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Mammillary Body and Hippocampal Injury After Acute Perinatal Arterial Ischemic Stroke

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Abstract

Background and Objectives

Perinatal arterial ischemic stroke (PAIS) affects approximately 1–2 per 5,000 full-term births and can result in long-term neurologic changes such as hemiparesis, epilepsy, and cognitive impairments, including memory difficulties. The mammillary bodies (MBs) and the hippocampi (HCs) are both important brain structures for memory, and they are affected in infants with perinatal asphyxia, but less is known about their involvement in PAIS. We performed a retrospective study to determine the prevalence of MB and HC injury and to assess whether the injury varied according to the arterial territory involved and the co-occurrence of perinatal asphyxia.

Methods

This retrospective study assessed the status of the MB and HC of 130 neonates born between 2005 and 2023 diagnosed with acute PAIS based on neonatal MRI. Involvement of the MB and HC was based on diffusion-weighted imaging and T1/T2-weighted imaging. The hemisphere and arterial territory involved in the PAIS were documented along with clinical measures and the presence of perinatal asphyxia and therapeutic hypothermia.

Results

MRI scans were acquired at a median of 5 days after birth. Most of the strokes involved the middle cerebral artery. The MB could be assessed in 127 of 130 patients with PAIS and were found to be injured in 21 (16.5%) of 127, with equivocal signal change in a further 27 (21%) of 127 patients. MB injury occurred more often with larger strokes and was almost exclusively found bilaterally. MB injury was more prevalent when there was co-occurring perinatal asphyxia (odds ratio 3.56). Only 6% (8/130) of patients with PAIS showed HC injury, which was typically unilateral and located within the primary arterial stroke area.

Discussion

MB injury is frequently present in neonates with PAIS, while HC injury occurs less often. The MB and HC injuries seem to be caused by different processes, with the HC injury typically resulting from the direct impact of the stroke. Understanding the mechanisms through which the MBs become injured in PAIS, both with and without perinatal asphyxia, is an important future goal.

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Glossary

ACA = anterior cerebral artery; **DWI** = diffusion-weighted imaging; **HC** = hippocampus; **MB** = mammillary body; **MCA** = middle cerebral artery; **NICU** = neonatal intensive care unit; **NSRU** = Neonatal Stroke Registry Utrecht; **OR** = odds ratio; **PAIS** = perinatal arterial ischemic stroke; **PCA** = posterior cerebral artery; **T1w** = T1-weighted; **T2w** = T2-weighted; **UMCU** = University Medical Center Utrecht.

Introduction

The newborn period is associated with a heightened risk of stroke, with perinatal arterial ischemic stroke (PAIS) affecting approximately 1–2 in 5,000 full-term neonates.[1-3] This can result in long-term neurologic impairments, including unilateral spastic cerebral palsy, epilepsy, and cognitive impairments.[4,5] At present, there are no clear early predictors of long-term cognitive outcomes,[6] highlighting the importance of identifying possible indicators for future monitoring.

The hippocampi (HCs) and mammillary bodies (MBs) are important brain structures for cognition and memory, and they are affected in several neurologic disorders, including perinatal asphyxia.[7-11] Furthermore, MB and HC injuries are associated with cognitive impairments at school age in children with perinatal asphyxia.[12] While the initial presentation and risk factors of perinatal asphyxia and PAIS vary,[13-15] there is a significant association between PAIS and perinatal hypoxic factors.[16] Moreover, similar inflammatory and apoptotic pathways are involved in the two conditions,[17] as well as the involvement of common subcortical gray matter regions.[15] Cognitive delay is also common in children after PAIS and perinatal asphyxia.[12,18] Given the involvement of the MB and HC in neonates with perinatal asphyxia, it is possible that these brain structures may also show vulnerability in neonates with PAIS. While there are previous reports of HC involvement in PAIS,[15,19] to our knowledge, the MBs have not been systematically assessed.

The primary aim of this study was to assess the incidence of MB and HC pathology in neonates with acute PAIS. The MBs receive their blood supply from branches of the posterior communicating artery and posterior cerebral artery, while the HC is supplied by both the posterior cerebral artery and the anterior choroidal artery.[20] We hypothesized two potential mechanisms for MB and HC injuries in this context: either direct ischemic injury due to compromised blood flow in their supplying arteries resulting in a unilateral injury pattern or global injury associated with PAIS, leading to bilateral MB and HC injuries regardless of the specific arterial territory involved. To differentiate between these mechanisms, we investigated whether MB and HC injuries were associated with the arterial territory involved in the stroke and whether MB and HC injuries were predominantly unilateral or bilateral. Finally, because perinatal asphyxia often co-occurs with PAIS and the MB and HC are known to be vulnerable to perinatal asphyxia, we sought to determine whether the observed MB and HC injuries were associated with perinatal asphyxia and vital parameters associated with perinatal asphyxia in this cohort.

