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Failla, Alberto

2022-11

Failla , A , Filatovaite , L , Wang , X , Vanhatalo , S , Dudink , J , de Vries , L S , Benders , M , Stevenson , N & Tataranno , M L 2022 , ' The relationship between interhemispheric synchrony, morphine and microstructural development of the corpus callosum in extremely preterm infants ' , Human Brain Mapping , vol. 43 , no. 16 , pp. 4914-4923 . https://doi.org/10.1002/hbm.26040

http://hdl.handle.net/10138/350358
https://doi.org/10.1002/hbm.26040

Received: 17 January 2022 | Revised: 15 July 2022 | Accepted: 25 July 2022

DOI: 10.1002/hbm.26040

RESEARCH ARTICLE

WILEY

The relationship between interhemispheric synchrony, morphine and microstructural development of the corpus callosum in extremely preterm infants

Alberto Failla[1] | Lauryna Filatovaite[1] | Xiaowan Wang[1] | Sampsa Vanhatalo[2,3] | Jeroen Dudink[1] | Linda S. de Vries[1] | Manon Benders[1] | Nathan Stevenson[4] | Maria Luisa Tataranno[1]

[1]Department of Neonatology, Wilhelmina Children's Hospital, Utrecht Medical Center, Utrecht, The Netherlands

[2]Department of Clinical Neurophysiology, BABA Center, Children's Hospital, HUS Diagnostics, Helsinki University Hospital, Helsinki, Finland

[3]Neuroscience Center, HiLife, University of Helsinki, Helsinki, Finland

[4]Brain Modelling Group, QIMR Berghofer Medical Research Institute, Brisbane, Australia

Correspondence
Maria Luisa Tataranno, Department of Neonatology, Wilhelmina Children's Hospital, Utrecht Medical Center, 3584 EA Utrecht, The Netherlands.
Email: m.l.tataranno-2@umcutrecht.nl

Funding information
European Commission - INFANS, Grant/Award Number: 813483

Abstract

The primary aim of this study is to examine whether bursting interhemispheric synchrony (bIHS) in the first week of life of infants born extremely preterm, is associated with microstructural development of the corpus callosum (CC) on term equivalent age magnetic resonance imaging scans. The secondary aim is to address the effects of analgesics such as morphine, on bIHS in extremely preterm infants. A total of 25 extremely preterm infants (gestational age [GA] < 28 weeks) were monitored with the continuous two-channel EEG during the first 72 h and after 1 week from birth. bIHS was analyzed using the activation synchrony index (ASI) algorithm. Microstructural development of the CC was assessed at ~ 30 and ~ 40 weeks of postmenstrual age (PMA) using fractional anisotropy (FA) measurements. Multivariable regression analyses were used to assess the primary and secondary aim. Analyses were adjusted for important clinical confounders: morphine, birth weight *z*-score, and white matter injury score. Due to the reduced sample size, only the most relevant variables, according to literature, were included. ASI was not significantly associated with FA of the CC at 30 weeks PMA and at 40 weeks PMA ($p > .5$). ASI was positively associated with the administration of morphine ($p < .05$). Early cortical synchrony may be affected by morphine and is not associated with the microstructural development of the CC. More studies are needed to evaluate the long-term effects of neonatal morphine treatment to optimize sedation in this high-risk population.

KEYWORDS
activation synchrony index, corpus callosum, diffusion tensor imaging, electroencephalography, fractional anisotropy

Abbreviations: ASI, activation synchrony index; AUC, area under the curve; bIHS, (bursting) interhemispheric synchrony; CC, corpus callosum; DTI, diffusion tensor imaging; E, epochs; FA, fractional anisotropy; FOV, fields of view; GA, gestational age; IQR, interquartile range; IVH, intraventricular hemorrhage; MRI, magnetic resonance imaging; NICU, neonatal intensive care unit; PDA, patent ductus arteriosus; PMA, postmenstrual age; TE, echo time; TEA, term equivalent age; TR, repetition time; WMI, white matter injury.

Alberto Faillo and Lauryna Filatovaite contributed equally to this study.

1 | INTRODUCTION

Early brain activity is important for brain development and later neurological outcome, as shown in vivo, in vitro, and in preterm neonates (for a review, Molnár et al., 2020). Electroencephalography (EEG) is a noninvasive and inexpensive bedside tool for evaluating the functional status of the newborn brain; it is assessed visually from the raw signals or from a time-compressed amplitude-integrated EEG (aEEG) trend display, both of which can be used for early prediction of neurodevelopmental outcomes in preterm neonates (Wikström et al., 2012). In the clinical tradition of EEG review, a key characteristic of a normal EEG is the visually observed interhemispheric synchrony (IHS) or simultaneity of bursting during quiet sleep (Lombroso, 1979). Since the introduction of a quantitative measure of bursting IHS (bIHS), known as activation synchrony index (ASI), several studies have shown its maturation during preterm development before term age (Benders et al., 2015; Koolen et al., 2017; Pavlidis et al., 2017; Räsänen et al., 2013). The emergence of bIHS of this kind is assumed to reflect growth and organization of interhemispheric connections via corpus callosum (CC; Scher, 1996; Vanhatalo & Kaila, 2006; Koolen et al., 2017; Kostović et al., 2019). This structure is essential and of highest importance for the development of later motor, perceptual, and cognitive functions, and due to its long myelinogenesis it is severely affected by preterm birth (Lean et al., 2019). Recently, a positive relationship was found between increased neuronal activity and structural brain development in preterm infants (measured as fractional anisotropy [FA] of the CC, right after birth; Tataranno et al., 2020). However, to this date, there are no studies available that investigated synchronicity as a predictive factor of a correct development of the CC. In this study, the authors aim to fill this gap in the literature by investigating early bIHS; as we suspect that this might play a predictive role in the later maturation of the CC. If true, this would allow practitioners to identify, early in their development, preterm infants at a higher risk of an abnormal neurological development.

