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1 **Early life factors and hippocampal functional connectivity in children with overweight/obesity**

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29 Running title: Early life factors and hippocampus in obesity.

31 **Summary**

32 **Objective:** We investigated association of anthropometric neonatal data (birth length and birth
33 weight) and breastfeeding practices (exclusive and any breastfeeding) with hippocampal
34 functional connectivity and its academic implication in children with overweight/obesity.

1 **Methods:** 96 children with overweight/obesity aged 8-11 years (10.01 ± 1.14) from the
2 ActiveBrains project were included in this cross-sectional study. Anthropometric neonatal data
3 were collected from birth records, whereas breastfeeding practices were reported by parents. A
4 3.0 Tesla Siemens Magnetom Tim Trio system was used to acquire T1-weighted and resting-
5 state functional magnetic resonance images. Academic performance was assessed by the
6 Woodcock-Muñoz standardized test. Hippocampal seed-based methods with post-hoc
7 regression analyses were performed. Analyses were considered significant when surpassing
8 Family-Wise Error corrections.

9 **Results:** Birth weight showed a positive association with the connectivity between the
10 hippocampus and the pre- and postcentral gyri, and the cerebellum. In addition, breastfeeding
11 was negatively associated with the connectivity between the hippocampus and the primary
12 motor cortex and the angular gyrus. Any breastfeeding in turn, showed a positive association
13 with the connectivity between the hippocampus and the middle temporal gyrus. None of the
14 connectivity outcomes related to early life factors was coupled with better academic abilities
15 (all $p > 0.05$).

16 **Conclusions:** Our findings suggest that birth weight at birth and breastfeeding are associated
17 with hippocampal connectivity in children with overweight/obesity. Despite this, how the
18 results relate of academic performance remain a matter of speculation. Our findings suggest
19 that clinicians should recognize the importance early life factors for potentially avoiding
20 consequences on offspring's brain development.

21

22 **Keywords:** birth weight; brain; breastfeeding; hippocampus; cognition, obesity.

1 1. INTRODUCTION

2 Environmental factors present in early life are particularly important for brain development ¹.
3 Early life factors such as anthropometric neonatal data (birth length and birth weight) and
4 breastfeeding practices (exclusive and any breastfeeding) might have long lasting
5 consequences in several key brain regions that support higher-level cognitive processes (i.e.,
6 planning, problem solving) later in life ²⁻⁴. Specifically, the hippocampus undergoes protracted
7 functional and structural development after birth, which likely influences everyday activities,
8 memory and learning, and in turn academic performance during childhood ^{1,5-7}.

9 There is extensive evidence of associations between early life factors and morphologic changes
10 of the hippocampus later in childhood ⁵. For example, low birth weight has been associated
11 with smaller hippocampal shape and volume in school-aged children ^{8,9}. Along these lines,
12 Anne et al. found that children that were born preterm and with very low birth weight had
13 smaller hippocampal subfield volumes than age-matched term-born controls at 9 years ¹⁰.
14 Interestingly, breastfed children showed larger hippocampal volumes at term equivalent age,
15 but this association was not evident at 7 years of age ¹¹. In contrast, recent evidence showed
16 that length of exclusive breastfeeding was associated with hippocampus volume, but was not
17 related to satiety or weight status ¹². To our knowledge, only one previous study was designed
18 to investigate the association of a single early life factor, birth weight, and the functioning of
19 limbic areas such as the hippocampus. This study demonstrated that birth weight was related
20 to more efficient limbic communication (meaning that information flow is maximized between
21 regions within a network) at rest in adolescents ¹³. Thus, in contrast to the extensive literature
22 on the relationship between early life factors and hippocampal structure ^{5,8-10,14}, few previous
23 studies have examined the relationship of early life factors with the function of the
24 hippocampus in children.

1 There is evidence, however, that resting state functional connectivity (rsFC) of other regions,
2 apart from the hippocampus, is sensitive to early life environment. For example, alterations in
3 rsFC are evident at birth in networks related to motor function, language, and executive
4 function ¹⁵. Previous studies on early life factors and rsFC have mostly focused on the
5 amygdala, because of its involvement in affective and cognitive processes later in life ^{16,17}.
6 However, the influence of early life factors on rsFC of the hippocampus is still poorly
7 understood ⁵. This represents an important gap in our understanding of how early life
8 environment relates to brain function because rsFC of the hippocampus has been related to
9 different health states in childhood and adulthood, including adolescent depression ¹⁸ and
10 schizophrenia in young adults ^{19,20} among others ¹⁹⁻²². In addition, hippocampal functional
11 connectivity may relate to several behavioral changes ²³, such as memory deficits ²⁴,
12 mathematical difficulties and nonverbal learning disabilities ²⁵, among others ^{26,27}, which may
13 have implications for academic performance during childhood. Further, the hippocampus is a
14 key region implicated in the regulation of food intake by detecting learned signals ²⁸, being
15 specifically important in a population with overweight/obesity, especially in youth when the
16 hippocampus is still in developments ²⁹.

