Growth in early infancy drives optimal brain functional connectivity which predicts cognitive flexibility in later childhood

Short title: Early growth predicts brain networks development

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Abstract

Functional brain network organization, measured by functional connectivity (FC), reflects key neurodevelopmental processes for healthy development. Early exposure to adversity, e.g. undernutrition, affects neurodevelopment, observable via disrupted FC, and leads to poorer outcomes from preschool age onward. We assessed longitudinally the impact of early growth trajectories on developmental FC in a rural Gambian population from age 5 to 24 months. To investigate how these early trajectories relate to later childhood outcomes, we assessed cognitive flexibility at 3-5 years. We observed that early physical growth before the fifth month of life drove optimal developmental trajectories of FC that in turn predicted cognitive flexibility at pre-school age. In contrast to previously studied developmental populations, this Gambian sample exhibited long-range interhemispheric FC that decreased with age. Our results highlight the measurable effects that poor growth in early infancy has on brain development and the subsequent impact on pre-school age cognitive development, underscoring the need for early life interventions throughout global settings of adversity.

Main Text

Introduction

The first 1000 days of life are of paramount importance for human brain (1) and body development (2, 3) Early adversity negatively impacts infant development, which leads to long-term consequences from delayed milestones in childhood development to lowered productivity at the individual and societal levels (4–6). Therefore, assessing trajectories of brain development in infants exposed to early adversity is essential to understand and mitigate effects on brain development and subsequent outcomes at school age and beyond (7, 8).

Early undernutrition engenders detrimental effects on a range of cognitive skills (9), with long-lasting effects through adulthood (10). Interventions using therapeutic feeding regimens and supplements have been shown to have a strong causal impact on childhood undernutrition, brain development, and subsequent neurodevelopmental outcomes (11–13). Despite numerous attempts of interventions, global rates of undernutrition remain high (14), with infants in low- and middle-income countries (LMICs) at greatest risk (15). Up to one-third of infants in LMICs are at risk of not meeting standard milestones of social, motor, linguistic, and cognitive development by pre-school age (16, 17), which can lead to lifelong consequences that impact social and economic development at individual, national, and global scales (18). Thus, it is essential to study neurodevelopment throughout the first two years in regions with high rates of undernutrition in order to optimize early interventions. To identify effective early interventions, we must first identify the mechanisms that link undernutrition to later cognitive outcomes. Effects of undernutrition on the developing brain may manifest through alterations in function brain networks (19), as shown in school-age children exposed to chronic poverty (20). However, our understanding of the relationships between early undernutrition, developmental trajectories of brain connectivity, and later cognitive outcomes is still very limited.

Functional brain network organization is commonly assessed with resting-state functional connectivity (FC) via temporal correlations in brain activity as measured with functional magnetic resonance imaging (fMRI) (21, 22). Based on data from fMRI, current models hypothesize that FC patterns mature throughout early development (23–27), where in typically developing brains, adult-like networks emerge over the first years of life as long-range functional connections between pre-frontal, parietal, temporal, and occipital regions become stronger and more selective (28–31). This maturation in FC has been shown to be related to the cascading maturation of myelination and synaptogenesis (32, 33) - fundamental processes for healthy brain development (34). Therefore, disrupted patterns of FC may reflect disrupted anatomical maturation of brain circuits and systems. For example, preterm infants with severe diffuse white matter impairment exhibited lower levels of FC in executive function networks compared with preterm infants with no or mild white matter impairment (35). Importantly, normative developmental patterns may be disrupted and even reversed in clinical conditions that impact development; e.g., increased short-range and reduced long-range FC have been observed in preterm infants (36) and in children with autism spectrum disorder (37, 38).

While widely used neuroimaging modalities such as fMRI can elucidate typical and atypical developmental trajectories, they are often poorly suited for lower resource neuroimaging contexts due to limited portability, need for specialized facilities, and the high cost associated with these methods (28, 36, 39). To address the need for neuroimaging studies in lower resource environments including (but not limited to) in LMICs, researchers have increasingly turned to more portable tools, including electroencephalography (EEG) (40), functional near-infrared spectroscopy (fNIRS) (41–43) and diffuse correlation spectroscopy (DCS) (44). These tools enable the creation of mobile neuroimaging laboratories that can be

deployed virtually anywhere, eliminating practical constraints imposed by costly and immobile methods. Indeed, research based in The Gambia, Guinea-Bissau, Bangladesh and Ivory Coast have established feasibility of these tools to perform brain imaging studies outside of a specialized lab environment, with recent studies reporting altered cortical physiology related to early adversity and undernutrition (40, 43–45). For example, using EEG, Xie and colleagues showed that in 2-3 year old Bangladeshi children, growth faltering was associated with FC in the theta and beta frequency bands, which was negatively related to children's IQ score at 4 years (46). With regards to fNIRS, it has a better spatial resolution and anatomical specificity than EEG, thus providing more precise and reliable localization of brain networks (47, 48). Moreover, fNIRS facilitates testing in awake and engaged infants, making the results more comparable with adult FC studies that are performed on awake participants (49). Given these unique strengths, fNIRS has been used to study FC in longitudinal studies in awake infants in high-resource settings (31), and the portability and the low cost of this equipment has fostered implementation outside conventional labs and in LMICs (50). Because fNIRS overcomes logistical (MRI) and resolution (EEG) challenges of other common modalities, we chose it for this study to assess early life brain health of children in LMIC exposed to early severe undernutrition.

The goal of the study was to investigate early physical growth in infancy, developmental trajectories of brain FC across the first two years of life, and cognitive outcome at school age in a longitudinal cohort of infants and children from rural Gambia, an environment with high rates of maternal and child undernutrition. Specifically, we aimed to: (i) investigate whether differences in physical growth through the first two years of life are related to FC at 24 months, and (ii) investigate if trajectories of early FC have an impact on cognitive outcome at pre-school age in these children. Data were collected from N=204 children as part of the Brain Imaging for Global Health project (BRIGHT; globalfnirs.org/the-bright-project), a longitudinal study examining infant development from term birth to 24-months of age. We acquired longitudinal measurements of brain FC in awake Gambian infant participants at five age points (5, 8, 12, 18, and 24 months) using fNIRS as the infants watched calming videos. We also acquired anthropometric measures (i.e., weight and length) as indicators of nutritional status at birth, at 7-to-14 days, one month of age, and at all five fNIRS data acquisition time points. Additionally, because developmental delays interfere with everyday functioning starting within the pre-school age range (16), we assessed cognitive flexibility, a core executive function (51), at ages three and five years. We hypothesized that (i) long-range FC would increase and short-range FC would decrease throughout the first two years of life, (ii) that early positive physical growth would significantly predict long-range FC, and that (iii) long-range FC strength would predict cognitive flexibility performance in pre-schoolers. Our results provide novel insights into the developmental trajectory of the functional organization of the developing brain, its relationship with nutritional adversity exposure and status, and the subsequent effects on preschool-age cognitive outcomes.

Results

Gambian infants exhibit robust FC development throughout first two years

The repeated assessments of brain FC throughout the first two years of life allowed us to analyse developmental trajectories of the maturation of early brain networks and how they relate to measures of infants' growth as well as to cognitive flexibility at preschool age (**Figure 1A**). To estimate how FC changed between 5-24 months of age in Gambian children, we performed linear mixed models (LMM) using data from the 132 infants with valid data for at least two time points. To increase statistical power while minimizing errors due to variability in head anatomy and cap placement, we divided the array into six sections and

calculated functional connectivity (FC) between all possible 21 interhemispheric, intrahemispheric, fronto-posterior, and crossed connections (**Figure SI1**). Results on the Fisher-z transformed correlation coefficients (z-RHO) of the oxygenated haemoglobin (HbO_2) showed that frontal interhemispheric FC decreased with age (F=11.03, p<0.001), and that left (F=5.8, p<0.001) and right fronto-middle (F=4.86, p<0.001) and right fronto-posterior (F=5.52, p<0.001) FC increased with age (**Figure 2**). Results on the z-RHO scores of the deoxygenated haemoglobin (HHb) largely agreed with the results on HbO_2 (**Table 1 and Figure 2**). Frontal interhemispheric FC significantly decreased with age, showing positive correlations at five months, and negative values at 24 months (**Figure 2B and Figure 2C**). The FC between fronto-middle and frontal-posterior that significantly increased with time showed strong anticorrelation values at early ages that gradually decreased but were still negative at 24 months (**Figure 2B and Figure 2C**). Notably, results from LMMs performed on data pre-processed without the global signal regression (GSR) (see results in SI, **Table SI1** and **Figure SI2**) confirmed a decreasing trajectory of the frontal interhemispheric FC and an increasing trajectory of bilateral short-range FC with age.

Early growth trajectories predict functional connectivity at 24 months

To investigate the impact of early nutritional status on FC at 24 months, we used multiple regression with the infant growth trajectory (delta weight for length z-score between time points, ΔWLZ) and FC at 24 months, adjusting for WLZ at birth or HCZ at 7/14 days. To maximise power, we considered only those FC that showed a statistically significant change with age. The ΔWLZ between birth/1 month and older ages positively predicted frontal interhemispheric FC at 24 months (ΔWLZ birth with older ages all p<0.02 FDR corrected, ΔWLZ 1 month with older ages all p<0.03, see **Figure SI3** for an example scatterplot) and negatively predicted left and right fronto-middle FC at 24 months (p<0.04). The ΔWLZ between birth and 1 month positively predicted right fronto-posterior FC at 24 months (p=0.006, FDR corrected) while ΔWLZ between 1 month and older ages negatively predicted right fronto-posterior FC at 24 months (all p<0.01, FDR corrected). Interestingly, ΔWLZ between 5/8/12/18 months and older ages did not show statistically significant impacts on any of the FC assessed (**Table 2 and Figure 3A**).

Early functional connectivity predicts cognitive flexibility at preschool age

To investigate whether FC early in life predicted cognitive flexibility at preschool age, we used multiple regression of FC across the first two years of life against later cognitive flexibility in preschoolers at three and five years. As per the analysis above, we focused on only those FC that showed a statistically significant change with age. Our results showed that frontal interhemispheric connectivity at 5 months (F(1,38)=4.21, p=0.047, R^2=0.102), left frontal-middle (F(1,33)=7.86, p=0.009, R^2=0.197) and right frontal-posterior FC at 12 months (F(1,38)=4.82, p=0.034, R^2=0.115) positively predicted cognitive flexibility in young preschoolers. Right frontal-posterior FC at 8 months negatively predicted cognitive flexibility in young preschoolers (F(1,32)=5.6, p=0.024, R^2=0.153). Frontal interhemispheric connectivity at 18 months (F(1,45)=4.82, p=0.030, R^2=0.115), and right frontal-posterior FC at 24 months (F(1,49)=4.85, p=0.032, R^2=0.092) positively predicted cognitive flexibility in older preschoolers. Left front-middle FC at 18 months negatively predicted cognitive flexibility in older preschoolers (F(1,45)=5.72, p=0.021, R^2=0.115). While these associations between early functional connectivity and cognitive flexibility at preschool age show promise, none of these survived FDR correction for multiple comparisons (**Figure 3B**), and so should be considered as preliminary and useful for hypothesis generation for future studies.

