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Sleep Stage Assessment in Very and Extremely Preterm Infants: Exploring the Relationship Between Behavioural Classification and Quantitative EEG Features

A Master's Thesis

Neuroscience and Cognition

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ABSTRACT

Objective Identification of sleep and wake states is important for the clinical and neurophysiological assessment of infants born preterm admitted to the neonatal intensive care unit (NICU). As the importance of different sleep stages on neurodevelopment and long-term prognosis is currently unravelling, there is a high demand for quantification of sleep stages. Electroencephalography (EEG), especially two-channel amplitude-integrated EEG, is a commonly used neuromonitoring tool in daily care of preterm infants due to its ease of use. The present study aims to investigate whether quantitative EEG (qEEG) features can differentiate the different stages of sleep in very and extremely preterm infants.

Methods Three-hour behavioural sleep observations were performed for 17 very and extremely preterm infants who were born before 30 weeks of gestation, within the first three days of life. The behavioural sleep stage classification scores were acquired using an observational score validated for preterm infants <30 weeks postmenstrual age. Several qEEG features were extracted from raw signals of the two-channel (a)EEG, including burst features (proportion spontaneous activity transients, SAT%; inter-SAT percentage, ISP; and inter-SAT interval, ISI), interhemispheric synchrony (Activation Synchrony Index, ASI) and absolute and relative spectral power of the delta frequency band. Differences of these qEEG features among different sleep stages were analysed by ANOVA.

Results Significant differences were found in several qEEG features at different sleep stages, mostly between Active Sleep (AS) and Quiet Sleep (QS). Specifically, the SAT%, ASI and absolute delta power of AS were significantly higher than that of QS, while ISP and ISI were higher for QS than for AS.

Conclusion Several qEEG features were identified that can differentiate different sleep stages, and thus, could be beneficial to the improvement of sleep stage classification. The present study sets the foundation for the development of an automatic sleep assessment tool using EEG for the very to extremely preterm population. Ultimately, this will eventually lead to the improvement of neurodevelopmental outcome in the very to extremely preterm infants in NICUs worldwide.

I. INTRODUCTION

A. The Vulnerable Preterm Infant

Unlike full-term infants, preterm born infants spend the majority of the late second and third trimesters developing in an incubator rather than within the protective environment of the mother's womb. In the womb, sleep is believed to be the major driver of neural activity in the foetus, and a process that is critical for neuronal survival, axonal guidance, and synapse maturation [3]. Therefore, sleep should be promoted and protected in neonatal intensive care units (NICUs), to protect the development of the vulnerable preterm brain. However, in the NICU, preterm infants are exposed to a myriad of extrinsic stimuli that radically alter their sleep-wake states, such as bright light, invasive procedures, loud noise and care giving activities [2], [4]. Specifically in the early stages of brain development, insufficient sleep in infants born preterm has been linked to impaired cognitive, psychomotor and behavioural development [5]–[7].

B. Preterm Sleep Organization

From 24-32 weeks gestational age (GA) onward, preterm sleep can be divided into different sleep stages, including Active Sleep (AS), Quiet Sleep (QS) and Intermediate Sleep (IS) [5] (a detailed overview of sleep stage development and maturation is given in Appendix I, supplemental text box). AS is characterized by eye movements, a high activity level of both body and face, sounds and rapid brain activity, [8], [9]. It is considered as the preterm precursor of the adult Rapid Eye Movement (REM)-sleep and is believed to be the sleep stage that most contributes to brain maturation [10]–[13]. QS on the other hand, as the preterm precursor of non-REM (NREM) sleep, is characterized by a low activity level with slower brain activity than AS [8], [9]. QS seems to be essential for consolidating AS-driven brain maturation and for preserving neural plasticity [10]. Lastly, Intermediate Sleep (IS) is known as a transitional stage between AS and QS [9]. Sleep deprivation, especially the deprivation of AS, is associated with adverse health consequences, such as behavioural problems, sleep disturbances and reduced cerebral cortical size later in life [14]–[16].

C. Sleep Assessment in Preterm Infants

Thus, assessment of sleep can be a valuable biomarker of long-term neurodevelopmental outcome. Furthermore, sleep and wake states could be used as a clinical assessment tool for the identification of optimal scheduling of elective interventions and care procedures, by preventing unnecessary interruption of the neural processes underlying sleep [17]. To this end, it is important that sleep stages can be classified in a reliable and valid way. The current gold standard to differentiate sleep stages in extremely to very preterm infants is commonly believed to be behavioural classification by bedside

observation [18], [19]. However, behavioural state classification is very labour-intensive and requires well-trained observers [20]–[22]. Another frequently-used sleep monitoring tool is polysomnography (PSG) [23], which combines vital sign measurement methods [19]. The vital signs are commonly retrieved via obtrusive methods, encompassing electrodes and cables, which are not well tolerated by the vulnerable preterm infant's skin [19]. Moreover, comprehensive PSG-monitoring is not readily available in most NICUs [24]. Until now, no reliable technique for automatic real-time bedside recognition of sleep stages in preterm infants is clinically available. While several techniques exist or are under development [22], [25]–[27], most are not yet clinically validated in preterm infants <30 weeks postmenstrual age (PMA).

Two-channel electroencephalography (EEG) monitoring is considered a valuable tool to monitor neurodevelopment in very preterm infants [28] and believed to be a useful to discriminate sleep stages [29]. Two-channel EEG (i.e., the raw signals retrieved from amplitude-integrated EEG [aEEG]) is routinely assessed in preterm infants by a growing number of NICUs, particularly for the first days of life. This makes two-channel (a)EEG an easily accessible technique, with the major advantage over other techniques that it allows around the clock recording and can be used already in very immature preterm infants [30]. Moreover, two-channel (a)EEG also seems a promising candidate for long-term future monitoring with the current development of less obtrusive dry electrodes [19], [31], [32].

Routinely, (a)EEG tracings are classified based on the background pattern (see Paragraph 1.5, The preterm EEG) [33] and visually observable features such as burst suppression [34], interhemispheric synchrony (IHS) [35] and seizures [36]. Though this visual approach is available at bedside, it is qualitative and subjective [37]. Recently however, quantitative approaches for raw EEG signals have been introduced [37], [38]. Taking a quantitative approach, two-channel (a)EEG might be a good candidate for an automatic and real-time bedside sleep stage recognition technique.

D. The Preterm EEG

During prematurity, brain activity and its corresponding EEG-recordings undergo rapid developmental changes [39], [40]. Even though the fully-developed sleep-wake cycling relies on the maturation of interconnected neural networks [1], [22], [41], preterm infants already express cyclicity of rudimentary sleep stages as early as 24 weeks PMA [42] (for a schematic overview of sleep and functional development, see Fig. 2). The preterm EEG is known to fluctuate between a more discontinuous background state (trace discontinue), and another background state with more continuous EEG activity [1], [39], [43]. While there is some scepticism, these two modes of activity may be readily seen as potential candidates to reflect sleep stages, respectively QS and AS [41]. At near-term age the EEG gets globally more continuous due to increasing influence of exogenous sensory driven input [43], with only minor relative changes in discontinuity between the background patterns of QS and non-QS states. Specific features regarding sleep stage organization occur only after 46-48 weeks' GA, with a transition

from neonatal sleep to infant sleep and a change in terminology from AS to REM and QS to NREM [44], [45]. Gradual reductions in REM-sleep take place, while NREM-sleep becomes more abundant [44]. This maturational shift corresponds to the increasing exogenous stimulation that the infant receives, which changes the emphasis from synaptic proliferation (during REM-sleep) to synaptic refinement and pruning (during NREM-sleep) [46], [47]. Within the same first 3 months of life, rapid maturation of the brain's electrical activities occurs. Neonatal patterns disappear, while sleep spindles and "adult-like" delta wave activity emerge. From this moment on, sleep stages are clearly recognizable in the EEG. However, research to quantitative EEG (qEEG) features linking to sleep stages in the preterm period is still ongoing, and clear consensus is yet to be achieved.

E. Current State of Literature, Relevance, and Research Question

A growing body of literature has investigated the organization of neonatal sleep EEG, typically finding differences in the time domain (e.g., in continuity) between sleep stages [1], [37], [39], [41], [43], [48]. Most literature has focused on the activity bursts, also known as **spontaneous activity transients (SAT)**, and found a higher proportion of SATs (SAT%), more frequent but shorter periods between the SATs (i.e. inter-SAT intervals; ISI), and higher amplitude bursts for AS compared to QS [1], [41], [48] (for an overview of burst features, see Fig. 1). Research about other characteristics in the preterm EEG, such as synchrony of bursts and the frequency content of the signal (i.e., spectral power), is still quite limited.