Methods

Study Population

For this retrospective cohort study, we selected a subset of 138 neonates from the Neonatal Stroke Registry Utrecht (NSRU; METC number 21-660), a research registry with clinical and imaging data that was approved by the medical ethical board of the University Medical Center Utrecht (UMCU), the Netherlands. All parents and/or patients gave written informed consent for the NSRU. The study population involved neonates born at >34 weeks of gestation and admitted to the neonatal intensive care unit (NICU) at the Wilhelmina Children's Hospital of the UMCU, the Netherlands, between 2005 and 2023. We included neonates who received a diagnosis of acute PAIS based on an MRI scan acquired during the neonatal period. Acute PAIS was based on changes in diffusion restriction in the stroke lesion, typically seen within 4–10 days after the event. Exclusions were made when there was evidence of other substantial brain injuries (e.g., hemorrhages) and if no changes in diffusion restriction in the stroke lesion were visible on MRI because of pseudonormalization. Brain MRI was performed for clinical purposes in all patients, to diagnose PAIS, and to enhance long-term neurodevelopmental prognosis and outcomes.

Clinical information about the labor and neonatal period was collected from the NSRU. A subset of neonates was diagnosed with perinatal asphyxia, which was defined as a 5-minute Apgar score ≤5, the need for resuscitation and/or mechanical ventilation during ≥10 minutes postpartum, pH <7.1, umbilical cord base excess <−16 mmol/L, or lactate >10.0 mmol/L in umbilical cord blood gas analysis or arterial, venous, or capillary blood gas analysis <1 hour after delivery.[21]

MRI

Due to the broad inclusion period, a variability in MRI acquisition was present, including the scanners and protocols. MRI scans were acquired using either a 1.5T or 3T whole-body system (Philips Medical Systems, Best, the Netherlands); scanning protocols have been described previously[22,23] and included T1-weighted (T1w) IR sequence on 1.5T, T2-weighted (T2w) imaging, and diffusion-weighted imaging (DWI). The T2w sequences had a maximum of 2-

mm slice thickness to ensure that the MBs could be visualized and to reduce partial volume effect.

MRI Data Assessment

A neonatologist experienced in neonatal brain MRI (J.D.) determined the arterial territory of the stroke according to Govaert et al.[24] MB and HC involvement was assessed by an experienced pediatric radiologist (M.L.). To measure reliability, a subset of 58 (45%) of 130 cases was assessed by researchers with expertise in neonatal neurology (J.D., L.M.B.). Where possible, the MB and HC were assessed in all three planes on both T1w and T2w sequences and an axial DWI trace map (b = 800 or b = 1,000). The MB and HC were assessed as either "Normal" (no signal intensity changes on T1/T2/DWI, no swelling), "Equivocal" (only mild signal intensity changes on T1w and T2w or only swelling on T2w without any T1/T2/DWI signal intensity changes), or "Injured" (high signal intensity on DWI and/or a combination of 2 out of 3 of low T1w signal, high T2w signal, and/or swelling) (Figures 1 and 2). It was also noted whether any signal change was unilateral or bilateral and whether the MB and HC were part of the primary lesioned area.

Statistical Analyses

Statistical analyses were performed with SPSS, version 29 (IBM SPSS Statistics; IBM Corp., Armonk, NY). Continuous clinical parameters were presented as median and interquartile range because of non-normality of the data, and categorical data were depicted as numbers and percentages. Regarding the primary outcome of the incidence of MB and HC injuries, numbers and percentages of the available study cohort were given. Regarding the secondary outcome, clinical and MRI characteristics were compared for subgroups divided by the co-occurrence of perinatal asphyxia by either independent t, Mann–Whitney U, Fisher exact, or χ^2 tests and by type of MB injury (none, equivocal, or injured) by 1-way analysis of variance, χ^2 tests, or Kruskal-Wallis tests. The association between MB and HC injuries and perinatal asphyxia and arterial territory was assessed by χ^2 tests and with a Fisher exact test for comparing main or partial middle cerebral artery (MCA) strokes and perforator, cortical, anterior cerebral artery (ACA), and posterior cerebral artery (PCA) strokes. A dichotomous variable for MB injury (clear bilateral injury vs other and equivocal bilateral injury vs other) was further used to assess the correlation between vital parameters and MB injury. Cohen κ was used to assess inter-rater reliability. We handled missing data by pairwise deletion. α levels were set at 0.05.