Discussion

This study investigated how early adversity via undernutrition drives longitudinal changes in brain functional connectivity at five time points throughout the first two years of life and how these developmental trajectories are associated with cognitive flexibility at preschool age. To capture the rapid neural development that takes place during early years in community LMIC settings we used fNIRS to assess brain connectivity (22, 24, 26, 31). Additionally, we recorded brain activity while infants were awake, thus making the results more comparable with those from adult studies. Our results show: (i) healthy growth trajectories during early infancy are crucial for healthy FC at 24 months; (ii) our cohort of Gambian infants exhibit atypical developmental trajectories of functional connectivity (FC) in over the first two years of life; and (iii) FC during the first two years of life may predict cognitive flexibility at preschool age.

First, we found that while left and right intra-hemispheric fronto-middle and right frontal-posterior FC increased with age, frontal inter-hemispheric FC decreased with age. Additionally, we observed long-range right frontal-posterior FC increased with age and exhibited positive correlations at 24 months, as consistent with previous infant studies (29, 31). Previous findings from typically developing infants measured in the US showed that long-range FC gradually strengthened with age, indicating maturing of functional networks that span distant brain regions (28, 29). Moreover, the presence of inter-hemispheric connections are known to indicate healthy development of FC (52) consistent with the continuous development of the corpus callosum from infancy until early adulthood (53). Conversely, decreased inter-hemispheric FC has been observed where development can follow a more divergent and/or heterogeneous path, such as in autism (54). The observed decrease in frontal inter-hemispheric FC with increasing age may be due to the exposure to early life undernutrition adversity. Finally, our results indicated that the Gambian infant bilateral fronto-middle FC exhibited anticorrelation at five months, and then gradually became less negative with age. This pattern suggests that this fronto-middle FC may become in sync later after the second year of life. The fNIRS array covers regions over the front and middle cortical regions, putatively belonging to the default mode network and fronto-parietal network, which are normally anticorrelated (55), suggesting that the earlier observed anticorrelation pattern might indeed be adaptive. While further investigations are needed to confirm these findings, these results highlight a general disruption of the integration/segregation process of network development among the infants in our study (56).

Second, a central goal of the current work was to investigate whether infant nutritional status, as measured by change in weight for length z-scores (ΔWLZ), was associated with functional connectivity at 24 months. First, we observed that ΔWLZ between neonatal time points and older ages positively predicted frontal interhemispheric FC at 24 months, which supports the idea that the decreasing interhemispheric connectivity observed with age might be atypical, as greater increases in ΔWLZ were found to be positively associated with stronger interhemispheric FC. This is consistent with research showing an impact of nutritional status on long-range connections and brain networks development (57–59) and the decreased long-range FC found in preterm babies with faltering growth (60–62). A common feature of the observed associations between ΔWLZ and FC is that they were statistically significant only when ΔWLZ was calculated including measures collected neonatally (i.e., at birth or one month of age). Changes in growth later in development (i.e. ΔWLZ between 5, 8, 12, or 18 months and older ages) did not show statistically significant impacts on any of the FC assessed. This provides support for the hypothesis that undernutrition during the first months of life is more impactful on brain development than

undernutrition occurring later in infancy and early childhood. While the impact of undernutrition on brain development has been documented in LMICs (46), herein, we provided empirical evidence that growth faltering specifically in infants younger than five months of age impacts observable development of functional brain networks in the second year of life. This result also suggests that early undernutrition has a lasting impact on subsequent cognitive skills, even in children who show subsequent catch-up growth.

The other primary aim of this study was to investigate whether early FC development was related to cognitive flexibility in preschoolers at three and five years of age. Long range FC (interhemispheric and frontal-posterior) positively predicted performance in the cognitive flexibility task both in younger and older preschoolers. This is consistent with the fact that the integration of distant brain regions fosters cognitive development, especially in prefrontal cortex (63, 64), possibly driven by maturation within the fronto-parietal network that has been widely documented as a primary neural underpinning of cognitive flexibility (65). Additionally, left fronto-middle FC at 12 months positively predicted cognitive flexibility in young preschoolers, but the same connection at 18 months negatively predicted cognitive flexibility in older preschoolers. This shift in direction of the relationship between FC and cognitive flexibility might indicate the shift between short-range and long-range FC during development, where short-range FC still promotes cognitive development early in life, while becoming less impactful as the child ages possibly because children develop a wider range of strategies to support cognitive flexibility as they age. A recent review on the neural underpinnings of cognitive flexibility highlighted that right-lateralized activations underlying this skill increased with age, while activations over the left hemisphere decreased with age (51). Our findings are consistent with this view, suggesting an increase in functional specialisation and segregation with development.

Some limitations of the current study merit discussion. Although the BRIGHT sample is one of the largest longitudinal neuroimaging studies in LMIC to assess longitudinally this many age points during the first two years of life, the sample size may still be underpowered to assess the modest effects of the regression models. Second, additional factors may affect FC that were not considered in our models. Applying more advanced statistical modelling methods and structural equation modelling analyses may provide greater insight with further investigations in contexts of adversity and, in turn, establish which outcomes are predicted by FC. That said, results from the regression models were statistically robust, as most survived FDR correction for multiple comparisons, and were significant after including HCZ at 7-14 days and WLZ at birth in the models. It is also worth noting that in line with previous FC fNIRS studies (31, 66), linear mixed model results on the HbO_2 and the HHb signals showed consistency in the FC changes with age measured in the two chromophores, and similar trajectories when removing the global signal regression step from the pre-processing, suggesting that the results were reliable and statistically robust. Future collaborations between projects studying the impact of adversity on brain development in both high resource and LMIC settings are crucial to advance the field and therefore inform efficient strategies – including the timing - of intervention. Even given the large sample in our study, we were underpowered to test for group comparisons between sets of infants with distinct undernutrition growth profiles, e.g., infants with early poor growth that later resolved and infants with standard growth early that had a poor growth later. We were also underpowered to test the associations between early growth and FC on clinically undernourished infants (defined as having ΔWLZ two standard deviations below the mean). However, our findings provide a solid foundation for future research on the impacts of undernutrition during the first months of life on later brain health and cognitive abilities throughout childhood.

In conclusion, herein we showed that, as expected, positive growth trajectories during the first few months of life were positively associated with stronger interhemispheric frontal FC and negatively associated with bilateral short-range FC. Interestingly, while Gambian infants had expected increasing left and right intra-hemispheric fronto-middle and right frontal-posterior FC with age, frontal inter-hemispheric FC decreased with age, which is inconsistent with extant data from other populations studied in Europe and US. Of particular note, growth during the first few months after birth showed a significant impact on brain health, as measured with FC, while later growth changes were not related to variability in FC. Moreover, long-range FC predicted the performance on a cognitive flexibility task at preschool age. Taken together, our results show the impact that early growth has on functional brain development, which in turn affects cognitive flexibility in preschoolers. Finally, this study emphasizes the importance of the timing of interventions, for which benefits are visible at preschool age and beyond.

Materials and Methods

Experimental Design

Our study investigated the developmental trajectory of brain health as assessed with functional connectivity (FC) in Gambian infants and its relationship with early growth, as a proxy for nutritional status, and later cognitive outcomes. To do, so we estimated FC from data acquired with fNIRS longitudinally at five time points between age five and 24 months in the context of the BRIGHT Project. The BRIGHT project sought to provide brain function-for-age curves from the UK and Gambian infants and to gain insight into the effects that issues related to living in low-resource settings may have on brain development. We then tested for relationships between brain FC at age 24 months with measures of early growth, as indexed by changes in weight-for-length z-scores assessed at birth, at one month of age, and at each of the four subsequent visits (details provided below). We also tested for associations between brain FC at each time point with later cognitive outcome, as indexed by cognitive flexibility assessed ain three- and five-year-olds preschool-age children (**Figure 1A**). Ethical approval was granted by the joint Gambia Government - MRC Unit the Gambia Ethics Committee, and written informed consent was obtained from all parents prior to participation.

Participants

We used data from N=204 infants recruited in rural community settings of The Gambia as part of the BRIGHT project. The study was a prospective, longitudinal study with fNIRS assessments planned for awake infants at ages 5, 8, 12, 18 and 24 months. All infants were born full term (37–42 weeks gestation). fNIRS data were additionally acquired from 1-month-old infants while asleep. However, as sleep stages may impact connectivity measures (98), these data are not part of the current analysis. Reasons for exclusion from the fNIRS analysis were: i) fussiness; ii) experimental errors (missing pictures of the headgear placement or any other technical issue); iii) poor headgear placement (see supplementary information for *Assessment of headgear placement*); iv) low-quality data (more than 40% of the channels excluded as previously done (67–70)). **Table SI2** summarises the details of the included and excluded participants for each visit, showing an attrition rate within the standard range for infant fNIRS studies (71) (see **Table 3** for the demographic information of the participants included in the analyses at each age).

Anthropometric measures

Measurements of length, weight, and head circumference (HC) were collected on all infants at birth, at 7/14 days, 1 month, and in all visits from 5 to 24 months, by trained field workers using calibrated tools. Length was measured using a Harpenden Infantometer length board (Holtain Ltd) to a precision of 0.1 cm. Weight was obtained using an electronic baby scale (Model 336, SECA) to a precision of 0.01 kg. Finally, HC was measured around the maximum circumference of the head (forehead to occiput) using stretch-proof measuring tape (Model 201, SECA) to the nearest 0.1 cm. Each measure was taken in triplicate and the mean of the three measures was used in analyses.

fNIRS data acquisition

fNIRS data were collected using the NTS topography system (Gowerlabs Ltd., London, UK). This system uses two continuous wavelengths of near-infrared light (780 and 850 nm) to detect changes in HbO_2 and HHb concentrations, using a sampling rate of 10 Hz (72).

Infants were assessed using a custom-built fNIRS headgear consisting of two arrays, including 14 sources and 12 detectors to create a total of 34 channels, covering bilateral frontal, inferior-frontal and temporal regions, with a source-detector separation of 2 cm (**Figure 1B** and **Figure 1C**).

For each infant, head measurements were taken to enable the alignment of the headgear with the 10–20 placement coordinates (73). Over the front of the head, the headgear was placed so that a vertical red line marking the centre of the band aligned with the nasion with the silicon band laying just above, and in line with, the eyebrows. The headgear was placed so that the reference optode (the third lower optode from the back) was placed over the tragus.

To model for the headgear placement offline, photographs of the participants (one from the front and one from either side) were taken after the headgear was placed and again at the end of the fNIRS session. To assess the headgear placement, photographs of the participants had to be sufficiently good in quality (i.e. not blurred) and with the camera pointing at the ear, at a position directly perpendicular to the infant's anatomical reference on the side of the head being photographed.