The brain of extremely preterm infants is susceptible to external disturbances, and previous literature suggests that the majority of the neurodevelopmental impairments experienced by these infants is caused by the injury in the white matter microstructure (Dudink et al., 2008; Kim et al., 2016). The CC is one of the last brain structures to develop and it is especially vulnerable due to its long myelinogenesis throughout the last trimester (Lean et al., 2019). Specifically, in the second half of gestation, the CC's length increases by 150% and commissural fibers start to appear in the rostral part of the CC (Malinger & Zakut, 1993).

As the establishment of a higher degree of bIHS develops with progressive synaptogenesis in the CC (Koolen et al., 2017; Vecchierini et al., 2007), it seems likely that early and repeated monitoring of the aEEG synchrony (bIHS) may be predictive of its later maturation. Several studies investigated the relationship between magnetic resonance imaging (MRI) and EEG measurements in preterm infants (Benders et al., 2015; Tataranno et al., 2018). These studies, although establishing a direct link, and a possibly predictive role, between electrophysiological measurement of brain activity in preterm infants and structural measures, focused on bursts (SATs, SATs rate, and inter-SAT interval) of activity in a very short postnatal period (24 or 48 h). However, the link between early aEEG IHS and the later development of the CC has not been investigated yet.

The involvement of subcortical areas, different from callosal structures, has been proposed as a possible alternative mechanism in the development of early bIHS in preterm infants (Pfurtscheller et al., 2005; Scher, 1996; Stenberg, 1973). However, while acknowledging alternative proposal, considering the positive relationship between early neural activity and callosal development (Tataranno et al., 2020) and a possible nonlinear trend in the development of bIHS (Scher, 1996), we aim to investigate the role of the CC in this early stage of preterm development.

Moreover, after birth, extremely preterm neonates admitted to the neonatal intensive care unit (NICU) are usually exposed to up to 15 invasive procedures a day (e.g., intubation and mechanical ventilation) (Roofthooft et al., 2014). Thus, the NICU can become a very stressful and painful environment for them (Duerden et al., 2018). For these reasons, the use of sedative medications, such as morphine, is fundamental in the NICU, even if there are insufficient data recommending the routine use of opioids for pain management (Bellù et al., 2010).

Recent literature investigating the analgesic role of morphine on brain development, found a negative effect on cerebellar growth at term equivalent age (TEA; Tataranno et al., 2020), and an alteration of the normal cortical activity soon after the analgesic was dispensed in preterm neonates (Tataranno et al., 2020). Thus, the aim of this study is twofold. First, we aim to explore the association between aEEG/EEG's early synchrony patterns and the later microstructural development of the CC—assessed at 30 and 40 weeks of GA with diffusion tensor imaging (DTI)—in order to determine whether early aEEG/EEG measurement can be used as proxy tool alongside its bedside use to assess later microstructural development of the CC. Concurrently, we are interested in investigating in more details the effects of the use of analgesics, such as morphine, on the IHS of extremely preterm infants within 1 week after birth.

2 | MATERIALS AND METHODS

2.1 | Study population

A total of 61 extremely preterm neonates (GA < 28 weeks), born between May 2008 and November 2010, and admitted to the NICU of the Wilhelmina Children's Hospital (Utrecht, The Netherlands) were enrolled into this retrospective study. Patients were selected from a larger NEOBRAIN study cohort (www.clinicaltrials.gov/ct2/show/NCT00544895; Tataranno et al., 2018, 2020). Written parental consents and an approval from the medical ethical review committee were obtained for the NEOBRAIN study and a separate approval was obtained for this retrospective investigation. According to the standard clinical care and the NEOBRAIN study protocol, all infants were monitored with the continuous two-channel aEEG during the first 72 h of life and at 1 week after birth. Furthermore, they also underwent serial MRI of the brain at around 30 and 40 weeks of postmenstrual age (PMA). Only extremely preterm neonates (born <28 weeks of gestation) with complete aEEG traces and high-quality

DTI were included, reducing the final cohort to 25 neonates (Figure S1). Data of clinical conditions, such as intraventricular hemorrhage (IVH), white matter injury (WMI), periventricular leukomalacia, patent ductus arteriosus (PDA) both surgically and medically treated, cerebellar hemorrhage, necrotizing enterocolitis, bronchopulmonary dysplasia, and culture-proven sepsis were also recorded. IVH was diagnosed according to the classification described by Papile et al. (1978). WMI was assessed by two experienced neonatologists using a scoring system devised by Kidokoro et al. (2013). Since morphine administration has been previously found to depress brain activity (Tataranno et al., 2020), the dosage of morphine administered over the first week after birth was also collected (Table 1). Patient data were anonymized prior to analysis, and the study was approved by the medical ethical review committee of the University Medical Centre, Utrecht.

