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TESI DI DOTTORATO

Sleep as a window for evaluating neurodevelopmental outcome: which impact on the brain of preterm infants? A prospective study on prematurity from a biological neuropsychiatry perspective

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To Ambra Luce, the new light of my life

To all the children of the world, who should never suffer

To my father & to the Rita(s) of my life, which guided me to becoming the woman I am

"E se a cambiare fossero le storie che ci insegnano da bambini? Se anziché farli addormentare sognandosi soli contro il mondo e l'uno contro l'altro dessimo loro avventure dove diventare potenti insieme?" – *Noi siamo Tempesta, 2019 Michela Murgia*

PREFACE

My PhD project has focused on finding relationships between sleep architecture and neurodevelopment among very low birth weight (VLBW) preterm infants, who, despite the great advances in neonatology, still survive carrying a wide spectrum of comorbidities, among which many interest the neuropsychiatry field, resulting in a consistent clinical and social burden.

It was a challenging task, since it was the first time that in our hospital was performed a study on neonatal EEG, with all the issues related to the start of a novel project: the purchase of specific equipment, deployment of resources, the running-in of evaluations both in terms of the quality of the examinations, the built-up of clinical/diagnostic workouts and the return to the parents of the patients involved.

All of this, which we already knew would be an onerous task in three years, has been slowed down by the COVID-19 pandemic, which, both in terms of restrictions due to, and in terms of bureaucratic slowdowns, has meant that registration of subjects has begun in November 2021. This unpleasant inconvenience gave me the clinical task of dealing with the mental health of children and adolescents during the pandemic period, and from this activity resulted 4 scientific papers as first author (single or shared) and 1 as co-author on a large international cohort, many academic and public talks and 2 reports for the Italian public community. Moreover, for the COVID-19 pandemic, even though I could not program an abroad research fellowship during my doctorate (initially due to the confinement and then due to clinical necessities), I had the chance to actively cooperate with the Psychotrauma Unit in Nice (France).

By the way, despite these difficulties and the remaining short period of time, I was able to collect data from 28 VLBW preterm neonates concerning at least a 24-hours videopolysomnographic (videoPSG) recording between at 34 weeks of post-menstrual age (PMA), a behavioural and visual assessment at 35 weeks PMA and at term equivalent age (TEA) and a follow-up (at 6 months of corrected age). In this work preliminary results from a sample of 10 VLBW are presented. Qualitative analysis on sleep distribution assessed with the videoPSG for 24hrs, its correlation early and mid-term neurological assessment and to brain lesions are presented. In this sample it was possible observing that VLBW infants show different trends in sleep patterns

during the 24 hours, that sleep states correlate with neurobehavioral state at 35 weeks PMA, at TEA and at 6 months of corrected age and that severe-moderate brain lesions impair sleep quantity, distribution and quality.
The reported results of this thesis are only the first step of a much more complex project that have just started his loading and that I aim to pursue during my research fellowship.

Thanks to this PhD project I had also the opportunity to build a strong collaboration with the Italian Institute of Technology (which resulted in a first paper on an animal model of prematurity which is about to be submitted on Science Translational Medicine by dr. Alberto Potenzieri and dr. Laura Cancedda), and I started collaborating with the engineering Department of Informatics, Bioengineering, Robotics and Systems of the University of Genova (in the person of prof. Gabriele Arnulfo and his working team with whom I have been a visiting fellow at the BABA Center of Sampsa Vanhatalo in Helsinki, Finland).
I have also started sowing the seeds for academic collaborations with national and international partners among which I count the participation in a large European project (ENSEMBLE-II).

During this three-year path, I had the opportunity to improve my skills and knowledge on the physiology of infants' sleep, the pathophysiology of preterms' neurodevelopmental disorders both from a clinical and preclinical point of view, the early developmental neuropsychology (such as early visual, neurological and neurobehavioral neonatal assessment), and the early identification of biological markers of risky conditions related to preterm birth (such as brain lesions). The understanding of pathogenetic mechanisms and the possibility of identifying infants at higher risk for neurological and neurodevelopmental problems finds reason in the possibility of preventing, where possible, the conditions that increase their risk and facilitating early neuroprotective and rehabilitative interventions. These collateral research interests resulted in published papers and abstracts or are about to result in the submission of written scientific contributions (see page 38).

