Pediatric RESEARCH

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CLINICAL RESEARCH ARTICLE

The long-term effect of perinatal asphyxia on hippocampal volumes

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BACKGROUND: Hypoxic–ischemic encephalopathy (HIE) in term-born infants can lead to memory problems. The hippocampus is important for long-term episodic memory. The primary aim was to investigate the effect of HIE on hippocampal volumes in 9- to 10-year-old children. The secondary aim was to investigate the association between hippocampal volumes and previously found impaired memory and cognitive functions in the current cohort.

METHODS: In total 26 children with mild HIE, 26 with moderate HIE, and 37 controls were included. The intelligence quotient (IQ) and memory were tested. A 3D-volumetric MRI was obtained. Brain segmentation was performed for hippocampal volumes and intracranial volume. The differences in hippocampal volumes, memory, and IQ between the groups were determined. Multivariable linear regression analyses were performed, including hippocampal volume as a percentage of intracranial volume as a dependent variable.

RESULTS: Smaller hippocampal volumes were found in moderate HIE ($p < 0.001$), with a trend toward smaller volumes in mild HIE, compared to controls. In multivariable linear regression analysis, hippocampal volume as a percentage of intracranial volume was significantly associated with long-term visuospatial memory.

CONCLUSION: Children with moderate HIE had smaller hippocampal volumes than controls, with a trend toward smaller volumes following mild HIE. Reduced hippocampal volumes were associated with poorer long-term visuospatial memory.

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INTRODUCTION

The prevalence of hypoxic–ischemic encephalopathy (HIE) after perinatal asphyxia is 1.5 per 1000 live-born term neonates.[1] Despite the introduction of hypothermia, the current standard of care, still around 45% of the infants with HIE die or have neurological deficits, such as cerebral palsy (CP), epilepsy, or cognitive impairment.[2] This risk is especially increased in infants with moderate and severe HIE according to the Sarnat criteria.[3]

In the past, it was considered unlikely that cognitive deficits in the absence of CP could be due to HIE, but nowadays, it is widely accepted that infants with HIE can develop isolated cognitive deficits.[4] The first landmark paper looking at long-term cognitive outcome following HIE, found that survivors of moderate HIE who did not develop CP had similar receptive vocabulary and perceptual motor skill outcomes as controls, but showed marked delays in reading, spelling, and arithmetic.[5] Several studies confirmed these findings and it became more accepted that survivors of HIE are at increased risk of cognitive impairment, even in the absence of motor deficits.[4,6–9] In addition, several studies have now shown that even children with mild HIE experience more memory problems than controls, as well as behavioral and attention problems.[10–13]

We have shown in a previous publication using the same cohort, that HIE especially affects long-term episodic memory, verbal working memory, and learning which are all associated with the degree of HIE.[10] Several studies, in different populations, have shown that (episodic) memory impairment might be related to smaller hippocampal volumes.[14,15] The hippocampus is a specific brain structure that is specifically vulnerable to hypoxia. In addition, some small sample-sized studies suggested smaller hippocampal volumes in HIE compared to controls.[6,8,16] However, these groups were heterogeneous and the relation between the hippocampus and memory functioning following HIE has not been fully elucidated.

The primary aim of this study is to evaluate the effect of neonatal HIE on hippocampal volumes in 9- to 10-year-old children. The secondary aim is to investigate whether these hippocampal volumes are associated with the previously found

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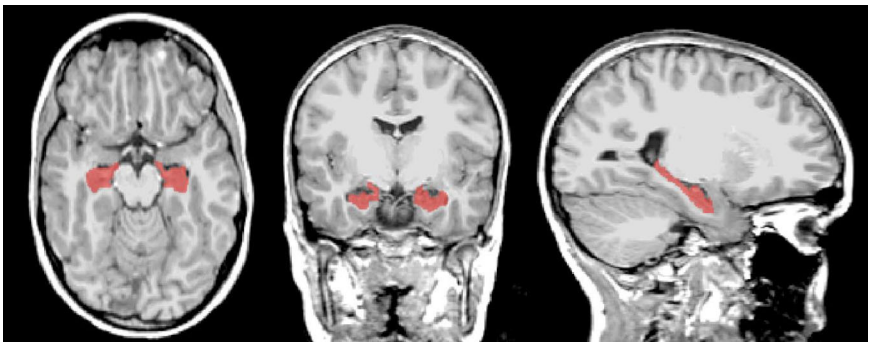

Fig. 1 Regions of interest for automated brain segmentation of the hippocampus. Left image: transverse view, middle: coronal view, and right: sagittal view

impaired memory and cognitive functions in HIE in the current cohort.[10]