2.2 | aEEG monitoring and analysis

As soon as possible after birth (between 4 and 6 h after birth), continuous two-channel aEEG/EEG recording, at a sampling rate of 256 Hz, was started as standard of care. aEEG/EEG continued for at least 72 h after birth and repeated at 1 week of life. Infants were monitored using the BrainZ monitor (BRM3, BrainZ; Natus CA, Seattle, USA). According to the international 10–20 EEG classification, subcutaneous needle electrodes were placed in frontoparietal (F3, F4, P3, P4) positions, and a central electrode (Cz) has been chosen as a reference. Analyze 2.0 (BrainZ Instruments Ltd, Pakuranga Manukau, New Zealand) and Matlab R2019b (The MathWorks, Inc., Natick, MA) were used to analyze the aEEG/EEG traces. Analysis by the Analyze 2.0 package was used to read any annotations at the time of recording, evaluate the impedance of recording electrodes and identify artifacts/seizures. In all infants, 3–5 epochs (E) of 5 min were manually selected during a period of active sleep, as suggested by Koolen et al. (2017) and Räsänen et al. (2013). This process was completed for four different timepoints: 24 h ± 1 (E24), 48 h ± 1 (E48), and 72 h ± 1 (E72), 1 week (W1) after birth, and the median value among the selected epochs was calculated for each timepoint. We decided to select active sleep instead of quiet sleep as in extremely preterm infants, bursting activity tends to have a longer interburst intervals in quiet sleep and we would have failed to pick the most active moments of cortical activation. On the contrary, during active sleep, the cortex is most active (Räsänen et al., 2013). bIHS of brain activity was analyzed using the previously developed and validated ASI algorithm (Koolen et al., 2017; Räsänen et al., 2013). The higher the values of ASI, the stronger the relationship between the signals, and, thus, the synchrony of the brain activity between the cortical areas.

2.3 | MRI acquisition

MRI, with DTI, was performed at 30 and 40 weeks PMA as standard of care at the Wilhelmina Children Hospital. At 30 weeks PMA, MRI was performed using an MRI-compatible incubator (Dräger MR Incubator [Dräger, Lübeck, Germany] and later a Nomag IC 3.0 [LammersMedical Technology, Lübeck, Germany], with a dedicated neonatal head coil). Both the 30 and the 40 weeks MRI were performed on a 3 T MR system (Achieva, Philips Medical Systems, Best, The Netherlands), the latter using a SENSE head coil. In order to reduce movement artifacts, the infants were positioned in a vacuum pillow. During the entire MRI procedure, all infants were monitored for changes in their heart rate, oxygen saturation, respiratory rate and were sedated with oral chloral hydrate (30 mg/kg at 30 PMA and 50–60 mg/kg at 40 PMA), if needed. In addition, all neonates received two layers of hearing protection, including earmuffs (EM's Kids, Brisbane, Australia) and minimuffs (Natus Medical Incorporated, San Carlos, CA). At both timepoints (30 and 40 weeks of PMA), regular clinical protocol included coronal T1-, T2-weighted images (at 30 weeks: repetition time [TR] = 10.085 ms, echo time (TE) = 120 ms, slice thickness = 2 mm, at 40 weeks: TR = 4.847–6.293 ms, TE = 120–150 ms, slice thickness = 1.2 mm) and DTI images. The DTI images were acquired with single—shot spin—echo planar imaging sequence covering the whole brain (TR = 5685 ms, TE = 70 ms, fields of view = 180 × 146 mm, acquisition matrix = 128 × 102, reconstruction matrix = 128 × 128, slice thickness = 2 mm without gap). Diffusion gradients were applied in 32 noncollinear directions (b value = 800 s/mm^2) and included one nondiffusion-weighted scan (b value = 0 s/mm^2).

2.4 | DTI image analysis

Qualitative and quantitative analyses of the DTI images were completed while using the diffusion MRI toolbox Explore DTI (http://www.exploredti.com/; Leemans & Jones, 2009). The preprocessing and postprocessing procedures were performed according to the following steps: (i) Visual inspection of the scans; (ii) Exclusion of poor quality images; (iii) Correction for head movements and eddy current induced distortions (reorienting the B-matrix; Leemans & Jones, 2009); (iv) Diffusion tensor model fitting using a robust fitting approach (REKINDLE) for outlier detection and reweighted linear least squares estimation, after removal of data outliers (Leemans & Jones, 2009); (v) Registration to the JHU neonatal atlas (publicly available at: http://cmrm.med.jhmi.edu; Oishi et al., 2011), which has been shown to be a reliable tool to assess the microstructural development of the preterm brain (Kersbergen et al., 2014). All DTI images from all neonates were visually inspected, in order to check the quality of registration; (vi) Computation of FA values after registration. FA represents the variance of the eigenvalues, in the range of 0 (isotropic diffusion) to 1 (complete anisotropy), and reflects the organization of the white matter fibers and their structural integrity. FA was calculated in 122 brain regions, generated by the JHU atlas, but only FA values of the CC (FA of the CC) were used in this study.

2.5 | Morphine data

Morphine was administered on clinical indication, that is, sedation, intubation, mechanical ventilation, and so on. Morphine