17 Therefore, this background highlights the importance of examining early life development of
18 hippocampal rsFC and its academic implications in children, and particularly in children with
19 overweight/obesity, who show altered hippocampal connectivity and worse executive function
20 and academic performance ³⁰⁻³⁵. In the present study, we investigated the associations of early
21 life factors such as anthropometric neonatal data (i.e., birth length and birth weight) and
22 breastfeeding practices (i.e., exclusive and any breastfeeding) with hippocampal rsFC, and ii)
23 tested whether connectivity related to early life factors is associated with long-term academic
24 performance in children with overweight/obesity.

1 **2. METHODS**

2 **Participants**

3 We included 96 children with overweight/obesity (categorized based on World Obesity
4 Federation cut-off points, which consist on an extrapolation to younger ages of the established
5 cut-points in adults, BMI 25 and 30) ^{36,37} aged 8-11 years from the ActiveBrains project ³⁸
6 (www.profitth.ugr.es/activebrains) with completed and valid measures of early life factors,
7 brain connectivity and academic performance variables. Briefly, inclusion criteria were (a) no
8 physical disabilities or neurological disorders; (b) absence of menstruation at the time of the
9 baseline assessment, (c) right-handedness, and (d) no present Attention-Deficit Hyperactivity
10 Disorder. This study is based on a cross-sectional analysis of baseline data prior to
11 randomization to an exercise intervention. Parents or legal guardians were informed of the
12 purpose of the ActiveBrains study and written informed consents were obtained. The project
13 was approved by the Human Research Ethics Committee of the University of Granada, and
14 was registered in ClinicalTrials.gov (identifier: NCT02295072).

15 **Measures**

16 **Early life factors**

17 Weight (kg) and length (cm) at birth were collected from health records (i.e., physical medical
18 record that parents had with the offspring's perinatal information). In addition, parents were
19 asked the following questions: *(i) for how long (months) did the child receive only breast milk*
20 *(neither formula or other liquid or solid)?*, as an indicator of exclusive breastfeeding, and *(ii)*
21 *for how long (months) did the child receive any breast milk (combined with other liquid,*
22 *formula, or solid)?*, as an indicator of any breastfeeding ³⁹. All measure were used as
23 continuous variables.

1 **Academic performance**

2 The version III of the Woodcock-Muñoz Tests of Achievement was used as a measure of
3 academic performance (i.e., Spanish version of the Woodcock-Johnson III) ⁴⁰. A trained
4 member of the research staff administered the test for each child. The full administration time
5 was between 100 to 120 min. We included standard score indicators of reading, writing
6 mathematics and total achievement in the present analysis ⁴¹.

7 **Covariates**

8 Gestational age (weeks) was collected from health records; peak height velocity (PHV) was
9 used as an indicator of pubertal maturity status in childhood and was obtained through the
10 Moore et al. equation ⁴²; PHV offset was computed by the difference between PHV and
11 chronological age. Parents reported their maximum completed level of education and answers
12 were categorized as: none of the parents had a university degree, one of the parents had a
13 university degree or both parents had a university degree, based on our previous studies ^{4,39}.
14 Children's cardiorespiratory fitness (CRF) was estimated through the 20-meter shuttle-run test
15 and maximal oxygen consumption (VO₂max, mL/kg/min) was calculated using the Lèger
16 equation⁴³.

17

18 **Resting state functional MRI (rsfMRI)**

19 *MRI data acquisition and preprocessing*

20 Images were collected using a 3.0 Tesla Siemens Magnetom Tim Trio system (Siemens
21 Medical Solutions, Erlangen, Germany) with a 32-channel head coil. The complete procedure
22 was published in a previous work of the present sample ⁴⁴. High-resolution T1-weighted images

1 were acquired using a 3D magnetization-prepared rapid gradient-echo (MPRAGE) protocol.
2 The parameters were as follows: repetition time (TR) = 2300 ms, echo time (TE) = 3.1 ms,
3 inversion time (TI) = 900 ms, flip angle = 9°, field of view (FOV) = 256 x 256, acquisition
4 matrix= 320 x 320, 208 slices, resolution = 0.8 x 0.8 x 0.8 mm, and scan duration of 6 min and
5 34 s. The rsfMRI was composed of a series of 160 scans acquired using a Gradient Echo Pulse
6 Sequence while participants rested with eyes closed. The parameters were as follows: TR =
7 1000 ms, TE = 25 ms, flip angle = 80°, FOV = 240 mm, acquisition matrix= 240 x 240, 35
8 slices, resolution = 3.5 x 3.5 x 3.5 mm, and scan duration of 5 min and 25 s.