METHODS

Study population

This study is a substudy of a larger follow-up cohort, and other results of this cohort have been previously published by van Handel et al. and van Kooij et al.[10,11,17,18] All participants were born at term (37–42 weeks of gestation) between 1993 and 1997 in the Wilhelmina Children's Hospital with HIE following presumed perinatal asphyxia. Perinatal asphyxia was diagnosed when at least three of the following criteria were met: signs of fetal distress (late decelerations on fetal monitoring or meconium-staining amniotic fluid), Apgar score below seven at 5 min postpartum, arterial umbilical pH < 7.10, delayed onset of spontaneous respiration, or multi-organ failure.[10,11] Exclusion criteria were small for gestational age, neurological malformations, congenital or chronic diseases influencing outcome, maternal substance use during pregnancy, and focal damage with total loss of hippocampal volume.

Children with HIE were divided into two groups. Mild HIE (HIE1) was defined as recovery within 24 h and a normal EEG, corresponding with Sarnat stage I.[3] Moderate HIE (HIE2) was defined as no recovery within 24 h and an abnormal EEG, the presence of neonatal seizures, corresponding with Sarnat stage 2.[3] Infants with severe HIE were not included in this study. All infants were born and admitted to our level three neonatal intensive care unit before therapeutic hypothermia became standard of care.

Children with the same sex and age, attending regular schools without any special help, were invited as controls. Parents provided details on the perinatal history to exclude children with complications around delivery or referral to a hospital in the first month after birth.

Informed parental consent and child assent were obtained for all participants according to the Declaration of Helsinki. The Ethical Committee of the University Medical Center Utrecht approved the study.

MRI and volumetric measurements

An MRI was obtained between the age of 9 and 10 years, using a 1.5-T Philips system. Brain segmentation was performed using coronal 3D-T1-weighted images (TR: 30 ms; TE: 4.6 ms, and slice thickness: 1.5 mm). The left and right hippocampal volume and the total intracranial volume (ICV) were segmented using FreeSurfer, https://surfer.nmr.mgh.harvard.edu/.[19](Fig. 1).

The total intracranial volume is the volume of the brain, including the ventricles and extracerebral space. The total hippocampal volume is the sum of the right and left hippocampal volume. The total hippocampal volume was divided by ICV to correct for any differences in ICV, referred to as "percentage hippocampus/ICV".

Memory and intelligence quotient tests

For a comprehensive description of the tests, we refer to a previously published study of this cohort.[10] In summary, the following neuropsychological tests were performed:

1. Short-term memory:
   a. Verbal short-term memory: Digit Span forward task of the Wechsler Intelligence Scale for Children, Dutch version (WISC-III-NL).[20]
   b. Visuospatial short-term memory: Spatial Memory test of the Kaufman-Assessment Battery for Children (KABC).[21]
2. Verbal working memory: Digit Span backward task of the WISC-III-NL.[20]
3. Long-term episodic memory:
   a. Verbal long-term memory: Rey Auditory Verbal Learning Test (RAVLT).[22]
   b. Visuospatial long-term memory: Rey Visual Design Learning Test (RVDLT) and Rey Complex Figure Test (RCFT).[23]
   c. Verbal associative learning: Learning Names of the Revisie Amsterdamse Kinderintelligentie Test (RAKIT; Amsterdam Child Intelligence Test Revised).[24]
4. Intelligence quotient (IQ): the Intelligence Scale for Children, WISC-III-NL.[25]

Statistical analyses

To detect the differences in baseline characteristics, one-way ANOVA and χ^2 tests were performed. The ANOVA test with Bonferroni post hoc test, or the Kruskal–Wallis-H test with post hoc test when appropriate, was used to compare hippocampal volumes, memory, and IQ between the groups. We tested asymmetry in hippocampal volumes with a paired-T test. Multivariable linear regression was performed to determine the association between HIE, age, sex, and hippocampal volumes. Further, we performed univariate and multivariable linear regression analyses to estimate the association between hippocampal volumes and memory and IQ. The percentage hippocampus/ICV, group, age, sex, socioeconomic status based on educational level of the mother (SES), and interaction terms were initially included in the multivariable model. Interaction terms were included to determine whether the effect of hippocampal volume on memory and IQ differed between boys/girls, controls/HIE1/HIE2, SES categories, and/or different ages. p-values > 0.1 were used to remove and p-values < 0.05 to enter the predictors stepwise and bidirectionally in the model. For multivariable linear regression, we

combined SES categories to limit the number of predictors (Table 1). Statistical analysis was performed using SPSS version 21, and R version 3.1.2. p-values < 0.05 were considered statistically significant. All p-values were corrected for multiple comparisons using the Bonferroni correction.

