



Association between sleep stages and brain microstructure in preterm infants: Insights from DTI analysis

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ABSTRACT

Study objectives: The aim of this study was to investigate the relationship between sleep stages and neural microstructure – measured using diffusion tensor imaging – of the posterior limb of the internal capsule and corticospinal tract in preterm infants.

Methods: A retrospective cohort of 50 preterm infants born between 24 + 4 and 29 + 3 weeks gestational age was included in the study. Sleep stages were continuously measured for 5–7 consecutive days between 29 + 0 and 31 + 6 weeks postmenstrual age using an in-house-developed, and recently published, automated sleep staging algorithm based on routinely measured heart rate and respiratory rate. Additionally, a diffusion tensor imaging scan was conducted at term equivalent age as part of standard care. Region of interest analysis of the posterior limb of the internal capsule was performed, and tractography was used to analyze the corticospinal tract. The association between sleep and white matter microstructure of the posterior limb of the internal capsule and corticospinal tract was examined using a multiple linear regression model, adjusted for potential confounders.

Results: The results of the analyses revealed an interaction effect between sleep stage and days of invasive ventilation on the fractional anisotropy of the left and right posterior limb of the internal capsule ($\beta = 0.04$, FDR-adjusted $p = 0.001$ and $\beta = 0.04$, FDR-adjusted $p = 0.02$, respectively). Furthermore, an interaction effect between sleep stage and days of invasive ventilation was observed for the radial diffusivity of the mean of the left and right PLIC ($\beta = -4.1e-05$, FDR-adjusted $p = 0.04$).

Conclusions: Previous research has shown that, in very preterm infants, invasive ventilation has a negative effect on white matter tract maturation throughout the brain. A positive association between active sleep and white matter microstructure of the posterior limb of the internal capsule, may indicate a protective role of sleep in this vulnerable population.

1. Introduction

Preterm infants are born during a crucial period of brain maturation. Moreover, preterm birth is associated with negative consequences for long- and short-term neurodevelopment [1,2]. The negative effect of preterm birth on white matter integrity is quantifiable in vivo by calculating the fractional anisotropy (FA) from diffusion MRI (dMRI). FA assessment has shown that white matter microstructure is compromised in preterm infants, even in the case of only mild white matter injury visible from conventional neuroimaging techniques (e.g., ultrasound) [3]. This dysmaturation of the white matter microstructure in preterm infants is thought to be related mainly to a lack of myelination by (pre)

oligodendrocytes and consequently impaired axonal development [3].

In preterm infants at risk for white matter injury, cerebral MRI scans are commonly performed in clinical practice at term equivalent age (TEA). The posterior limb of the internal capsule (PLIC) and corticospinal tract (CST) are among the areas with the greatest predictive value for future neurodevelopmental outcomes in infants born preterm [4–6]. Both the PLIC and the CST are primarily involved in motor outcomes [7, 8]. Several perinatal and neonatal factors are known to influence the development of the PLIC and CST, including gestational age at birth, birth weight, sex, illness severity, and nutrition [9–13]. Notably, white matter injury is often found in infants after prolonged mechanical ventilation [9], possibly due to increased immaturity, increased risk of

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inflammation, and long-term intensive care [9,14]. Even in the absence of evident or visible brain injury, these mechanically ventilated infants are more at risk for long-term developmental problems, including motor development [15,16].

Another factor that may be related to PLIC and CST development is sleep quality and quantity [17]. In infants, sleep consists of two main stages: active sleep (AS; the neonatal equivalent of REM sleep in adults) and quiet sleep (QS; the neonatal equivalent of non-REM sleep in adults) [18]. An increasing number of animal studies link AS to sensorimotor development [19–24]. During AS, the limbs of animals tend to show endogenously generated myoclonic movements or twitches. These movements have been linked to functional connectivity development. Infants exhibit similar motor activity—or twitches—during sleep and mainly during AS [25]. Furthermore, in infants, AS has been related to spontaneous activity transients (SATs) that may drive brain development [26].