I have also had the chance to implement my research activity with the clinical practice at the Child Neuropsychiatry Unit and at the Neonatal Intensive Care Unit of our Institute. I thus refined my clinical experience in the early assessment and diagnosis of at-risk infants and children, in the clinical neurophysiology evaluations and in the management of any kind of

neurodevelopmental disability (both neurological and psychiatric), grasping the direct effects of the observations that emerged in the various research projects.

I worked together with wonderful specialists, who helped and supported me in my clinical and research work, sharing with me knowledge and precious moments also of fun. Among them, to dr. Deborah Preiti and dr. Alberto Potenzieri goes my affectionate thanks for supporting me and sustaining me even in difficult times.

For all these opportunities, I thank my two professors, who continue to give me support both in terms of equipment and of working/scientific trust.

However, my passionate thank goes to my companion Luca, my mother, my two sisters and brother, and my friends (in particular Laura and Chiara) for the unconditioned beloved support they gave me even when I dealt difficult moments of struggling among my love for research, the need and necessity for keeping my economic independency, the unpredictable changes encountered, the loneliness felt at times, the sad family illnesses, and the exciting experience of becoming a mother while trying to preserve my job in the research field.

While I am finishing this thesis my daughter Ambra has started toddling. I interpreted it as a good omen for the growing of this project.

Genova, 05/09/2023

"Quante volte ve lo devo dire..! La vita non è perfetta, le vite nei film sono perfette. Belle o brutte, ma perfette. Nei film non ci sono tempi morti, mai" – *Radiofreccia*

ABSTRACT

Background. Newborns and infants spend most of their time sleeping an immature sleep, which allows brain maturation a good neurodevelopment. Preterm birth is associated with abnormal brain development and alterations in later-in-life sleep patterns, carrying a high social burden, even when not accompanied by major neurological damages. Which impact prematurity itself can have on early sleep architecture, which influence it can have on well-known adverse outcomes, and which role brain lesions play in determining sleep patterns in preterm infants are still matter of debate.

This exploratory pilot study aimed to describe the distribution of sleep states among very low birth weight (VLBW) infants, and to correlate it with neurobehavioral assessment at 35 weeks of post-menstrual age (PMA), and to observe if these persisted at term equivalent age (TEA) and at 6 months of corrected age (CA). Secondly, it aimed to assess if the presence of a major or minor brain lesion detected at MRI can affect sleep duration, distribution and quality.

Methods. 10 VLBW were assessed at 34±2 weeks PMA with a 24-hours video-polysomnographic recording and received a neurobehavioral examination at the moment of the recording and at TEA (with Neonatal Behavior Assessment Scale; Hammersmith Neurological Neonatal Examination, and the neonatal visual battery). They were followed-up at 6 months CA with Griffiths' Mental Development Scale III edition. Analysis of sleep stages distribution and spectra was conducted.

Results. Total sleep time and total amount of transitional sleep (TS) significantly positively correlated with neurological, and neurobehavioral assessment at 34 weeks PMA, at TEA and with neurodevelopment at 6 months CA, while Sleep Onset Active Sleep (SOAS) had a negative association. Infants carrying severe-moderate brain lesions showed lower Total Sleep time (66.9% ± 7.39 vs 72.2% ± 3.52, $p = 0.047$) accompanied by a higher prevalence of SOAS (23.9% ± 10.2 vs 12.26% ± 5.5 $p = 0.048$), and showed a gradient for higher power of posterior slow activity (slow δ and δ) during SOAS from the posterior cerebral regions.

Conclusions. Understanding sleep mechanism among preterm infants might provide future therapeutic/management strategies, which need to encompass sleep care. Further analyses with larger samples and more complex methods are claimed.

SUMMARY

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INTRODUCTION

Sleep

Sleep is a complex and vital neuroendocrine function, that could be defined as a reversible behavioral state, essential for maintaining optimal performance, well-being, and mental health (Carskadon, 2011). Arousal and sleep are dynamic physiologic processes regulated through a complex network of activation and suppression of cortical and sub-cortical pathways, and are finely regulated by inner homeostatic, circadian and ultradian rhythms (Bathory & Tomopoulos, 2017).

Two processes regulate sleep/awake cycles: an increase in hypnogenic substances throughout the day, which drive the homeostatic need for sleep (homeostatic process), and a synchronisation of neurons located in the suprachiasmatic nucleus in the ventral hypothalamus to daily exogenous environmental cues (for example light), also called zeitgebers, which activate photoreceptors in the retina and inhibit pineal gland secretion of sleep promoting hormone melatonin. Sleep itself has a cyclic organization (ultradian rhythms): it alternates between rapid eye movement sleep (REM) and non-rapid eye movement sleep (NREM) (Bathory et al. 2017).