Results

We identified 138 eligible neonates from the registry. After exclusions due to pseudonormalization (i.e., scans not being acquired during the optimal time window; n = 6), scan availability (n = 1), and extensive additional hemorrhage (n = 1), 130 term neonates were included in the final analyses.

Almost one-third of neonates had perinatal asphyxia (n = 37, 28%), although only 8 (6%) met the criteria to receive therapeutic hypothermia (Table 1). None of the neonates received other treatments as part of an intervention study before undergoing the neonatal MRI. Neonates presented with clinical seizures in most of the cases (77%). Other presenting symptoms included infection, symptomatic hypoglycemia, or subclinical seizures on amplitude-integrated EEG (amplitude-integrated EEG was performed as part of standard care after a surgical procedure). Seven percent of patients did not show any of the abovementioned clinical signs, and PAIS was visualized during cerebral ultrasound or MRI performed as part of routine clinical care because of other comorbidities present. Neonatal MRI confirmed the presence of acute PAIS in all patients.

MRI Findings

Most of the neonates underwent 3T MRI (n = 95/130, 73%), which was performed at a median of 5 days after birth (Table 2). Across the whole cohort, the MCA was most often involved; within this category, the main, middle, posterior, and perforator branches were most frequently affected (16%, 18%, 20%, and 20%, respectively) with involvement of the anterior MCA or one of the cortical branches being less frequent (8% and 11%, respectively). Nine patients (7%) had a PCA stroke, and 1 patient (1%) had an ACA stroke. Over half of the patients had concomitant injury to the basal ganglia (63%) or thalamus (52%).

MB Injury

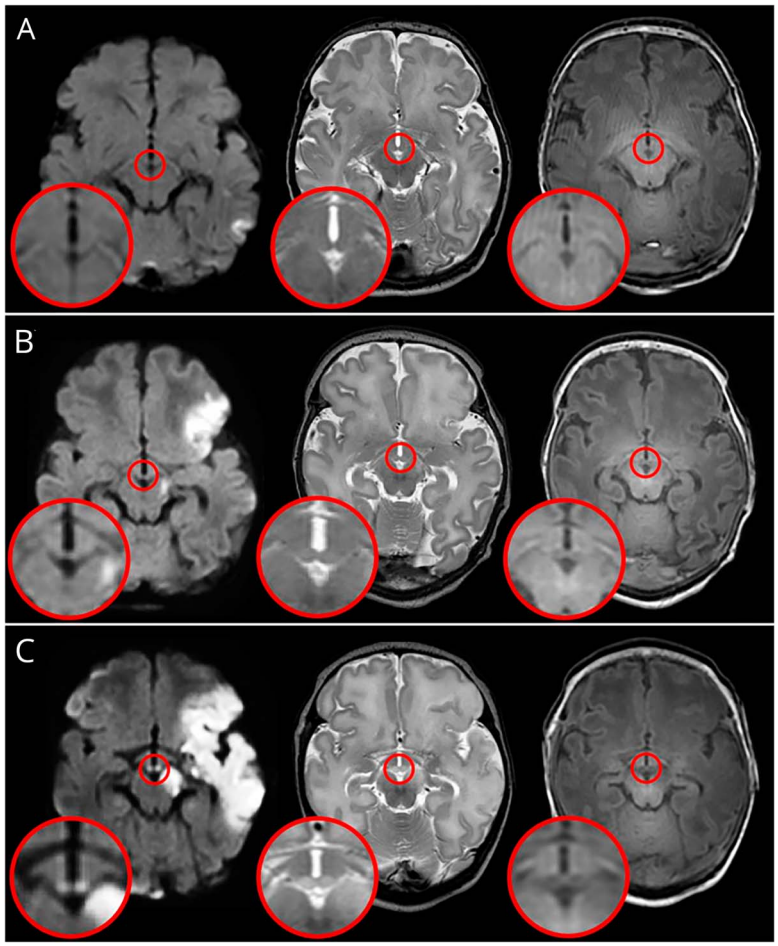
The MBs of three patients were not visible on the T1w and T2w sequences because of technical restrictions, resulting in 127 patients in whom the MBs were assessed (eTables 1 and 2) and 130 in whom the HCs were assessed. Cohen κ values for inter-rater reliability were 0.90, 0.93, and 0.93 for the assessment of ipsilesional, contralesional, and bilateral MB injury, respectively, and 0.90, 0.65, and 1.0 for the assessment of ipsilesional, contralesional, and bilateral HC injury.