Synchrony reflects a proper transfer of information from one hemisphere to the other [49], and is observed as coincidence of electrical activity [38]. It has been a key element in the visual assessment of the preterm EEG [50], reflecting the EEG maturation and development of the connection between the two hemispheres [43]. Only some minor results point to higher synchrony of bursts in AS compared to QS [51], [52], while others found a higher synchrony in QS compared to AS [53].

Spectral power is also useful for brain monitoring, showing consistent maturational changes in EEG in preterm infants, indicating functional brain maturation [54]. The frequency spectrum can be divided in four frequency bands, comprising from low to high the delta (δ), theta (θ), alpha (α), and beta (β) frequency band. Mainly the delta frequency band seems to yield the highest power in neonates, ranging from 95%-85% of total power from very preterm (<32 weeks PMA) to term age [55]. Spectral power analysis also might have the potential to discriminate between sleep stages. While findings on the role of both the relative and absolute frequency power in the different sleep stages are contradictory [56]–[58], mainly changes in the delta frequency are found between sleep stages [56], [58], [59].

However, most of the above-mentioned literature (with the exception of literature about burst features) has focused on preterm infants older than 30 weeks PMA [29], [38], [57]–[59], while research in the younger preterm infant is lacking. This is a shortcoming of the existing literature, as promoting sleep is likely to yield the highest return in the early phases of brain development [5], [14], [15].

Moreover, the behavioural sleep state assessment used in these studies is barely done using long-lasting observations with a validated behavioural sleep score, questioning the reliability of existing work. With advancing knowledge about the importance of sleep in preterm infants, there is a growing demand for a reliable, automated, real-time sleep monitoring tool. Therefore, the aim of this study is to correlate sleep behaviour scores to simultaneous quantitative two-channel EEG features in extremely to very preterm infants of <30 weeks PMA admitted to the NICU. It is hypothesized that a higher SAT% is found in AS compared to QS, while the ISIs are expected to be longer in QS and inter-SAT percentage (ISP) to be higher in QS compared to AS. Similarly, it is expected that burst synchrony is higher during AS. Lastly, both absolute and relative power of the delta frequency could be relevant markers to distinguish sleep stages, but how these differ between the sleep stages is yet to be determined.

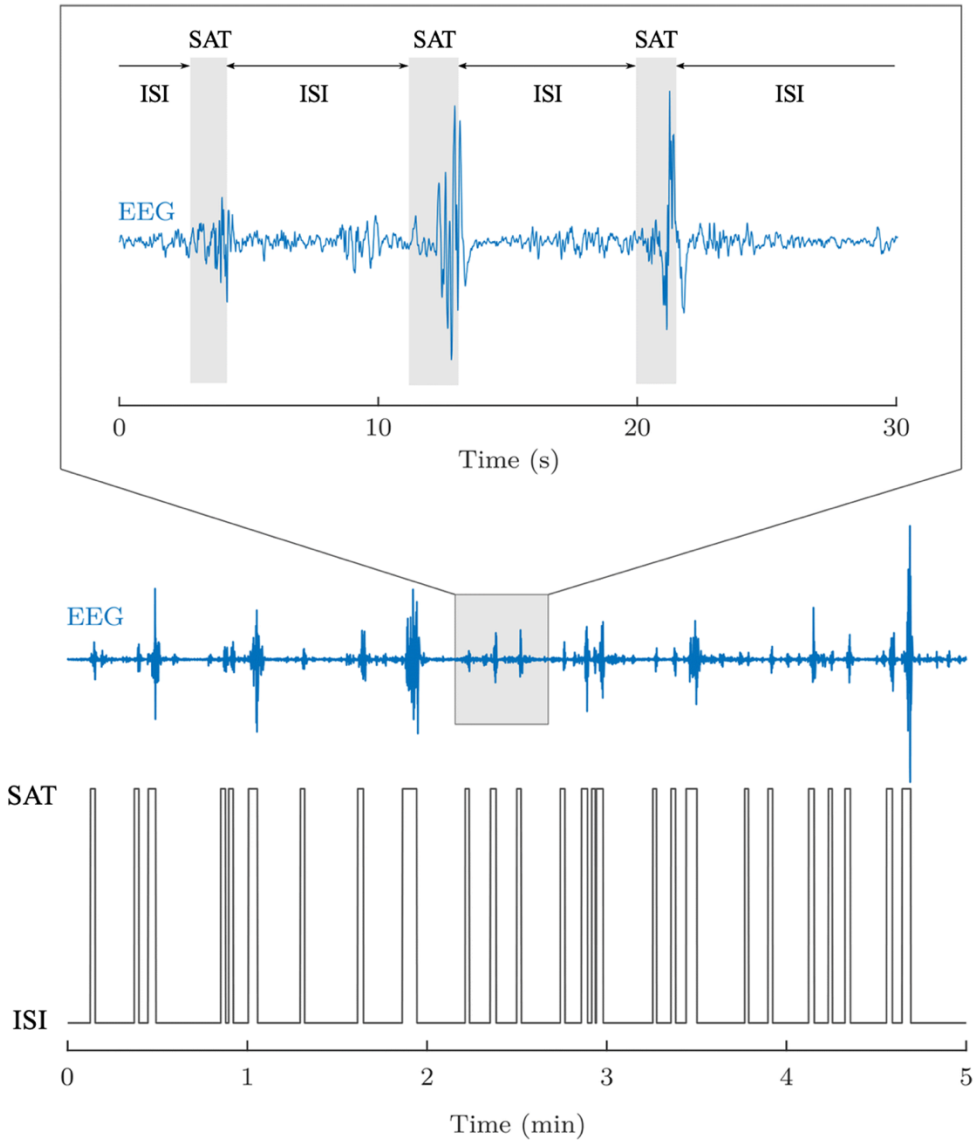


Fig. 1. Visualization of spontaneous activity transients (SAT) and inter-SAT intervals (ISI) in the preterm EEG. At the bottom, a 5-minute EEG-segment and the output of a SAT detection algorithm are presented. On top, a 30s epoch of the 5-minute EEG-segment is shown, with labelled SATs and ISIs. Adapted from De Wel and colleagues [60].

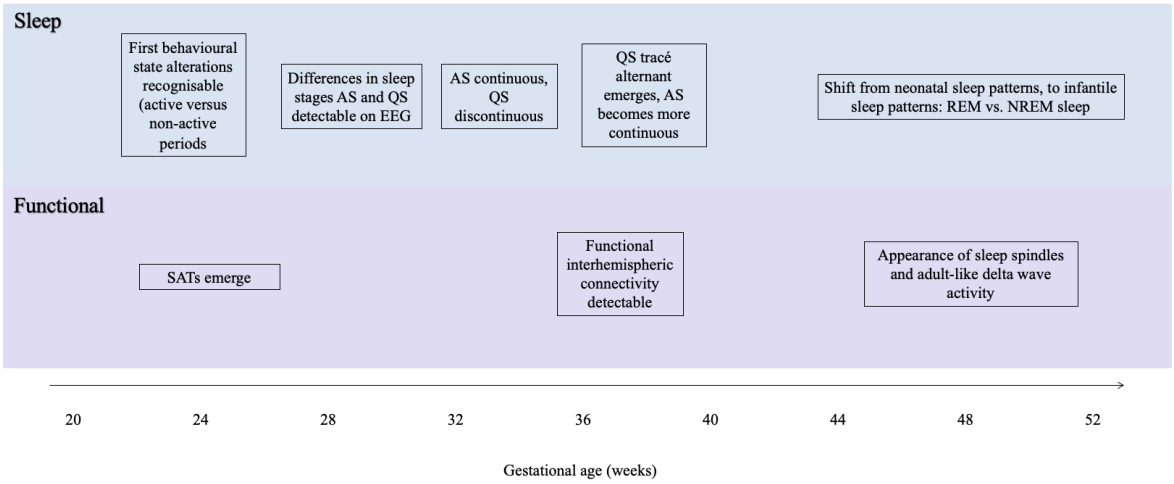


Fig. 2. The parallel development of sleep and functional networks in the developing brain. Approximate time points of major markers in sleep and functional development. Adapted from Uchitel and colleagues [4]. List of abbreviations: Active Sleep (AS), Quiet Sleep (QS), Electroencephalography (EEG), Rapid Eye Movement (REM), non-REM (NREM), Spontaneous Activity Transient (SAT).