Sleep undergoes changes from foetal to adult life, with specific patterns typical for age, associated with the development, maturation, and connectivity within neural networks (Dereymaeker et al., 2017). In last years the crucial role of both NREM (Non-Rapid Eye Movement) and REM sleep in neuronal plasticity has been established (de Vivo et al., 2017). REM sleep has been linked to brain development during the neonatal period by pruning and scaling synapses, enhancing learning and memory processes and allowing acquisition of

sensory-motor, visual, and adaptive behavioral skills (Blumberg et al., 2022; Frank & Heller, 2019).

Newborns and infants spend most of their time sleeping an immature REM sleep, conventionally named Active Sleep (AS) because presence of many movements during this phase, alternated with the calmer behavioural sleep state of Quiet Sleep (QS), equivalent to NREM sleep. Active sleep consists of low amplitude high frequency electroencephalogram (EEG) patterns that are similar to those seen during wakefulness. Newborns enter sleep cycles frequently through AS till 3-6 months of age. Quiet Sleep is characterised by high voltage low amplitude EEG activity, the absence of eye movements, higher muscle tone, regular heart rate and respiration, and a characteristic tracè-alternant pattern on EEG, characterised by bursts of slow waves intermixed with sharp waves and periods of relative quiescence with very low-amplitude activity, which usually disappears by 3–4 weeks of age (Bennet et al., 2018; Dereymaeker et al., 2017; Grigg-Damberger, 2016).

Sleep has also a strong effect on endocrine function, by regulating most of the hormonal secretions per se, by the circadian processes, and by behavioral changes. Sleep per se has a marked influence on levels of some hormones produced by the pituitary axes, especially the growth hormone (and one of its products, such as the Insulin-like growth factor type 1, IGF-1) and the cortisol, but the mechanisms through which sleep affects circulating hormonal levels are poorly understood (Morris et al., 2012). Literature suggests that sleep deprivations can alter the hypothalamic-pituitary axes, in particular the functioning of hypothalamic-pituitary-adrenal (HPA) axis, therefore changing its basal activity, influencing the stress responsiveness, immunity and neuroplasticity (van Dalfsen & Markus, 2018).

Since chronic sleep loss has been linked to aberrant wiring, chronic sleep disruption prevents proper refinement of mature neural circuits during early developmental stages and may predispose to neurodevelopmental and neurobehavioral problems (Bellesi et al., 2018).

The environmental and genetic mechanisms underlying sleep, which can give insights on its molecular substrates and its pathologies, remain poorly understood, a part from what concerns circadian rhythms, which rely on cell-autonomous processes (Ashbrook et al., 2020; Jan et al., 2020).

Brain vulnerability of preterm infants

Preterm birth has been defined as any birth before 37 weeks completed weeks of gestation and it affects 1 over 10 newborns each year (Howson et al., 2013). The causes of preterm birth is still matter of concern, but research supports a possible role of inflammatory, immunological, metabolic, endocrine, tissue remodelling, vascular, and endothelial pathways, with both some grade of inheritability and a higher prevalence of de novo mutations (Mead et al., 2023). Despite the great improvement in neonatal cares both in developed and developing countries, premature birth is still a common cause of morbidity, that includes neurological sequelae, neurodevelopmental disorders, and psychiatric complaints, thus requiring long-term health care assistance (Kroll et al., 2018; Mento & Nosarti, 2015).

Brain development is a major concern in premature infants, as prematurity disrupts the normal neurogenesis that happens in-utero. Neuronal precursors come from germinal matrices (intraventricular and cerebellar), two stem structure starting their development respectively since the 7^{th} and the 9^{th} week of gestation, reaching their maximum volume at

25 weeks, and withering subsequently at different stages. The germinal matrices are intrinsically vulnerable due to the limited astrocyte end-feet coverage of microvessels, the reduced expression of fibronectin, and the immature tight junctions, which allow neural proliferation and migration, therefore predisposing, in case of premature birth, to brain damage or to some grade of abnormal brain development in its ultrastructure (Ramenghi, 2015). Recently, we have been able to demonstrate that even small bleedings coming from these structures (intraventricular and cerebellar haemorrhages, IVH and CBH respectively) affect neurodevelopment of very preterm infants (Uccella et al., 2023).