The MBs were classified as Injured in 21 (16%) of the 127 neonates while, in 27 (21%), the MBs were classified as Equivocal (Figure 1; eTables 1 and 2 provide the observed alterations on neuroimaging sequences). Only 2 patients showed unilateral Injured MB (1 patient with ipsilesional and 1 patient with contralesional injury); the remainder showed bilateral MB injury classification. Nearly all patients with MB injury had a unilateral stroke (n = 19/21); in the 2 remaining patients, both hemispheres were affected by the stroke. All the injured MBs fell outside the primary stroke area. There was a trend for neonates with Equivocal MB injury to be scanned earlier than those with Injured MB (day 4 vs day 6; H (2, N = 127) = 5.32; p = 0.070). Injury to the basal ganglia or thalamus was not related to MB injury (Table 2).

HC Injury

The HCs were assessed in 130 neonates and were rated as Injured in eight (6%). In six patients, HC injury was unilateral and present in the same hemisphere as the stroke while the remaining patients showed bilateral HC injury (Figure 2). In

Figure 1 Examples of MR Images With Normal, Equivocal, and Injured MBs in Neonates With PAIS



Panels A–C show (left to right) axial diffusion-weighted MRI, T2w MRI, and T1w MRI with red circles highlighting the MB. (A) MRI of a full-term neonate diagnosed with a left posterior branch MCA stroke, showing normal MB. (B) MRI of a full-term neonate infant diagnosed with a left anterior branch MCA stroke, showing equivocal bilateral MB injury, visible as hyperintensity on T2w and hypointensity on T1w MRI, without visible diffusion restriction on DWI. (C) MRI of a full-term neonate diagnosed with a left main MCA stroke showing bilateral MB injury visible on all 3 sequences. DWI = diffusion-weighted imaging; MB = mammillary body; MCA = middle cerebral artery; T1w = T1-weighted; T2w = T2-weighted.

five of the six patients with unilateral HC injury, the HC was part of the primary stroke area. In the two patients with bilateral HC injury, the HC was not located within the primary stroke area. The cases with HC and MB injuries partly overlapped: six out of eight cases with HC injury had some form of MB injury, although the MBs were classified as Equivocal in four of them.

Patients with bilateral MB injury more often showed a lower Apgar score at 1 minute, presented less often with seizures as the primary symptom, and were more often diagnosed with perinatal asphyxia (Table 3). None of the clinical or MRI characteristics was significantly different for cases with and without unilateral or bilateral HC injury (results not shown).

Arterial Territories vs Injury

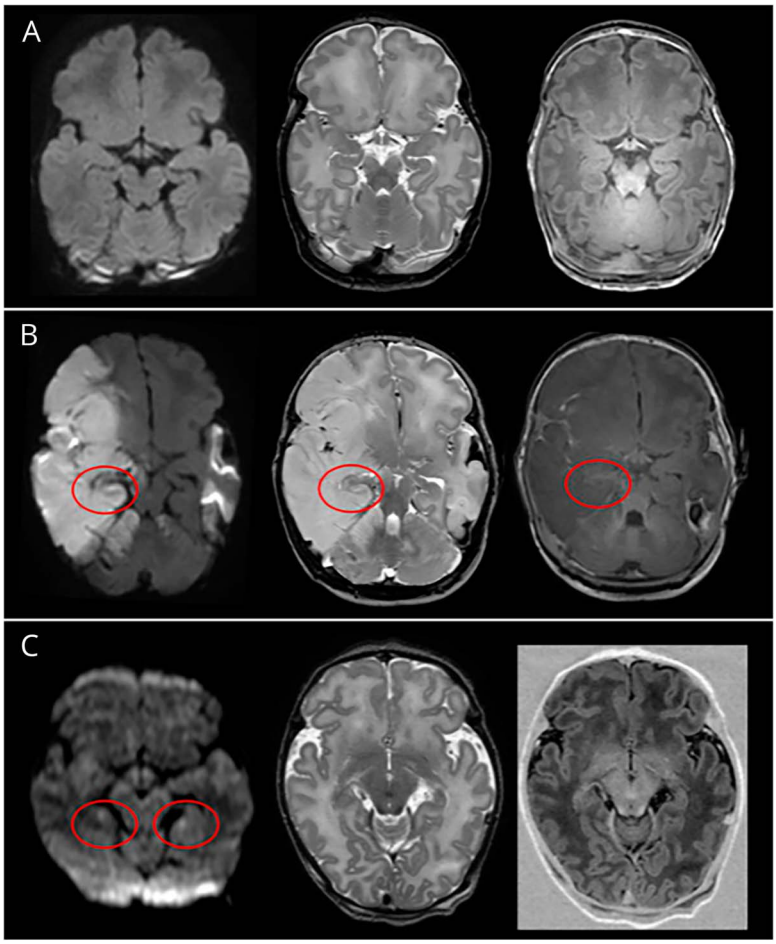
A borderline relationship between MB injury and arterial territory was observed (p = 0.070), and the distribution of data was consistent with more frequent MB involvement in patients with a larger stroke subtype (Table 2; eTable 3). After grouping the strokes into main or partial branch MCA involvement (i.e., main MCA and anterior, middle, posterior branch MCA; n = 79) or smaller strokes including perforator, cortical, ACA, or PCA stroke (n = 49), a significant difference in the incidence of MB injury (p = 0.015) was observed, with MB injury occurring more frequently in the main or partial branch MCA strokes. This difference in distribution across arterial territories was only apparent for the Injured MB group, not the Equivocal group (Table 2).