Infants were excluded from further analysis if the band was excessively high over the front above the eyebrows, by checking the displacement of the lower edge of the band with respect of the eyebrows with a "traffic-light" code (red, orange, green). Three experimenters independently classified the infants' frontal headgear placement, and discrepancies were discussed until agreement was reached. Channels of those infants with valid placement over the front but horizontal lateral displacement between the ideal and the actual position of the reference (optode marked in green in **Figure 1B**) equal or greater than 1.6 cm were renumbered, so that each channel was shifted either backward or forward one full channel location in space

At each visit, the fNIRS data acquisition took place while infants sat on their parent's lap. The parent was instructed to refrain from interacting with the infant during the stimuli presentation. Videos were of male and female Gambian adults singing nursery rhymes in Mandinka (for 70 seconds) and videos of toys in action (for 60 seconds), video blocks alternated, with each type shown to the infant twice, with the aim of keeping the infant awake, calm, attentive and as still as possible. The functional connectivity (FC) paradigm formed part of a greater battery of fNIRS paradigms that ran in a continuous and consistent order across all infants. The fNIRS FC acquisition lasted for a maximum of 520 seconds (two rounds of 260 seconds each) or were stopped as soon as the participant became fussy. Stimuli were presented using a MATLAB® custom-written stimulus presentation framework, Task Engine (sites.google.com/site/taskenginedoc) and Psychtoolbox on an Apple Macintosh computer.

fNIRS preprocessing and data-analysis

Data analyses were carried out using in-house programmes developed in MATLAB (MathWorks, Natick, MA) (**Figure SI4** summarises fNIRS data preprocessing steps). Stringent multi-level data-quality assessments were used to ensure all data included in the group were of adequate quality. First, low-quality channels based on physiological indicators of quality were pruned using QT-NIRS (https://github.com/lpollonini/qt-nirs). The software takes advantage of the fact that the cardiac pulsation is also recorded in addition to haemodynamic activity associated with brain-driven variance including task-based responses and those contributing to functional connectivity (74), and quantifies its strength in the spectral and temporal domains with two measures: the Scalp Coupling Index (SCI) and the Peak Spectral Power (PSP) (for more details, see (75). For each infant, data quality was assessed channel-by-channel, and SCI and PSP were calculated with sliding 3-second windows (threshold SCI=0.70, threshold PSP=0.1, empirically defined and based on (76)) (75). Channels that had both SCI and PSP below threshold for more than 70% of the windows (75), were excluded from further pre-processing steps.

Raw intensity data were converted to optical density via a logarithm after dividing by

429 the temporal mean of each channel (*hmrIntensity2OD.m* function from Homer2 tool (77)) and
430 bandpass filtered (0.009 – 3 Hz) (*hmrBandpassFilt.m* function from Homer2 (77)). Using
431 first a wide filter leaves in the signal contamination from the high bandwidth information (i.e.
432 respiration, pulse, etc.) that we aim to regress out next. To help manage the systemic arterial
433 signal that is well known to contaminate fNIRS signals (78), we regressed out the mean of
434 the signals across the array, a method also known as global signal regression (GSR) (79, 80)
435 (Results of analysis performed also without GSR are reported in the Supplementary
436 Materials). Hereafter, optical density data underwent a second bandpass filtered (0.009 – 0.08
437 Hz), as previously described (47). Motion artefacts were then rejected using global variance
438 of temporal derivatives (GVTD, STD=5) in the NeuroDOT toolbox
439 (https://www.nitrc.org/projects/neurodot/) (81) (**Figure SI5** show how we tested the effect of
440 different STD thresholds of GVTD on data inclusion). To further manage potential motion
441 contamination of the data, we additionally removed five seconds before and after each motion
442 artefact, and only chunks of data that were at least 20 seconds long were retained.
443 Optical density data were converted to relative concentrations of haemoglobin using
444 the modified Beer–Lambert law (*hmrOD2Conc.m* function from Homer2 (77)). Differential
445 path length factors were calculated (82) and adapted to wavelengths (780 and 850 nm) and
446 ages (5 months = 5.24 and 4.25; 8 months = 5.26 and 4.26; 12 months = 5.27 and 4.28 ;18
447 months = 5.30 and 4.30; 24 months = 5.32 and 4.32).
448 To increase the statistical power of our analysis and to reduce the number of multiple
449 comparisons for all statistical analysis to investigate developmental changes in connectivity,
450 we averaged the concentration changes of the channels that survived pre-processing into
451 sections. The sections were established via the 17 channels of each hemisphere which were
452 grouped into front, middle and back (for a total of six regions) based on a previous co-
453 registration of the BRIGHT fNIRS arrays onto age-appropriate templates (68) (**Figure SI1**).
454 Each section represented a reasonable estimate of an anatomically consistent array coverage
455 across the group, and was used to increase statistical power while minimizing errors due to
456 variability in head anatomy and cap placement. For each participant, the Pearson-r correlation
457 matrix between all the sections was calculated for both HbO_2 and HHb, resulting in a 6×6
458 matrix of section-pair correlations. We then applied a Fisher z-transformation on the
459 correlation matrix for further statistical analyses. Infants with at least 250 seconds of valid
460 data after pre-processing were considered for further analyses. To choose the optimal
461 minimum amount of valid data, per each infant at each time point for both the HbO_2 and HHb
462 signal, we correlated the connectivity matrices estimated in the first portion of data with the
463 one in the last portion of data by increasing the seconds of data considered (i.e. the first 60
464 seconds with the last 60 seconds, the first 100 seconds with the last 100 seconds, the first 120
465 seconds with the last 120 seconds, etc.). We found that correlating the first and the last 250
466 seconds provided the highest percentage of infants with strong correlation between the first
467 and the last portion of data, suggesting that FC patterns reached a usable stability after 250
468 seconds (**Figure SI6**).
469
470 **Cognitive flexibility at preschool age**
471 Gambian infants from BRIGHT were cross-sectionally assessed at the age of 3 or 5 years for
472 cognitive flexibility using the "card sorting" task from the tablet-based Early Years Toolbox
473 (83) (http://www.eytoolbox.com.au). In this task, children were asked to sort cards (i.e. red
474 rabbits or blue boats) either by colour (red or blue) or shape (rabbits or boats), and to flexibly
475 switch back and forth between these rules (84).
476 Due to disruptions to field work as a result of a political crisis in December 2016 -
477 January 2017 that forced us to pause recruitment, data were collected in two ranges of
478 preschool ages, younger preschoolers (age mean ± SD=47.96 ± 2.77 months) and older

479 preschoolers (age mean ± SD=57.58 ± 2.11 months) (85).
480
481 **Statistical Analyses**
482 To investigate developmental trajectories of functional connectivity between 5 and 24
483 months, we ran linear mixed models (86) which is the standard statistical test to analyse
484 repeated measures and longitudinal data with dropouts (87). Statistical analysis was
485 performed with SPSS Version 28.0 statistic software package. Compared to repeated
486 measures ANOVA, linear mixed models account for within-participant dependence and allow
487 for missing data by using only information from the individual at the other visits (88). All
488 possible interhemispheric, intrahemispheric, fronto-posterior, and crossed connections
489 between the six regions were inserted as dependent variables in a linear mixed model, for a
490 total of 21 linear mixed models. Functional connectivity was modelled as the dependent
491 variable as a function of age with a random participant effect and random errors. Intercept
492 and age were fixed effects, while within-participant dependence was modelled as a random
493 effect (89). This same procedure was used in other longitudinal studies that explored
494 developmental changes over time (31, 90, 91). The linear mixed model included the 132
495 infants who had valid data for at least two visits (55 infants contributed with data from two
496 visits, 51 infants contributed with data from three visits, 22 infants contributed with data from
497 four visits, four infants contributed with data from all of the five visits). The type of
498 covariance between the observations was specified as Autoregression (AR) as two measures
499 close in time of the same participant are likely to be correlated (92). To ensure statistical
500 reliability, significant results from the linear mixed models were corrected for multiple
501 comparisons using Bonferroni correction (p=0.00238).
502 Anthropometric measures were converted to age and sex adjusted z-scores that are
503 based on World Health Organization Child Growth Standards (93). Weight-for-Length
504 (WLZ) and Head Circumference-for-age (HCAZ) z-scores were computed. Delta WLZ
505 (ΔWLZ) were calculated for various periods (0-1 months, 0-5 months, etc.) by subtracting the
506 z-score at the later age from that at the previous age. The use of delta z-scores as outcome
507 measures allowed us to assess the impact of positive or negative deviation from the expected
508 growth trajectories. To investigate the impact of undernutrition on FC development, we used
509 ΔWLZ as independent variables in regression analyses on HbO_2 (as the chromophore with
510 the highest signal-to-noise ratio) FC at 24 months. To maximise power, we considered only
511 those FC that showed a statistically significant change with age. These analyses were
512 adjusted by WLZ at birth and HCAZ at 7/14 days. We used HCZ measured at 7/14 days and
513 not HCZ at birth as HC measures at birth are unreliable following vaginal delivery.
514 Therefore, HCAZ at 7/14 days is a more accurate head measure than HCAZ at birth. To
515 ensure statistical reliability, results from the regression analyses on each FC were corrected
516 for multiple comparisons using false discovery rate (FDR) (94).
517 To investigate whether FC early in life predicted cognitive flexibility at preschool
518 age, we regressed FC that showed a significant change across the first two years of life
519 against later cognitive flexibility.
520

521 **References**

522 1. H. J. Kang, *et al.*, Spatio-temporal transcriptome of the human brain. *Nature* **478**, 483–
523 489 (2011).
524 2. M. M. Black, *et al.*, Early childhood development coming of age: science through the
525 life course. *Lancet* **389**, 77–90 (2017).
526 3. M. S. Goyal, M. E. Raichle, Gene expression-based modeling of human cortical
527 synaptic density. *Proc. Natl. Acad. Sci.* **110**, 6571–6576 (2013).
528 4. C. A. Nelson, *The effects of early adversity on neurobehavioral development*
529 (Psychology Press, 2000).
530 5. N. H. Rod, *et al.*, Trajectories of childhood adversity and mortality in early adulthood:
531 a population-based cohort study. *Lancet* **396**, 489–497 (2020).
532 6. P. Kim, *et al.*, Effects of childhood poverty and chronic stress on emotion regulatory
533 brain function in adulthood. *Proc. Natl. Acad. Sci.* **110**, 18442–18447 (2013).
534 7. C. W. Kuzawa, *et al.*, Metabolic costs and evolutionary implications of human brain
535 development. *Proc. Natl. Acad. Sci.* **111**, 13010–13015 (2014).
536 8. R. A. Thompson, C. A. Nelson, Developmental science and the media: Early brain
537 development. *Am. Psychol.* **56**, 5 (2001).
538 9. K. K. Kesari, R. Handa, R. Prasad, Effect of undernutrition on cognitive development
539 of children. *Int. J. Food, Nutr. Public Heal.* **3**, 133–148 (2010).
540 10. C. G. Victora, *et al.*, Maternal and child undernutrition: consequences for adult health
541 and human capital. *Lancet* (2008) https:/doi.org/10.1016/S0140-6736(07)61692-4.
542 11. S. M. Grantham-McGregor, C. A. Powell, S. P. Walker, J. H. Himes, Nutritional
543 supplementation, psychosocial stimulation, and mental development of stunted
544 children: the Jamaican Study. *Lancet* **338**, 1–5 (1991).
545 12. S. E. Cusick, M. K. Georgieff, Nutrient supplementation and neurodevelopment:
546 timing is the key. *Arch. Pediatr. Adolesc. Med.* **166**, 481–482 (2012).
547 13. H. McKay, L. Sinisterra, A. McKay, H. Gomez, P. Lloreda, Improving cognitive
548 ability in chronically deprived children. *Science (80-.).* **200**, 270–278 (1978).
549 14. S. E. Moore, Using longitudinal data to understand nutrition and health interactions in
550 rural Gambia. *Ann. Hum. Biol.* **47**, 125–131 (2020).
551 15. H. M. Nabwera, A. J. Fulford, S. E. Moore, A. M. Prentice, Growth faltering in rural
552 Gambian children after four decades of interventions: a retrospective cohort study.
553 *Lancet Glob. Heal.* **5**, e208–e216 (2017).
554 16. D. C. McCoy, *et al.*, Early Childhood Developmental Status in Low- and Middle-
555 Income Countries: National, Regional, and Global Prevalence Estimates Using
556 Predictive Modeling. *PLoS Med.* (2016) https:/doi.org/10.1371/journal.pmed.1002034.
557 17. R. Martorell, The nature of child malnutrition and its long-term implications. *Food*
558 *Nutr. Bull.* **20**, 288–292 (1999).
559 18. J. Hoddinott, J. A. Maluccio, J. R. Behrman, R. Flores, R. Martorell, Effect of a
560 nutrition intervention during early childhood on economic productivity in Guatemalan
561 adults. *Lancet* **371**, 411–416 (2008).
562 19. M. M. Black, "Impact of nutrition on growth, brain, and cognition" in *Recent Research*
563 *in Nutrition and Growth*, (Karger Publishers, 2018), pp. 185–195.
564 20. R. K. Sripada, J. E. Swain, G. W. Evans, R. C. Welsh, I. Liberzon, Childhood poverty
565 and stress reactivity are associated with aberrant functional connectivity in default
566 mode network. *Neuropsychopharmacology* **39**, 2244–2251 (2014).
567 21. B. Biswal, F. Zerrin Yetkin, V. M. Haughton, J. S. Hyde, Functional connectivity in
568 the motor cortex of resting human brain using echo-planar MRI. *Magn. Reson. Med.*
569 **34**, 537–541 (1995).
570 22. W. Gao, *et al.*, Evidence on the emergence of the brain's default network from 2-