Although the connection between sleep and brain development has been increasingly understood in animal studies, human research has not yet reached a clear conclusion on a causal relationship. It is currently unclear whether healthier (preterm) infants have a more typical brain structure, leading to a more mature sleep pattern, or if a more mature sleep pattern supports early neurodevelopmental processes. Nevertheless, researchers suspect that sleep may be a significant factor in preterm brain development, particularly in sensorimotor networks such as the CST, based on animal studies and circumstantial evidence in humans [19–24].

To further elucidate the association between sleep and early human brain development, the aim of the current study was to investigate the association between sleep stages (AS, QS) and microstructural white matter development. Specifically, this association was investigated in the context of other well-known factors that influence sensorimotor development, such as GA at birth, PMA at scan, sex, birth weight, and days of invasive ventilation during NICU stay [9–13].

We hypothesize that more AS is associated with better microstructural white matter development, operationalized as greater fractional anisotropy (FA). We expect this association in the CST—mostly the PLIC—as animal research has shown an association between AS and the development of the CST [20]. Furthermore, CST and PLIC development are highly predictive of neurodevelopmental outcome in preterm human infants [4–6].

2. Methods

2.1. Participants

All extremely to very preterm infants (born at <30 weeks GA) were considered for the study. Infants were included if cardiorespiratory data were recorded for 5–7 consecutive days between 29 + 0 and 31 + 6 weeks PMA. Furthermore, infants were only included if they had DTI scans at term equivalent age without overt artifacts. Infants with overt brain injury (defined as the presence of an intraventricular hemorrhage grade III, arterial ischemic stroke, venous infarction, posthemorrhagic ventricular dilatation, or (cystic) periventricular leukomalacia) were excluded. Finally, infants who received mechanical ventilation or sedatives while 5–7 days of cardiorespiratory data were acquired, were excluded. Infants who received mechanical ventilation or sedatives outside this period were not excluded.

All enrolled infants were admitted to the NICU of the Wilhelmina Children's Hospital (Utrecht, The Netherlands). DTI data were acquired between January 2019 and February 2021. Permission to use patient data for our study was granted by the Medical Research Ethics Committee (MREC) of the University Medical Center (UMC) Utrecht (No. 21-066-C). Since MRI scanning at TEA and routine heart and respiratory rate monitoring are part of standard medical care for infants in our NICU who are born before 28 weeks GA, the MREC Utrecht waived the need for written parental consent. All the data were analyzed in a pseudo-

anonymous format.

2.2. Data acquisition

2.2.1. Sleep stage assessment

For all infants, heart and respiratory rates were measured with a standard bedside patient monitor (IntelliVue MP70; Philips Medical Systems, Böblingen, Germany) and stored at 0.4 Hz using software developed in-house (BedBase, UMC Utrecht, the Netherlands). These parameters were used to assess sleep stages for each recorded minute with an automated sleep stage classifier, which is based on heart and respiratory rate signals. For an extensive explanation of the development of the automated sleep stage classifier, see Wang et al. [27].

To address data gaps within these 5–7 days during which cardiorespiratory parameters were calculated, backfilling was employed, followed by forward filling. To ensure the reliability of the results, any segments with more than 10 min of data missing were excluded from the subsequent analyses. Based on the results of the algorithm, infants' sleep architecture could be described by the total sleep time per 24 h, active sleep percentage of the total sleep time, quiet sleep percentage of the total sleep time, and percentage of awake time.

2.2.2. DTI acquisition

For infants in the study cohort, MRI was performed at TEA (range 39+0–42 + 6 weeks PMA) on a clinical 3-T Philips Achieva scanner (Philips Healthcare, Best, The Netherlands) with a SENSE head coil. Diffusion-weighted images were obtained in the axial plane using diffusion gradients applied in 45 phase-encoding directions with a b-value of 800 s/mm², as well as one direction with a b-value of 0 s/mm².