There was no significant relationship between either unilateral or bilateral HC injury and arterial territory (ipsilesional HC injury: p = 0.780; contralesional and bilateral HC injury: p = 0.632). The two patients with bilateral HC injury and the one patient with equivocal bilateral HC injury all had a stroke involving the middle branch MCA. All patients with ipsilesional HC injury had strokes in the MCA territory: main MCA (n = 3), posterior branch MCA (n = 1), middle branch MCA (n = 1), and perforator branch (n = 1).

Perinatal Asphyxia

In this cohort of 130 patients with PAIS, 37 (28%) had co-occurring perinatal asphyxia. Perinatal asphyxia did not seem

Figure 2 Examples of MR Images With Normal, Unilaterally, and Bilaterally Injured HCs in Neonates With PAIS



Panels A–C show (left to right) axial diffusion-weighted MRI, T2-weighted MRI, and T1-weighted MRI (inversion recovery MRI in figure C). (A) MRI of a late term neonate diagnosed with a left posterior branch MCA stroke, showing normal HC. (B) MRI of a full-term neonate diagnosed with a right main MCA and PCA stroke, showing unilateral HC injury as part of the primary stroke lesion visible on all sequences (red circles). (C) MRI of a full-term neonate diagnosed with a left middle branch MCA stroke, showing bilateral HC injury visible as diffusion restriction on DWI (red circle). DWI = diffusion-weighted imaging; HC = hippocampus; MCA = middle cerebral artery; PCA = posterior cerebral artery.

to relate to the stroke subtype either when using the individual categories (p = 0.721), or when the stroke subtype was grouped according to involvement of main or partial branch MCA vs other arterial territories (p = 0.842).

A higher incidence of MB injury was observed in patients with PAIS with co-occurring perinatal asphyxia compared with those without perinatal asphyxia (p = 0.006). Although this pattern could be observed for both the Injury and Equivocal MB groups, a significant effect was observed for the Injured group only (Injured group, odds ratio [OR] 3.56, 95% CI 1.36–9.37; Equivocal group, OR 1.67, 95% CI 0.68–4.12). Of the eight patients with HC injury, three patients with unilateral HC injury but none with bilateral HC injury had accompanying perinatal asphyxia. The incidence of basal ganglia or thalamus injury between patients with and without perinatal asphyxia did not differ (p = 0.434 and 0.560, respectively).

Clinical Parameters

No difference in clinical parameters was observed between patients with main or partial branch MCA strokes and those with the other stroke subtypes when considering the cohort as a whole. However, when considering the patients with PAIS without perinatal asphyxia, those with a main or partial branch MCA stroke had a lower umbilical cord pH (n = 36, median 7.20 vs 7.25, p = 0.027), and a trend toward lower Apgar scores at 1 minute (n = 83, median 8 vs 9, p = 0.091).

Compared with patients with PAIS with Normal or Equivocal MB, those with Injured MB had significantly lower Apgar scores at 1 minute (n = 118, median 4 vs 8, p = 0.031; 5 minutes: n = 120, median 7 vs 8, p = 0.070; 10 minutes: n = 102, median 8.5 vs 9, p = 0.197), a significantly lower umbilical cord pH (n = 69, median 7.08 vs 7.18, p = 0.024), and a lower base excess (n = 56, median −14.5 vs −8.0, p = 0.046). By contrast, no difference was observed in any of these clinical parameters when comparing patients with Equivocal MB injury with those with Normal MB (all $p > 0.5$). When patients with PAIS and co-occurring perinatal asphyxia were excluded from the comparisons, no difference in circulatory measures between patients with Injured and Normal MB were observed (all $p > 0.4$). No differences were found in circulatory parameters between patients with and without HC injury.