II. METHODS

A. Study Population

A total of 17 very and extremely preterm infants (<30 weeks PMA), admitted to the Neonatal Intensive Care Unit (NICU) of the Wilhelmina Children's Hospital (Utrecht, The Netherlands), were enrolled in this study. All neonates received continuous two-channel (a)EEG monitoring during their first 72 hours of life and were medically stable at times of study. Written informed consents were obtained from parents before enrolment. Exclusion criteria were: infants suffering from congenital malformations, seizures, major brain damage or brain abnormalities, among which an IVH grade 3 or 4, and infants of whom the mother used recreational drugs during pregnancy. Infants that received invasive respiratory support, only were included if a) they were stable at times of observation; b) other behavioural features were clearly visible, e.g., they did not receive phototherapy. Clinical characteristics of the included patients were shown in Table I. The research protocol was presented to the Medical Research Ethics Committee (METC), who confirmed that the Medical Research Involving Human Subjects Act (WMO) does not apply to this study.

TABLE I
FREQUENCE OF OCCURRENCE OF PATIENT CHARACTERISTICS

Characteristic	**N / mean (SD / range)**
Gender	
Male	10
Female	7
Mean GA in weeks + days (SD in days)	27 + 5 (± 8 days)
Mean PMA at observation in weeks + days (SD in days)	28 + 0 (± 9 days)
Birth weight in grams (SD in grams)	1112.04 (209.18)
Mean Apgar score (range)	7 (1-10)
Medication during observation	
Coffein	17
Glucose	16
Benzylpeniciline	8
Gentamincine	5
NaCl + Heparine	13
Intralipid 20%	15
TPN	15
$NaHCO^3$	4

Study medication	1
Respiratory support	
NCPAP	8
NIPPV	7
SIPPV-VG	1
Flowsnor	1
Phototherapy	11
Location	
Ward	14
Box	3
Sleeping position*	
Prone	1
Supine	4
Lateral	15

For GA, PMA and birth weight means (SD) are displayed instead of n. For Apgar score mean (range). *Two different positions noted for n=3 patients, as they changed position during observation. List of abbreviations: gestational age (GA), postmenstrual age (PMA), standard deviation (SD), natrium chloride (NaCl), total parenteral nutrition (TPN), sodium bicarbonate ($NaHCO^3$), nasal continuous positive airway pressure (NCPAP), nasal intermittent positive pressure synchronised intermittent positive pressure ventilation (NCPAP), nasal intermittent positive pressure ventilation (NIPPV), ventilation (NIPPV), synchronised intermittent positive pressure ventilation with volume guarantee (SIPPV-VG).

B. Data Acquisition

1) Sleep stage classification

For observational sleep stage classification, a validated in-house developed behavioural state classification score was used [9] (See Appendix II for observation form and III for ethogram). The observations were performed for three consecutive hours, consisting of 180 one-minute epochs, between 9 AM and 7 PM. All observations were done within the first 72 hours after birth and while the two-channel (a)EEG was connected. To distinguish between behavioural states (AS, QS, IS and wake; W), it was determined for each one-minute epoch whether the eyes were open, and whether REM was visible. Secondly, facial movement, body movement, and sounds were observed. Thirdly, respiration rate and heart rate were observed every 15 seconds (i.e., four times per epoch). Respiration rate and heartrate were monitored using Philips Intellivue MP70 Neonatal monitors (Koninklijke Philips N.V., Eindhoven, The Netherlands). Lastly, a confidence score was assigned to each epoch. With this score, the observer indicated whether he was highly confident (1), moderately confident (0) or not confident (-1) about the classified sleep stage of that epoch. For a summary of characteristics per sleep stage, see Table IV, Appendix IV. For a detailed explanation of the score, see [9].

2) (a)EEG monitoring

Following local protocol, all neonates born <30 weeks GA were submitted to a one- or two-channel (a)EEG for the first 72 hours after birth. Two-channel (a)EEG is used when infants weigh around 800 grams or more, which was the case for all infants in this study. Subdermal EEG needle electrodes (Natus, Seattle, WA, USA) were used. Needles were placed at F3, F4, P3 and P4. The reference electrode was placed either between the two most frontal electrodes, or on the forehead, depending on the distance between the two most frontal electrodes. The signal was acquired using Brainz monitors (BRM2/BRM3; Natus, Seattle, WA, USA). For data analysis, the non-time compressed raw data at a sampling rate of 256 Hz was used.

C. Data Analysis

1) Sleep stage classification

After collection of observational behavioural state data, data was smoothed following protocol [9]. The general idea of smoothing is to make sure all features are interpreted correctly. Epochs with a moderate (0) to low (-1) confidence score were reassessed based on the information recorded on the observation form, the manual and discussion with a supervising researcher (EG). When observational data complied more to another stage than initially classified, the stage was reclassified as such. Also, periods with fluctuations were re-evaluated. In case epoch-by-epoch fluctuations of AS and QS occurred, and this happened between a period of at one side AS and the other side QS, the full period was reclassified as IS. Lastly, each epoch was checked against the 3-minutes before and the 3-minutes after and was smoothed accordingly. For example, if one epoch of QS occurred within a period of AS, that epoch was smoothed to AS. Epochs with high (1) confidence scores were only smoothed with caution, acknowledging that real-time classification can help putting behaviour into context. Only smoothed observational data were included for analysis. For illustration of the raw observational data and data after smoothing, see Fig. 3.

Fig. 3. Example of smoothing of observational sleep stage classification. a) Raw observational data for sleep stage classification; b) sleep stage classification after smoothing.

2) Quantitative EEG feature extraction

The raw EEG-data was first pre-processed (removing artefacts and filtering) before feature extraction. Based on the pre-processed data, three types of qEEG features were calculated for each one-minute epoch. The first type of features, the burst features, are event-based EEG measures related to the degree of continuity of the signal. The burst features consist of SAT%, ISP and ISI. Higher SAT%, lower ISP and shorter ISI indicate more continuity. SAT% is defined as the number of SATs during a block length of a one-minute epoch [41]. ISP is defined percentage of inter-SAT duration during a block length of a one-minute epoch. Lastly, ISI is defined as the interval duration of the last inter-SAT interval. Synchrony of bursts was calculated using the Activation Synchrony Index (ASI) [53]. The ASI is based on the statistical measurement of the temporal delay between two signal energies, taking two bipolar derivations in the left and right hemisphere as input to processing. If energy envelopes were co-incident, which is clinically perceived as synchronous, this led to a high ASI value. Finally, spectral delta power is described as the distribution of signal power over delta frequency. The absolute power of the delta band is the integral of all of the power values within its frequency range (0.5-3 Hz). Relative delta power is estimated as the ratio of the absolute power in the delta band divided by the total energy within the whole frequency range (0.5–30 Hz), comprising the delta (δ, 0.5–3 Hz), theta (θ, 3–8 Hz), alpha (α, 8–15 Hz), and beta (β, 15–30 Hz) frequency band. Burst features (i.e., SAT%, ISI and ISP) were calculated using SignalBase software (SignalBase® v10.6, University Medical Center Utrecht, The Netherlands). ASI, relative and absolute delta power were retrieved from in-house developed Matlab scripts (MathWorks Inc., Natick, MA, USA).

D. Statistical Analysis

First, descriptive analyses were performed for each qEEG feature of each behavioural state. Then, differences between the four states (i.e., AS, QS, IS and W) were examined for each qEEG feature using a one-way Analysis of Variance (ANOVA) test and Tukey post-hoc testing. To investigate the influence of EEG channel (left/right), the interaction between channel and sleep states was tested by using a two-way ANOVA and Tukey post-hoc testing. All statistical analyses were performed using Python (v3.8.5).

III. RESULTS

A. Descriptives

In total, 2967 minutes of data were acquired, of which 164 minutes (6%) were missing data due to interruptions of the behavioural observations (e.g., by elective care treatments). Smoothed observational data consisted of 1435 minutes of AS epochs (48%), 961 minutes of QS (32%), 311 minutes of IS (11%), and 96 minutes of W (3%). For each qEEG feature, the mean and standard deviation (SD) were calculated per behavioural state, see Table II.