2.3. Data analysis

2.3.1. Quality assessment

After DTI acquisition, image quality was visually assessed by an experienced neonatologist and neuroscientist (JD), and only scans without large movement artifacts or overt brain injury were used for subsequent analysis.

From the obtained DTI data, brain tissue was extracted using the Brain Extraction Tool (BET) from FSL [28]. The data were corrected for subject motion and eddy current distortion using ExploreDTI [29]. Subsequently, quality assessment was performed by assessing the average residuals for each DTI scan, as well as outlier profiles for each volume. In the case of a volume with more than 10 % outliers, a maximum of 4 vol were removed with the aim of improving image quality. Overall, for eight patients, 1–4 vol (median = 1) were removed.

2.3.2. Region of interest analysis

ROI analysis of the PLIC was performed via ExploreDTI following the regular procedure described in the manual [30]. The ROI was placed manually. The placement was determined using Wycoco et al. [31] and the Fiber Tract-based Atlas of Human White Matter Anatomy by Wakana et al. [32] as guidelines. Going through the slices superior to inferior, the first brain slice that showed an easily identifiable bilateral PLIC was chosen from an axial view (Fig. 1). The ROI was drawn on a black-and-white view to minimize bias in the simulated and probable directions created to view the scan.

After assessment by an experienced neonatologist and neuroscientist (JD), an additional AND ROI was drawn to maximize the possibility of including the CST, which is located in the posterior part of the PLIC.

2.3.3. Tractography

Tractography of the CST was performed via ExploreDTI following the procedure described in the manual [30]. First, whole-brain tractography was performed using a deterministic streamline approach based on the DTI model of ExploreDTI [29]. To optimize the fiber tracking and adjust for the size and maturation level of preterm infants, the following

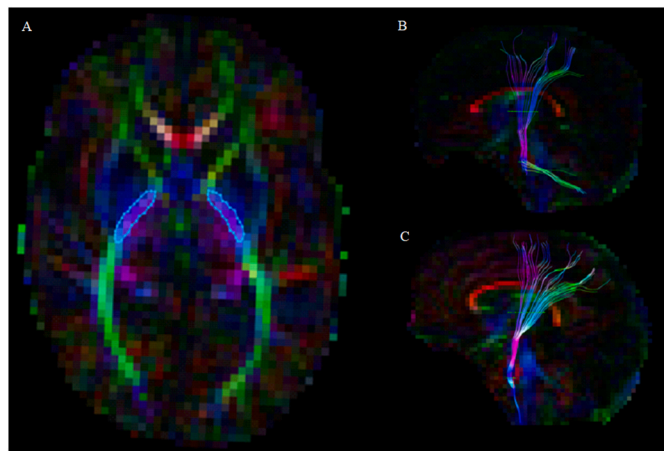


Fig. 1. (A) Axial view of a color-coded FA map, with the PLIC ROI outlined in blue. (B) Sagittal view of the extracted CST using an ROI at the PLIC and pons region with (C) and without (B) a ‘NOT’ ROI placed to exclude fibers projecting from the brainstem to the cerebellum. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

thresholds were used: minimum FA value of 0.1, curvature angle of $<45^\circ$ and seed point resolution of $2 \times 2 \times 2 \text{ mm}^3$ [33].

Two seed ROIs were chosen for specific reconstruction of the CSTs. First, the PLIC was used at the level of the lateral ventricles. Second, an ROI was drawn at the midlevel of the pons, the middle and superior cerebellar peduncles can be observed. Finally, a ‘NOT’ ROI was drawn on the coronal plane at the pons level to exclude fibers projecting in the posterior direction. All ROIs were placed separately for the left and right hemispheres.

After placing the ROIs, fiber tracking was performed from the whole-brain tractography file. An example in the sagittal view is shown in Fig. 1.

2.4. Statistical analysis

All the statistical analyses were performed in RStudio (version 2023.03.1). The percentage AS of total sleep time (%AS) was used as an independent variable. GA at birth, PMA at scan, assigned sex, birthweight in grams, and days on invasive ventilation during stay (DOV) were assessed as potential covariates. Assigned sex was excluded as a covariate because of the equal division between girls and boys in our sample and the lack of difference between %AS in boys versus girls. Furthermore, birthweight was excluded as a covariate due to its strong correlation with GA at birth. No outliers were found outside the natural range.