9 Preprocessing steps were carried out in FMRIB's Software Library (FSL) version 5.0.7. The
10 following steps were applied: (i) skull-stripping using brain extraction tool (BET), (ii) spatial
11 normalization of the MPRAGE structural image to Montreal Neurological Institute (MNI)
12 space, (iii) alignment of all rsfMRI frames to correct for head motion during the scan, (iv) co-
13 registration to each participant's MPRAGE structural image and spatial normalization to MNI
14 space, (v) the rsfMRI time courses were then band-pass filtered (0.1–0.01 Hz) to attenuate
15 respiration and other physiological noise and to focus on signal frequencies associated with
16 intrinsic connectivity, (vi) six affine transformation parameters from the alignment process, as
17 well as the mean time courses from the brain parenchyma including white matter tissue and
18 ventricles were included as covariates to further account for motion and physiological noise.
19 We visually checked each individual image for acquisition artifacts, and one child was
20 excluded due to visual image corruption.

21 *Seed creation and resting-state functional connectivity analyses*

22

23 FMRIB's Integrated Registration and Segmentation Tool (FIRST) in FSL 5.0.7 was used for
24 seed creation. FIRST is a semi-automated model based subcortical segmentation tool that use

1 a Bayesian framework from shape and appearance models obtained from manually segmented
2 images from the Center for Morphometric Analysis, Massachusetts General Hospital, Boston,
3 MA, USA ³³. Briefly, two-stage affine registration is run to register the MPRAGE to standard
4 MNI space using 12 degrees of freedom with 1 mm resolution and uses a subcortical mask to
5 exclude voxels not corresponding (outside) to subcortical regions. Then, subcortical regions,
6 including hippocampus, are separately segmented for each hemisphere. Manual volumetric
7 region labels are parameterized as surface meshes and modeled as a point distribution model.
8 The final segmentations of the hippocampus seeds were visually checked for quality control
9 by one rater, and when there was any questionable segmentation, a second rater checked the
10 images. In addition, after calculating framewise displacement, no further exclusions were
11 performed (all framewise displacement <0.2). In a first-level (single-subject), whole-brain
12 voxel-wise functional connectivity network maps were created for left and right hippocampal
13 seeds, for each participant using the pre-processed rsfMRI data. The residualized parameter
14 estimate rsFC maps were converted to z scores (via Fishers r to z transformation) to achieve
15 normality and were entered into higher level analyses.

16 **Statistical analysis**

17 Descriptive data by sex are presented as mean and standard deviation (SD) for continuous
18 variables as well as number of cases (n) and percentage (%) for categorical variables. In
19 second-level (group) imaging analysis, the residualized parameter estimate rsFC maps were
20 then included in two separate linear regression models to identify regions where connectivity
21 with the right and left hippocampus was explained by each early life factors (birth weight, birth
22 length, months of exclusive or any breastfeeding). Sex, gestational age, PHV, parental
23 educational level and CRF were included as covariates in each of the second-level models. All
24 the variables were mean centered prior to inclusion in the models. Correction for multiple

1 comparisons was performed using FSL's automatic FEAT cluster-based thresholding at an
2 alpha of $p < .05$, which is a method of Family-Wise Error correction based on Gaussian Random
3 Field Theory. To further evaluate the link with academic performance, we performed
4 regression analyses between this variable and the signal from those regions showing a
5 significant association with the early-life factors in the preceding analyses. Signal extraction
6 represents the strength of the functional connectivity between the seeds (left and right
7 hippocampus) and the voxels showing a significant association with early-life factors. A false
8 discovery rate (FDR) was applied using the Benjamini and Hochberg method ($q < .05$).
9 Sensitivity analyses were carried out excluding children born preterm (gestational age < 37
10 weeks, $n=17$). Sensitivity analyses were carried out excluding children born preterm
11 (gestational age < 37 weeks, $n=17$). Descriptive and statistical analysis corresponding to
12 extracted rsFC signal were performed in R (version 3.6.1; R Foundation for Statistical
13 Computing, Vienna, Austria), and statistical significance was set at $p < .05$.

14 **3. RESULTS**

15 **Table 1** shows the descriptive characteristics of the study sample. Higher birth weight was
16 associated with increased rsFC between the left hippocampus and 2 clusters corresponding to
17 the left precentral and postcentral gyri ($k=267$ and $k=382$ respectively; **Table 2, Figure 1.A**).
18 In addition, higher birth weight was associated with increased rsFC between the right
19 hippocampus and 3 clusters corresponding to the left and right postcentral gyri and cerebellum
20 (k ranged from 258 to 421; **Table 2, Figure 1.A**). Birth length was not associated with
21 connectivity of the hippocampus.