TABLE II

DESCRIPTIVES FOR QEEG FEATURES SEPERATED PER BEHAVIOURAL STATE

	SAT%_left		**ISP_left**		**ISI_left**		**Absδ_left**		**Relδ_left**	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Sleep state										
AS	6.360	1.959	46.186	22.057	3.573	3.992	1099.837	818.968	0.912	0.070
QS	5.968	1.891	56.295	20.171	5.041	5.582	965.229	703.933	0.916	0.060
IS	6.166	2.160	54.453	19.626	4.434	4.788	977.356	710.517	0.912	0.070
W	6.177	2.027	35.789	18.317	2.553	2.813	861.644	586.633	0.855	0.109

	SAT%_right		**ISP_right**		**ISI_right**		**Absδ_right**		**Relδ_right**	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Sleep state										
AS	6.467	2.147	48.300	22.902	4.043	5.502	1074.980	956.919	0.917	0.064
QS	6.155	1.903	57.982	18.609	5.124	5.403	899.706	661.505	0.923	0.054
IS	6.429	2.205	56.288	19.877	4.901	5.607	921.368	739.425	0.922	0.054
W	5.780	2.235	34.038	19.859	2.670	2.565	914.705	938.511	0.854	0.117

	ASI	
	Mean	*SD*
Sleep state		
AS	6.817	4.834
QS	4.759	3.087
IS	5.865	3.748
W	4.113	1.709

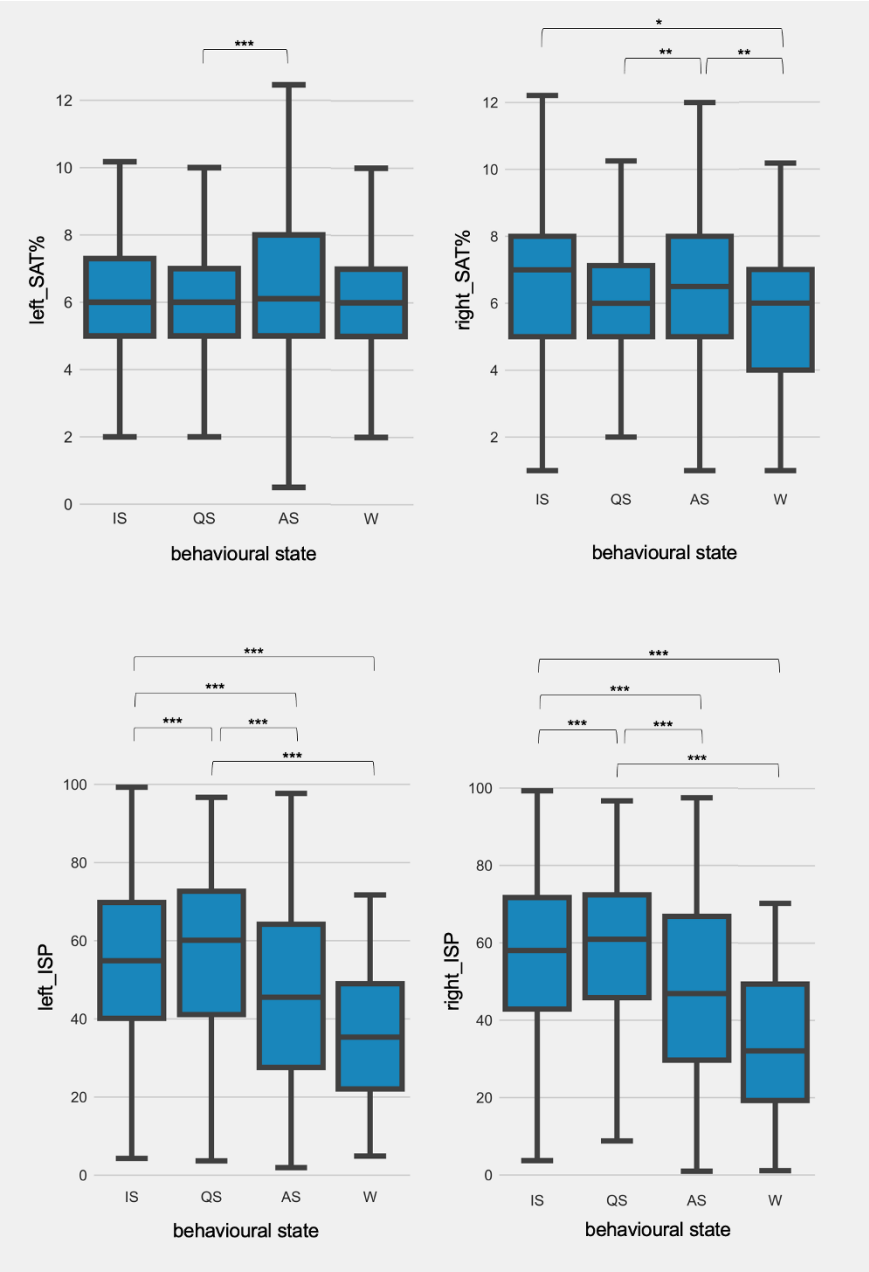
Means and standard deviations (SD) are displayed for both the left (top panel) and the right (middle panel) channel for burst features (i.e., SAT%, ISP & ISI) and for absolute and relative spectral power of the delta frequency. Moreover, the means and SDs for the interhemispheric ASI (bottom panel) are displayed. List of abbreviations: Active Sleep (AS), Quiet Sleep (QS), Intermediate Sleep (IS), wake state (W), standard deviation (SD), proportion of Spontaneous Activity Transients (SAT%), inter-SAT percentage (ISP), inter-SAT interval (ISI), absolute delta power (Absδ), relative delta power (Relδ), Activation Synchrony Index (ASI).

B. qEEG Features

1) Burst features

The three burst features SAT%, ISP and ISI significantly differed between the behavioural states, for both the left (respectively $F(3, 2782) = 7.623$, $p < .001$; $F(3, 2782) = 63.228$, $p < .001$; $F(3, 2782) = 23.147$, $p < .001$) and the right (respectively $F(3, 2782) = 7.623$, $p < .001$; $F(3, 2782) = 67.754$, $p <$

.001; F(3, 2782) = 11.811, $p < .001$) channel (see Table III for ANOVA output). Using a Tukey post-hoc, SAT% showed higher for AS compared to QS ($p < 0.001$ for left channel; $p = 0.002$ for right channel). ISP and ISI were higher for QS compared to AS, with a $p < 0.001$ for both features and each channel. For boxplots of burst features with differences between all behavioural states indicated, see Fig. 4. For output of Tukey post-hoc analyses for all behavioural states, see Table V, Appendix V.



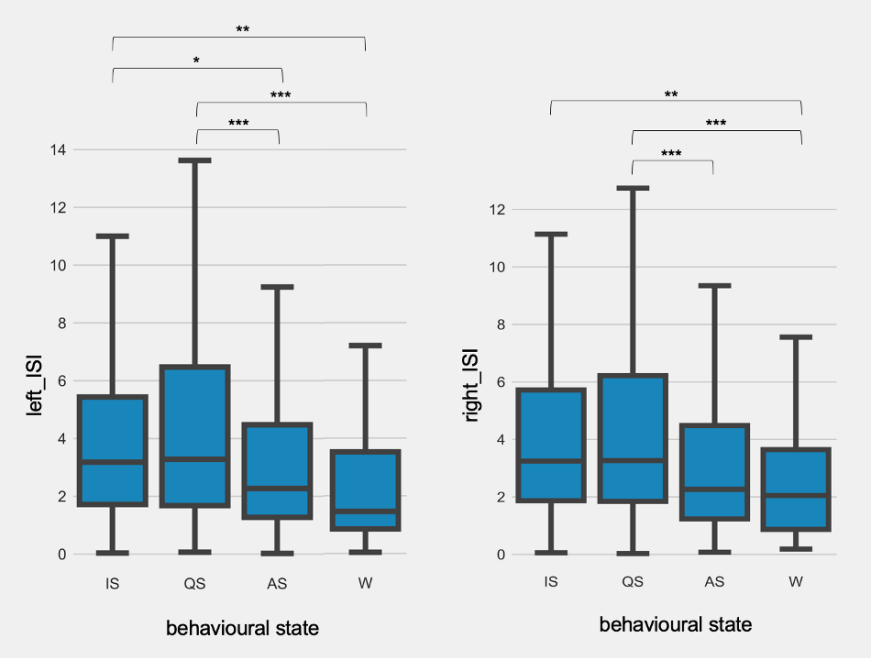


Fig. 4. Boxplots for each burst feature: SAT% in top panel, ISP in middle panel and ISI in lower panel. Boxplots most left are for values from the left channel, boxplots most right for values from right channel. Significances are displayed with * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. List of abbreviations: proportion of Spontaneous Activity Transients (SAT%), inter-SAT percentage (ISP), inter-SAT interval (ISI), Active Sleep (AS), Quiet Sleep (QS), Intermediate Sleep (IS), wake state (W).

TABLE III

ANOVA OUTPUT FOR EACH QEEG FEATURE

Feature	Channel	df	sum square	mean square	F	p-value
SAT%	Left	3	88.042	29.347	7.623	<0.001***
	Right	3	88.132	29.377	6.813	<0.001***
ISP	Left	3	83990.670	27996.890	63.228	<0.001***
	Right	3	90362.660	30120.890	67.754	<0.001***
ISI	Left	3	1504.716	501.572	23.148	<0.001***
	Right	3	1035.811	345.270	11.811	<0.001***
Absδ	Left	3	10809157.000	3603052.000	6.208	<0.001***
	Right	3	15146550.000	5048850.000	7.155	<0.001***
Relδ	Left	3	0.232	0.077	16.455	<0.001***
	Right	3	0.300	0.100	26.242	<0.001***
ASI	Interhemi	3	1449.322	483.107	28.938	<0.001***

List of abbreviations: proportion of Spontaneous Activity Transients (SAT%), inter-SAT percentage (ISP), inter-SAT interval (ISI), absolute delta power (Absδ), relative delta power (Relδ), Activation Synchrony Index (ASI), interhemispheric (interhemi), degrees of freedom (df).