RESULTS

Study population

In total, 164 full-term infants with HIE were admitted to the Neonatal Intensive Care Unit of the Wilhelmina Children's hospital in Utrecht between 1993 and 1997. In the neonatal period, 46 children died, including all children with severe HIE. Of the 118 survivors, 81 were examined at 9–10 years of age. Of the children not examined, six were too severely affected to participate, seven could not be traced (HIE1 $n = 5$, HIE2 $n = 2$), and the parents of 24 children (HIE1 $n = 13$, HIE2 $n = 11$) refused to participate, mainly because MRI was part of the protocol. Fifty-three controls were included.

An MRI was obtained and was of sufficient quality for volumetric analysis in 26 of the 34 HIE1 infants, 27 of the 47 HIE2 infants, and 37 of the 53 controls. In the other children, MRIs could not be obtained due to fear during or before the MRI ($n = 11$), were of insufficient quality due to movement artifacts ($n = 31$), or not available for segmentation ($n = 2$). One additional child with HIE2 was excluded from volumetric analysis because of a perinatal arterial ischemic stroke, leading to total destruction of the hippocampus. There were no significant differences in baseline characteristics or the degree of HIE between the children whose MRI was used for volumetric analysis and the children of whom no MRI was obtained or whose MRI was of insufficient quality.

Table 1. Patient characteristics of the HIE1, HIE2, and control group

Characteristics	Controls ($n = 37$)	HIE1 ($n = 26$)	HIE2 ($n = 26$)	p-value
Gender, n (%)				0.92
Male	19 (51.4)	13 (50)	12 (46.2)	
Female	18 (48.6)	13 (50)	14 (53.8)	
CP, n (%)	0 (0)	2 (7.7)	2 (7.7)	0.23
Age at follow-up, mean years ± SDS	10.07 ± 0.43	9.80 ± 0.47	9.77 ± 0.55	0.03[a]
SES 5 categories[b], median (range)	4 (2–5)	4 (1–5)	4 (1–5)	0.14
Incidence of epilepsy at school age, n (%)	0 (0.0)	0 (0.0)	1 (4.0)	0.30

[a]There was a significant trend toward a lower age in the HIE groups ($p = 0.03$), but there were no significant differences in the post hoc tests between the groups

[b]Socioeconomic status (=educational level of the mother): 1=no education or primary school, 2=lower technical or vocational training, 3=lower secondary education, 4=higher secondary education, and 5=higher education, e.g., university. Category 1, 2, and 3 were combined for multivariable linear regression analyses

Patient characteristics are shown in Table 1. The children with CP were able to perform all tests that were part of the protocol. On conventional imaging, none of the children had pathological lesions of the hippocampus, such as mesial temporal sclerosis.

Hippocampal volumes in HIE

The total hippocampal volumes in HIE2 were significantly smaller than in controls, with a trend toward smaller volumes in HIE1 (controls: 8.7 ± 0.8 ml; HIE1: 8.2 ± 0.9 ml; and HIE2: 7.6 ± 1.3 ml, $p < 0.001$, Fig. 2a). The hippocampal volume in HIE2 was 12.6% smaller than in the controls. No significant difference in volumes was observed between the HIE1 and HIE2 group.

The total ICV was similar in the three groups (controls: 1498 ml ± 179 ml; HIE1: 1456 ± 144 ml; and HIE2: 1432 ± 205 ml, p = 1.000, Fig. 2b). When correcting for ICV, differences in the percentage hippocampus/ICV were observed between the three groups, with smaller volumes in HIE (controls 0.58% ± 0.05; HIE1: 0.56% ± 0.06; and HIE2: 0.53% ± 0.07; $p = 0.012$, Fig. 2c). Again, HIE2 and controls differed significantly in the post hoc tests. After correcting for ICV, the difference between HIE2 and controls was 8.6%.

Girls had a significantly larger percentage hippocampus/ICV compared to boys in the total group (boys: 0.55% ± 0.06; girls: 0.58% ± 0.06; $p = 0.012$).

Multivariable linear regression with the percentage hippocampus/ICV as a dependent variable and HIE1/HIE2/control, age, and sex as independent variables showed that HIE2 and sex were significantly associated with hippocampal volume. HIE2 was associated with a reduction of the percentage hippocampus/ICV of 0.054% (95% CI −0.083%–0.025%), HIE1 with a reduction of 0.021% (95% CI −0.050%–0.008%), and being a girl with an increase of 0.035% (95% CI 0.011%–0.069%).