week-old to 2-year-old healthy pediatric subjects. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 6790–6795 (2009).

23. W. Gao, *et al.*, Evidence on the emergence of the brain's default network from 2-week-old to 2-year-old healthy pediatric subjects. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 6790–5 (2009).
24. W. Gao, S. Alcauter, J. K. Smith, J. Gilmore, W. Lin, Development of human brain cortical network architecture during infancy. *Brain Struct Funct* **220**, 1173–1186 (2015).
25. P. Fransson, *et al.*, Spontaneous brain activity in the newborn brain during natural sleep-an fMRI study in infants born at full term. *Pediatr. Res.* **66**, 301–305 (2009).
26. P. Fransson, *et al.*, Resting-state networks in the infant brain. *Proc. Natl. Acad. Sci. U. S. A.* **104**, 15531–15536 (2007).
27. D. Asis-Cruz, M. Bouyssi-Kobar, I. Evangelou, G. Vezina, C. Limperopoulos, Functional properties of resting state networks in healthy full-term newborns. *Sci. Rep.* **5**, 1–15 (2015).
28. Smyser, C.D., A. Z. Snyder, J. J. Neil, Functional Connectivity MRI in Infants: Exploration of the Functional Organization of the Developing Brain. *Neuroimage* **1**, 1437–1452 (2011).
29. E. Damaraju, A. Caprihan, J. Lowe, Functional connectvity in the developing brain: A longitudinal study from 4 to 9 months of age. *Neuroimage* **84**, 1–28 (2014).
30. W. Gao, W. Lin, K. Grewen, J. H. Gilmore, Functional connectivity of the infant human brain: Plastic and modifiable. *Neuroscientist* **23**, 169–184 (2017).
31. C. Bulgarelli, *et al.*, The developmental trajectory of fronto-temporoparietal connectivity as a proxy of the default mode network: a longitudinal fNIRS investigation. *Hum. Brain Mapp.* (2020) https:/doi.org/10.1002/hbm.24974.
32. R. F. Betzel, *et al.*, Changes in structural and functional connectivity among resting-state networks across the human lifespan. *Neuroimage* **102**, 345–357 (2014).
33. C. J. Honey, *et al.*, Predicting human resting-state functional connectivity from structural connectivity. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 2035–40 (2009).
34. J. Dubois, *et al.*, The early development of brain white matter: A review of imaging studies in fetuses, newborns and infants. *Neuroscience* **276**, 48–71 (2014).
35. L. He, N. A. Parikh, Aberrant Executive and Frontoparietal Functional Connectivity in Very Preterm Infants With Diffuse White Matter Abnormalities. *Pediatr. Neurol.* **53**, 330–337 (2015).
36. C. D. Smyser, *et al.*, Longitudinal analysis of neural network development in preterm infants. *Cereb. Cortex* **20**, 2852–2862 (2010).
37. S. Khan, *et al.*, Local and long-range functional connectivity is reduced in concert in autism spectrum disorders. *Proc. Natl. Acad. Sci.* **110**, 3107–3112 (2013).
38. D. R. Dajani, L. Q. Uddin, Local brain connectivity across development in autism spectrum disorder: A cross-sectional investigation. *Autism Res.* **9**, 43–54 (2016).
39. F. X. Shen, *et al.*, Emerging ethical issues raised by highly portable MRI research in remote and resource-limited international settings. *Neuroimage* **238**, 118210 (2021).
40. S. K. G. Jensen, *et al.*, Neural correlates of early adversity among Bangladeshi infants. *Sci. Rep.* **9**, 1–10 (2019).
41. S. Lloyd-Fox, *et al.*, Cortical specialisation to social stimuli from the first days to the second year of life: A rural Gambian cohort. *Dev. Cogn. Neurosci.* **25**, 92–104 (2017).
42. A. Blasi, S. Lloyd-Fox, L. Katus, C. E. Elwell, fNIRS for tracking brain development in the context of global health projects in *Photonics*, (MDPI, 2019), p. 89.
43. S. Lloyd-Fox, *et al.*, Habituation and novelty detection fNIRS brain responses in 5-and 8-month-old infants: The Gambia and UK. *Dev. Sci.* **22**, e12817 (2019).

44. S. B. Roberts, *et al.*, A pilot randomized controlled trial of a new supplementary food designed to enhance cognitive performance during prevention and treatment of malnutrition in childhood. *Curr. Dev. Nutr.* **1**, e000885 (2017).
45. K. K. Jasińska, S. Guei, Neuroimaging field methods using functional near infrared spectroscopy (NIRS) neuroimaging to study global child development: Rural sub-Saharan Africa. *J. Vis. Exp.* **2018**, 1–11 (2018).
46. W. Xie, *et al.*, Growth faltering is associated with altered brain functional connectivity and cognitive outcomes in urban Bangladeshi children exposed to early adversity. *BMC Med.* **17**, 199 (2019).
47. A. T. Eggebrecht, *et al.*, Mapping distributed brain function and networks with diffuse optical tomography. *Nat. Photonics* **8**, 448–454 (2014).
48. S. L. Ferradal, *et al.*, Functional imaging of the developing brain at the bedside using diffuse optical tomography. *Cereb. cortex* **26**, 1558–1568 (2016).
49. A. Mitra, *et al.*, Resting-state fMRI in sleeping infants more closely resembles adult sleep than adult wakefulness. *PLoS One*, 1–19 (2017).
50. A. Blasi, S. Lloyd-fox, L. Katus, C. E. Elwell, fNIRS for Tracking Brain Development in the Context of Global Health Projects. *Photonics* **6**, 1–10 (2019).
51. D. R. Dajani, L. Q. Uddin, Demystifying cognitive flexibility: Implications for clinical and developmental neuroscience. *Trends Neurosci.* **38**, 571–578 (2015).
52. S. L. Ferradal, *et al.*, Functional Imaging of the Developing Brain at the Bedside Using Diffuse Optical Tomography. *Cereb. cortex*, 1–11 (2015).
53. E. Luders, P. M. Thompson, A. W. Toga, The development of the corpus callosum in the healthy human brain. *J. Neurosci.* **30**, 10985–10990 (2010).
54. J. S. Anderson, *et al.*, Decreased interhemispheric functional connectivity in autism. *Cereb. cortex* **21**, 1134–1146 (2011).
55. M. D. Fox, *et al.*, The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc. Natl. Acad. Sci. U. S. A.* **102**, 9673–8 (2005).
56. D. A. Fair, *et al.*, A method for using blocked and event-related fMRI data to study "resting state" functional connectivity. *Neuroimage* **35**, 396–405 (2007).
57. T. Talukdar, M. K. Zamroziewicz, C. E. Zwilling, A. K. Barbey, Nutrient biomarkers shape individual differences in functional brain connectivity: Evidence from omega-3 PUFAs. *Hum. Brain Mapp.* **40**, 1887–1897 (2019).
58. C. Algarin, *et al.*, Differences on brain connectivity in adulthood are present in subjects with iron deficiency anemia in infancy. *Front. Aging Neurosci.* **9**, 54 (2017).
59. M. K. Zamroziewicz, M. T. Talukdar, C. E. Zwilling, A. K. Barbey, Nutritional status, brain network organization, and general intelligence. *Neuroimage* **161**, 241–250 (2017).
60. P. G. Grieve, *et al.*, EEG functional connectivity in term age extremely low birth weight infants. *Clin. Neurophysiol.* **119**, 2712–2720 (2008).
61. N. Padilla, *et al.*, Intrinsic functional connectivity in preterm infants with fetal growth restriction evaluated at 12 months corrected age. *Cereb. Cortex* **27**, 4750–4758 (2017).
62. E. G. Duerden, *et al.*, Early protein intake predicts functional connectivity and neurocognition in preterm born children. *Sci. Rep.* **11**, 1–8 (2021).
63. R. Case, The role of the frontal lobes in the regulation of cognitive development. *Brain Cogn.* **20**, 51–73 (1992).
64. L. J. Hearne, J. B. Mattingley, L. Cocchi, Functional brain networks related to individual differences in human intelligence at rest. *Sci. Rep.* **6**, 1–8 (2016).
65. L. Q. Uddin, Cognitive and behavioural flexibility: neural mechanisms and clinical considerations. *Nat. Rev. Neurosci.* **22**, 167–179 (2021).
66. B. R. White, *et al.*, Resting-state functional connectivity in the human brain revealed