Moreover, preterm infants are also exposed to brain vulnerability per-se, which is related both to other well-known brain lesions of prematurity such as periventricular leukomalacia, highly decreased in prevalence during last decades, and its etiologically related lesions (punctate white matter lesions), only detectable by bran Magnetic Resonance Imaging (MRI), which are associated to adverse outcome (Guo et al., 2017; Malova et al., 2023) and, of more challenging interest, to the higher risk for cerebral damage (Ment & Vohr, 2008).

The abnormal brain development occurring in premature infants is complex and it is a tricky matter of concern for neonatologists, obstetricians, and child neurologists, since the higher prevalence in this population of neurodevelopmental disorders (intellectual disabilities, autism spectrum disorders, attention deficit disorders), once severe brain lesions (and, therefore, cerebral palsies) are excluded. Several studies have documented white matter (in particular microglia) and grey matter injury (both neurons and interneurons) among preterm infants (Krishnan et al., 2017; Stolp et al., 2019). The underlying pathological mechanisms is still unclear, but likely to be multifactorial and to involve as strong predictors neuroinflammation, genetic/epigenetic factors, and social environment (Erdei et al., 2020; Fumagalli et al., 2018; Holloway et al., 2021; Provenzi & Montirosso, 2015).

Moreover, preterm birth implies an abrupt premature dissociation of the endocrine maternal-placental-foetal unit, resulting in a withdrawal of maternal/placental hormones such as insulin-like growth factor 1 (IGF-1) and its binding proteins (especially IGFBP-3), leptin, thyroid hormones, steroids, and estrogen, therefore leading to multiple endocrine disruptions, resembling a panhypopituitarism along with the increased release of stress hormones. This has been linked not only to impaired body growth and composition but also to adverse metabolic programming, and neurodevelopmental disorders (Möllers et al., 2022; Sullivan et al., 2017). In particular, the dysregulation of the HPA axis (at the level of a deficient pituitary responsiveness to exogenous corticotropin-releasing hormone, an insufficient 11β-hydroxylase activity, and a interconversion between cortisol and inert cortisone) and the impaired secretion of IGF-1 (a peptide hormone with mitogenic, antiapoptotic, and metabolic functions which plays a fundamental role in promoting the growth and differentiation of several cell types, including brain cells) have been designed as possible major actors of the neurobehavioral problems associated with prematurity (Fernandez & Torres-Alemán, 2012; Finken et al., 2017; Hellström et al., 2016). An animal model for preterm birth has been ideated and developed by doctor Alberto Potenzieri (from the team of doctor Laura Cancedda, Italian Institute of Technology, Genova – Italy) and our team (Potenzieri, Uccella et al, *under submission to Sci Trasl Med*) confirms this hypothesis.

Sleep and prematurity

Premature birth disrupts also sleep homeostasis and its associated neuroendocrine processes (Dereymaeker et al., 2017). Effects of prematurity on sleep and, vice versa, consequences of

sleep alterations, also related to the environmental stressors of the Neonatal Intensive Care Unit (NICU) can be detrimental, having significant implications for their development, behavior, and overall health. Moreover, preterm babies seem to have more frequent sleep disorders in childhood, related often to social and attention problems: long term effects of sleep disturbances on prematurity and role of destroyed sleep in enhancing abnormal neurodevelopment is still poorly understood (Caravale et al., 2017).

It is unclear which impact can have prematurity itself (and its related, even minor, brain lesions) on early life sleep architecture, which kind of outcome sleep alteration can lead, and which kind of interventions could be done to prevent unfavourable outcomes (Bennet et al., 2018).

Understanding the pathological basis of sleep alterations in this vulnerable population is essential for early diagnosis and personalized treatment strategies.

Preterms' behavioral states are a source of interest. For instance, the role of AS in determining neurodevelopmental outcome among very preterm infants is intuitable, but yet unexplored (Calciolari & Montirosso, 2011; Cirelli & Tononi, 2015; Gogou et al., 2019). A recent study on very preterm infants conducted at term of equivalent age demonstrated different possible roles AS and QS at this age and the presence of alterations of connectivity between AS and QS in occipital regions (and their correlation with poor visual and social outcome at two years of age) (Tokariev et al., 2019).

Transitional states are also poorly investigated but can be sources of information for understanding the neurodevelopmental trajectories of these babies (Uchitel et al., 2022).