Memory and IQ in HIE

Short-term memory, verbal working memory, and total verbal long-term memory were comparable between children following HIE and controls and no difference was found between boys and girls. Long-term episodic memory (verbal long-term memory, visuospatial long-term memory, and verbal associative learning) was significantly impaired in children with HIE2 compared to the controls. The IQ of the controls was significantly higher than that of children with HIE. The IQ in children with HIE2 was not found to be significantly lower than 100 in a one-sample-T test ($p = 0.068$) (Supplemental Table S1). IQ was not significantly different in boys and girls (boys: 104 ± 19; girls: 100 ± 18; $p = 0.292$).

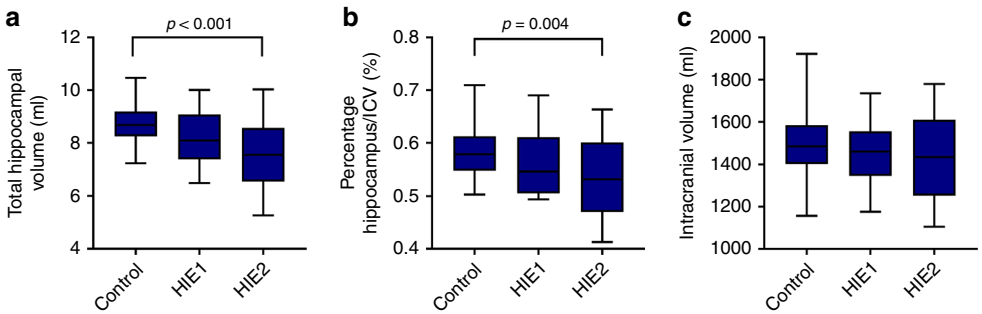

Fig. 2 Overview of hippocampal volumes in controls, HIE1 and HIE2. The total hippocampal volumes (**a**) were significantly smaller in the HIE2 group compared to controls, but the volumes of the HIE1 group were not significantly different. The total hippocampal volume as a percentage of ICV (**b**) was smaller in the HIE2 group compared to controls, but again the HIE1 group did not differ significantly. The intracranial volumes (**c**) were similar in the three groups

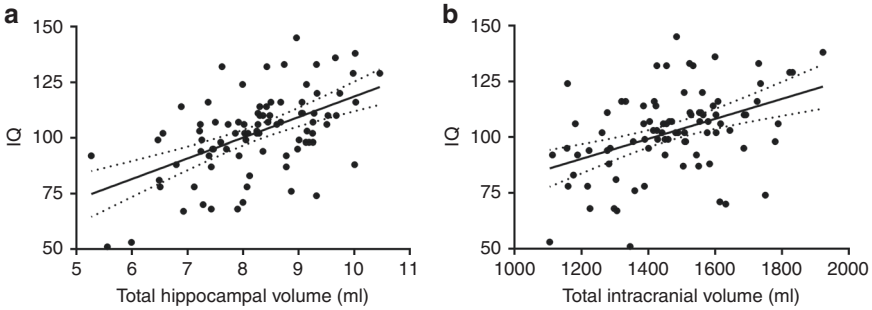

Fig. 3 The total hippocampal volume (**a**) and the total intracranial volume (**b**) were both significantly associated with IQ. The Pearson's correlation coefficient between IQ and hippocampal volume was 0.52 ($p < 0.001$) and between IQ and intracranial volume 0.43 ($p < 0.001$)

Table 2. Predictors included in the final linear regression model per memory or IQ test

Test	Group[a]	Percentage hippocampus/ICV	Age	SES[b]	Gender	Age* percentage hippocampus/ICV	Group*SES
Verbal short-term memory				x			
Visuospatial short-term memory				x	x		
Verbal working memory							
Verbal long-term memory (immediate)	x						
Verbal long-term memory (total)	x						
Verbal long-term memory (delayed)	x						
Visuospatial long-term memory (immediate)	x	x	x			x	
Visuospatial long-term memory (total)	x	x	x			x	
Visuospatial long-term memory (delayed; RVDLT RECALL)	x	x					
Visuospatial long-term memory (delayed; RFCT RECALL)	x						
Verbal associative learning (immediate)	x			x			x
Verbal associative learning (total)	x						
IQ	x			x			

[a]Group: HIE1, HIE2, controls.[b] Socio-economic status (educational level of mother): 1=no education or primary school, 2=lower technical or vocational training, 3=lower secondary education, 4=higher secondary education, 5= higher education e.g. university. Category 1, 2 and 3 were combined for multivariable linear regression.