22 Longer and exclusive breastfeeding was associated with decreased rsFC of the left
23 hippocampus with the left angular gyri ($k=369$; **Table 2, Figure 1.B**) and of the right
24 hippocampus with the primary motor cortex ($k=250$; **Table 2, Figure 1.B**). Any breastfeeding

1 was not associated with rsFC of the left hippocampus, but was positively associated with rsFC
2 between the right hippocampus and middle temporal gyrus ($k=329$ **Table 2, Figure 1.C**). The
3 results after excluding children born preterm from the analyses did not substantially change
4 (see Table S1).

5 **Post-hoc analyses**

6 **Table 3** shows the association between the early-life factor-related hippocampal rsFC and
7 academic performance. There was a positive association between the connectivity of the right
8 hippocampus with left primary motor cortex and mathematics ($\beta= 0.225$; $p=0.019$), although
9 this association disappeared after FDR correction.

10 **4. DISCUSSION**

11 Our main finding suggests that early life factors were related to hippocampal connectivity in
12 children with overweight/obesity. Specifically, birth weight, but not birth length, was
13 associated with greater hippocampal rsFC. In addition, longer exclusive breastfeeding was
14 associated with diminished hippocampal rsFC, and any breastfeeding was related to greater
15 hippocampal rsFC. However, hippocampal rsFC was not coupled with better academic
16 abilities. The present study expands the knowledge about the influence of early life factors on
17 hippocampal structure by showing that birth weight and breastfeeding relate to hippocampal
18 functional connectivity later in life, but its behavioral implications remain unknown.

19 There are several potential mechanisms for how the targeted early life factors influence rsFC
20 in the hippocampus of school-aged children. At a molecular level, low birth weight has been
21 previously associated with hippocampal gene expression; this is related to neuronal maturation,
22 transcription and apoptosis, which may in turn influence connectivity⁴⁵. Also, previous studies

1 suggested that the hippocampus may be highly vulnerable during fetal environment with
2 functional consequences even at a period of hippocampal growth peak, around 9-11 years^{5,46,47}.

3 The hippocampus is a key region involved in learning and memory as well as in higher order
4 cognitive processes. Previous structural brain studies showed that compared with term-born
5 controls, premature-born babies had reduced hippocampal volume during childhood^{8,9}, and
6 even during adulthood; and this was coupled with cognitive consequences^{48,49}. Functional
7 studies in adults showed that the activation of the hippocampus was associated with BMI and
8 in turn, worse decision making, suggesting that body weight is closely tied to both brain
9 function and cognitive outcomes⁵⁰. In addition, disrupted hippocampal connectivity during
10 adolescence, mainly within the limbic network, was partially explained by birth weight¹³. In
11 the present study, birth weight was mainly associated with greater functional connectivity
12 between the hippocampus and frontal and parietal regions, namely, precentral and postcentral
13 gyri. Both precentral and postcentral gyri are related to sensorimotor and somatosensory
14 systems, and have been recognized as being disrupted in obesity^{51,52}. Specifically, while the
15 precentral gyrus participates in planning and execution, the postcentral gyrus is a key related-
16 obesity region involved in sensory processing and taste processing in children and adolescents
17 with excess weight⁵³. Decreased activation in the precentral and postcentral gyrus has been
18 associated with loneliness⁵¹ and cue reactivity scores⁵⁴, both behaviors that might be affected
19 in obesity during childhood^{55,56}. In addition, we found that birth weight was related to greater
20 rsFC between the right hippocampus and left cerebellum, and we previously showed that both
21 birth weight and birth length were also associated with cerebellar volume³⁹. Further, the
22 cerebellum is functionally heterogeneous, and specifically the association focused on left
23 posterior cerebellar lobule, which is involved mainly in spatial task, emotion and executive
24 function⁵⁷. In this line, the hippocampal-cerebellar functional connectivity has a key role in
25 supporting spatial navigation in different neurological disorders, suggesting a possible clinical

1 implication of reduced hippocampal-cerebellar connectivity ⁵⁸. Collectively, our results
2 support the role of birth weight on functional connectivity between the hippocampus and
3 sensorimotor and motor regions in children with overweight/obesity. However, future studies
4 should examine the influence of anthropometric neonatal indicators (i.e., birth weight/length)
5 on functional connectivity in larger samples using graph- based network analysis of rsfMRI to
6 expand our understanding of early life development of the brain as a complex network.

7 Surprisingly, we found that exclusive breastfeeding was associated with a diminished
8 connectivity between the hippocampus and the angular gyrus, and the primary motor cortex.
9 Greater hippocampal-angular gyrus connectivity has been related to episodic memory, and
10 reduced communication between these regions might lead to reduced ability to creatively think
11 and to imagine an episodic future event in young adults (age range 19 to 26 y) ⁵⁹. However, we
12 did not find any negative academic impact in relation to the lower hippocampal-angular gyrus
13 connectivity in the present childhood sample. Consequently, while these unexpected results
14 might be explained by the possible functional reorganization in response to early life adversity
15 of offspring's, future studies including other variables (e.g., better characterization of early life
16 stress or diet, among others) that help to understand this result are needed ^{60,61}. In addition, any
17 breastfeeding was associated with greater connectivity between hippocampus and middle
18 temporal gyrus. A recent study has shown that connectivity between the hippocampus and
19 middle temporal gyrus is a critical neural basis for novelty and usefulness processing during
20 concept construction, perceptual motor system (including precentral and postcentral gyrus) ⁶²,
21 and linguistic and nonlinguistic semantic-level processes or memory ⁶³. However, we did not
22 find a relationship between hippocampal connectivity and behavior (i.e., academic
23 performance) in this sample of children with overweight/obesity. This lack of association,
24 however, does not preclude the possibility that these networks are implicated in other
25 unmeasured behavioral or cognitive processes.