2) Interhemispheric synchrony

Interhemispheric synchrony between bursts also differed significantly between the behavioural states ($F(3, 1486) = 28.938$, $p < .001$; see Table III for full ANOVA output). Tukey post-hoc testing showed a significant difference ($p < 0.001$) for ASI between AS and QS, with a higher synchrony for AS compared to QS. For all significant differences between behavioural states, see Fig. 5 for boxplots and Table V, Appendix V for output of Tukey post-hoc analyses.

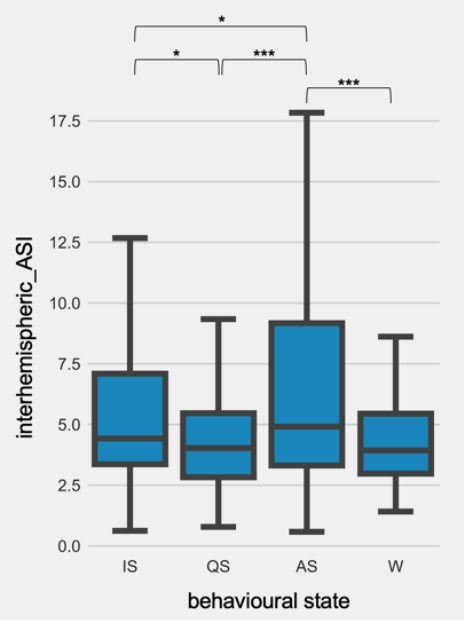


Fig. 5. Boxplots for interhemispheric ASI. Significances are displayed with * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. List of abbreviations: Activation Synchrony Index (ASI), Active Sleep (AS), Quiet Sleep (QS), Intermediate Sleep (IS), wake state (W).

3) Spectral power of the delta frequency

Spectral power of the delta frequency yielded the highest relative power of all frequency bands in the EEG-signal, covering around 90% or the total power during each behavioural state (for means per state, see Table II). Both absolute ($F(3, 2095) = 6.208$, $p < .001$ for left channel; $F(3, 2095) = 7.155$, $p < .001$ for right channel) and relative delta power ($F(3, 2095) = 16.455$, $p < .001$ for left channel; $F(3, 2095) = 26.244$, $p < .001$ for right channel) showed significant differences between behavioural states for each channel (see Table III for full ANOVA output). Absolute delta power was significantly higher for AS than for QS, with a p-value of 0.001 for the left channel and a p-value lower than 0.001 for the right channel. There was no difference found between the sleep stages for relative delta power. However, each sleep stage showed a significant difference with the awake state at $p < .001$ in both channels, with the sleep stages yielding a higher relative delta power compared to the awake state. For all significant differences between behavioural states, see Fig. 6 for boxplots and Table V, Appendix V for output of Tukey post-hoc analyses.

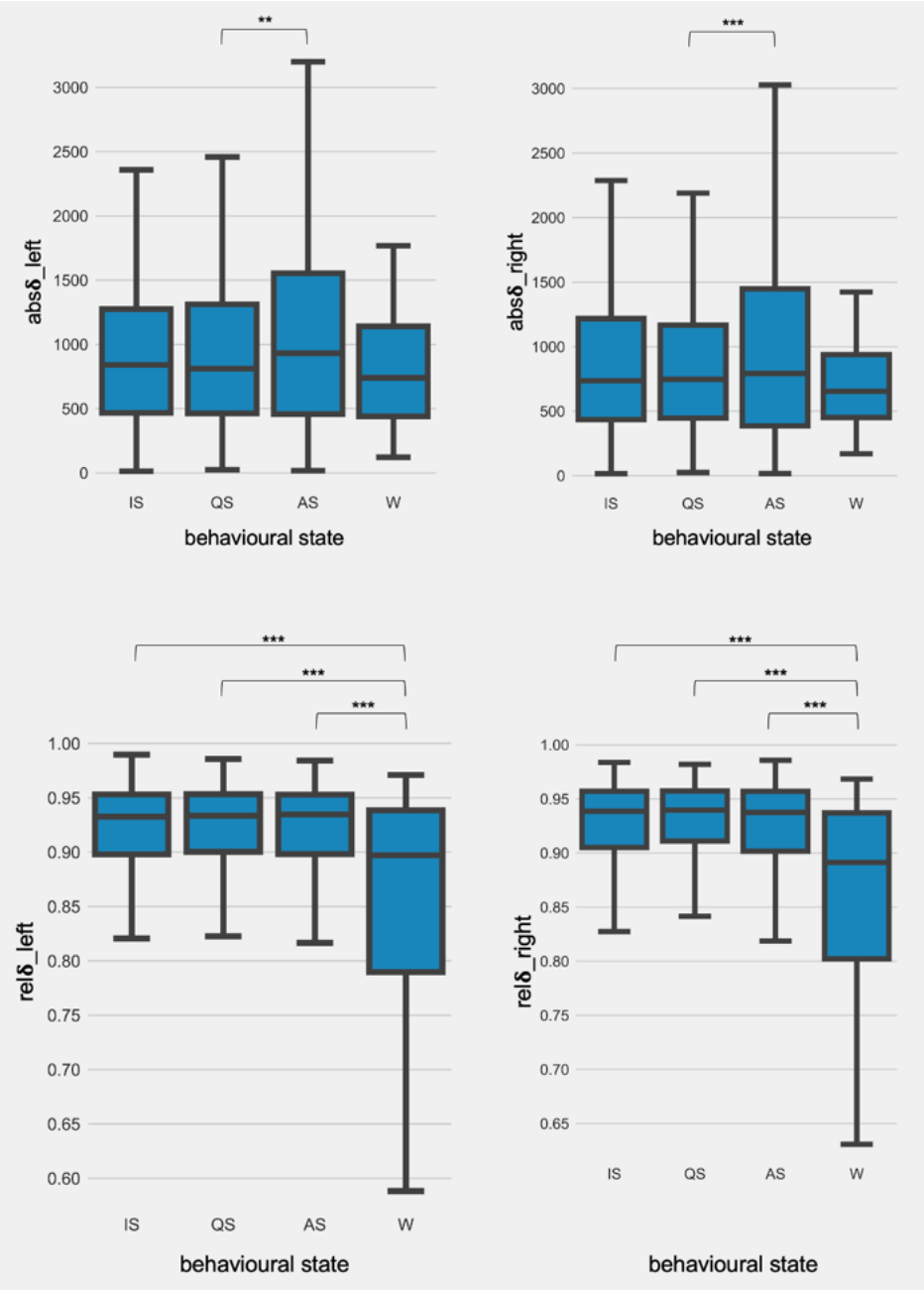


Fig. 6. Boxplots for spectral delta power: absolute delta power is displayed in top panel, relative delta power in lower channel. Boxplots most left are for values from the left channel, boxplots most right for values from right channel. Significances are displayed with * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. List of abbreviations: Absolute delta power (absδ), relative delta power (relδ), Active Sleep (AS), Quiet Sleep (QS), Intermediate Sleep (IS), wake state (W)

3.2.4 Effect of EEG channel

The interaction between channel and sleep stages was tested. No significant effect was found for SAT% ($p = 0.223$), ISP ($p = 0.674$), ISI ($p = 0.594$), absolute delta power ($p = 0.787$), and relative delta power ($p = 0.825$).

IV. DISCUSSION

A. Summary of Findings

Several quantitative EEG features are found to be able to differentiate between sleep stages in the extremely to very (<30 weeks' PMA) preterm infants admitted to the NICU. First, we found a higher SAT% in AS compared to QS, while ISP and ISI were higher for QS compared to AS. Second, we found a higher synchrony of bursts in AS compared to QS. Lastly, absolute delta power showed higher values for AS compared to QS. To our knowledge, this is the first time multiple qEEG features are extracted from two-channel (a)EEG and correlated with simultaneously performed long-lasting observations using a validated behavioural state classification score.