The association between hippocampal volumes and cognition

In univariate linear regression, both hippocampal volume and ICV were positively associated with IQ (Fig. 3), but the percentage hippocampus/ICV was not. All long-term episodic memory tests (verbal long-term memory, visuospatial long-term memory, and verbal associative learning) and visuospatial short-term memory were significantly associated with the percentage hippocampus/ICV (data not shown). In multivariable linear regression, the degree of HIE was significantly associated with all long-term episodic memory and IQ models. The percentage hippocampus/ICV was a significant predictor for visuospatial long-term memory (Table 2). Table 3 shows the models for the memory tests for which the hippocampal volume was a significant predictor. The socio-economic status and HIE were significantly associated with IQ ($IQ = 104.23 \pm 10.58 \times HIE1 \pm 18.76 \times HIE2 + 4.69 \times SES2 + 15.17 \times SES3$). For IQ analysis, the percentage hippocampus/ICV was corrected for the age of testing, but was not a significant predictor.

DISCUSSION

In the current study, we reported the hippocampal volumes and the association with cognitive outcome at the age of 9–10 years in a cohort of children who suffered HIE following presumed perinatal asphyxia. We first showed that hippocampal volumes in children with a history of moderate HIE were significantly smaller compared to controls, with a trend toward smaller volumes following mild HIE. As previously reported, infants with moderate HIE were found to have poorer long-term episodic memory and lower IQ scores than controls.[10] Finally, multivariable linear regression showed that the hippocampal volume as a percentage of ICV was positively associated with visuospatial long-term memory, suggesting that poor visuospatial long-term memory following HIE can be mediated by hippocampal damage.

To the best of our knowledge, this is the largest study investigating the effect of neonatal HIE on hippocampal volumes, but smaller studies have been published that are in agreement with our finding that children with HIE have reduced bilateral hippocampal volumes.[6,8,26] For example, Gadian and colleagues examined a smaller group of children and found that severe acute hypoxia was associated with reduced hippocampal volumes and memory problems. This was reported in six school-aged children with memory problems who had experienced hypoxia at, or shortly after birth and showed bilateral reduction of hippocampal volumes of ~40%.[6,8] Using MR spectroscopy, Mañeru et al.[26] found lower N-acetyl aspartate concentrations and N-acetyl

Table 3. The influence of the percentage hippocampus/ICV on memory in multivariable linear regression analysis

Possible items in the model	Model visuospatial long-term memory (immediate)[a]	Model visuospatial long-term memory (total)[a]	Model visuospatial long-term memory (delayed)[a]
Intercept	115.04 ($p = 0.028$)	471.23 ($p = 0.021$)	3.84 ($p = 0.234$)
HIE1	−1.54 ($p = 0.024$)	−5.87 ($p = 0.028$)	−1.16 ($p = 0.129$)
HIE2	−2.10 ($p = 0.004$)	−8.52 ($p = 0.003$)	−2.27 ($p = 0.006$)
Age	−11.31 ($p = 0.031$)	−45.46 ($p = 0.028$)	n/a
Percentage hippocampus/ICV	−177.17 ($p = 0.055$)	−808.30 ($p = 0.026$)	9.95 ($p = 0.082$)
Age*percentage hippocampus/ICV	19.06 ($p = 0.041$)	84.58 ($p = 0.021$)	n/a

[a]ß-coefficients and *p*-values are shown for the variables included in the final models

aspartate/choline ratios in the anterior hippocampus of adolescents following HIE, indicating biochemical damage.[7] Furthermore, they also confirmed that infants with moderate HIE have bilateral hippocampal atrophy. The trend of decreased hippocampal volumes in mild HIE has not been published previously. This finding might explain that several studies found decreased cognitive and memory functions after even mild HIE compared to controls.[12] Besides perinatal asphyxia, other conditions leading to neonatal hypoxia, namely acute respiratory distress (ARD),[27] prematurity,[28] and congenital heart disease,[29] have been associated with decreased hippocampal volumes later in life. Furthermore, smaller hippocampal volumes were found in 12-year-old extracorporeal membrane oxygenation (ECMO) survivors compared to controls.[30]

The underlying mechanism explaining the vulnerability of the hippocampus to hypoxia is unknown. It has been hypothesized that the high expression and potentiation of N-methyl-D-aspartate (NMDA) receptors in the hippocampus lead to this higher vulnerability.[31–34] During hypoxia, there is an excessive release of glutamate, leading to NMDA activation.[31] NMDA activation causes an influx of calcium into the cell, resulting in neuronal death by the induction of apoptosis and necrosis.[31] Furthermore, NMDA activation induces astrogliosis and microglial reactions, leading to impaired recovery of neurons.[32]