TABLE 1 Baseline characteristics of the study population

Baseline characteristics of the study population	$n = 25$
Male/female, *n* (%)	17/8 (68%)
GA (weeks), mean (SD)	26.24 (0.88)
Birth weight, mean (g) (SD)	962 (153)
Birth weight *z*-score, mean (SD)	−0.318 (1.15)
HC, mean (cm) (SD)	24.73 (1.44)
Apgar score 1 min, median (IQR)	5 (2–7)
Apgar score 5 min, median (IQR)	8 (6–9)
PMA at 30 weeks scan (weeks), mean (min–max)	30.59 (28.71–32)
PMA at 40 weeks scan (weeks), mean (min–max)	41.26 (40.0–42.71)
Hypoglycemia (<2.5 mmoL/L)	
Once, *n* (%)	5 (20%)
Hypotension (MABP < GA)	
None	10 (40%)
Treatment: inotropes, *n* (%)	7 (28%)
Treatment: fluids and/or inotropes, *n* (%)	8 (32%)
IVH	
No, *n* (%)	17 (68%)
Yes (Grades I and II), *n* (%)	5 (20%)
Yes (Grade III), *n* (%)	3 (12%)
WMI	
No, *n* (%)	19 (76%)
Mild abnormality, *n* (%)	3 (12%)
Moderate–severe abnormality, *n* (%)	3 (12%)
PHVD	
No, *n* (%)	21 (84%)
Yes, *n* (%)	4 (16%)
PVL	
7 days of flaring or more, *n* (%)	23 (92%)
Flaring evolving into small frontoparietal cysts, *n* (%)	1 (4%)
Extensive cysts, *n* (%)	1 (4%)
PDA	
Yes, but no treatment needed	1 (4%)
Conservatively treated, *n* (%)	9 (36%)
Surgically treated, *n* (%)	2 (8%)
Cerebellar hemorrhage at TEA	
Normal	24 (96%)
< 6 punctate lesions, *n* (%)	1 (4%)
Necrotizing enterocolitis, *n* (%)	1 (4%)
Bronchopulmonary dysplasia, *n* (%)	13 (52%)
Culture-proven sepsis, *n* (%)	8 (32%)
Received morphine within 72 h after birth, *n* (%)	12 (48%)
Morphine dosage until Week 1	
Yes condition (mean)	1.12 mg/kg/day

Notes: Seven days of flaring for PVL refers to an appearance of relative increased echogenicity in periventricular white matter, persisting for at least 7 days but not undergoing cystic degeneration.
Abbreviations: GA, gestational age; HC, head circumference; IQR, interquartile range; IVH, intraventricular hemorrhage; MAPB, mean arterial blood pressure; PDA, patent ductus arteriosus; PHVD, posthemorrhagic ventricular dilatation; PMA, postmenstrual age; PVL, periventricular leukomalacia; TEA, term equivalent age; WMI, white matter injury.

administration (Yes/No) was marked during the first week of life. For the neonates who received morphine for 1 or more days, the final morphine dosage was calculated as the average of mg/kg/day over the first 7 days after birth (Table 1). Data were retrospectively obtained from patients' charts.

2.6 | Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows v 25.0 (IBM Corp., Armonk, NY) and MATLAB 2019b (The MathWorks, Inc., Natick, MA). FA values for both left and right side of the CC were corrected for PMA at the time of MRI, as already described by Kersbergen et al. (2014). A paired sample *t*-test was performed to determine the difference between FA of the left versus right side of the CC. Because no significant differences were found ($p > .05$), the average values of the FA of the CC at 30 and 40 weeks scan were used for all subjects. The reason to include just FA values as a measure of white matter development is two-folded: on the one hand, due to the reduced size of our sample, we decided—following past literature's findings—to include only the most powerful white matter measure to our analysis model; whereas, on the other hand, FA is the most sensitive measure for evaluating WM variances in preterm babies (Dudink et al., 2008).

The mean value of ASI at the four timepoints was compared using a repeated measure ANOVA and tested for significant difference. However, no significant increase of ASI over the four timepoints of interest was revealed from this statistical test. Instead, a single value of ASI was calculated for each participant using the area under the curve (AUC) analysis of the timepoints of interest: E24, E48, E72, and W1. A multivariate linear regression model was used to test the association of ASI (AUC) and FA of the CC over two timepoints (30 and 40 weeks of PMA).

An independent sample *t*-test was used also to compare whether morphine administration (dosage) significantly differed from the patients to which no morphine was delivered during the first week of life.

First, a linear regression analysis model was used to determine whether there was an age-dependent (GA) statistically significant difference in the AUC values of our cohort. Subsequently, in our multivariate regression analysis model—due to the small sample size—only the most influential clinical factors, selected based on the previous studies (Kersbergen et al., 2014; Tataranno et al., 2018), were included as covariates. We investigated four different multivariable analyses, where we included birth weight *z*-score, WMI score, morphine, ASI (as a single value among the four timepoints), and FA of the CC, either at 30 or at 40 weeks scan. In particular, morphine was investigated as morphine administration (Yes/No) during the first week of life, or morphine dosage (i.e., the total amount of morphine administered during the first 7 days of life).

In order to investigate the effect of morphine on the IHS of extremely preterm infants, two multivariate linear regression analysis models included birth weight *z*-score, and either morphine administration (Yes/No) in the first week of life, or morphine dosage in the first week after birth, and ASI values (as a single value among the four timepoints). A *p*-value < .05 was accepted as a cutoff for significance.

3 | RESULTS

3.1 | Population

Sixty-one neonates were initially enrolled in this study (GA < 28 weeks). Twenty-eight premature babies were excluded for various reasons, including death, no parental consent and low quality of the MRI scans. Moreover, a further eight patients were excluded because they displayed damaged and/or short aEEG recordings. Eventually, 25 extremely preterm neonates (GA min 24^{+5}–max 27^{+6} weeks) were included in the final cohort of this study. A linear regression model was used to examine whether there was a statistically significant relationship between GA and AUC values in our cohort. The results of this model showed that there is not an age-dependent (GA) difference in the AUC values of our cohort ($t = -0.576$, $p = .570$). Baseline characteristics of the study population are presented in Table 1. MRI, with DTI, was completed at a mean PMA of 31 (SD: 6 days; range 28–32) weeks and at a mean PMA of 41 (SD: 4 days; range 40–42) weeks. The majority of the neonates were scored as not having white matter abnormalities, and only six had either mild or moderate/severe white matter injuries. For the neonates with signs of hypotension, the mean dopamine dosage was 6 μg/kg/min. None of the included infants received any other medication, that has been proven to influence brain activity, except morphine. In particular, 12 neonates out of 25 (48%) received morphine during the first week of life.