Assumptions (multicollinearity, outliers, and normal distribution) were tested. Furthermore, the difference in FA and MD between the left and right hemispheres was assessed for the PLIC and CST.

Finally, multiple linear regression was performed for all covariates, followed by multiple linear regression for %AS and all covariates. To further explore the effect of the DOV on the relationship between %AS and DTI microstructure, moderation analyses were performed with %AS and the DOV. For this moderation analysis, a cutoff of 7 DOVs was considered clinically relevant [34]. Finally, to estimate clinical relevance, the general weekly increase in FA values per brain region was used as a reference. [35].

3. Results

3.1. Demographics

A total of 61 extremely to very preterm infants were included. During

quality assessment 11 infants were excluded due to insufficient data quality (n = 5) or missing or corrupt data (n = 6), leaving a cohort of 50 infants.

The demographic and clinical information of the participants are summarized in Tables 1 and 2. Among infants who received mechanical ventilation during their NICU stay, significantly less time was spent in AS (49.4 % vs. 44.4 %, $t(43.7) = 2.45$, $p = 0.02$, 95 % CI = [0.010.09]), but this was not the case for W (10.2 % vs. 9.6 %, $t(35.5) = 1.07$, $p = 0.29$, 95 % CI = [-0.000.02]) between 29 and 32 weeks PMA.

No significant difference was found in the AS% or W% between male and female infants. Finally, birth weight and GA at birth were not associated with AS% or W%.

3.2. Region of interest analysis of the PLIC

No outliers were found, so all analyses were performed with 50 subjects. All assumptions for linear regression were checked and adhered to.

Compared with that of the right PLIC, the FA of the left PLIC was significantly greater ($t(49) = 2.18$, $p = 0.03$, 95 % CI = [0.000.02]). No significant difference was found between the MD of the left and right PLIC ($t(49) = -1.82$, $p = 0.08$, 95 % CI [-0.000.00]).

3.2.1. Models for fractional anisotropy

3.2.1.1. Left PLIC. The FA of the left PLIC could not be predicted using a model that included the DOV, GA at birth, and PMA at scan ($F(3,46) = 0.62$, $p = 0.60$; $\text{adj-R}^2 = -0.02$). Adding AS to the model did not significantly improve the predictive power ($F(1,45) = 4.00$, $p = 0.052$). Furthermore, the model with AS, GA at birth, PMA at scan, and DOV did not reach a significant level ($F(4,45) = 1.49$, $p = 0.22$; $\text{adj-R}^2 = 0.04$).

Moderation analysis revealed a significant interaction effect of DOV on the AS and FA values of the mean PLIC ($F(5,44) = 3.28$, $p = 0.01$; $\text{adj-R}^2 = 0.19$). Adding this interaction to the model improved the predictive power ($F(1,44) = 9.34$, $p = 0.004$).

3.2.1.2. Right PLIC. The FA of the right PLIC could not be predicted using a model that included the DOV, GA at birth, and PMA at scan ($F(3,46) = 0.42$, $p = 0.74$; $\text{adj-R}^2 = -0.04$). Adding AS to the model did not significantly improve the predictive power ($F(1,45) = 2.48$, $p = 0.12$). Furthermore, the model with AS, GA at birth, PMA at scan, and DOV did not reach a significant level ($F(4,45) = 0.94$, $p = 0.45$; $\text{adj-R}^2 = -0.005$).

Moderation analysis revealed a significant interaction effect of DOV on the AS and FA values of the mean PLIC (see Table 3, model performance; $F(5,44) = 2.48$, $p = 0.05$; $\text{adj-R}^2 = 0.13$). Adding this interaction to the model improved the predictive power ($F(1,44) = 8.04$, $p = 0.007$).