The children reported in this cohort were part of a larger study population whose neuropsychological performance has been published previously.[10] In the current subgroup of children who underwent MRI, a significantly impaired IQ and long-term episodic memory in the moderate HIE group was found compared to controls. This is in line with the previously reported data, although we now had smaller subgroups.[10] Also, a trend of impaired memory functioning in mild HIE was found for some long-term episodic memory subtests. With a mean of 112, IQ scores in controls were significantly higher than in children with HIE and significantly higher than the reference standard of 100, which is likely to be explained by the fact that their mothers had a higher educational level than the average population. This might partially explain the difference in IQ between children with HIE and healthy controls.

It is well known that infants with HIE have an increased risk of developing cognitive deficits, even in the absence of motor deficits.[4,5] In a follow-up study of Robertson et al.,[5] 40% of the children with moderate HIE without neurological deficits had an impaired IQ and delayed learning skills at 8 years of age in comparison to controls. Similarly, Steinman et al.[35] showed that 9% of the children following HIE without motor problems had a verbal IQ < 70 at 4 years of age. Besides IQ, memory also has been shown to be affected in children with HIE.[9,10] Mañeru et al.[9] found that children with moderate HIE have impaired verbal and visual memory compared to mild HIE and controls. It was thought for many years that only infants with moderate and severe HIE are at risk for cognitive impairment.[4–6,8,9,26] However, a possible trend of impaired memory function and IQ in mild HIE has been suggested recently.[10,12,17] In a large Swedish cohort, even infants with Apgar scores <7 without encephalopathy had an increased risk of an impaired IQ at 18 years of age.[36]

The observed predominant deficits in episodic memory in our cohort are in line with the literature.[6,8,10,26] Semantic memory is relatively spared in HIE. It is hypothesized that semantic memory depends on the hippocampus and the temporal cortices, but that a spared temporal cortex is sufficient to compensate for hippocampal damage and to develop normal semantic memory functions.[37] On the contrary, episodic memory seems to depend fully on the development of the hippocampus and is therefore more affected by hippocampal damage.[37,38]

To study the association between HIE, hippocampal volumes, and cognition, we first showed that HIE is an important predictor of hippocampal volumes using multivariable linear regression analysis. Next, we studied how the hippocampal volume is associated with IQ and memory using multivariable linear regression. Based on previous reports, we expected reduced hippocampal volumes to be associated with long-term episodic memory impairment.[6,38] However, after correcting for age, sex, and SES, the percentage hippocampus/ICV was only strongly associated with the episodic subtests for visuospatial long-term memory. In a larger cohort, the effect of hippocampal volume on other subtests for long-term episodic memory would also have been significant. The effect of hippocampal volume on IQ and memory analyzed with multivariable linear regression has not been investigated in HIE before, but has been tested in other neonatal populations. In congenital heart diseases, smaller hippocampal volumes were strongly associated with impaired IQ, verbal, and working memory[29] and in ECMO survivors with verbal long-term memory which is a subfield of long-term episodic memory.[32] It is of interest that there seems to be an association between hippocampal volumes and memory in different neonatal groups experiencing hypoxia, but that the affected subfields differ.

Some limitations need to be addressed. Although this is the largest study investigating the effect of HIE on hippocampal volumes, the sample size was too small to perform multivariable linear regression in HIE1 and HIE2 separately. In addition, no severely affected children were included, because they either died in the neonatal period or were too severely impaired to be part of the study. The association between hippocampal damage, HIE, and memory might be even stronger, including this subgroup. Further, segmentation with Freesurfer has some limitations that might influence the quality of the segmentation: Freesurfer uses T1-weighted images that have less contrast between gray and white matter than T2-weighted images, and the automated segmentation method is based on the resolution of 3-T images instead of the 1.5-T images that were used.[39] Segmentation of the

images with less contrast or a lower resolution might influence the quality of the segmentation. Based on the quality of the scans, it was not feasible to measure the volumes of specific subregions of the hippocampus and to relate these to the different memory fields. The segmentation of subregions might have given additional information, since the posterior subregions of the hippocampus might be more important for episodic memory than the anterior subregion.[40] Furthermore, previous studies have shown that hippocampal volumes segmented by Freesurfer are larger than manual segmentations.[41] However, automatic segmentation with Freesurfer is better reproducible and if the volume of the hippocampus is overestimated, this applies to all three groups so that it remains feasible to compare the groups.