3.2 | Early interhemispheric (a)synchrony of cortical activity

Changes of the ASI during the first week after birth (ASI at 24 h, Median = 4.74, interquartile range [IQR] = 1.06; ASI at 48 h, median = 4.69, IQR = 1.53; ASI at 72 h, median = 4.81, IQR = 1.94; ASI at Week 1, median = 4.69, IQR = 2.12) is shown in Figure 1a. No significant increase in ASI in the first week after birth was found (Wilks' Lambda = 0.679, $p > .05$). The AUC values of ASI over the four timepoints of interest (E24, E48, E72, and EW1) have a median value of $M = 10.40$ (IQR = 16.06; Min.: 4.99; Max.: 38.31).

3.3 | Microstructural development of the CC

Growth of the FA of the CC between 30 (mean = 0.25 [SD = 0.03]) and 40 (mean = 0.29 [SD = 0.03]) weeks scans is shown in Figure 1b. A *t*-test did show a significant difference in the FA of the CC at the two times of measurements; specifically, the analysis showed a higher FA of the CC at 40 weeks (Roland et al., 2017; $t = -5.055$, $p < .0001$).

3.4 | Relationship between interhemispheric (a) synchrony and microstructural development of the CC

The multivariable linear regression analysis was used to test the relationship between ASI and FA of the CC at 30 and 40 weeks scans. In particular, the values of ASI were calculated as the area under the curve over the four timepoints of interest (E24, E48, E72, and EW1). No significant association was found between ASI (AUC) and FA of the CC at 30 weeks (Table 2). No significant association was found, either, between ASI (AUC) and FA of the CC at 40 weeks (Table 2). Other clinical factors (birth weight *z*-score, WMI score, dosage of morphine) did not show a significant association with the microstructural development of the CC at 30 and 40 weeks PMA (Table 2). In particular, two different models were created to account for morphine. In Table 2, we represented morphine as a dichotomous value, Yes-Morphine and No-Morphine conditions during the first week of life; the scatter plots are shown in Figure 2b. Moreover, morphine was represented also as a continuous value of the analgesic administration during the first 7 days after birth (Table 3); the scatter plots are represented in Figure 2c,d.

3.5 | Relationship between interhemispheric (a) synchrony index and morphine administration

A multivariable linear regression analysis was used also to test the relationship between ASI and Morphine administration. ASI values were computed as the AUC over the four timepoints of interest (E24, E48, E72, and EW1); while morphine was computed in two different ways. One model considered morphine until 1 week of life as a dichotomous (Yes/No) variable; instead, in the second model, morphine was computed as a continuous variable of the total amount of analgesic administration during the first week of life. A significant association was found in both of our models (Table 3). Specifically, a significant positive association was found between the AUC values of ASI and morphine (Table 3). The multivariable regression model