with diffuse optical tomography. *Neuroimage* **47**, 148–156 (2009).
67. C. Bulgarelli, *et al.*, Standardising an infant fNIRS analysis pipeline to investigate neurodevelopment in global health. *Biophotonics Congr. Biomed. Opt. 2020 (Translational, Microsc. OCT, OTS, BRAIN)* (2020).
68. L. H. Collins-Jones, *et al.*, Longitudinal infant fNIRS channel-space analyses are robust to variability parameters at the group-level: An image reconstruction investigation. *Neuroimage* **237**, 118068 (2021).
69. L. Katus, *et al.*, Longitudinal fNIRS and EEG metrics of habituation and novelty detection are correlated in 1–18-month-old infants. *Neuroimage* **274**, 120153 (2023).
70. S. Lloyd-Fox, *et al.*, Habituation and novelty detection fNIRS brain responses in 5- and 8-month-old infants: The Gambia and UK. *Dev. Sci.*, e12817 (2019).
71. S. Baek, *et al.*, Attrition rate in infant fNIRS research: A meta-analysis. *Infancy* **28**, 507–531 (2023).
72. N. L. Everdell, *et al.*, A frequency multiplexed near-infrared topography system for imaging functional activation in the brain. *Rev. Sci. Instrum.* **76** (2005).
73. S. Lloyd-Fox, *et al.*, Coregistering functional near-infrared spectroscopy with underlying cortical areas in infants. *Neurophotonics* **1**, 025006 (2014).
74. I. Tachtsidis, F. Scholkmann, False positives and false negatives in functional NIRS: 7 issues, challenges and the way forward. *J. Biomed. Opt.*, 1–12 (2015).
75. S. M. Hernandez, L. Pollonini, NIRSplot: A Tool for Quality Assessment of fNIRS Scans. *Biophotonics Congr. Biomed. Opt. 2020 (Translational, Microsc. OCT, OTS, BRAIN)* (2020).
76. S. Montero Hernandez, J. Gervain, L. Pollonini, Data Quality Assessment for Infant fNIRS Data. *Poster Present. fNIRS 2022 Bienn. Meet. Soc. fNIRS, Boston, USA* (2022).
77. T. J. Huppert, S. G. Diamond, M. A. Franceschini, D. A. Boas, HomER: a review of time-series analysis methods for near-infrared spectroscopy of the brain. *Appl. Opt.* **48**, 280–298 (2009).
78. N. M. Gregg, B. R. White, B. W. Zeff, A. J. Berger, J. P. Culver, Brain specificity of diffuse optical imaging: improvements from superficial signal regression and tomography. *Front. Neuroenergetics* **2**, 14 (2010).
79. M. A. Yücel, *et al.*, Best practices for fNIRS publications. *Neurophotonics* **8**, 12101 (2021).
80. K. Murphy, M. D. Fox, Towards a consensus regarding global signal regression for resting state functional connectivity MRI. *Neuroimage* **154**, 169–173 (2017).
81. A. Sherafati, *et al.*, Global Motion Detection and Censoring in High-Density Diffuse Optical Tomography (2020).
82. F. Scholkmann, M. Wolf, General equation for the differential pathlength factor of the frontal human head depending on wavelength and age. *J. Biomed. Opt.* **18**, 105004 (2013).
83. S. J. Howard, E. Melhuish, An early years toolbox for assessing early executive function, language, self-regulation, and social development: Validity, reliability, and preliminary norms. *J. Psychoeduc. Assess.* **35**, 255–275 (2017).
84. B. Milosavljevic, *et al.*, Executive functioning skills and their environmental predictors among pre-school aged children in South Africa and The Gambia. *Dev. Sci.* (2023).
85. S. Lloyd-Fox, *et al.*, The Brain Imaging for Global Health (BRIGHT) Study: Cohort Study Protocol. *Gates Open Res.* **7** (2023).
86. A. L. Oberg, D. W. Mahoney, Linear mixed effects models. *Top. Biostat.*, 213–234 (2007).
87. A. Cnaan, N. M. Laird, P. Slasor, Using the general linear mixed model to analyse

unbalanced repeated measures and longitudinal data. *Stat. Med.* **16**, 2349–2380 (1997).
88. C. Krueger, L. Tian, A comparison of the general linear mixed model and repeated measures ANOVA using a dataset with multiple missing data points. *Biol. Res. Nurs.* **6**, 151–157 (2004).
89. L. Meteyard, R. A. I. Davies, Best practice guidance for linear mixed-effects models in psychological science. *J. Mem. Lang.* **112**, 104092 (2020).
90. N. H. Pusponegoro, K. A. Notodiputro, B. Sartono, Linear mixed model for analyzing longitudinal data: A simulation study of children growth differences. *Procedia Comput. Sci.* **116**, 284–291 (2017).
91. L. M. Wierenga, *et al.*, A multisample study of longitudinal changes in brain network architecture in 4–13-year-old children. *Hum. Brain Mapp.* **39**, 157–170 (2018).
92. J. P. Selig, T. D. Little, Autoregressive and Cross-Lagged Panel Analysis for Longitudinal Data. *Handb. Dev. Res. Methods.* (2012).
93. WHO Multicentre Growth Reference Study Group, *WHO Child Growth Standards: Length/height-for-age, weight-for-age, weight-for- length, weight-for-height, and body mass index-for-age: Methods and development.*, World Health Organization, Ed. (2006).
94. Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. B* **57**, 289–300 (1995).
95. S. Lloyd-Fox, *et al.*, Cortical specialisation to social stimuli from the first days to the second year of life: A rural Gambian cohort. *Dev. Cogn. Neurosci.* **25**, 92–104 (2017).

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Author contribution:
Conceptualization: CB, SEM, SLF, CEE, ATE
Methodology: CB, AB, SMC, BM, SEM, SLF, CEE, ATE
Investigation: SMC, GG, EM, ET, TF, LA
Visualization: CB, ATE
Supervision: SEM, SLF, CEE, ATE
Writing-original draft: CB, ATE
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Competing interests: Chiara Bulgarelli is a research consultant for Gowerlabs Ltd., the company that produces the NTS optical topography system used in this work.

Data and materials availability: Data supporting this paper will be made available subject to established data sharing agreements.

Figures and Tables

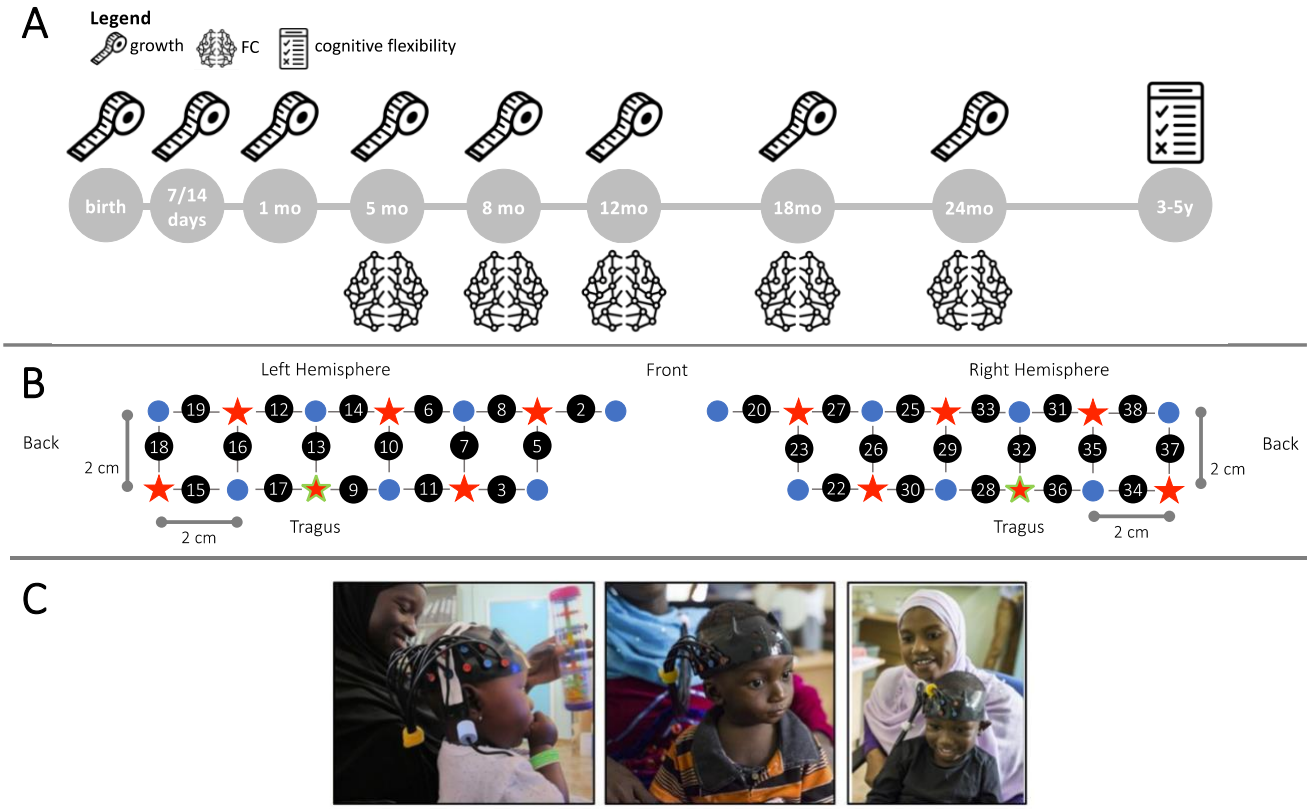

Figure 1. Experimental design. (**A**) Measures taken in the BRIGHT project used in this work. The measuring tape represents anthropometric measures, the brain represents fNIRS FC and the test represents the cognitive flexibility assessment. (**B**) Schematic representation of the spatial layout of the fNIRS array. Sources are marked with red stars, detectors are marked with blue circles, channels are marked with grey lines and numbered with black circles. The channels/optodes used as a reference for the tragus are highlighted in green. (**C**) Participants undergoing fNIRS testing at different ages. Image reproduced with permission from (95).

Table 1. FC that significantly changed with age. Results are displayed in terms of estimated betas, standard errors and p values that survived Bonferroni correction.

FC	*F*	*p*	Baseline (5 months) Betas (SE), *p*	5-8 change Betas (SE), *p*	5-12 change Betas (SE), *p*	5-18 change Betas (SE), *p*	5-24 change Betas (SE), *p*
HbO_2							
Frontal interhemispheric	11.03	<0.001	0.28 (0.03), <0.001	-0.09 (0.06), 0.12	-0.20 (0.05), <0.001	-0.25 (0.05), <0.001	-0.33 (0.05), <0.001
Left fronto-middle	5.8	<0.001	-0.28 (0.04), <0.001	0.06 (0.06), 0.293	0.09 (0.05), 0.102	0.19 (0.05), <0.001	0.23 (0.05), <0.001
Right fronto-middle	4.86	<0.001	-0.70 (0.29), 0.018	0.01 (0.05), 0.982	0.05 (0.05), 0.288	0.13 (0.05), 0.007	0.19 (0.05), <0.001
Right fronto-posterior	5.52	<0.001	-0.20 (0.03), <0.001	0.16 (0.06), 0.005	0.14 (0.05), 0.006	0.20 (0.05), <0.001	0.22 (0.05), <0.001
HHb							
Frontal interhemispheric	6.59	<0.001	0.32 (0.03), <0.001	-0.09 (0.06), 0.107	-0.22 (0.05), <0.001	-0.23 (0.05), <0.001	-0.22 (0.05), <0.001
Cross left fronto-right middle	5.17	<0.001	-0.32 (0.04), <0.001	0.06 (0.06), 0.328	0.07 (0.05), 0.188	0.19 (0.05), <0.001	0.22 (0.05), <0.001
Right fronto-middle	5.55	<0.001	-0.78 (0.32), 0.036	0.01 (0.06), 0.86	0.05 (0.05), 0.301	0.16 (0.05), 0.002	0.21 (0.05), <0.001
Right fronto-posterior	5.38	<0.001	-0.24 (0.04), <0.001	0.10 (0.06) 0.073	0.08 (0.05) 0.133	0.23 (0.05), <0.001	0.02 (0.05), <0.001