Table 1 Patient Characteristics for the Whole PAIS Cohort (Total Cohort); PAIS, No PA; and PAIS and PA

Patient characteristics	Total cohort (n = 130)	PAIS, no PA (n = 93)	PAIS and PA (n = 37)	*p* Value
Female sex, n (%)	62 (48)	51 (55)	11 (30)	**0.012**
Gestational age, wk, median (IQR)	40.4 (2.1)	40.1 (2.0)	40.7 (1.9)	0.063
Born preterm, n (%)	5 (4)	3 (3)	2 (5)	0.623
Birth weight (n = 128), g, median (IQR)	3,470 (637)	3,450 (619)	3,479 (730)	0.889
Delivery mode, n (%)				0.061
Vaginal	83 (64)	63 (68)	20 (54)	
Emergency cesarean	38 (30)	22 (24)	16 (43)	
Elective cesarean	9 (7)	8 (9)	1 (3)	
1-minute Apgar score (n = 118), median (IQR)	7 (5)	8 (3)	3 (6)	**<0.001**
5-minute Apgar score (n = 120), median (IQR)	8 (3)	9 (2)	5 (3)	**<0.001**
10-minute Apgar score (n = 102), median (IQR)	9 (2)	10 (1)	7 (3)	**<0.001**
Umbilical cord pH (n = 69), median (IQR)	7.17 (0.17)	7.22 (0.12)	7.06 (0.17)	**<0.001**
Umbilical cord base excess (n = 56), median (IQR)	−9.0 (8)	−6.4 (4.1)	−14.5 (8.2)	**<0.001**
Presenting symptoms, n (%)				**<0.001**
Seizures	100 (77)	80 (86)	20 (54)	
Encephalopathy	14 (11)	0 (0)	14 (38)	
Abnormalities seen in ultrasound or MRI	9 (7)	7 (8)	2 (5)	
Subclinical seizures on aEEG	1 (1)	1 (1)	0 (0)	
Infection	4 (3)	4 (4)	0 (0)	
Symptomatic hypoglycemia	2 (2)	1 (1)	1 (3)	
Age at onset of symptoms (n = 122), d, median (IQR)	1 (1)	1 (2)	0 (0)	**<0.001**
Therapeutic hypothermia, n (%)	8 (6)	0 (0)	8 (22)	**<0.001**

Abbreviations: aEEG = amplitude-integrated EEG; IQR = interquartile range; PAIS = perinatal arterial ischemic stroke; PAIS, no PA = patients with PAIS without perinatal asphyxia; PAIS and PA = patients with PAIS and perinatal asphyxia.
PA was defined as 5-minute Apgar score ≤5, resuscitation, mechanical ventilation during ≥10 minutes postpartum, pH <7.1, base excess <–16 mmol/L, or lactate >10.0 mmol/L in umbilical cord blood gas analysis or arterial, venous, or capillary blood gas analysis <1 hour after delivery. Not all data were available for all patients. For measures with missing data, the number of patients with available data is given in the row header. Significant p values are in bold.

Discussion

We performed a retrospective study to assess the involvement of the MB and HC in 130 neonates with PAIS. This is the first study describing MB injury after acute PAIS. We identified MB injury (i.e., clear injury across multiple MR sequences) in 16% of all patients, and further 21% of patients had equivocal MB changes (mild signal change or swelling on T2-weighted scans). In all but two patients, the injury was observed bilaterally. HC involvement was observed less often after acute PAIS, with only eight patients (6%) showing injury and, in five of them, the HC was part of the primary stroke lesion. The prevalence of HC injury in our cohort was slightly lower than the 13% previously reported in a study involving 22 patients with PAIS.[15] Over half of the patients had concomitant injury to the basal ganglia and thalamus, but there was no relationship between basal ganglia/thalamus injury and the presence of MB injury.