The interaction (Fig. 2) showed that in individuals with relatively more DOV (≥ 7 DOV), a positive association existed between AS and FA, whereas no association was present for individuals with relatively low DOV (< 7 DOV).

3.2.2. Models for radial diffusivity

The mean RD of the PLIC could not be predicted using a model including DOV, GA at birth, and PMA at scan ($F(3,46) = 0.54$, $p = 0.66$; $\text{adj-R}^2 = -0.03$). Adding AS to the model did not significantly improve

Table 1
Sleep characteristics.

	Sample (n = 50)
Total time per patient, minutes, mean (SD; range)	9772 (647; 7465–10081)
TST/24 h, minutes, mean (SD; range)	1297 (25; 1246–1371)
%AS/TST, mean (SD; range)	47 % (8 %; 28–66 %)
%QS/TST, mean (SD; range)	53 % (8 %; 34–72 %)
%W, mean (SD; range)	10 % (2 %; 5–13 %)

Table 2
Patient characteristics.

	Sample (n = 50)
Sex	
Female, n (%)	26 (52 %)
Male, n (%)	24 (48 %)
GA at birth, weeks, mean (SD; range)	27.2 (1.3; 24.6–29.4)
Birth weight, grams, mean (SD)	973 (206)
PMA at start of automated sleep, assessment weeks, mean (SD; range)	29.85 (0.55; 29.00–31.14)
PMA at time of MRI scan, weeks, mean (SD; range)	41.10 (0.64; 39.86–42.86)
Infants with invasive respiratory support during NICU stay, n (%)	24 (43.6 %)
SIPPV, days, mean (SD; range)	2.8 (5.7; 0–27)
SIMV, days, mean (SD; range)	0.5 (1.5; 0–7)
HFO, days, mean (SD; range)	1.0 (3.5; 0–24)
Apgar score	
1 min, median (Q1, Q3)	5 (3, 7)
5 min, median (Q1, Q3)	9 (8, 9)
10 min, median (Q1, Q3)	9 (8, 9)

the predictive power ($F(1,45) = 3.04$, $p = 0.09$). Furthermore, the model with AS, GA at birth, PMA at scan, and DOV did not reach a significant level ($F(4,45) = 1.18$, $p = 0.33$; $\text{adj-R}^2 = 0.01$).

Moderation analysis revealed a significant interaction effect of DOV on the AS and RD values of the mean PLIC (see Table 3, model performance; $F(5,44) = 2.32$, $p = 0.06$; $\text{adj-R}^2 = 0.12$). Adding this interaction to the model improved the predictive power ($F(1,44) = 6.32$, $p = 0.02$).

Analyses of the AD and MD of the PLIC did not yield significant results (see Table 3).

3.3. Tractography of the corticospinal tract

No outliers were found, so all analyses were performed with 50 subjects. All assumptions for linear regression were checked and adhered to. No significant differences were found in the FA, MD, AD, or RD of the left or right CST. Therefore, all subsequent analyses were performed with the mean value between the left and right sides. Analyses of the FA, MD, RD, or AD of the CST did not yield significant results.

4. Discussion

The current research included preterm infant sleep stages as a modifying variable in microstructural white matter analysis using DTI at TEA. The results of our study showed that infants who spend more days on invasive respiratory support have a stronger positive association between active sleep and white matter microstructure in the PLIC at TEA.

We performed ROI analysis in the PLIC and tractography analysis in the CST. The PLIC was chosen a priori because previous quantitative MRI evaluations at TEA showed that the PLIC has high predictive power for future neurodevelopmental outcomes [4]. A significant difference of 0.008 was found between the FA values of the left and right PLIC, which corresponds to approximately 4 days according to the general FA increase—or slope—of the PLIC, as reported by Rogers et al. [35] This finding is in line with previous findings that the left PLIC matures faster [38]. An interaction effect was found between AS and DOV for FA of both the left and right PLIC.