This study shows that children with mild and moderate HIE can experience long-term episodic memory problems, which is associated with hippocampal damage. If hippocampal volumes can be measured in infancy, we may be able to use these measurements to predict memory problems, and intervene at a younger age. More research is essential to determine at what age hippocampal atrophy first becomes visible. To answer this question, a follow-up study with sequential MRI scans of the brain of neonates with HIE is necessary, using a higher field strength of 3.0 T instead of 1.5 T. Additionally, an even larger follow-up study is necessary to investigate the memory problems in mild HIE and the exact association between hippocampal volumes and all fields of episodic memory. Further, research on the relation between episodic memory and the subregions of the hippocampus would provide us with an even more specific understanding of the long-term effect of HIE on hippocampal volumes and memory and the functional neuroanatomy of the hippocampus.

In conclusion, children with HIE have decreased hippocampal volumes, as well as impaired long-term episodic memory at the age of 9–10 years. Furthermore, hippocampal volumes are associated with long-term visuospatial memory impairment.

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AUTHOR CONTRIBUTIONS

All authors have met the Pediatric Research authorship requirements.

ADDITIONAL INFORMATION

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REFERENCES

1. Kurinczuk, J. J., White-Koning, M. & Badawi, N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. *Early Hum. Dev.* **86**, 329–338 (2010).
2. Azzopardi, D. et al. Moderate hypothermia to treat perinatal asphyxial encephalopathy. *N. Engl. J. Med.* **361**, 1349–1358 (2009).
3. Sarnat, H. B. & Sarnat, M. S. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. *Arch. Neurol.* **33**, 696–705 (1976).
4. Gonzalez, F. F. & Miller, S. P. Does perinatal asphyxia impair cognitive function without cerebral palsy? *Arch. Dis. Child. Fetal Neonatal Ed.* **91**, 454–459 (2006).
5. Robertson, C., Finer, N. & Grace, M. School performance of survivors of neonatal encephalopathy associated with birth asphyxia at term. *J. Pediatr.* **114**, 753–760 (1989).
6. Gadian, D. G. et al. Developmental amnesia associated with early hypoxic–ischaemic injury. *Brain* **123**, 499–507 (2000).
7. Mañeru, C. et al. H-MR spectroscopy is sensitive to subtle effects of perinatal asphyxia. *Neurology* **57**, 1115–1118 (2001).
8. Vargha-Khadem F. et al. Developmental amnesia: effect of age at injury. *Proc. Natl Acad. Sci. USA* **100**, 10055–60 (2003).
9. Maneru, C., Junque, C., Botet, F., Tallada, M. & Guardia, J. Neuropsychological long-term sequelae of perinatal asphyxia. *Brain Inj.* **15**, 1029–1029 (2001).
10. van Handel, M., de Sonneville, L., de Vries, L. S., Jongmans, M. J. & Swaab, H. Specific memory impairment following neonatal encephalopathy in term-born children. *Dev. Neuropsychol.* **37**, 30–50 (2012).
11. van Handel, M., Swaab, H., de Vries, L. S. & Jongmans, M. J. Behavioral outcome in children with a history of neonatal encephalopathy following perinatal asphyxia. *J. Pediatr. Psychol.* **35**, 286–295 (2010).
12. Murray D. M., O'Connor C. M., Ryan C. A., Korotchikova I., & Boylan G. B. Early EEG grade and outcome at 5 years after mild neonatal hypoxic ischemic encephalopathy. *Pediatrics* **138**, e20160659 (2016).
13. Hayes, B. C. et al. Neurodevelopmental outcome in survivors of hypoxic ischemic encephalopathy without cerebral palsy. *Eur. J. Pediatr.* **177**, 19–32 (2018).
14. Isaacs, E. B. et al. Developmental amnesia and its relationship to degree of hippocampal atrophy. *PNAS* **100**, 13060–13063 (2003).
15. Allen, J. S., Tranel, D., Bruss, J. & Damasio, H. Correlations between regional brain volumes and memory performance in anoxia. *J. Clin. Exp. Neuropsychol.* **28**, 457–476 (2006).
16. Vargha-Khadem, F. et al. Differential effects of early hippocampal pathology on episodic and semantic memory. *Science* **277**, 376–380 (1997).