Most of the patients with PAIS had a stroke in the MCA territory, with an MCA perforator and posterior branch of the MCA being most frequently involved.[5] Up to half of all patients with a substantial MCA stroke showed an abnormal MB signal on MRI: 23% of these patients had clear MB injury, and further 24% had equivocal injury. In comparison, a multisite study of infants with perinatal asphyxia found an abnormal MB signal in approximately 40% of patients.[9]

In nearly all patients in whom MB injury was present, the injury was bilateral. By contrast, most cases involving the HC were unilateral, and in those cases, the HC was mainly included within the stroke territory. The greater prevalence of MB injury compared with HC injury, as well as the different pattern of unilateral/bilateral presentation, indicates that these brain structures are being affected by different mechanisms; that is, the HCs are directly affected by the stroke while

Table 2 MRI Characteristics for the Total Cohort and Groups Separated According to MB Status

MRI characteristics	Total cohort (n = 130)	Normal MBs (n = 79)	Equivocal MB injury (n = 27)	Injured MB (n = 21)	*p* Value
Age at neonatal MRI, d, median (IQR)	5 (3)	5 (3)	4 (2)	6 (2)	0.070
Side of the stroke, n (%)					0.267
Left	83 (64)	50 (63)	17 (63)	15 (70)	
Right	43 (33)	28 (35)	9 (33)	4 (19)	
Bilateral	4 (3)	1 (1)	1 (4)	2 (10)	
Arterial territory, n (%)					0.070
Perforator artery	26 (20)	20 (25)	3 (11)	2 (10)	
Posterior branch MCA	26 (20)	17 (22)	4 (15)	5 (24)	
Middle branch MCA	23 (18)	11 (14)	6 (22)	5 (24)	
Cortical artery	14 (11)	13 (17)	1 (4)	0 (0)	
Main MCA	21 (16)	10 (13)	7 (26)	4 (19)	
Anterior branch MCA	10 (8)	4 (5)	2 (7)	4 (19)	
ACA	1 (1)	0 (0)	1 (4)	0 (0)	
PCA	9 (7)	4 (5)	3 (11)	1 (5)	
Combined arterial territory					**0.015**
Perforator, cortical, ACA, PCA		37 (78)	8 (16)	3 (6)	
Main or partial branch MCA		42 (53)	19 (24)	18 (23)	
Basal ganglia involvement	82 (63)	38 (48)	10 (37)	9 (43)	0.595
Thalamus involvement	68 (52)	38 (48)	14 (52)	14 (67)	0.318

Abbreviations: ACA = anterior cerebral artery; IQR = interquartile range; MB = mammillary body; MCA = middle cerebral artery; PCA = posterior cerebral artery.
Not all data were available for all patients. Statistical analyses were performed using the 3 mammillary body scoring subgroups. The total cohort data are provided for comparison.

the MBs are affected through co-occurring and/or secondary processes.

Given the previous reports of MB injury in cases of perinatal asphyxia,[8,9] we wanted to assess whether MB injury observed in PAIS was related to the presence of perinatal asphyxia. In our study, 28% of patients with PAIS had perinatal asphyxia although only eight patients with PAIS (6%) met the criteria to receive therapeutic hypothermia. Previous studies reported around 8%–10% of cases with co-occurring perinatal asphyxia.[14,25] The difference in prevalence could be linked to the defined criteria used for perinatal asphyxia because the definition published by Cowan et al.[21] is less strict than the definitions published by American College of Obstetricians and Gynecologists.[26] For example, a large multinational cohort study found that significant early neonatal resuscitation (assisted ventilation, chest compressions, intubation, medications) was documented in 30% of cases but the term birth asphyxia was only used in 8% of cases.[14,25] In this study, patients with perinatal asphyxia were equally distributed across the stroke categories and had a higher incidence of Injured MB (30% vs 11%). A similar, but less pronounced, pattern was observed in those patients with Equivocal MB injury (27% vs 18%). While perinatal asphyxia likely contributes to the MB injury observed in PAIS, MB injury is not exclusively observed in neonates with co-occurring perinatal asphyxia. Furthermore, perinatal asphyxia does not always result in MB injury. As such, more than one underlying factor may be responsible for MB injury in PAIS, including additional clinical factors such as seizures and infections, and these may interact with individual differences in inflammation and oxidative stress responses.

The MBs are supplied with blood through the posterior communicating artery, which extends to the PCA; strokes involving the PCA might, therefore, be expected to result in higher rates of MB injury. When considering the subset of patients with a PCA stroke, there was evidence of abnormal MB signal in 50% of patients, which is similar to the incidence in larger strokes. However, as only eight patients in this cohort suffered a PCA stroke, the current findings are only indicative with larger numbers needed to make any definite statements.