The interaction effect between AS and DOV for the FA of the PLIC showed that for infants with more DOV, a positive association existed between AS and FA, whereas this association was not significant for infants with fewer DOVs. In the group that spent 7 or more days on mechanical ventilation, a 9 percent point increase in AS was related to a 0.015-point increase in FA (for median DOV = 13). A 0.015-point increase in FA corresponds to 1 PMA week according to the general FA increase—or slope—in the PLIC, as reported by Rogers et al. [35].

Table 3
Model performance.

		Covariate performance		Model performance			Model improvement
			β (corr. p)	F-stat	p	adj-R ²	p
FA Left PLIC	DOV	–0.00 (0.49)	0.62	0.60	–0.02		
	GA at birth	–0.00 (0.72)					
	PMA at scan	0.00 (0.49)					
	DOV	–0.00 (0.55)	1.49	0.22	0.04	0.052	
	GA at birth	–0.00 (0.62)					
	PMA at scan	0.00 (0.42)					
	%AS	0.12 (0.21)					
	DOV	–0.02 (0.001**)	3.28	0.013*	0.19	0.004**	
	GA at birth	0.00 (0.70)					
	PMA at scan	0.00 (0.48)					
	%AS	0.03 (0.70)					
	DOV*	0.04 (0.001**)					
FA right PLIC	DOV	–0.00 (0.59)	0.42	0.74	–0.04		
	GA at birth	–0.00 (0.59)					
	PMA at scan	0.00 (0.59)					
	DOV	–0.00 (0.58)	0.94	0.45	–0.00	0.12	
	GA at birth	–0.00 (0.58)					
	PMA at scan	0.00 (0.51)					
	%AS	0.10 (0.49)					
	DOV	–0.02 (0.02*)	2.48	0.05*	0.13	0.007**	
	GA at birth	0.00 (0.87)					
	PMA at scan	0.00 (0.58)					
	%AS	0.01 (0.87)					
	DOV*	0.04 (0.02*)					
RD mean PLIC	DOV	1.3e-07 (0.84)	0.53	0.66	–0.03		
	GA at birth	2.4e-07 (0.84)					
	PMA at scan	–1.6e-06 (0.64)					
	DOV	2.6e-08 (0.97)	1.18	0.33	0.01	0.09	
	GA at birth	3.1e-07 (0.86)					
	PMA at scan	–1.8e-06 (0.30)					
	%AS	–1.2e-04 (0.30)					
	DOV	1.8e-05 (0.04*)	2.32	0.06	0.12	0.02*	
	GA at birth	–1.9e-07 (0.78)					
	PMA at scan	–1.5e-06 (0.34)					
	%AS	–3.4e-05 (0.78)					
	DOV*	–4.1e-05 (0.04*)					

(continued on next page)

Table 3 (continued)

		Covariate performance		Model performance		Model improvement	
		β (corr. p)	F-stat	p	adj-R ²	p	
MD mean PLIC	DOV	−4.5e-07 (0.72)	0.77	0.52	−0.01		
	GA at birth	8.2e-09 (0.98)					
	PMA at scan	−5.1e-07 (0.75)					
	DOV	−4.9e-07 (0.56)	0.88	0.48	−0.01	0.28	
	GA at birth	3.7e-08 (0.92)					
	PMA at scan	−5.9e-07 (0.58)					
	%AS	−4.6e-05 (0.56)					
	DOV	3.7e-06 (0.74)	0.86	0.51	−0.01	0.37	
	GA at birth	−7.7e-08 (0.86)					
	PMA at scan	−5.2e-07 (0.74)					
	%AS	−2.6e-05 (0.74)					
	DOV*	−9.4e-06 (0.74)					
	%AS						
AD mean PLIC	DOV	−1.6e-06 (0.20)	1.42	0.25	0.02		
	GA at birth	−4.5e-07 (0.63)					
	PMA at scan	1.7e-06 (0.49)					
	DOV	−1.5e-06 (0.33)	1.37	0.26	0.03	0.28	
	GA at birth	−5.1e-07 (0.58)					
	PMA at scan	1.9e-06 (0.37)					
	%AS	1.0e-04 (0.37)					
	DOV	−2.5e-05 (0.053)	2.36	0.06	0.12	0.02*	
	GA at birth	1.4e-07 (0.92)					
	PMA at scan	1.5e-06 (0.61)					
	%AS	−1.0e-05 (0.92)					
	DOV*	5.4e-05 (0.053)					
	%AS						