Only a few studies assessed the early sleep states organization and ultradian sleep distribution among premature infants (Biagioni et al., 2005; Curzi-Dascalova et al., 1993).

Quite recently, American Academy of Sleep Medicine has revised the sleep staging classifications for infants and the novel criteria open new perspective of sleep research in the above mentioned fields (Grigg-Damberger, 2016).

Study aims

The primary aim of this study was to describe the distribution of sleep states among very preterm infants during a 24-hours video-polysomnographic recording, to evaluate the influence of sleep states on neurobehavioral assessment at 35 weeks of post-menstrual age (PMA), and to observe if these persisted at term equivalent age (TEA) and at 6 months of corrected age (CA).

A second aim was to assess if the presence of a major or minor brain lesion detected at MRI can affect sleep duration and distribution.

Finally, this study aimed to explore the effects of brain lesions of prematurity across sleep stages using EEG power spectral analysis, in the hypothesis that the presence of a brain lesion may also have an effect on brain maturation as examined by sleep EEG quantitative analysis. In this manuscript preliminary exploratory analyses are reported.

MATERIALS AND METHODS

This is a prospective cross-sectional study. Prior to starting this study, Ethical Committee Approval has been achieved (PN-CGM study, CERL number approval 0028224/21 of the 6/10/2021), and parents provided informed consent for the study.

Subjects

Subjects born very preterm with a birth weight under 1500 grams (Very Low Birth Weight, VLBW) and hospitalized at the Neonatal Intensive Care Unit of the Gaslini Children Hospital, Genova (Italy) from November 2021 were considered eligible for the study. The ones carrying known or discovered genetic/metabolic and malformative disorders were excluded.

Patients started the protocol once they became hemodynamically and respiratory stable and free of sedations (such as morphine, barbiturates, and benzodiazepines) at 34 +/-2 weeks of post menstrual age (PMA).

Prenatal, intrapartum, and neonatal data were collected after the scoring of the neurophysiological and neurobehavioral assessment and included information collected as already noted in previous studies (Uccella et al., 2023). The following categories have been included: a) prenatal information (including twinship, monochoriality, twin-to-twin transfusion, use of assisted reproductive technologies, gestational diabetes, maternal hypertension, metrorrhagia and/or placental abruption, premature prolonged rupture of membranes (pPROM), intrauterine growth restriction (IUGR), complete antenatal steroid course); b) intrapartum information (type of delivery, Apgar score at 1st and 5th minute, birth weight, gestational age, sex); c) neonatal information (need for invasive or non-invasive

ventilation and their duration, surfactant use, early onset sepsis [bacteraemia or bacterial meningitis occurring at 72 hours], late onset sepsis [worsening of clinical conditions occurring at >72 hours treated with antibiotic therapy], necrotising enterocolitis, surgical treatments, presence of retinopathy of prematurity (ROP) stage III-IV); d) presence of anatomically detectable brain lesions at brain MRI performed at term TEA during spontaneous sleep using the "feed and wrap" technique (Ibrahim et al., 2015) on a 3T MR scanner (InteraAchieva 2.6; Philips, Best, The Netherlands) using a dedicated pediatric head/spine coil. Brain lesions were categorized in in mild, moderate or severe IVH or CBH or mild, moderate or severe white matter lesions (WML). For details on MRI protocol and classification, see our previous study (Malova et al., 2023, p. 20).

For this study aims, we divided in presence of mild (IVH grade I or II according to modified Volpe classification, punctate WML situated only posteriorly to the mid ventricle line, punctate CBH) or moderate/severe lesions (IVH grade III or IV, punctate WML situated anteriorly to the mid ventricle line, periventricular leukomalacia, limited or massive CBH).