Table 3 Patient Characteristics for Neonates With PAIS According to MB Injury

Patient characteristics	Normal MBs (n = 79)	Equivocal MB injury (n = 27)	Injured MB (n = 21)	*p* Value
Female sex, n (%)	36 (46)	14 (52)	10 (48)	0.852
Gestational age, wk, median (IQR)	40.4 (2.0)	40.0 (2.1)	40.3 (2.3)	0.649
Born preterm, n (%)	3 (4)	1 (4)	1 (5)	0.977
Birth weight (n = 125), g, median (IQR)	3,470 (630)	3,488 (723)	3,390 (800)	0.979
Delivery mode, n (%)				0.692
Vaginal	48 (61)	18 (67)	14 (67)	
Emergency cesarean	24 (30)	7 (26)	7 (33)	
Elective cesarean	7 (9)	2 (7)	0 (0)	
1-minute Apgar score (n = 116), median (IQR)	8 (3)	6 (4)	4 (6)	0.052
5-minute Apgar score (n = 118), median (IQR)	8 (3)	8 (2)	7 (5)	0.163
10-minute Apgar score (n = 101), median (IQR)	9 (2)	9 (3)	9 (4)	0.372
Umbilical cord pH (n = 68), median (IQR)	7.17 (0.16)	7.19 (0.24)	7.08 (0.21)	0.078
Umbilical cord base excess (n = 55), median (IQR)	−7.6 (5.8)	−9.1 (8.0)	−14.5 (10.4)	0.108
Presenting symptoms, n (%)				**0.014**
Seizures	66 (84)	22 (82)	11 (52)	
Encephalopathy	3 (4)	3 (11)	7 (33)	
Abnormalities seen in ultrasound or MRI	6 (8)	1 (4)	1 (5)	
Subclinical seizures on aEEG	1 (1)	0 (0)	0 (0)	
Infection	2 (3)	0 (0)	2 (10)	
Symptomatic hypoglycemia	1 (1)	1 (4)	0 (0)	
Age at onset of symptoms (n = 120), d, median (IQR)	1 (1)	0 (1)	0 (1)	0.557
Perinatal asphyxia, n (%)	15 (19)	10 (37)	11 (52)	**0.006**
Therapeutic hypothermia, n (%)	4 (5)	2 (7)	2 (10)	0.730

Abbreviations: aEEG = amplitude-integrated EEG; IQR = interquartile range; MB = mammillary body; PAIS = perinatal arterial ischemic stroke.
Perinatal asphyxia was defined as 5-minute Apgar score ≤5, resuscitation, mechanical ventilation during ≥10 minutes postpartum, pH <7.1, base excess <–16 mmol/L, or lactate >10.0 mmol/L in umbilical cord blood gas analysis or arterial, venous, or capillary blood gas analysis <1 hour after delivery. Not all data were available for all patients. For measures with missing data, the number of patients with available data is given in the row header.

While our retrospective study design enabled us to cover a longer time frame, it results in some limitations because of data availability. For example, the study only included neonates admitted to the NICU and so neonates with no neurologic symptoms but with (more minor) infarcts were not included in the study. Given the higher prevalence of MB injury in patients with larger strokes, the rate of MB injury may be lower once these patients with milder injury are considered. A further limitation is that even with 130 neonates included in this study, because of the variation in arterial territory involved and missing data for some of the clinical parameters, some of the comparisons are likely to have been underpowered. This highlights the need for larger—preferably multisite—studies in the future and studies that include neurodevelopmental outcomes. While neonatal MB injury in perinatal asphyxia has been associated with cognitive impairments at school age,[12] it is important to determine whether a similar pattern is found in patients with PAIS and whether differences in cognitive outcomes are observed in patients with MB injury, with and without perinatal asphyxia. A further limitation is that, in this study, only scans with acute injury were assessed; therefore, we cannot determine whether the patients with "Equivocal" MB injury result in atrophy at a later time point, especially because the patients with Equivocal MB injury tended to be scanned earlier than those with more definitive MB injury. Routinely assessing the MB in neonates with PAIS would enable patients with equivocal findings to be followed up, thereby determining the prognostic power of equivocal injury for later MB atrophy and neurodevelopmental outcomes.

Given the importance of the MB for memory function,[10,27-29] the prevalence of MB injury in this study suggests a possible early prognostic tool to inform caregivers and potentially target those patients with PAIS at high risk of cognitive

impairment in childhood. A better understanding of the mechanisms through which the MBs become injured in PAIS, both with and without co-occurring perinatal asphyxia, may help identify potential neuroprotective therapies and help improve long-term outcomes.

Author Contributions

M. Lequin: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. L.M. Baak: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. J. Dudink: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. S. Vann: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. F. Groenendaal: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. L. de Vries: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. M.J.N.L. Benders: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. C. Nijboer: drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data. N. Wagenaar: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. N.E. van der Aa: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

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