B. Burst Features

We successfully replicated the findings of existing literature on burst features [1], [41], [48]. Palmu and colleagues [41] found a higher mean SAT% for AS compared to QS (in a preterm group of 25.9–32.7 weeks CA), while André and colleagues [1] also acknowledged a longer ISI in QS compared to AS in preterm infants. SATs are the most salient feature of the preterm EEG and can already be observed at 23-24 weeks conceptional age (CA) [39], [61], the age at which the earliest preterm born infants survive at the NICU. SATs are likely to be crucial for early brain development [39], [62]. Seen the hand-in-hand relationship between brain development and sleep [10]–[13], it is not surprising that SATs are more prevalent during AS, the sleep stage mainly responsible for brain maturation. The cumulative amount of SATs even seems to be the main difference between the EEG background patterns corresponding to either AS and QS in early preterm life [41]. During prematurity, brain activity and the corresponding EEG undergo rapid developmental changes [1], [39]. Near term age, the distinction of SAT and ISI waveforms diminish, eventually disappearing after term age [40]. Using the burst features described in this study to distinguish sleep stages is therefore suboptimal in a population older than 30 weeks PMA, beyond the age group of this study.

C. Synchrony

Next to measures in the temporal domain, we also included a feature reflecting spatial coordination, or synchrony, between the two EEG channels: ASI. Even though interhemispheric connections are not yet fully developed in the current preterm sample, synchrony can already be seen as early as 26 weeks CA [49]. In the present study we observed synchrony between bursts, with AS yielding a higher synchrony of bursts than QS. In contrast to our finding, Räsänen and colleagues found lower levels of synchrony in AS compared to QS, also using ASI as measure for synchrony [53]. This could be explained by the dependence of ASI on the temporal fluctuation of power, which is less clear during continuous activity

[1]. Räsänen and colleagues [53] studied a subgroup of preterm infants averaging a conceptional age of 30.4 weeks. At 30-31 weeks CA, the EEG background pattern of AS is nearly continuous, making it hard for ASI to recognize synchronicity [52]. However, before 30 weeks, AS still shows a more discontinuous background pattern with temporal fluctuation of power. This might explain why in the present study ASI was able to recognize synchronicity during AS. That Räsänen and colleagues [53] found lower levels of synchrony in AS might therefore be more a technical shortcoming of the parameter ASI for infants older than 30 weeks PMA, than that there is really a lower synchrony in AS compared to QS.

That we found a higher synchronicity during AS compared to QS is not surprising. AS consists of more bursts (among which SATs) than QS, and bursts have proven to be already roughly coincident before the development of interhemispheric connections [39], [49]. Particularly the high-amplitude bursts, which mainly appear during AS, tend to be highly synchronous already from 24 weeks GA onward [1], [51], [52]. It should be addressed however, that there could be a degree of paradoxical hypersynchrony in the present sample, because bursts tend to get more precise and consistent in temporal synchrony after appearance of interhemispheric connections, which only happens at 35 weeks' CA [39]. The synchrony of bursts observed before this time, may therefore be mediated by mechanisms at midbrain and brainstem level. Räsänen and colleagues [53] did not find this paradoxical hypersynchrony. However, this might thus be explained by the inability of ASI to recognise synchrony during continuous periods rather than that there was no synchrony during AS in these infants.

Lastly, there seems to be a maturational effect for synchrony. Synchrony exists for high amplitude bursts up to 28 weeks GA, but after this age it gets more asynchronous, until synchrony increases again from 31 weeks on [1]. At full term, asynchrony is consistently absent in all behavioural states. It is important to take this maturational effect of synchrony into account when interpreting existing literature. This might also explain the difference in findings between the present study, where we also included infants < 28 weeks PMA, and the study of Räsänen and colleagues [53], where the subgroup averaged a conceptional age of 30.4 weeks.

D. Spectral Power of the Delta Frequency

Lastly, we explored the changes in the frequency domain over the different sleep stages, using spectral power frequencies. Of all frequencies measured in the preterm infants of the present study, the highest absolute spectral power could be found in the delta band, fitting well into existing literature [55]. Moreover, we found a higher absolute delta power for AS compared to QS. This is in agreeance with existing literature [57]–[59], however, all these studies have been done in older preterm to term aged infants.

The higher absolute delta power in AS compared to QS might be explained by the presence of SATs. SATs are EEG events that in the frequency domain are characterized by the presence of multiple

frequencies within the range of 0.1-30 Hz and are hence also called multiband events [62]. The most common SAT-event is the delta brush, a transient pattern characterized by a slow delta wave (0.3-1.5 Hz) with superimposed fast frequency spindles in the alpha-beta range (8-25 Hz) [63], [64]. This might explain why absolute power of the delta frequency is higher for AS compared to QS, where SATs (among which thus delta brushes) are more prevalent. However, this might indicate that absolute alpha and beta power are also increased in AS compared to QS, but this should be investigated in future research.

No significant difference in relative power between the sleep stages was found, which is contradictory to existing literature. In a neonatal sample of 24.8-45.4 weeks PMA, Koolen and colleagues [26] found that relative delta power in the lower frequency range (0.5-1Hz) discriminates sleep stages very well, with AS yielding a higher relative delta power than QS. The present study did not make a distinction between a lower frequency range of delta (0.5-1 Hz) and a higher frequency range (1-4Hz) like Koolen and colleagues [26] did, which might explain the differences in findings. However, to get more reliable results, both absolute and relative spectral power indices should be included in future studies to establish the findings of Koolen and colleagues [26]. To our knowledge, no other studies to relative power in AS versus QS have been done in a preterm sample <30 weeks PMA.

It should be mentioned that findings in a sample older than 30 weeks PMA cannot be fully compared to the preterm sample (<30 weeks PMA) of the present study. Spectral power is known to show a maturational trend in the preterm EEG [54], [56], [58]. In general, the absolute spectral power in the lower frequency range (e.g. in the delta band) tends to decrease with increasing PMA, while relative spectral power shifts towards the higher frequency ranges [54], [58]. Maturational effects were also demonstrated during the sleep stage AS. First, absolute delta power decreases with advancing age during AS [56]. Second, relative power shows an opposite maturational trend for the lower and upper frequencies of the delta band: while relative power of frequencies ≤1Hz decreases with age, relative power of frequencies 2-3Hz increases during AS [55]. Taking these known maturational trends of spectral power into account, an elaborate study in different preterm age groups is a necessity to establish the direction of relationship between spectral power and sleep stages.

E. Sleep Stages Versus the Awake State

With exception of relative delta power, each qEEG feature has proven to be able to distinguish between AS and QS. However, we have not always found significant differences in qEEG features for AS versus W. This might be explained by the difficulty to reliably differentiate wakefulness from AS, as the states are not clearly discernible on the EEG in infants younger than 30 weeks GA [5], [45]. Moreover, the states have behavioural similarities, including irregular respirations [65] and similar movements and postural patterns [66]. The only difference between AS and W is that the eyes may be open in W and

are closed in AS [67]. These behavioural similarities make it difficult to distinguish sleep from wake, and in clinical practice the probable consequent misinterpretation can have detrimental consequences. Nurses might easily mistake AS for wakefulness, consequently plan elective care during an AS period, and unintentionally disturb brain development [10]–[13]. It is not surprising that the question how to distinguish sleep and wakefulness is one that sleep researchers want to address for a long time. Results of the present study indicate that relative delta power might be able to differentiate sleep and wakefulness, as it was significantly lower for wake compared to each of the sleep stages. However, more research is necessary to establish this finding.

Also, the qEEG features had some difficulties in distinguishing IS from other sleep stages. This could possibly be explained by the definition of IS, being an intermediate (i.e., transitional) stage between AS and QS. IS was scored when epochs that were in between an AS and QS period did not show clear characteristics of either AS or QS but showed characteristics of both sleep stages instead. This might have led to IS data being too similar to both AS and QS, which made it difficult to find clear differences in qEEG features.

F. Strengths of the Present Study

Taken together, the present study serves as a foundation for future studies, providing a novel method to assess behavioural states in very to extremely preterm infants (< 30 weeks PMA) using two-channel (a)EEG. The study stands out from existing literature by doing three-hour long bed-side observations using an observational score validated for preterm infants <30 weeks PMA. This in-house developed observational score [9] is the result from a profound revision of existing observational sleep state classification scores [18], resulting in the most optimal score for assessing sleep stages. Moreover, the present study was done in a relatively healthy and clinically relevant sample, using a large quantity of data. Lastly, the inclusion of multiple qEEG features next to the well-known burst features makes the present study stand out from existing literature. The quantitative features have proven to be able to distinguish sleep stages, offering a less time-consuming possibility to assess sleep than using behavioural scoring, while being more objective than the visual assessment of qualitative EEG features.

G. Limitations of the Present Study

Despite its strengths, the present study also has its limitations. Although IS is used in multiple behavioural sleep state classification scores as a transition phase between AS and QS [68], [69], there is no consensus on the existence of IS as a real, physiologically existing sleep stage. This means that the inclusion of IS might have led to a bias in the data.