Neurophysiological assessment

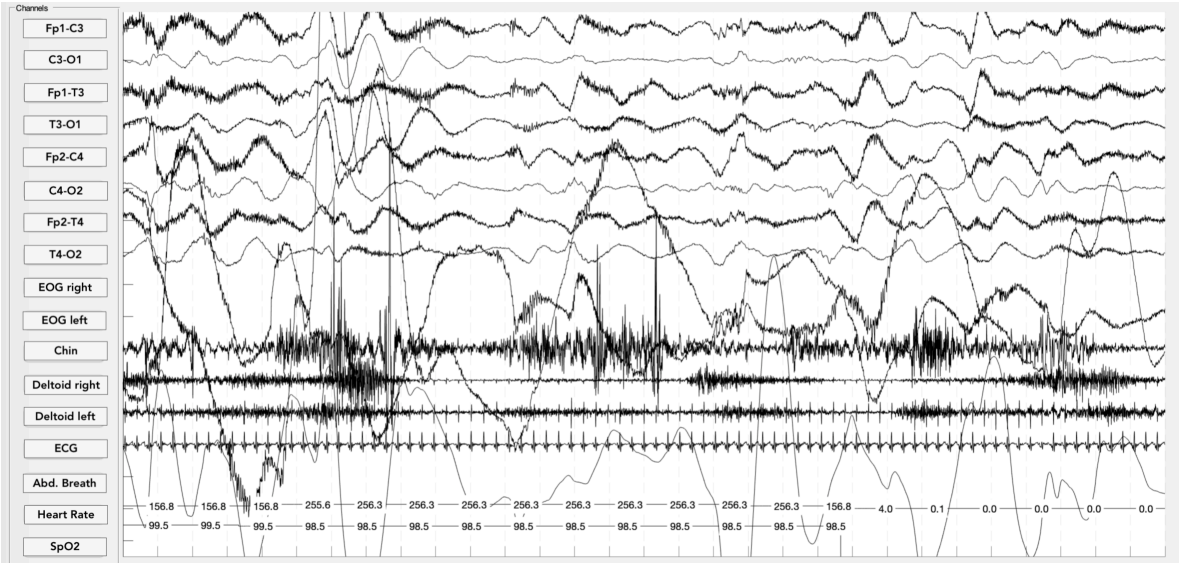
Video-polysmonographic assessments were obtained with a Brain QUICK® (Micromed) machine.

Registrations were conducted for 24 consecutive hours starting at 8.00 am. Ten scalp silver electrodes were placed according the International 10-20 system (Fp1, Fp2, C3, C4, T3, T4 O1, O2, a physical reference and a ground electrode, accompanied by two electroculograms, the submental electromyogram, the cardiac and the abdominal breath electrodes, two electromyograms placed at the deltoids and a saturimetry probe (André et al., 2010). Electroencephalogram activity sampling frequency was set at 512 Hz.

Electrode assembly was performed in respect of individualized care measures appropriate for age (Stjerna et al., 2012). Recordings were acquired and then manually scored by the same clinical (SU), blinded for the medical history of the patient, in 30-seconds epochs, as proposed by the American Academy of Sleep Medicine, in order to determine active/quiet wakefulness, Active Sleep (AS), Quiet Sleep (QS) or Transitional Sleep (TS).

Active wakefulness (AW) was defined by a behaviour corresponding to 5-6 Brazelton state: high muscle tone and movements, eyes generally open (eyes are allowed to be closed if infant is being fed or is crying/yawning). It is linked to an electroencephalogram (EEG) characterized by numerous movements' artifacts, a continuous pattern, and irregular respiration and heart rate (*Figure 1*).

Figure 1. Example of active wakefulness in a very preterm infant recorded at 32.5 weeks PMA