17. Van Kooij, B. J. M. et al. Serial MRI and neurodevelopmental outcome in 9- to 10-year-old children with neonatal encephalopathy. *J. Pediatr.* **157**, 221–227 (2010).
18. Van Kooij, B. J. M. et al. Corpus callosum size in relation to motor performance in 9- to 10-year-old children with neonatal encephalopathy. *Pediatr. Res.* **63**, 103–108 (2008).
19. Fischl, B. et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* **33**, 341–355 (2002).
20. Kort, W. et al. *WISC-III Nl.: Wechsler Intelligence Scale for Children. Handleiding en Verantwoording* (Harcourt Test Publ., Amsterdam, 2005).
21. Kaufman, A. S. & Kaufman, N. L. *Kaufman Assessment Battery for Children* (Am Guid Serv, Circ Pines, MN, 1983).
22. Kalverboer, A. F. & Deelman, G. B. *De 15-Woorden Test [The 15 Words Test]* (Acad Ziekenh Groningen, Groningen, Netherlands, 1986).
23. Spreen, O., Risser, A. & Edgell, D. *Developmental Neuropsychology* (Oxford Univ Press, New York, NY, 1995).
24. Bleichrodt, N., Drenth, P. J. D., Zaal, J. & Resing, W. C. M. *RAKIT: Revisie Amsterdamse kinderintelligentietest [Revision Amsterdam Children's Intelligence Test]* (Swets & Zeitlinger, Lisse, 1996).
25. Sattler, J. M. *Assessment of Children* (J M Sattler Publ Inc., San Diego, CA, 1988).
26. Mañeru C. et al. Hippocampal damage in PA residual hippocampal atrophy in asphyxiated term neonates. *J. Neuroimaging* **13**, 68–74 (2003).
27. Cooper, J. M. et al. Neonatal hypoxia, hippocampal atrophy, and memory impairment: evidence of a causal sequence. *Cereb. Cortex* **25**, 1469–1476 (2015).
28. Guo, T. et al. Automatic segmentation of the hippocampus for preterm neonates from early-in-life to term-equivalent age. *Neuroimage Clin.* **9**, 176–193 (2015).
29. Latal, B. et al. Hippocampal volume reduction is associated with intellectual functions in adolescents with congenital heart disease. *Pediatr. Res.* **80**, 531–537 (2016).
30. Schiller, R. M. et al. Neonatal critical illness and development: white matter and hippocampus alterations in school-age neonatal extracorporeal membrane oxygenation survivors. *Dev. Med. Child Neurol.* **59**, 304–310 (2017).
31. McDonald, J. W. & Johnston, M. V. Physiological and pathophysiological roles of excitatory amino acids during central nervous system development. *Brain Res. Rev.* **15**, 41–70 (1990).
32. Bernal, F. N., Saura, J., Ojuel, J. L. & Mahy, N. Differential vulnerability of hippocampus, basal ganglia, and prefrontal cortex to long-term NMDA excitotoxicity. *Exp. Neurol.* **161**, 686–695 (2000).
33. Gee, C. E. et al. NMDA receptors and the differential ischemic vulnerability of hippocampal neurons. *Eur. J. Neurosci.* **23**, 2595–2603 (2006).
34. Dos-Anjos, S. et al. Transient global ischemia in rat brain promotes different NMDA receptor regulation depending on the brain structure studied. *Neurochem. Int.* **54**, 180–185 (2009).
35. Steinman, K. J., Gorno-tempini, M. L. & Glidden, D. V. Neonatal watershed brain injury on MRI correlates with verbal IQ at four years. *Pediatrics* **123**, 1025–1030 (2009).
36. Odd, D. E., Rasmussen, F., Gunnell, D., Lewis, G. & Whitelaw, A. A cohort study of low Apgar scores and cognitive outcomes. *Arch. Dis. Child. Fetal Neonatal Ed.* **93**, 115–120 (2008).
37. Mishkin, M., Suzuki, W. A., Gadian, D. G. & Vargha-Khadem, F. Hierarchical organization of cognitive memory. *Philos. Trans. R. Soc. B Biol. Sci.* **352**, 1461–1467 (1997).

38. de Haan, M., Mishkin, M., Baldeweg, T. & Vargha-Khadem, F. Human memory development and its dysfunction after early hippocampal injury. *Trends Neurosci.* **29**, 374–381 (2006).
39. Wisse, L. E. M., Biessels, G. J. & Geerlings, M. I. A critical appraisal of the hippocampal subfield segmentation package in FreeSurfer. *Front. Aging Neurosci.* **6**, 261 (2014).
40. Greicius, M. D. et al. Regional analysis of hippocampal activation during memory encoding and retrieval: fMRI study. *Hippocampus* **13**, 164–174 (2003).
41. Pipitone, J. et al. Multi-atlas segmentation of the whole hippocampus and subfields using multiple automatically generated templates. *Neuroimage* **101**, 494–512 (2014).