1 Collectively, we did not find any links with academic achievement indicators of the
2 connectivity previously relate with early life factors. Similarly, a previous study with the
3 present sample on early life factors and gray matter volume also failed to find any link with
4 academic achievement scores ³⁹. Thus, it is possible that in our sample of children with
5 overweight/obesity, the lack of variability in terms of adiposity may mask an association of
6 functional network connectivity with academic achievement ⁶⁴. Further research examining the
7 academic implications of the early life factors-hippocampal rsFC comparing normal-weight
8 children and those with overweight/obesity is needed.

9 The present study expands the existing literature, which has mainly examined morphologic
10 associations of the hippocampus with early life factors, by demonstrating that early life factors
11 such as birth weight and breastfeeding practices also relate to hippocampal connectivity in
12 children with overweight/obesity. However, some limitations of this study should be
13 acknowledged. The retrospective cross-sectional design precludes our ability to draw causal
14 interpretations between the targeted early life factors and rsFC of the hippocampus. In addition,
15 parents self-report of breastfeeding practices did not clarify whether the practices were
16 formula-fed, and parents could have potentially misunderstood the meaning of “exclusiveness”
17 of breastfeeding ⁶⁵, which may explain, in part, the unexpected association of breastfeeding
18 practices. Also, we obtained relatively short resting-state fMRI scans. The reason for this was
19 that children are susceptible to more movement and cannot tolerate long scanning times ⁶⁶.
20 Despite this, similar scan time has shown to be reliable in populations with similar
21 characteristics ⁶⁶. Lastly, we focused on a specific limited age range (i.e., children aged 8-11 y)
22 and population of children with overweight/obesity, and the lack of a normal weight group
23 limit the extrapolation of our findings to other age or weight status groups. In addition, we did
24 not collect information about feeding or hunger status during the MRI session, and different
25 pattern of feeding has may alter brain connectivity ⁶⁷. Additionally, we did not collect

1 information about dietary patterns of mothers, stress or birth complication, which has been
2 associated to hippocampal features of offspring ⁶. The current findings provide new insights
3 and prompt questions for future studies to further understand hippocampal functional
4 connectivity in this population. The study has the strength in that it provides a unique
5 contribution to the literature on early life programming of hippocampal functional connectivity
6 in children with overweight/obesity. Clinical relevance of this findings is to help clinicians to
7 recognize the importance of anthropometric neonatal data and infant feeding for potentially
8 avoiding the negative consequences brain development. Therefore, interventions aiming at
9 improving prenatal health and promoting breastfeeding in infancy may be implications for
10 influencing hippocampal connectivity later in life.

11 In conclusion, our findings suggest that birth weight at birth and breastfeeding practices are
12 associated with hippocampal functional connectivity in children with overweight or obesity at
13 ages 8-11. Future research on the association of these changes with a greater range of
14 behavioral outcomes is needed.

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11 Granada, Spain.

12 **Conflict of interest** Authors declare no conflict of interest.

13

14 **Consent to participate** All participant signed the consent statement prior their inclusion in the
15 study.

16 **Ethical approval** The ActiveBrains project was registered in ClinicalTrials.gov (identifier:
17 NCT02295072) and was approved by the Ethics Committee on Human Research of the
18 University of Granada.

19

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Table 1. Characteristics of study sample.

Table 2. Significant clusters in the association of early life factors associated with hippocampal connectivity in the left and right seeds.

Table 3. Associations of early-life factor-related hippocampal connectivity with academic performance*.