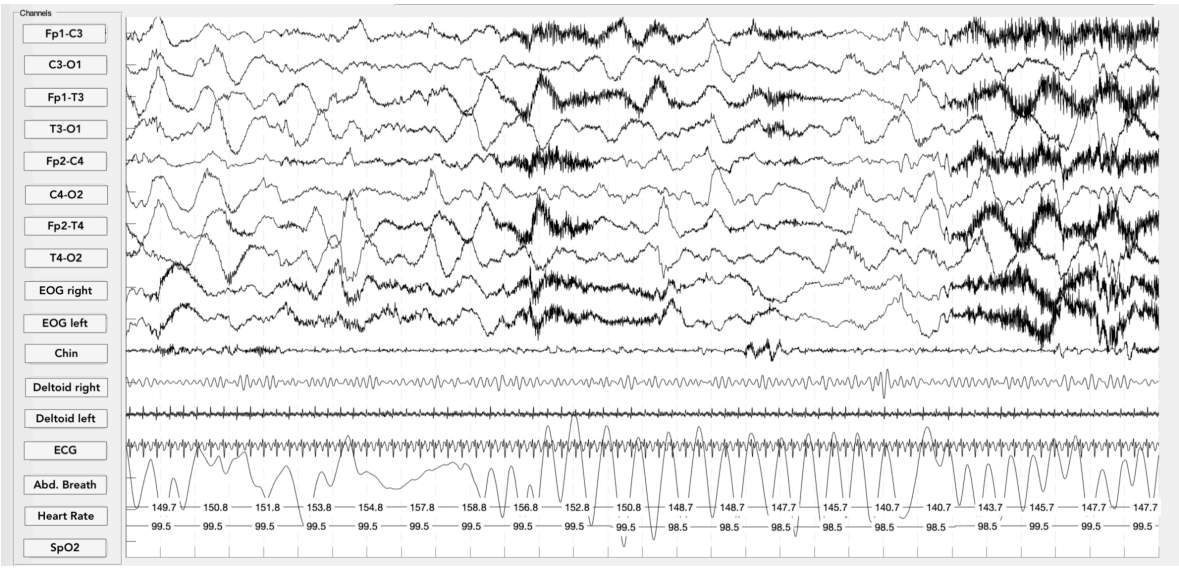


Legend. Note high muscular activity overlaying the rapid EEG activity.

Quiet wakefulness (QW) was defined by Brazelton state 4: attention focused, eyes wide open, low muscular activity (but present), a continuous EEG pattern (low voltage irregular or

mixed) and a low motor activity. Transitional state of drowsiness/semi-dozing (Brazelton stage 3) featured by open but dull and heavy-lidded, or blinking, variable activity level with some startles, delayed reactive to sensory stimuli, and a continuous EEG pattern characterized by theta and delta activity at times (low voltage irregular or mixed patterns) was also scored as QW (*Figure 2*).

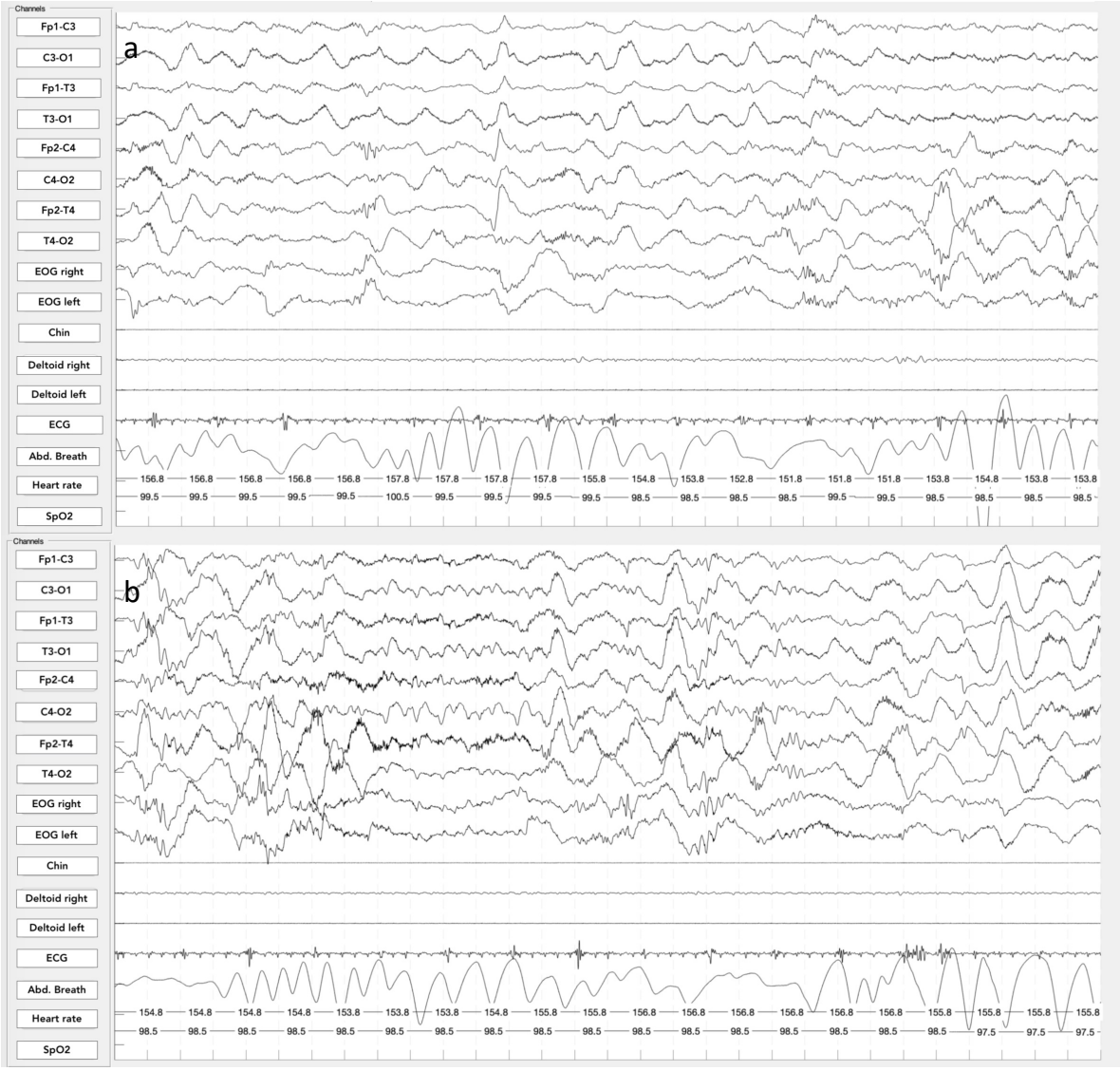
Figure 2. Example of quiet wakefulness in a very preterm infant recorded at 32.5 weeks PMA