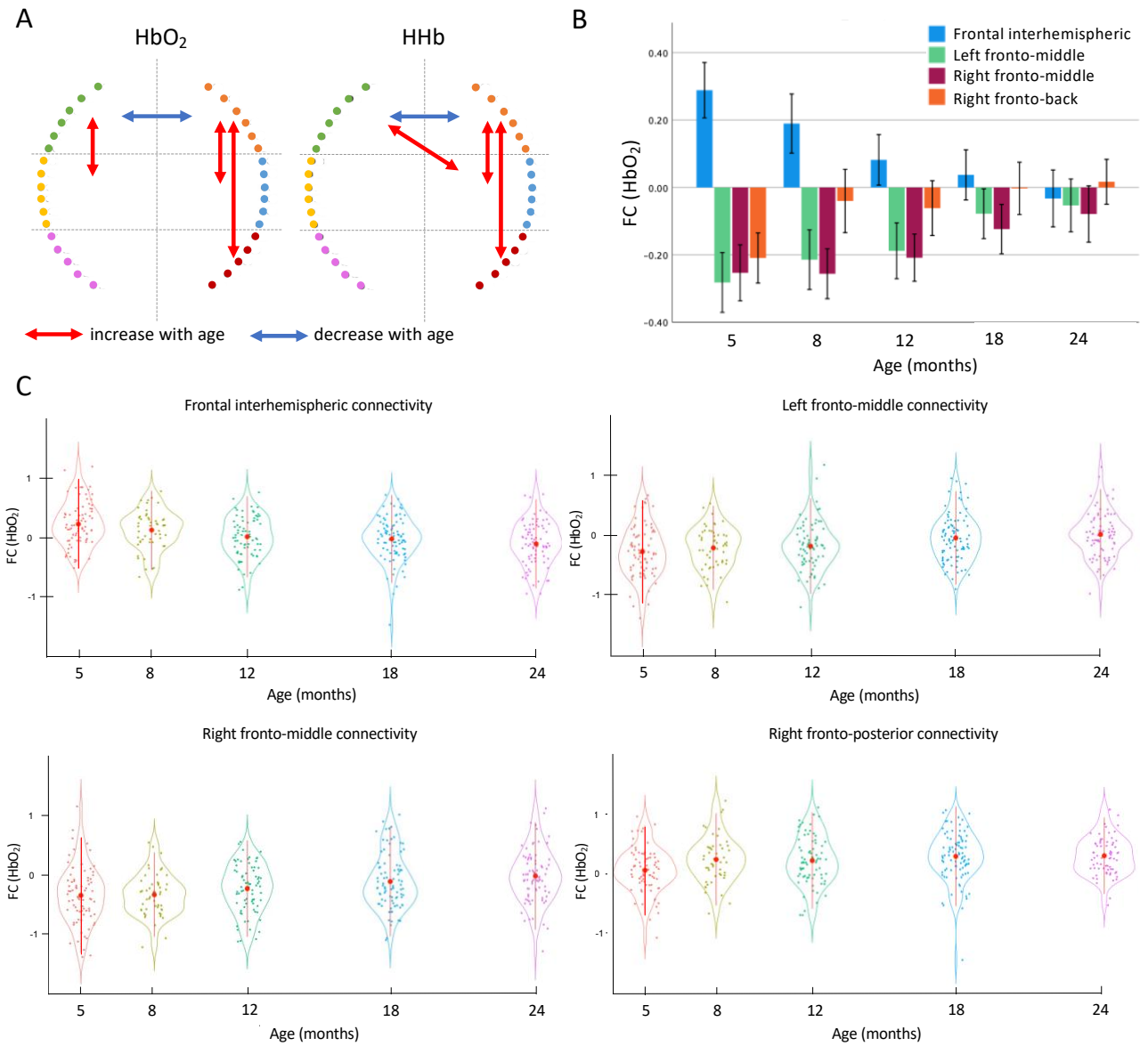

Figure 2. Linear mixed models results showing FC that displayed a statistically significant change with age. (**A**) Significant results of the linear mixed model, blue indicates connections that decreased with age, red indicates connections that increase with age. (**B**) Mean and standard error of the mean (SE) of the functional connections that changed with age. Error bars are 1 SE. (**C**) Violin plot showing the mean ± SD (red circles and lines) and the individual variability (coloured dots) of the FC that showed a change with time.

Table 2. Results of the regression analyses of the effect of ΔWLZ on FC at 24 months. Significant positive associations are in green, significant negative associations are in orange, and non-significant (NS) associations are in blue; * indicates regressions that are still significant after correcting for HCAZ at 7/14 days and ** indicates regressions that are still significant after correcting for neonatal HCAZ and WLZ. Regressions that survived FDR correction for multiple comparisons are in bold.

Frontal interhemispheric FC at 24 mo							
ΔWLZ	**birth**	**1 mo**	**5 mo**	**8 mo**	**12 mo**	**18 mo**	**24 mo**
birth		NS	***F*(1,68)=8.69, p=0.004*, R^2=0.102**	***F*(1,67)=7.65, p=0.007*, R^2=0.104**	***F*(1,69)=10.4, p=0.002**, R^2=0.121**	***F*(1,68)=10.98, p=0.001**, R^2=0.142**	***F*(1,59)=5.40, p=0.024*, R^2=0.085**
1 mo			*F*(1,77)=5.59, p=0.021**, R^2=0.069	NS	*F*(1,78)=4.74, p=0.032**, R^2=0.058	*F*(1,75)=4.45, p=0.038**, R^2=0.057	NS
5 mo				NS	NS	NS	NS
8 mo					NS	NS	NS
12 mo						NS	NS
18 mo							NS
24 mo							

Left fronto-middle FC at 24 mo							
ΔWLZ	**birth**	**1 mo**	**5 mo**	**8 mo**	**12 mo**	**18 mo**	**24 mo**
birth		NS	NS	*F*(1,67)=4.07, p=0.048**, R^2=0.058	NS	NS	*F*(1,59)=4.24, p=0.044**, R^2=0.068
1 mo			NS	NS	NS	NS	NS
5 mo				NS	NS	NS	NS
8 mo					NS	NS	NS
12 mo						NS	NS
18 mo							NS
24 mo							

Right fronto-middle FC at 24 mo							
ΔWLZ	**birth**	**1 mo**	**5 mo**	**8 mo**	**12 mo**	**18 mo**	**24 mo**
birth		NS	NS	*F*(1,67)=4.74, p=0.033, R^2=0.067	NS	*F*(1,68)=5.29, p=0.024**, R^2=0.073	NS
1 mo			*F*(1,77)=4.26, p=0.042**, R^2=0.053	*F*(1,75)=6.66, p=0.012**, R^2=0.083	NS	*F*(1,75)=5.33, p=0.024**, R^2=0.067	NS
5 mo				NS	NS	NS	NS
8 mo					NS	NS	NS
12 mo						NS	NS
18 mo							NS
24 mo							

Right frontal-posterior FC at 24 mo							
ΔWLZ	**birth**	**1 mo**	**5 mo**	**8 mo**	**12 mo**	**18 mo**	**24 mo**
birth		***F*(1,66)=8.26, p=0.006**, R^2=0.113**	NS	NS	NS	NS	NS
1 mo			*F*(1,69)=4.86, p=0.031*, R^2=0.067	***F*(1,68)=6.98, p=0.01**, R^2=0.094**	***F*(1,71)=8.86, p=0.004**, R^2=0.112**	***F*(1,67)=7.72, p=0.007**, R^2=0.105**	***F*(1,57)=7.03, p=0.01**, R^2=0.112**
5 mo				NS	NS	NS	NS
8 mo					NS	NS	NS
12 mo						NS	NS
18 mo							NS
24 mo							

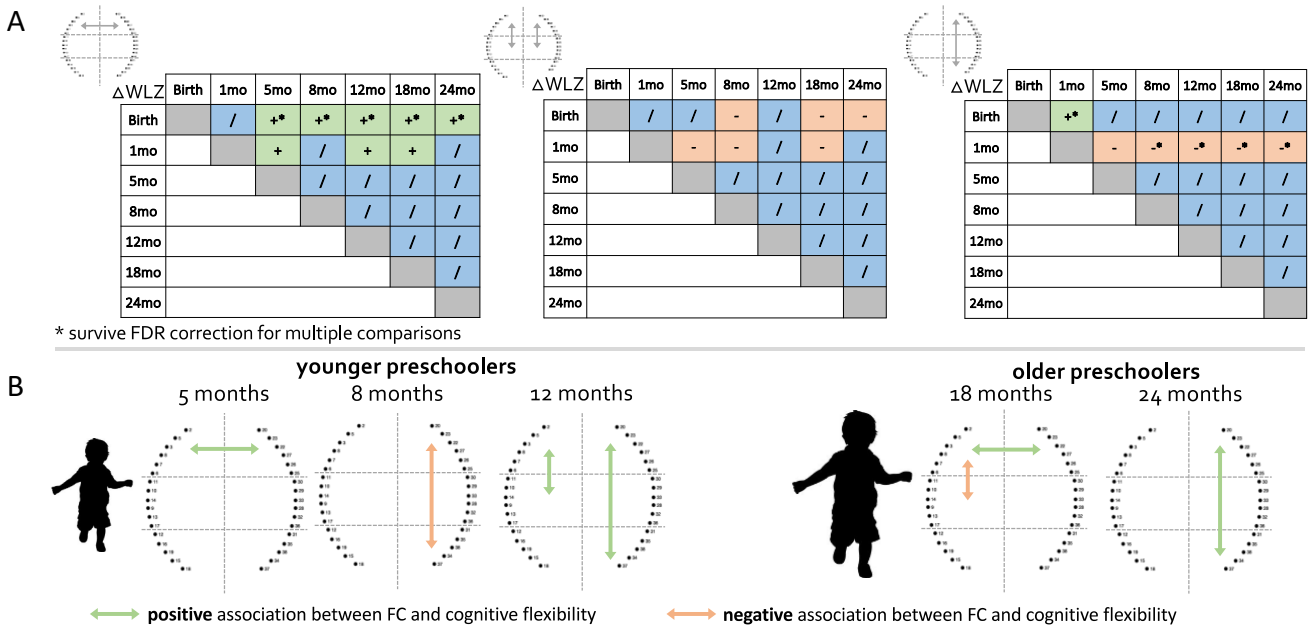

Figure 3. **Associations between FC, early growth and later cognitive flexibility**. (**A**) Significant positive associations are in green, significant negative associations are in orange, and non-significant associations are in blue. * indicates regressions that survived FDR correction for multiple comparisons. (**B**) Schematic representations of the early FC connections shown to predict cognitive flexibility in preschoolers. Significant positive associations are in green, significant negative associations are in orange.

Table 3. Demographic information per each age.

