entirety. Table 2 briefly describes measures used at enrolment only. I suggest describing each questionnaire, the rationale for choosing that specific questionnaire and any adaptation done for the study reality. State if the questionnaire is validated or not for the study sample and inform on future plans to calculate internal consistency and other measures of validity.
The manuscript is a lacking a proposed analysis plan for the data being collected and hypothesis.

Discussion
Some of my suggestions to include in the introduction, for example, a justification for some measures being used, are present in the discussion section. I suggest incorporating/moving them to the introduction. The reader will benefit from this information in the introduction to follow the rationale of the study being presented in the subsequent sections. There are also some methods information that should be moved to the methods sections. There is a mention of an executive function task that is not described in the methods.
I suggest giving more focus on elaborating in the discussion the expected outcomes of the study, what benefits it will bring in terms of interventions and creation of new knowledge, and the overall impact of the study.

Is the work clearly and accurately presented and does it cite the current literature?
Partly

Is the study design appropriate and is the work technically sound?
Partly

Are sufficient details of methods and analysis provided to allow replication by others?
No

If applicable, is the statistical analysis and its interpretation appropriate?

Partly

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Gene by enviroment interaction, early life adversity, neurodevelopment

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.