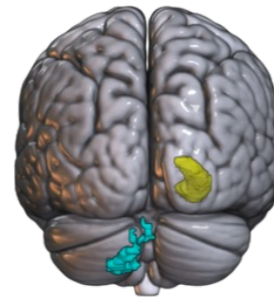
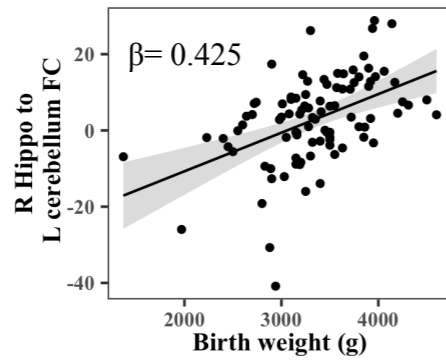
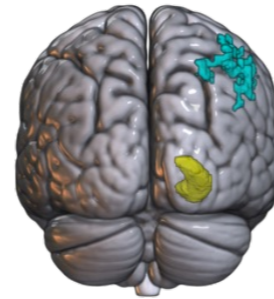
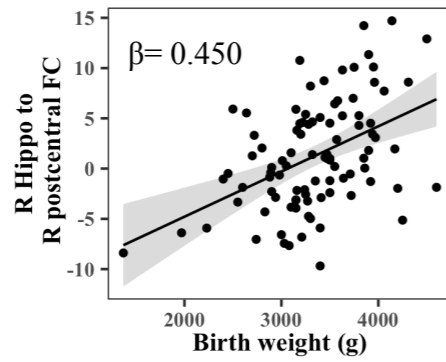
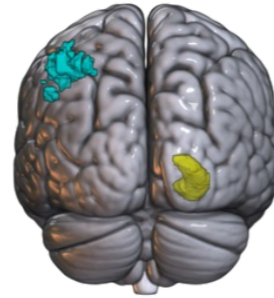
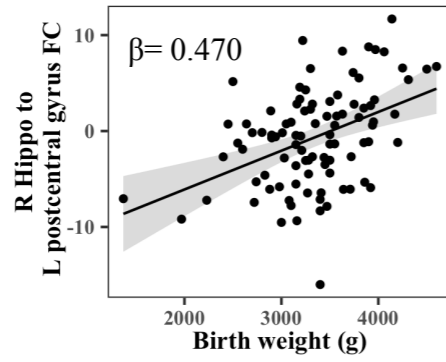
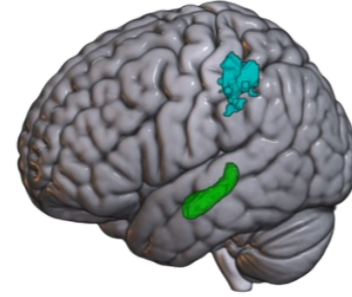
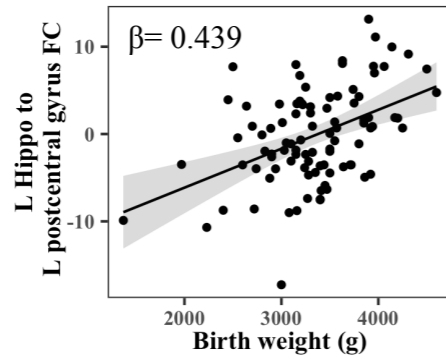
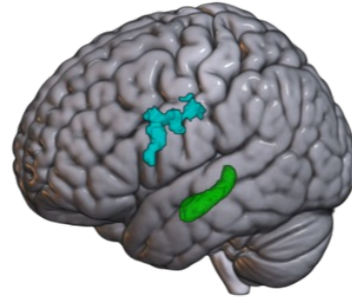
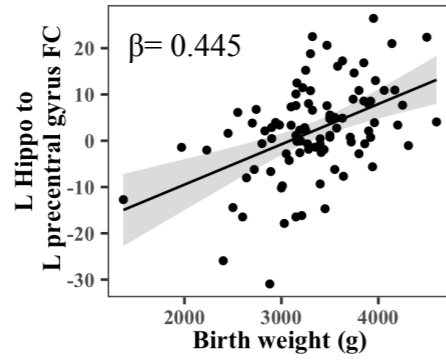
Figure. 1. Clusters showing association between early life factors and left and right hippocampal resting state functional connectivity. The color represent seed for left (green) and right hippocampi (yellow), as well as positive (light blue) and negative (red) association. Figures A shows birth weight cluster associated with left and right hippocampal seed. Figure B and C shows exclusive and any breastfeeding with left and right hippocampal seed, respectively. L: left, R: Right, FC: functional connectivity.

Table 1. Characteristics of study sample.