Age	Age in days (mean±SD)	Sex (M, F)	WLZ z-scores (mean±SD)
5 months	158.92±9.91	45, 42	-0.24±0.95
8 months	249.34±17.97	28, 25	-0.24±1.01
12 months	372.92±14.75	42, 40	-0.58±1.05
18 months	560.37±24.38	48, 49	-0.85±0.91
24 months	746.61±23.25	49, 47	-0.44±0.99

Supplementary Materials for

Growth in early infancy drives optimal brain functional connectivity which predicts cognitive flexibility in later childhood

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This PDF file includes:

Supplementary Results:
Linear mixed model results showing the developmental trajectories of functional connectivity in Gambian infants over the first 2 years of life age (fNIRS pre-processing without global signal regression) 2

Supplementary Figures:
Figure SI1 – Schematic representation of the fNIRS array and the functional connections tested 3
Figure SI2 - Linear mixed models result showing FC that displayed a statistically significant change with age (fNIRS pre-processing without global signal regression) 4
Figure SI3 – An example scatterplot of the association between ΔWLZ and FC at 24 months 5
Figure SI4 – fNIRS preprocessing steps 6
Figure SI5 – The effect of different thresholds for GVTD for motion detecion and minium valid data after pre-processing on data inclusion 7
Figure SI 6 – Strength of correlation between FC in the first and last portion of data 8

Supplementary Tables:
Table SI1 - FC that significantly changed with age (fNIRS pre-processing without global signal regression) 9
Table SI2 - Characteristics of included and excluded participants and seconds of data included in the analyses at each age 10

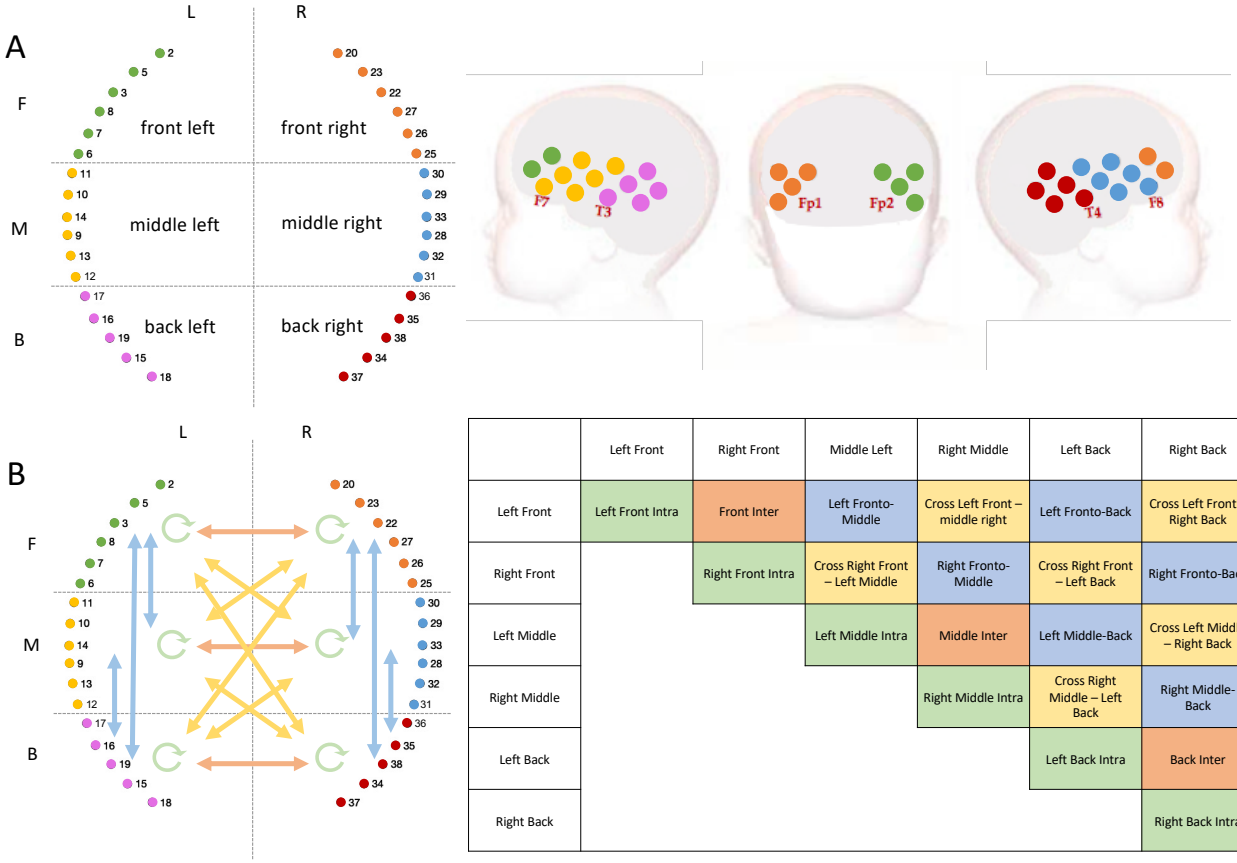
Supplementary Results

Linear mixed model results showing the developmental trajectories of functional connectivity in Gambian infants over the first 2 years of life age (fNIRS pre-processing without global signal regression)

To rule out that the global signal regression (GSR) performed as part of our fNIRS preprocessing might had an impact on our results, we re-processed the data without this step. All the other steps were kept the same. Hereafter, we reperformed the linear mixed models (LMM) on all the possible 21 interhemispheric, intrahemispheric, fronto-posterior, and crossed connections.

Results on the Fisher-z transformed correlation coefficients (z-RHO scores) on the oxygenated haemoglobin (HbO_2) showed that left ($F=10.4$, $p<0.001$) and right fronto-middle ($F=4.82$, $p<0.001$) FC increased with age. Results on the Fisher-z transformed correlation coefficients (z-RHO scores) on the deoxygenated haemoglobin (HHb) showed that showed that frontal interhemispheric FC decreased with age ($F=3.5$, $p<0.002$) (**Table SI1** and **Figure SI2**).

Supplementary Figures



	Left Front	Right Front	Middle Left	Right Middle	Left Back	Right Back
Left Front	Left Front Intra	Front Inter	Left Fronto-Middle	Cross Left Front – middle right	Left Fronto-Back	Cross Left Front – Right Back
Right Front		Right Front Intra	Cross Right Front – Left Middle	Right Fronto-Middle	Cross Right Front – Left Back	Right Fronto-Back
Left Middle			Left Middle Intra	Middle Inter	Left Middle-Back	Cross Left Middle – Right Back
Right Middle				Right Middle Intra	Cross Right Middle – Left Back	Right Middle-Back
Left Back					Left Back Intra	Back Inter
Right Back						Right Back Intra

Figure SI1. Schematic representation of the fNIRS array and the functional connections tested. (**A**) Each dot represents a channel, colours on the left plot corresponds to colours on the right on the baby's head. 6 sections. (**B**) The 21 connections tested in the linear mixed models. Interhemispheric connections are in orange, intrahemispheric connections are in green, fronto-posterior are in blue, and crossing interhemispheric connections are in yellow.

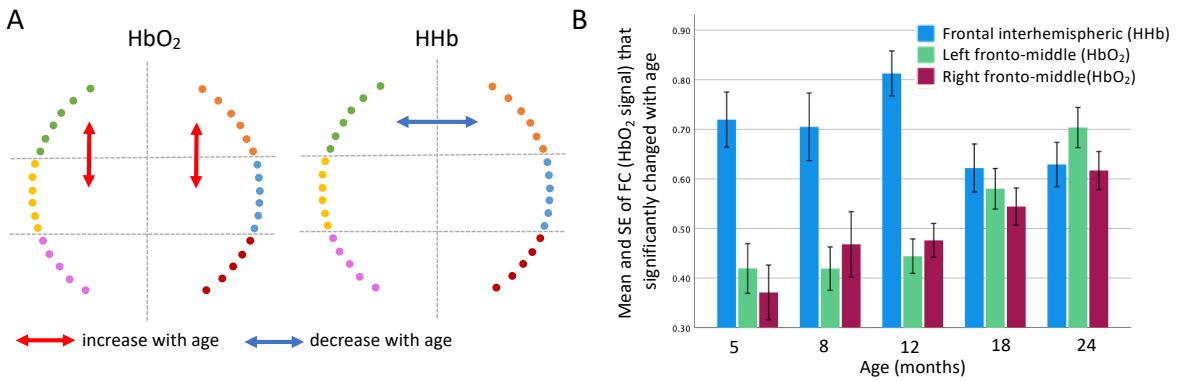


Figure SI2. Linear mixed models result showing FC that displayed a statistically significant change with age. (**A**) Results of the linear mixed model, blue indicates connections that decreased with age, red indicates connections that increase with age. (**B**) Mean and SE of the functional connections that changed with age. Error bars are 1 SE.

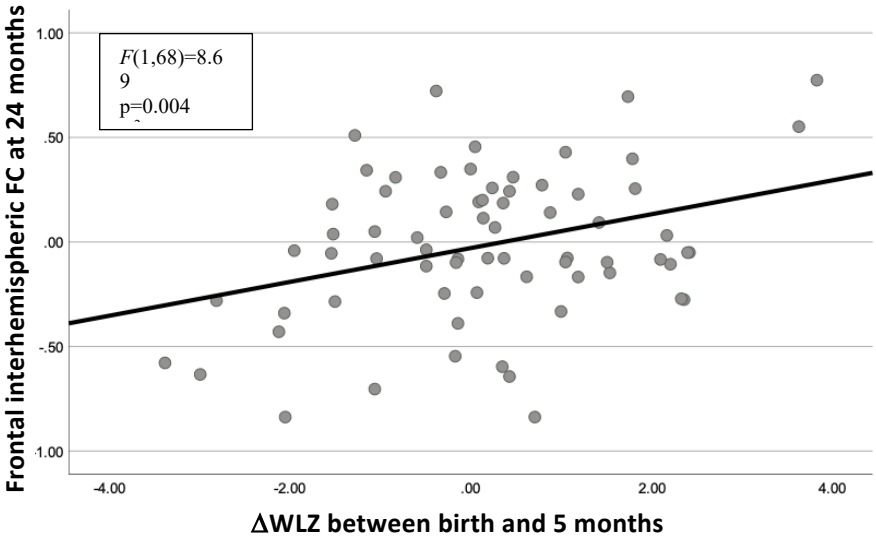


Figure SI3. An example scatterplot of the association between ΔWLZ and FC at 24 months. Scatterplot of the association between ΔWLZ between birth and 5 months and frontal interhemispheric connectivity at 24 months. The black line represents the line of best fit.