A second limitation is the restricted visibility of behaviours in several infants. In eleven infants, eyes were covered for phototherapy, restricting the visibility of the eyes and limiting the visibility of

facial movements. Also, one infant received invasive ventilation, resulting in the inability to assess respiration regularity. These conditions led to less observational features available to confidently score sleep stages. However, it is unfeasible to only include infants for whom each feature is fully visible, as extremely to very preterm infants of <30 weeks PMA often receive either phototherapy or invasive ventilation.

The present study also has its technical limitations. Although a simple pre-processing procedure was done for raw EEG data, the extracted qEEG features could still have been biased by artefacts. Artefacts can have multiple origins, and can either be physiological of nature, or external to the human body [70], [71]. Artefacts can imitate nearly all types of EEG patterns, and can as such seriously affect results, eventually leading to misinterpretation of data [71]. The simple pre-processing performed in the present study might have been insufficient to remove all artefacts, rendering the analysis inacceptable if too many artefacts were missed.

Finally, EEG data was collected using only a minimal number of channels (i.e., two). This could have led to a limited availability of information. For example, Koolen and colleagues [26] did find differences in direction of ASI between two different sets of bipolar channels: while bipolar ASI C3O1-C4O2 was higher in AS, they found bipolar ASI Fp1C3-Fp2C4 to be higher in QS. Taking only two channels, the present study might have missed valuable information about the potential role of the qEEG features in sleep stage differentiation.

H. Recommendations for Future Research

For future research, it is recommended to investigate the effects of age, considering both birth- and postnatal age. All qEEG features are prone to maturational effects [39], [40], [54], [56], [58], which might yield different outcome for different age groups. Also, sleep stages tend to be better discernible by 28-30 weeks of gestational age [1], [5], [10] (more details are given in Appendix I, supplemental text box). This means it might be worthwhile to split the present sample in a group of <28 weeks PMA and 28-30 weeks PMA, while also including an age group >30 weeks PMA. Next, it could be interesting to explore more qEEG features linking to sleep, improving confidentiality of state differentiation. For optimal classification, a balance between minimizing the number of features and maximizing classification accuracy should be aimed for [26]. In order to find the most optimal combination of features, more exploration to potential features differing between sleep stages should be done. Also, the current features should be critically reconsidered. For example, spectral delta power could be divided in a lower frequency range (0.5-1Hz) and a higher range (1-3Hz). Seen the findings of Koolen and colleagues [26], and our finding that 90% of the spectral power originates from the delta band, splitting the delta band in a lower and upper range might gain valuable information.

Lastly, seen the limitations of the present study, it is recommended to exclude IS from future analyses, perform more extensive pre-processing of data for artefact removal, and use multichannel

EEG. This will eventually lead to more reliable and valuable results. When multichannel EEG turns out to be unfeasible in a preterm sample <30 weeks PMA, it is recommended to average channels of the two-channel (a)EEG in future studies instead. In the present study we did not find any effect of channel, meaning that features might appear similarly in the two hemispheres (apart from ASI, which is an interhemispheric measure). Averaging channels gives more robust results, reducing the impact of short-duration distortions of the signal [60].

Taken everything together, this will eventually lead to an increased possibility of the quantitative EEG to distinguish sleep stages. Ultimately, this gives rise to the development of a state-of-the-art deep-learning algorithm for automatic sleep stage classification. The development of such a sleep monitoring tool has the potential to provide a more objective measure than the qualitative, visually assessed sleep stages by bed-side observations, while being less labour-intensive than behavioural sleep stage classification by bedside observation but without being overly obtrusive. Finally, the present study even sets the foundation for long-term future monitoring of sleep stages in infants born preterm. The current gold standards of subdermal needle and normal gel electrodes are not useful for long-term monitoring [19]. But with the advancing techniques of dry electrodes for preterm EEG [31], long-term monitoring by EEG seems more practicable in the future.

I. Conclusion

To conclude, SAT%, ASI and absolute delta power were higher for AS compared to QS, while ISP and ISI were higher for QS in preterm infants <30 weeks PMA, which was consistent with our hypotheses. These findings confirmed the importance of quantitative EEG in the distinguishment of sleep stages in very and extremely preterm infants. Our research will hopefully lead to the development of an automatic sleep assessment tool for the very to extremely preterm population. Eventually, the goal is to the improve clinical practice, reduce the interruption of preterm sleep, and improve neurodevelopmental outcome in the very to extremely preterm samples in NICUs worldwide.

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APPENDIX I

SUPPLEMENTAL TEXT BOX

Sleep stage development and brain maturation

Neonatal sleep is a state that involves endogenous driven brain activity, crucial for neuronal survival and guidance of brain networks [5], and starts to develop early in life [72]. Rudimentary sleep stage cycling has already been described in extremely preterm born infants, at 24 weeks postmenstrual age (PMA) [42], [51]. Neonatal sleep is characterized by alternating periods of Active Sleep (AS) and Quiet Sleep (QS). AS is known to provide endogenous neuronal activation, which provides the growth of neural networks and neurosensory systems in preterm infants, who have limited waking experiences and thus limited exogenous sensory stimulation [5], [47]. In its earliest form, these endogenous stimulation encompasses cell firings that soon begin after a neuron has differentiated into a specific cell type [73]. As the infant approaches 29-30 weeks PMA [48], the sensory and central brain systems begin to propagate synchronous oscillations, which connects the areas essential for neurosensory development [73]. When these neural networks (located throughout the cortex, thalamus and brainstem) are sufficiently interconnected, sleep-wake cycling becomes more established. Accordingly, sleep-specific cortical phenomena can be recognized in the preterm EEG from 30 weeks PMA on [22], [41].

Patterns of endogenous stimulation only occur during AS and provide crucial input for the development of long-term circuitry [10], [73]. However, QS is essential for the preservation of brain plasticity and the consolidation of those processes [10]. Hippocampal processing of external stimuli occurs during QS [73], and the progression from QS to AS (i.e., sleep wake cycling), is necessary for memory processing [5], [73].

When sleep is uninterrupted, preterm infants spend around 90% of the time asleep (some studies even report a 97% [74]), of which AS makes up 40-60% at the early stages of brain development [75]. As the infant matures, the percentage of QS increases, both at the expense of the sleep stage called Intermediate Sleep (IS) and of AS. IS mainly appears in extremely and very preterm infants, and disappears when sleep stage cycling gets more established, from 30 weeks PMA onward [5]. The increase in QS as the infant matures corresponds to its role in the organization of exogenous stimulation in the form of learning and memory consolidation [47], [73]: from preterm to term age, infants experience wakeful periods from 3% to 15% of the time [74], which means that exogenous stimulation gets more profound. This increase in QS near term age thus offers the brain the ability to re-wire itself to adjust to the increasing sensory exposures.

APPENDIX II

OBSERVATION FORM

The first page of the observation form used. For every minute the behaviours in the categories eyes, facial movement, body movement, sounds, heart rate and respiration rate were written down. Based on the observed behaviour, a sleep state got assessed at the end of each epoch. Lastly, a confidence score for sleep state classification was noted. For more information on this, see [9]. For an ethogram for the abbreviations, see Appendix III.

NICU room			
Location bed			
N of people present			
Type of crib			
Respiratory support			
Phototherapy			
Sleeping position			
N of beds occupied			
PMA at observation			
OBSERVATION			
Date:	**Starting time:**	**Ending time:**	

Min	**Sleep state***	**Eyes**	**Facial movement**	**Body movement**	**Sounds**	**HR**	**RR**	**Conf.**
180	A Q I	C CS CR OR O	$FM_{F,G,Si,E,M}$ Y FJ Sk Su FX	GM SM Tw Ji Sa Se Wr BX	So Sh H Sq C SX	Hrr Hri	Rrr Rri	
179	A Q I	C CS CR OR O	$FM_{F,G,Si,E,M}$ Y FJ Sk Su FX	GM SM Tw Ji Sa Se Wr BX	So Sh H Sq C SX	Hrr Hri	Rrr Rri	
178	A Q I	C CS CR OR O	$FM_{F,G,Si,E,M}$ Y FJ Sk Su FX	GM SM Tw Ji Sa Se Wr BX	So Sh H Sq C SX	Hrr Hri	Rrr Rri	
177	A Q I	C CS CR OR O	$FM_{F,G,Si,E,M}$ Y FJ Sk Su FX	GM SM Tw Ji Sa Se Wr BX	So Sh H Sq C SX	Hrr Hri	Rrr Rri	
176	A Q I	C CS CR OR O	$FM_{F,G,Si,E,M}$ Y FJ Sk Su FX	GM SM Tw Ji Sa Se Wr BX	So Sh H Sq C SX	Hrr Hri	Rrr Rri	
175	A Q I	C CS CR OR O	$FM_{F,G,Si,E,M}$ Y FJ Sk Su FX	GM SM Tw Ji Sa Se Wr BX	So Sh H Sq C SX	Hrr Hri	Rrr Rri	
174	A Q I	C CS CR OR O	$FM_{F,G,Si,E,M}$ Y FJ Sk Su FX	GM SM Tw Ji Sa Se Wr BX	So Sh H Sq C SX	Hrr Hri	Rrr Rri	
173	A Q I	C CS CR OR O	$FM_{F,G,Si,E,M}$ Y FJ Sk Su FX	GM SM Tw Ji Sa Se Wr BX	So Sh H Sq C SX	Hrr Hri	Rrr Rri	
172	A Q I	C CS CR OR O	$FM_{F,G,Si,E,M}$ Y FJ Sk Su FX	GM SM Tw Ji Sa Se Wr BX	So Sh H Sq C SX	Hrr Hri	Rrr Rri	
171	A Q I	C CS CR OR O	$FM_{F,G,Si,E,M}$ Y FJ Sk Su FX	GM SM Tw Ji Sa Se Wr BX	So Sh H Sq C SX	Hrr Hri	Rrr Rri	