Model performance of multiple linear regression for predicting the white matter microstructure of the PLIC. P values ≤ 0.05 are indicated with an asterisk (*). P values ≤ 0.01 are indicated with two asterisks (**). All p values are corrected using FDR-correction [36]. R² is adjusted using the Wherry formula [37].

The interaction between AS and DOV might indicate that AS plays a protective role in white matter microstructure. This theory is supported by the interaction effect between AS and DOV on RD in the PLIC, displaying a negative association between AS and RD in infants with 7 or more DOVs. Furthermore, the interpretation of white matter microstructure parameters is increasingly reliable if effects are found across different parameters [39]. A higher RD, combined with an unchanged AD, is associated with dysmyelination [40]. The association of AS with higher FA, lower RD, and unchanged AD points to a generally positive association between AS and white matter microstructure.

Mechanical ventilation has been related to decreased FA in the internal capsule at adolescence [9] and decreased fine and gross motor

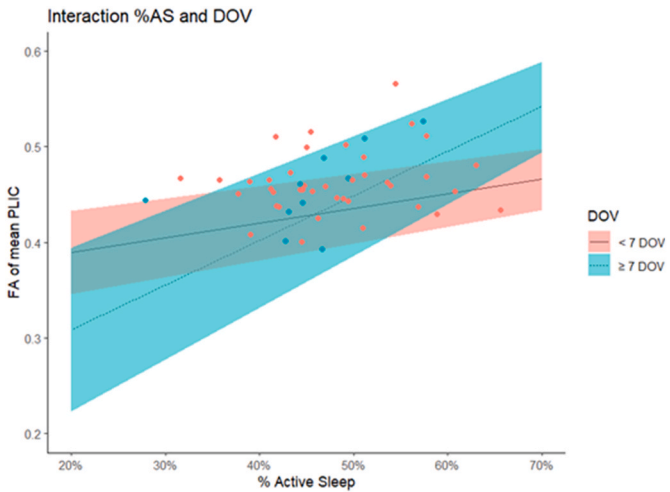


Fig. 2. Interaction between DOV and %AS for FA values of the mean of the left and right PLIC. The ‘high DOV’ group (n = 9) consisted of infants who spent 7 days or more on invasive ventilation. The ‘low DOV’ group (n = 41) consisted of infants who spent less than 7 days on invasive ventilation. Slopes were calculated using the median of each group (13 and 0 days on invasive ventilation respectively).

outcomes at two years of corrected age [41]. Furthermore, mechanical ventilation has been associated with decreased fiber bundle cross-section and fiber density and cross-section in the corticospinal tract [42].

Brain damage after mechanical ventilation is thought to be caused mainly by injury to developing oligodendrocytes [43]. Inflammation caused by ventilation-induced lung injury is thought to disrupt pre-oligodendrocyte development and, as a consequence, white matter integrity, including hypomyelination and impaired axonal development [3,14,44,45]. In addition, more days of mechanical ventilation coincides with increased use of medication that may influence brain development, such as steroids or sedation [46]. Finally, more days on mechanical ventilation as a preterm infant has been associated with neurodevelopmental impairment at two years of age [34,46,47].

In brief, white matter microstructure may be compromised by more DOVs and, related to this, increased illness severity. Previous studies have suggested that protective factors such as nutrition may be used to improve impaired brain development, including CST development, as a result of prolonged mechanical ventilation [48]. In addition to nutrition, sleep quality might also be a protective factor.