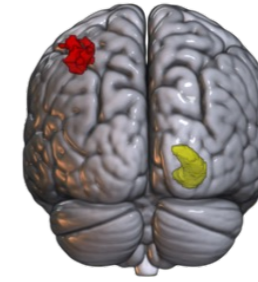
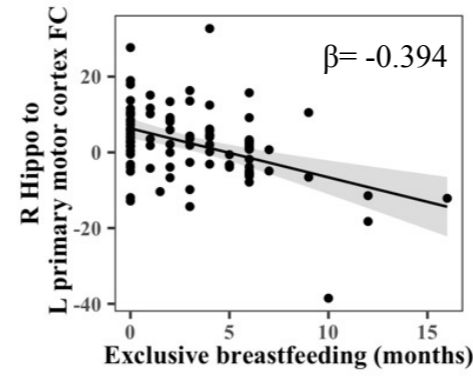
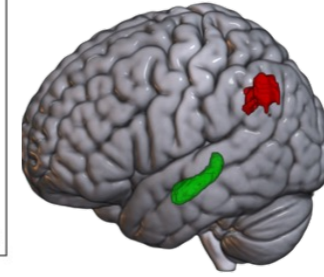
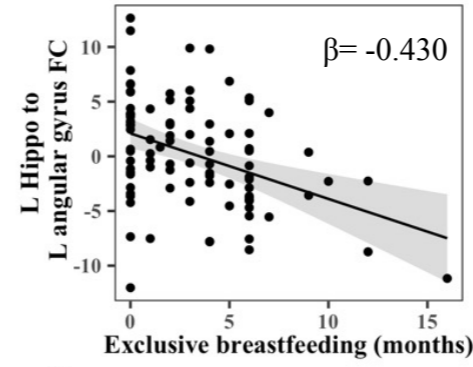
	<i>All</i>	<i>Boys</i>		<i>Girls</i>		
<i>N</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>	
Physical characteristics	96	60		36		
Age (yr)	10.01 ± 1.14	10.16 ± 1.14		9.78 ± 1.13		
Weight (kg)	55.65 ± 11.15	56.66 ± 10.69		53.97 ± 11.85		
Height (cm)	143.81 ± 8.32	144.69 ± 7.37		142.34 ± 9.64		
Peak height velocity offset (yr)	-2.33 ± 0.96	-2.65 ± 0.78		-1.80 ± 1.01		
Cardiorespiratory fitness (mL/kg/min)*	40.86 ± 2.77	40.84 ± 2.77		40.90 ± 2.82		
Body mass index (kg/m ²)	26.70 ± 3.69	26.90 ± 3.79		26.36 ± 3.53		
Body mass index category (n,%)	96	60		36		
Overweight	25 (26.0)	16 (26.7)		9 (25.0)		
Obesity type I	41 (42.7)	27 (45.0)		14 (38.9)		
Obesity type II	30 (31.2)	17 (28.3)		13 (36.1)		
Parental education university level (n,%)	96	60		36		
None of the parents	64 (66.7)	43 (71.7)		21 (58.3)		
One of the two parents	17 (17.7)	10 (16.7)		7 (19.4)		
Both parents	15 (15.6)	7 (11.7)		8 (22.2)		
Neonatal characteristics						
Birth weight (g) [1370-4600]	94	3343.72 ± 542.25	59	3358.98 ± 579.32	35	3318.00 ± 480.28
Birth length (cm) [40-57]	85	50.69 ± 2.68	57	50.61 ± 3.02	28	50.86 ± 1.84
Gestational age (week) [26-43]	96	38.62 ± 2.59	60	38.57 ± 2.59	36	38.70 ± 2.63
Breastfeeding practices (months)	92		59		33	
Exclusive breastfeeding [†] [0-16]		3.19 ± 3.24		3.53 ± 3.53		2.58 ± 2.59
Any breastfeeding [‡] [0-43]		7.06 ± 8.03		6.81 ± 7.18		7.50 ± 9.44
Academic performance (standard score)**	96		60		36	
Mathematics		102.02 ± 10.68		102.37 ± 11.23		101.44 ± 9.82
Reading		108.55 ± 12.92		108.33 ± 11.08		108.92 ± 15.67
Writing		113.99 ± 11.99		112.55 ± 11.91		116.39 ± 11.91
Total Achievement		109.49 ± 11.66		108.98 ± 10.67		110.33 ± 13.25

Values are mean ± SD or percentage. *Measured by the 20-m shuttle run test; [†]Months the child received only breast milk. [‡]Months the child received breast milk combined with other liquid, or solid. **Measured by the Bateria III Woodcock-Muñoz Tests of Achievement.

A. Birth weight



B. Exclusive breastfeeding



C. Any breastfeeding

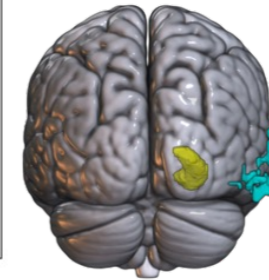
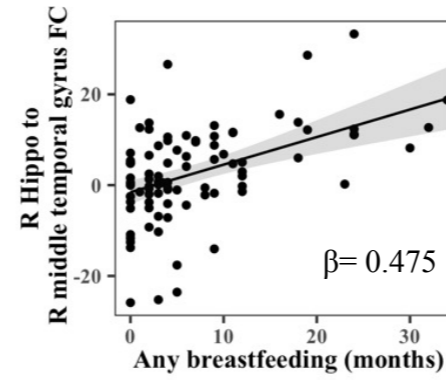


Table 3. Associations of early-life factor-related hippocampal connectivity with academic performance*.