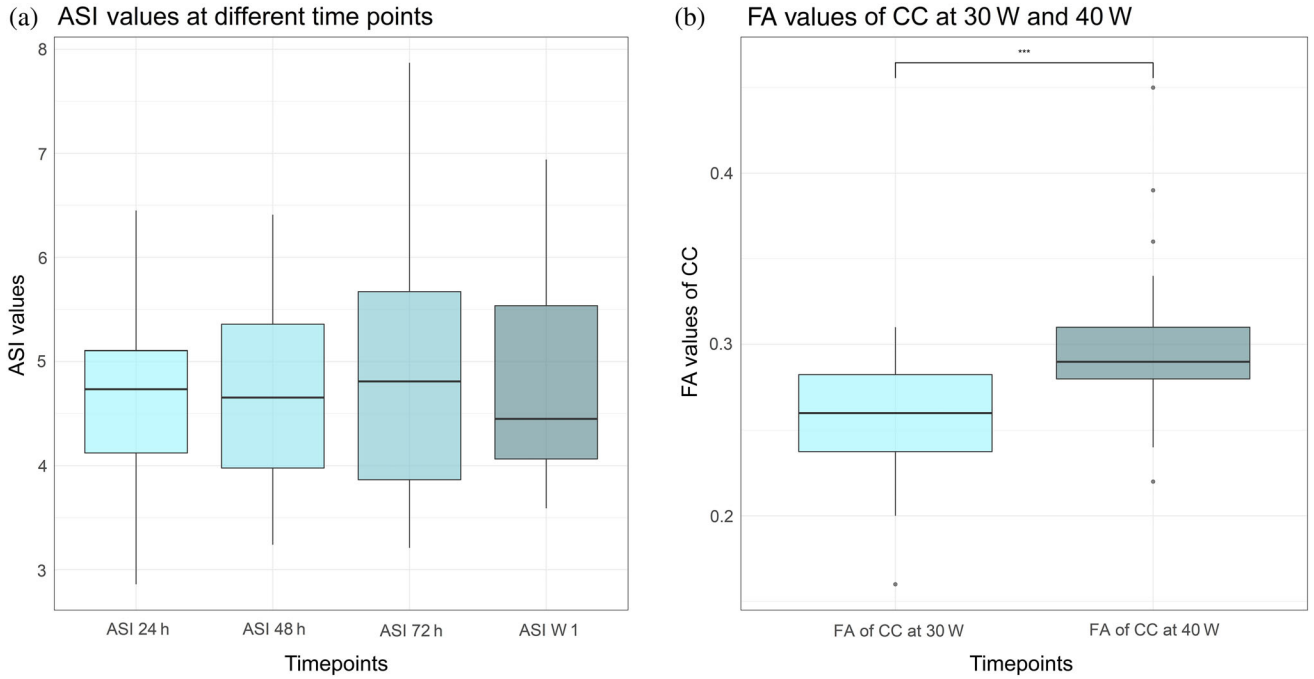

FIGURE 1 (a) Increase between 24 h and Week 1 for all 25 subjects are shown for activation synchrony index (ASI). Statistical analysis did not find significant differences between the different timepoints. (b) Fractional anisotropy (FA) of the corpus callosum (cc) at 30 and 40 weeks PMA for all 25 subjects. Statistical analysis did not find significant differences between the different timepoints. The FA values were corrected for postmenstrual age (PMA) at the time of the scan.

TABLE 2 ASI and microstructural development of the CC at 30 and 40 weeks scan

	FA of the CC (30 weeks; $n = 25$)	FA of the CC (30 weeks; $n = 25$)	FA of the CC (40 weeks; $n = 24$)	FA of the CC (40 weeks; $n = 24$)
	B (CI 95%) [p value]			
Morphine dosage until W1	-	−0.009 (−0.043; 0.025) [.630]	-	−0.019 (−0.057; 0.019) [.315]
Morphine Yes/No until W1	−0.007 (−0.047; 0.033) [.712]	-	−0.028 (−0.076; 0.021) [.25]	-
ASI, as AUC until W1	0.000 (−0.002; 0.002) [.776]	0.000 (−0.002; 0.003) [.676]	0.000 (−0.003; 0.002) [.930]	−0.000 (−0.003; 0.003) [.957]
WMI score	−0.020 (−0.045; 0.005) [.111]	−0.018 (−0.045; 0.009) [.170]	−0.015 (−0.047; 0.016) [.321]	−0.014 (−0.047; 0.019) [.386]
BW z-score	−0.002 (−0.018; 0.014) [.782]	−0.002 (−0.017; 0.013) [.788]	0.000 (−0.020; 0.019) [.968]	0.001 (−0.019; 0.020) [.941]

Notes: Rows are showing ASI and other clinical parameters included in the multivariable linear regression analysis. Columns show FA of the CC at 30 and 40 weeks scan. *b* Values with CI and *p* values are shown.
Abbreviations: ASI, activation synchrony index; BW, birth weight; CC, corpus callosum; CI, confidence intervals; FA, fractional anisotropy; WMI, white matter injury.

revealed that the administration of morphine until the first week of life resulted in higher value of ASI (computed as the AUC) (Table 3). The scatter plots are represented in Figure 2f. Moreover, morphine administration during the first 7 days after birth showed to result in higher AUC values for ASI (Table 3); scatter plots are shown in Figure 2e. Another clinical factor (birth weight z-score) was included in the model and did not show a significant association with the AUC of ASI values (Table 3).

4 | DISCUSSION

This study found no evidence that the early bIHS of brain activity, assessed with aEEG/EEG, is associated with microstructural development of the CC in extremely preterm infants. These findings suggest that the naturally occurring levels of cortical synchrony are robust to the varying levels of microstructural effects seen by prematurity (Koolen et al., 2017). However, morphine—a widely used analgesic