Nevertheless, infants who receive mechanical ventilation during their stay spend less time in AS. Therefore, the added benefit of spending more time in AS may have been greater in the ≥7 DOV group. Furthermore, ventilated preterm infants may experience more startles and twitches than nonventilated infants [49]. It is unclear whether this increase in reflexive movements is caused by respiratory support or by the fact that ventilated infants are more often placed in the supine position – as opposed to prone – during which more reflexive movements take place [50]. A greater density of twitches might cause more effective AS, resulting in a greater impact on brain development. However, in our sample, no infants were mechanically ventilated during sleep assessment. Therefore, it is unclear whether these speculations are applicable to the current data.

Some limitations and lessons learned during this research should be discussed. On the technical side, preterm brains are relatively small compared to the voxel size used. Therefore, DTI analyses in this group are often not highly specific. Therefore, the results for smaller structures should be interpreted carefully. Due to the relatively large voxel size, ROIs were drawn manually, as this approach was considered more reliable than using an atlas.

The current research included only the period between 29 and 32

weeks PMA because this period is important for brain development [51]. Furthermore, for preterm infants, the time in the NICU between 29 and 32 weeks is characterized by development and growth. The study setup could be improved in the future by monitoring sleep throughout the NICU stay, so sleep patterns can be investigated over the total time spent in the NICU. Finally, other influencing factors that are not considered in the current research – such as the effect of infection, nutrition, or positioning of the infant – should be corrected for in future analyses.

Since little research has been done on the association between white matter microstructure and sleep quality, no reliable sample size could be calculated. Although the current study yielded satisfactory results regarding effect sizes and clinical relevance, we suggest that future studies include more infants. In particular, the number of infants receiving 7 or more DOVs should increase, as the current subsample is too small to draw strong conclusions.

While increasing the sample size might improve the predictive power of the study, it remains unclear when increases in neonatal FA values are clinically meaningful. The current study used a general weekly increase in FA values per brain region based on one study as a reference. [33] However, this comparison only showed a linear increase in the FA value, without considering the clinical implications. Therefore, additional research should be conducted to provide insight into the association between sleep quality and quantity during the NICU stay and later neurodevelopmental outcomes.

Additionally, future research should investigate the difference in sleep quality and quantity between ventilated infants and nonventilated infants. Aside from a study by Chang et al. [50] and one by Karch et al. [49], the association between sleep stages and mechanical ventilation is little studied in preterm infants. Furthermore, the possible associations between the number of twitches, mechanical ventilation, and body position should be further investigated, preferably using EEG, video, and EMG data to specifically assess the number of SATs and twitches during AS.

Finally, to monitor the potential protective effect of AS, it is important to be able to continuously assess sleep stages in the NICU. In the neonatal field, sleep is increasingly considered in research and in clinical practice. This re-emerged interest is accelerated by new possibilities for monitoring sleep in infants [18,52]. Many researchers are working toward continuous monitoring of sleep stages in the NICU [53–56]. With these advancements in neonatal sleep monitoring, future research should focus on intervention studies to assess the feasibility and advantages of protecting sleep in the NICU.

5. Conclusion

In very preterm infants, invasive ventilation is associated with adverse impacts on the maturation of white matter tracts across the brain. Nevertheless, our research suggests that active sleep might serve a protective function in these high-risk infants, particularly by facilitating the development of the PLIC. Comprehending how sleep influences the microstructural development of the brain in preterm babies could be pivotal in devising specific non-pharmacological strategies to protect sleep to improve neurodevelopmental outcomes in this vulnerable group.

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CRediT authorship contribution statement

Eline R. de Groot: Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Xiaowan Wang:** Writing – review & editing, Software, Data curation. **Klaudia Wojtal:** Visualization,

Methodology, Data curation. **Els Janson:** Software, Resources, Methodology. **Thomas Alderliesten:** Writing – review & editing, Supervision. **Maria Luisa Tataranno:** Writing – review & editing, Supervision. **Manon J.N.L. Benders:** Writing – review & editing, Supervision. **Jerroen Dudink:** Writing – review & editing, Writing – original draft, Validation, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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