Early life factor	Seed	Cluster location	Mathematics			Reading			Writing			Total achievement		
			β	p-value	p-FDR	β	p-value	p-FDR	β	p-value	p-FDR	β	p-value	p-FDR
Birth weight	L hippocampus	L precentral gyrus	-0.006	0.949	0.949	-0.05	0.614	0.702	-0.006	0.948	0.948	-0.041	0.664	0.997
		L postcentral gyrus	0.185	0.051	0.204	0.117	0.228	0.633	-0.139	0.14	0.282	0.067	0.467	0.997
	R hippocampus	L cerebellum	0.079	0.412	0.593	-0.078	0.429	0.633	-0.122	0.202	0.323	-0.059	0.525	0.997
		R postcentral gyrus	0.098	0.309	0.593	0.092	0.347	0.633	-0.142	0.134	0.282	0.02	0.832	0.997
		L postcentral gyrus	0.072	0.445	0.593	0.069	0.475	0.633	-0.147	0.116	0.282	0.00	0.997	0.997
Exclusive breastfeeding	L hippocampus	L angular gyrus	-0.05	0.608	0.695	-0.037	0.711	0.711	0.08	0.405	0.540	-0.004	0.963	0.997
	R hippocampus	L primary motor cortex	0.225	0.019 [#]	0.152	0.176	0.075	0.600	0.051	0.598	0.683	0.181	0.054	0.432
Any breastfeeding	R hippocampus	R middle temporal gyrus	0.084	0.383	0.593	0.095	0.338	0.633	0.141	0.141	0.282	0.132	0.159	0.636

Values are standardized regression coefficients (β). Analyses were adjusted for sex, peak height velocity offset (years), parent education university level (neither/one/ both) and cardiorespiratory fitness (mL/kg/min). L: Left, R: Right *Measured with the Bateria III Woodcock-Muñoz Tests of achievement. FDR: False discovery rate. [#] This association disappears after FDR correction

Table 2. Significant clusters in the association of early life factors associated with hippocampal connectivity in the left and right seeds.

Explicative variable	Seed	Nature of association	Cluster location	Cluster size	Peak Z	Peak MNI coordinates
Birth weight	Left hippocampus	Positive	L precentral gyrus	267	3.81	-54, 10, 26
		Positive	L postcentral gyrus	382	4.34	-44, -36, 48
	Right hippocampus	Positive	L postcentral gyrus	421	3.86	-44, -40, 54
		Positive	R postcentral gyrus	409	4.28	54, -20, 44
		Positive	L cerebellum	258	4.02	-16, -80, -48
Exclusive breastfeeding	Left hippocampus	Negative	L angular gyrus	369	4.30	-52, -56, 40
	Right hippocampus	Negative	L primary motor cortex	250	4.21	-36, -24, 46
Any breastfeeding	Right hippocampus	Positive	R middle temporal gyrus	329	3.47	70, -26, -20

R: Right; L: Left; MNI: Montreal neurologic institute. Analyses were adjusted for sex, peak height velocity offset (years), parent education university level (neither/one/both), gestational age (weeks) and cardiorespiratory fitness (mL/kg/min). Birth length was not associated to hippocampal resting state functional connectivity.

Early life factors and hippocampal functional connectivity in children with overweight/obesity

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Table S1. Significant clusters in the association of early life factors associated with hippocampal connectivity including sensitivity analysis (Excluding preterm born, <37 weeks).

Explicative variable	Seed	Nature of association	Cluster location	Peak MNI coordinates	β	p-value	β -sens	p-value-sens
Birth weight	Left hippocampus	Positive	L precentral gyrus	-54, 10, 26	0.445	< 0.001	0.441	< 0.001
		Positive	L postcentral gyrus	-44, -36, 48	0.439	< 0.001	0.432	< 0.001
	Right hippocampus	Positive	L postcentral gyrus	-44, -40, 54	0.470	< 0.001	0.465	< 0.001
		Positive	R postcentral gyrus	54, -20, 44	0.450	< 0.001	0.400	0.001
		Positive	L cerebellum	-16, -80, -48	0.425	< 0.001	0.420	< 0.001
Exclusive breastfeeding	Left hippocampus	Negative	L angular gyrus	-52, -56, 40	-0.430	< 0.001	-0.453	< 0.001
	Right hippocampus	Negative	L primary motor cortex	-36, -24, 46	-0.394	< 0.001	-0.426	< 0.001
Any breastfeeding	Right hippocampus	Positive	R middle temporal gyrus	70, -26, -20	0.475	< 0.001	0.438	< 0.001

R: Right; L: Left; MNI: Montreal neurologic institute. Analyses were adjusted for sex, peak height velocity offset (years), parent education university level (neither/one/both), gestational age (weeks) and cardiorespiratory fitness (mL/kg/min). Birth length was not associated to hippocampal resting state functional connectivity. β -sens and p-value-sens corresponding to values of sensitivity analysis excluding preterm born (<37 weeks) children, n =17.