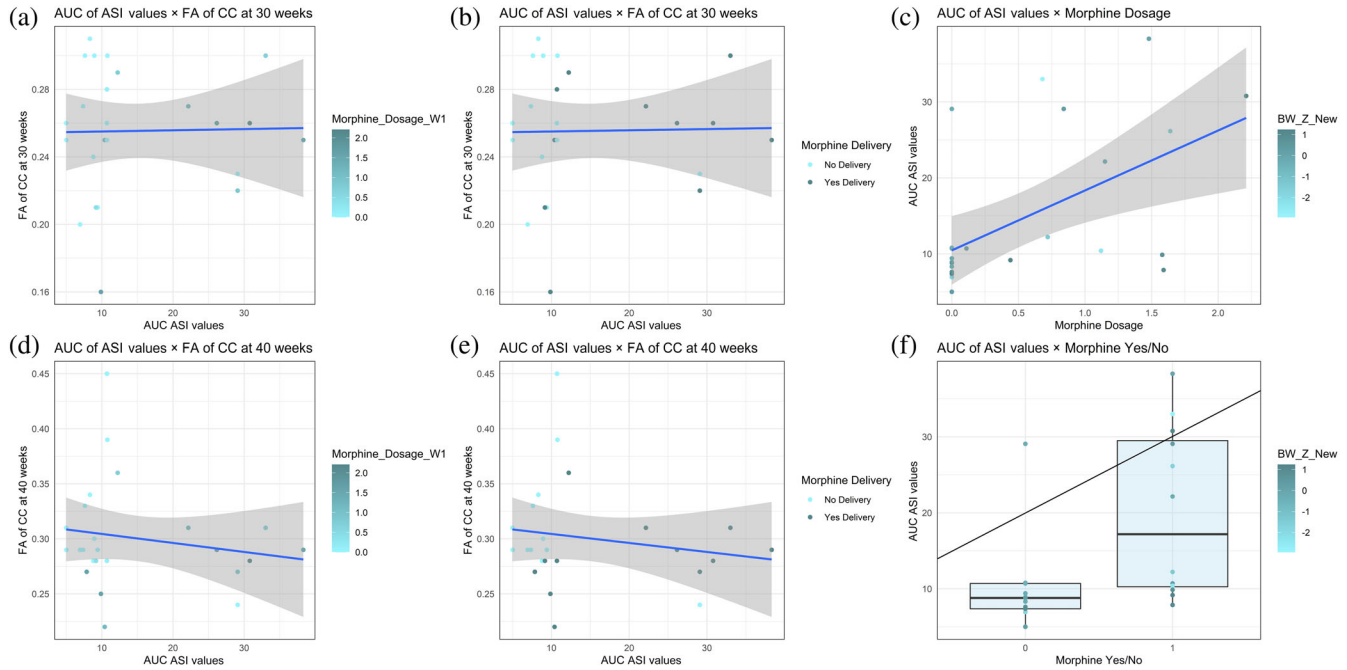

FIGURE 2 Multivariate linear regression analysis to investigate the association between fractional anisotropy (FA) of corpus callosum (CC) and ASI value; association of activation synchrony index (ASI) with FA of the CC at (a,b) 30 and (d,e) 40 weeks scan, in relation to morphine dosage (a,d) and morphine administration (b,e). Multivariate linear regression analysis including ASI of extremely preterm infants, birth weight *z*-scores (BW-Z) and either (c) morphine dosage or (f) morphine administration. Coefficients and confidence intervals, corrected for confounding clinical factors, are shown in Tables 2 and 3

TABLE 3 ASI and morphine until the first week of life, as either dosage or yes/no-measurement

	ASI (as AUC until W1; $n = 25$)	
	***b* (CI 95%) [*p* value]**	
Morphine dosage until W1	-	−1.719 (−4.936; 1.497) [.280]
Morphine Yes/No until W1	9.960 (2.545; 17.374) [.466]	-
BW *z*-score	−1.202 (−4.565; 2.162) [**.011**]	8.061 (2.926; 13.195) [**.004**]

Notes: Rows are showing Morphine and birth weight included in the multivariable linear regression analysis. Column shows ASI value as a single value until the first week of life. The *b* values with CI and *p* values are shown. Bold values indicate *p value* < .05.
Abbreviations: ASI, activation synchrony index; BW, birth weight; CI, confidence intervals.

treatment in the NICUs administered to the infants for clinical reasons, may affect interhemispheric communication, which adds to the growing body of evidence for morphine's subtle functional effect of potential developmental relevance (Tataranno et al., 2020).

The absence of a link between ASI and later CC's FA maybe due to maturational stage of infants in our work. Prior studies (Koolen et al., 2017; Scher, 1996) have shown a clear increase in ASI between 30 and 45 weeks PMA, which also correlates with the growth of cortico-cortical, callosal connections (Kostović et al., 2019). We found a negative correlation between ASI (measured as the AUC among the four timepoints of interest) and GA; however, we only followed a very short postnatal time frame at an earlier age. So, it is possible that the bIHS phenomenon measured with ASI in the early neonatal period does not yet reflect the underlying structural maturation since many other changes are going on during this vulnerable period (O'Toole et al., 2019; Scher, 1996).

4.1 | Neurobiology of postnatal adaptation and early structure development

We could not find significant association between early bIHS and microstructural development of the CC either at 30 or at 40 weeks PMA. As mentioned earlier, in human neonates, visually observed synchrony of brain activity has its early onset at around 30 weeks of gestation, and it gradually increases until TEA (Vanhatalo & Kaila, 2006). At term age, IHS is not uniform throughout the brain; being higher in the temporal region, followed by frontal, occipital, and parietal areas (Vanhatalo & Kaila, 2006).

The instantaneous development of bIHS between corresponding cortical regions from 34 weeks of gestation is thought to be a result of a rapid expansion of callosal connections, while the synchrony patterns before this period are caused by the thalamic/brainstem activity, thus loose and inconsistent (Kostović et al., 2019; Vanhatalo &

Kaila, 2006). For instance, around 26–27 weeks of GA it is possible to identify synchronous high-amplitude delta wave bursts in the occipital areas (Pavlidis et al., 2017); such an aEEG synchronous pattern is thought to be related to the immaturity of the cortex, while subcortical—thalamic control predominates and CC synaptogenesis is still ongoing (Koolen et al., 2017; Scher, 1996; Vecchierini et al., 2007).

Midbrain/brainstem early involvement and functional maturation, prior to superficial cortical structures, has already been seen directly in animals (guinea pigs; Stenberg, 1973) and indirectly observed in the heart rate variability, associated with bursts (a.k.a. SATs), in human preterm neonates (Pfurtscheller et al., 2005). In that early neonatal period (around 30 weeks of gestation), afferent fibers start to move toward the cortical plate and reach most brain areas, encouraging connections with the neurons of cortical layer IV (Kostović et al., 2019). Around 34 weeks of gestation, a robust development of commissural bundles, emerging from pyramidal neurons of cortical layer III, becomes visible, together with a rapid maturation of short cortico-cortical fibers (Kostović et al., 2019). As a result, the impact of subcortical activity on interhemispheric synchronization becomes less noticeable. This early predominance of subcortical control and the later development of the cortex provides a natural explanation for the significant changes in IHS as described by other groups (Kostović et al., 2019; Vanhatalo & Kaila, 2006) and a possible explanation of why it was not possible to find an association with later microstructural development in our cohort.