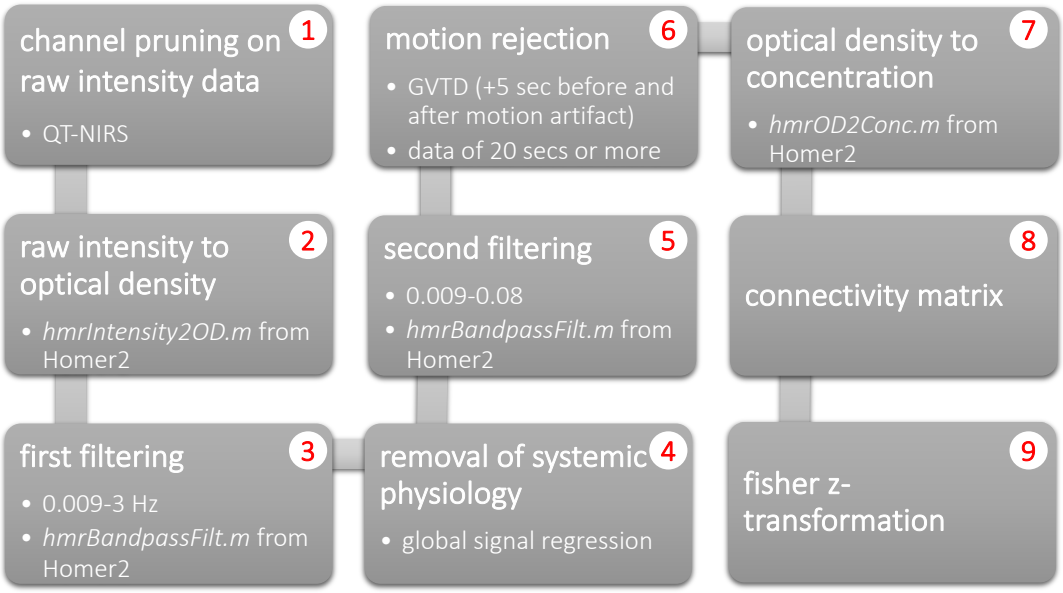


Figure SI4. fNIRS preprocessing steps. The column "infants included in the analyses" in Table 3 refers to those participants whose data survived these preprocessing steps.

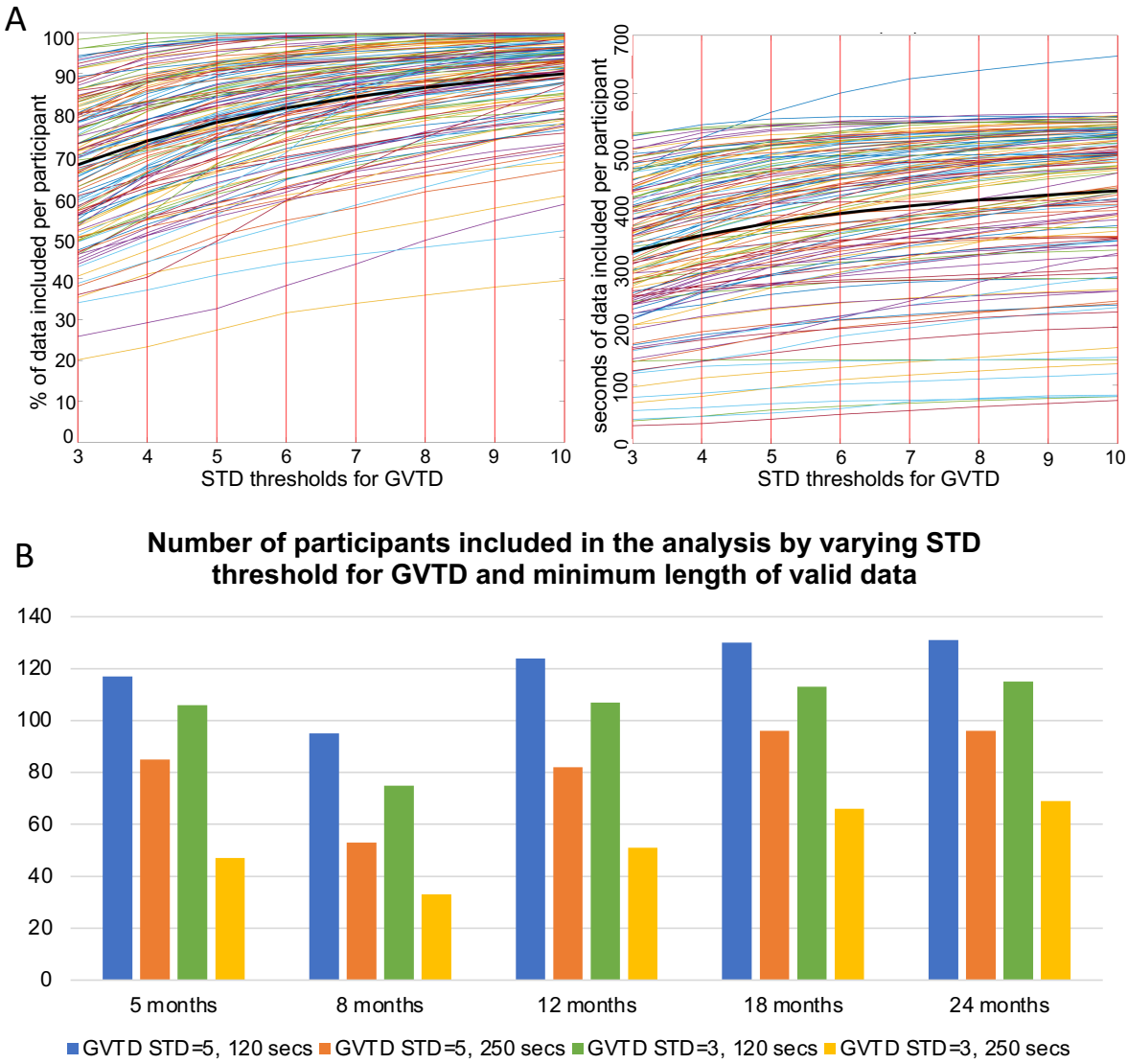


Figure SI5. **The effect of different thresholds for GVTD for motion detection and minimum valid data after pre-processing on data inclusion.** (A) Percentage of data included (left) and seconds of data included (right) per participant. Each line represents an infant, the black line represents the mean value. These graphs are reported from the 12 months sample as example. (B) Number of infants included in the LMM by varying STD threshold for GVTD and minimum length of valid data at different ages.

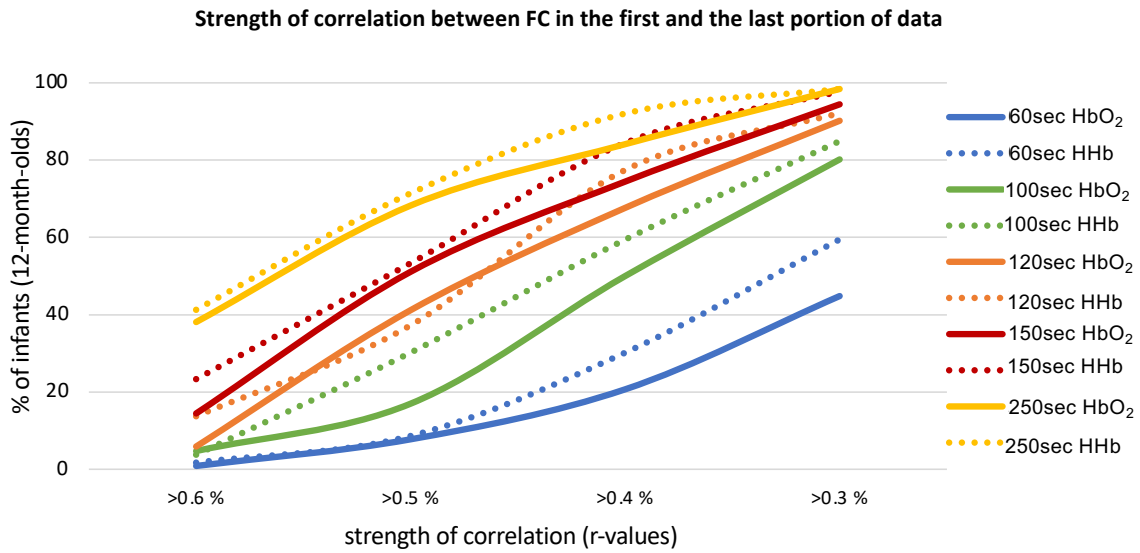


Figure SI6. **Strength of correlation between FC in the first and last portion of data**. This graph is reported from the age 12 month sample as example.

Supplementary Tables

Table SI1. FC that significantly changed with age (fNIRS pre-processing without global signal regression). Results are displayed in terms of estimated betas, standard errors and p values that survived Bonferroni correction.

FC	*F*	*p*	Baseline (5 months) Betas (SE), *p*	5-8 change Betas (SE), *p*	5-12 change Betas (SE), *p*	5-18 change Betas (SE), *p*	5-24 change Betas (SE), *p*
HbO_2							
Left fronto-middle	10.4	<0.001	-0.55 (0.29), <0.063	0.05 (0.05), 0.239	0.07 (0.04), 0.125	0.16 (0.04), <0.001	0.26 (0.04), <0.001
Right fronto-middle	4.82	<0.001	-0.24 (0.28), 0.398	0.03 (0.05), 0.535	0.05 (0.04), 0.246	0.08 (0.04), 0.051	0.18 (0.04), <0.001
HHb							
Frontal interhemispheric	3.5	0.002	0.18 (0.33) 0.591	-0.07 (0.06), 0.260	-0.07 (0.05), 0.214	-0.17 (0.05), 0.002	-0.17 (0.05), 0.002

Table SI2. Characteristics of included and excluded participants and seconds of data included in the analyses at each age. WD=withdrawn, D=deceased, MV=missed visit, DD=developmental delay, NIRS not undertaken = the participant was assessed but did want or could not perform the NIRS assessments, FC not undertaken = the participant was assessed with other NIRS task, but not FC, Fussed out = the participant wore the headband and the FC acquisition had started but the participant showed signs of fussiness soon after the start of the acquisition, MP=missing pictures of the headband placement, EM=missing event markers, TI=technical issues during the NIRS testing session.

Age	N - %	Not tested				NIRS not undertaken	FC not undertaken	**Infants with FC data**	Fussed out	Experimental errors			Headband Placement	Too many channels excluded	Not enough data after pre-processing	**Infants included in the analyses**	**Seconds of data included in the analyses (mean±SD)**
		WD	D	MV	DD					MP	EM	TI					
5 months	N	2	1	2	3	10	7	**179**	16	11	3	3	7	5	47	**87**	382.68±92.78
	%	0.98	0.49	0.98	1.47	4.90	3.43	**87.74**	8.93	5.39	1.67	1.67	5.58	2.79	26.25	**48.6**	
8 months	N	7	1	5	3	18	14	**156**	7	6	0	1	23	6	60	**53**	372.93±80.66
	%	3.43	0.49	2.45	1.47	8.82	6.86	**76.47**	4.48	3.84	0	0.64	14.7	3.84	38.46	**33.97**	
12 months	N	9	1	4	3	17	13	**157**	4	1	0	2	10	2	56	**82**	372.93±80.66
	%	4.41	0.49	1.96	1.47	8.33	6.37	**76.96**	2.54	0.63	0	1.27	6.36	1.27	35.66	**52.22**	
18 months	N	8	1	15	3	12	5	**160**	4	0	0	1	16	4	38	**97**	388.85±81.63
	%	3.92	0.49	7.35	1.47	5.88	2.45	**78.43**	2.5	0	0	0.62	10	2.5	23.75	**60.62**	
24 months	N	13	1	29	1	3	4	**153**	0	2	0	0	6	4	45	**96**	399.56±79.86
	%	6.37	0.49	14.2	0.49	1.47	1.96	**75**	0	1.30	0	0	3.92	2.61	29.41	**62.74**	