*A = Active Sleep, Q = Quiet Sleep, I = Intermediate Sleep. Wakefulness can also be classified, then leave this column empty

APPENDIX III

ETHOGRAM FOR ABBREVIATIONS OF OBSERVATION FORM

Behaviour	Specification	Abbreviation
Eyes	Closed	C
	REM (closed)	CR
	REM (open)	OR
	Open	O
Facial Movement	Facial Movement	FM
	• Frown	F
	• Grimace	G
	• Smile	Si
	• Eyebrow	E
	• Mouthing	M
	Yawn	Y
	Facial Jerk	FJ
	Smacking	Sk
	Sucking	Su
	No Facial movement	FX
Body Movement	Gross movement	GM
	Small movement	SM
	Twitch / Jerk	Tw
	Jitter	Ji
	Startle	Sa
	Stretch	Se
	Writhing	Wr
	No body movement	BX
Sounds	Sobs	So
	Sigh	Si
	Hiccup	H
	Squeal	Sq
	Cry	C
	No sound	SX
Heart Rate	Regular	Hrr
	Irregular	Hri
Respiration Rate	Regular	Rrr
	Irregular	Rri

APPENDIX IV

TABLE IV

SUMMARY OF CHARACTERISTICS PER SLEEP STAGE

	Active Sleep	**Quiet Sleep**	**Wake**
Eyes	Closed; REM (open or closed eyes).	Closed.	Open.
Body movements	Gross movements; Small movements.	Reflexive movements*; High muscle tension; No movements.	Gross movements; No movements.
Facial movements	Non-reflexive facial movements; Non-rhythmic mouth movements.	Reflexive facial movements; Rhythmic mouth movements; No facial movements.	All facial movements.
Sounds	Grunts; Distressed sounds; Reflexive sounds.	Sobs/Sighs; Reflexive sounds.	All sounds.
Heart rate	Irregular.	Regular.	Regular, but faster.
Respiratory frequency	Irregular.	Regular.	Regular, but faster.
Activity level	High.	Low.	Either high or low.

*Reflexive movements endorse (but are not limited to): jerks, twitches, jitter, and startles. Intermediate Sleep does not have specific characteristics but can show behaviours from all the other states listed. List of abbreviations: Rapid Eye Movement (REM). Adapted from [9].

APPENDIX V

TABLE V
RESULTS OF TUKEY POST-HOC TESTING

Feature	Channel	Diff levels		Diff means	Lower	Upper	q-value	p-value
SAT%	Left	IS	QS	0.197	-0.133	0.527	2.172	0.418
		IS	AS	0.194	-0.122	0.511	2.230	0.393
		IS	W	0.011	-0.578	0.600	0.068	>0.900
		QS	AS	0.391	0.180	0.602	6.747	<0.001***
		QS	W	0.208	-0.332	0.748	1.401	0.729
		AS	W	0.183	-0.349	0.715	1.252	0.788
	Right	IS	QS	0.274	-0.075	0.624	2.854	0.181
		IS	AS	0.038	-0.297	0.373	0.412	>0.900
		IS	W	0.649	0.025	1.272	3.781	0.038*
		QS	AS	0.312	0.089	0.535	5.087	0.002**
		QS	W	0.374	-0.197	0.946	2.382	0.332
		AS	W	0.687	0.124	1.250	4.435	0.009**
ISP	Left	IS	QS	1.842	-1.698	5.382	1.892	0.534
		IS	AS	8.266	4.872	11.661	8.853	<0.001***
		IS	W	18.664	12.343	24.984	10.735	<0.001***
		QS	AS	10.108	7.847	12.370	16.250	<0.001***
		QS	W	20.506	14.714	26.297	12.872	<0.001***
		AS	W	10.397	4.694	16.101	6.627	<0.001***
	Right	IS	QS	1.694	-1.853	5.240	1.736	0.596
		IS	AS	7.988	4.586	11.389	8.538	<0.001***
		IS	W	22.250	15.917	28.583	12.772	<0.001***
		QS	AS	9.681	7.415	11.947	15.532	<0.001***
		QS	W	23.943	18.141	29.746	15.000	<0.001***
		AS	W	14.262	8.547	19.977	9.072	<0.001***
ISI	Left	IS	QS	0.607	-0.175	1.390	2.820	0.190
		IS	AS	0.861	0.110	1.612	4.168	0.017*
		IS	W	1.880	0.482	3.278	4.889	0.003**
		QS	AS	1.468	0.968	1.968	10.677	<0.001***
		QS	W	2.488	1.207	3.769	7.059	<0.001***
		AS	W	1.019	-0.243	2.281	2.937	0.161
	Right	IS	QS	0.223	-0.685	1.131	0.891	>0.900
		IS	AS	0.859	-0.012	1.730	3.584	0.055
		IS	W	2.231	0.601	3.861	4.976	0.002**
		QS	AS	1.081	0.501	1.662	6.773	<0.001***
		QS	W	2.454	0.959	3.948	5.967	<0.001***

		AS	W	1.372	-0.100	2.845	3.388	0.078
Absδ	Left	IS	QS	12.128	-134.640	158.895	0.300	>0.900
		IS	AS	122.481	-18.742	263.704	3.154	0.116
		IS	W	115.713	-155.572	386.998	1.551	0.670
		QS	AS	134.609	40.642	228.575	5.209	0.001***
		QS	W	103.585	-146.378	353.548	1.507	0.687
		AS	W	238.194	-8.554	484.942	3.510	0.063
	Right	IS	QS	21.662	-140.164	183.489	0.487	>0.900
		IS	AS	153.612	-2.100	309.325	3.587	0.055
		IS	W	6.663	-292.456	305.783	0.081	>0.900
		QS	AS	175.275	71.667	278.882	6.151	<0.001***
		QS	W	14.999	-260.611	290.609	0.198	>0.900
		AS	W	160.276	-111.789	432.341	2.142	0.430
Relδ	Left	IS	QS	0.004	-0.009	0.017	1.040	0.872
		IS	AS	0.001	-0.012	0.014	0.229	>0.900
		IS	W	0.058	0.033	0.082	8.586	<0.001***
		QS	AS	0.005	-0.004	0.013	1.969	0.504
		QS	W	0.061	0.039	0.084	9.929	<0.001***
		AS	W	0.057	0.035	0.079	9.308	<0.001***
	Right	IS	QS	0.001	-0.011	0.013	0.279	>0.900
		IS	AS	0.005	-0.006	0.017	1.681	0.618
		IS	W	0.068	0.046	0.090	11.304	<0.001***
		QS	AS	0.006	-0.001	0.014	2.962	0.155
		QS	W	0.069	0.049	0.090	12.432	<0.001***
		AS	W	0.063	0.043	0.083	11.466	<0.001***
ASI	Both	IS	QS	1.106	0.190	2.023	4.389	0.010**
		IS	AS	0.952	0.064	1.841	3.899	0.030*
		IS	W	1.752	-0.130	3.635	3.386	0.079
		QS	AS	2.058	1.464	2.652	12.606	<0.001***
		QS	W	0.646	-1.117	2.409	1.333	0.756
		AS	W	2.705	0.956	4.453	5.627	<0.001***

Significances are displayed with * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$. List of abbreviations: difference of levels (Diff levels), difference of means (Diff means), lower boundary of 95% confidence interval (Lower), upper boundary of 95% confidence interval (Upper), Active Sleep (AS), Quiet Sleep (QS), Intermediate Sleep (IS), wake state (W), proportion of Spontaneous Activity Transients (SAT%), inter-SAT percentage (ISP), inter-SAT interval (ISI), Absolute delta power (Absδ), relative delta power (Relδ), Activation Synchrony Index (ASI).