4.2 | Effect of morphine on early IHS

This study showed for the first time that morphine affects bIHS; with bIHS showing a significant increase when the analgesic is being delivered. A large number of studies showed the detrimental effects of pain on the correct brain development of preterm infants (e.g., Duerden et al., 2018), and its long-term consequences on the cognitive and motor development (Valeri et al., 2015). In particular, preterm infants display a lower threshold and enhanced reflex in responses to touch, when compared with TEA infants. Therefore, correctly assessing and addressing stressful, and potentially painful, experiences in this vulnerable population is of great relevance. For these reasons, the use of analgesics and opioids, such as morphine, is of vital importance for the treatment of procedural pain. However, recent studies show that morphine can negatively affect brain activity (Tataranno et al., 2020), and no final conclusions are drawn on the consequences of morphine administration on brain development in extremely preterm infants (Tataranno et al., 2020).

The results of this study suggest that morphine might have an effect on IHS. Previous investigations determined that a higher synchrony between hemispheres was a key feature of normal development of the infant's brain (Lombroso, 1979); for instance, higher bIHS values are a sign of normal CC development (Dudink et al., 2008; Kostović et al., 2019); however, at later GA/PMA. This is the first study evaluating the effect of morphine on aEEG synchronicity in extremely preterm infants. Recently, the strict monitoring of pain and stress using pain scales and comfort scores in high-risk infants, has led to a more individualized and patient-tailored use of sedatives. The results of our study may suggest that correctly addressing the painful sensations experienced by infants using the right amount of sedative, may be beneficial for the increase of IHS. In particular, the administration of morphine showed a significant relation with the subsequent increase in ASI values, during the first week after birth. However, it can also be hypothesized that at this early stage, hypersynchronicity is still a predominant feature and more mature aEEGs paradoxically show a lower bIHS (Scher, 1996; Vecchierini et al., 2007). Preterms' IHS follows a U-shaped trend, indicating that older infants (around the 30th week of gestation) should have a sparser synchronicity index, when compared with more immature infants (GA < 30 weeks; Scher, 1996). For this reason, we suspect that the high IHS shown by those infants requiring morphine, might be a sign of delayed maturation, rather than a positive indication of successfully addressed painful experience.

Moreover, this study shows once again that brain activity is sensitive to external disturbances, and sedatives and these need to be considered when analyzing aEEG recordings.

Further research is necessary to investigate the role of morphine on ASI and its short-term and long-term effects. As mentioned earlier, few studies investigated the effect of lower bIHS values on cerebral injuries such as hypoxic ischemic encephalopathy or periventricular leukomalacia (Leroy-Terquem et al., 2017). However, the interaction between morphine, IHS and long-term outcome still remains controversial. Future research should also focus on this interaction in order to disentangle the three-folded relationship between morphine dosage (low or high dose), IHS and the further implications on a clinically normal development of brain activity in preterm infants.

4.3 | Limitations

This study has some limitations. First of all, the small sample size reduces the statistical power of our results, and we cannot completely exclude that the absence of association between bIHS and microstructure is not due to the small samples size. Moreover, the small cohort reduced the number of clinical confounders that could be included in the multivariate analysis; therefore, the analyses could not be corrected for all the co-variables. However, this study is performed on a relatively healthy cohort of extremely premature infants. Furthermore, it has been shown by Koolen et al. (2017) and Räsänen et al. (2013) that ASI measurement can be easily distorted by an inappropriate selection of the aEEG epochs. However, we focused on active sleep periods, and we excluded all periods with artifacts, seizures and other factors that may have distorted the signal. Besides, ASI requires only rough temporal coordination between hemispheres, thus other newly developed quantitative synchrony measurements, such as a phase lag index (van de Pol et al., 2018), might be also examined. The very limited number of channels used in this study as well as its montage could have also missed regional asynchrony patterns shown by

Koolen et al. (2017). Last, a limited number of brain structures, as investigated in this study, yields the possibility to underscore the effect of other white matter commissures, as it appears that all major commissural tracts are responsible for the IHS in preterm infants (Roland et al., 2017). Further prospective research with larger sample size, multichannel EEG recordings, more clinical confounders, and more brain structures under investigation, will be of highest importance in order to reveal a possible relationship between aEEG synchrony patterns (possibly at later stages of development) and microstructural brain development.

5 | CONCLUSIONS

This study and the interpretation of the results seem to suggest that early bIHS is robust to variation in upcoming microstructural development of the CC. Instead, morphine treatment was significantly correlated to an increase in ASI values early after birth. Thus, this study adds to the growing literature that the administration of sedative medications, such as morphine, should be considered when interpreting neonatal aEEG/EEG, as this may slow the natural trend of interhemispheric communication in preterm infants.

ACKNOWLEDGMENTS

The authors acknowledged the financial support of EU H2020 MSCA-ITN-2018: INtegrating Functional Assessment measures for Neonatal Safeguard (INFANS), funded by the European Commission under Grant Agreement no. 813483.

DATA AVAILABILITY STATEMENT

The data that support the findings of this article are available upon request.

ORCID

Alberto Failla https://orcid.org/0000-0003-4949-8500

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Failla, A., Filatovaite, L., Wang, X., Vanhatalo, S., Dudink, J., de Vries, L. S., Benders, M., Stevenson, N., & Tataranno, M. L. (2022). The relationship between interhemispheric synchrony, morphine and microstructural development of the corpus callosum in extremely preterm infants. *Human Brain Mapping*, *43*(16), 4914–4923. https://doi.org/10.1002/hbm.26040