Legend. Note the low mastoid/temporal muscular activity overlaying the rapid EEG trace and the mylohyoid activity associated to a suction movement.

Active sleep (AS) was defined by closed eyes with intermittent eyes movements, brief facial and body movements (twitches), isolated vocalizations, variable and augmented heart rate and breathing, and relatively continuous EEG compared with quiet sleep. Mylohyoid tone is absent or briefly present (*Figure 3a*). Sleep Onset Active Sleep (SOAS) was usually scored if the baby fell asleep during AS. Mixed pattern is often seen during SOAS, low voltage irregular and, sometimes, high voltage slows patterns are frequently seen during AS) (*Figure 3b*).

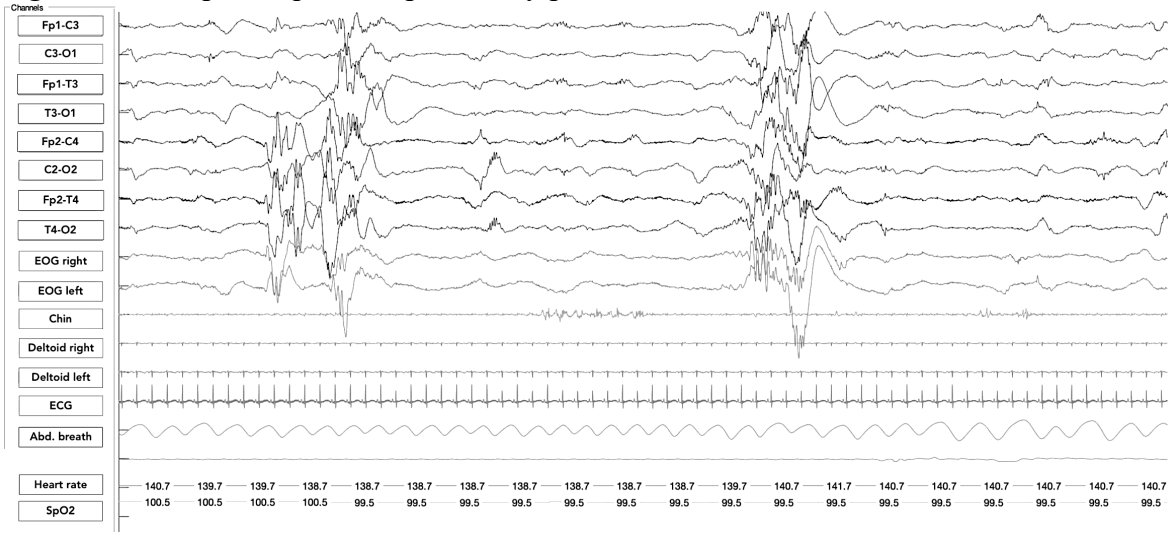
Figure 3. Example of sleep onset active sleep (3a) and active sleep (3b)in a very preterm infant recorded at 32.5 weeks PMA



Legend. Note the continuous activity, the irregular breath and heart rate in both 3a and 3b. SOAS usually is believed to have also more REMs, present in this page. Note on 3b a muscular twitch of the cheeks.

Quiet sleep (QS) was defined by closed eyes, poor movements, deep breathing and regular heart rate, and discontinuous EEG pattern (trace alternant) compared with active sleep and wakefulness. Mylohyoid tone is present (*Figure 4*).

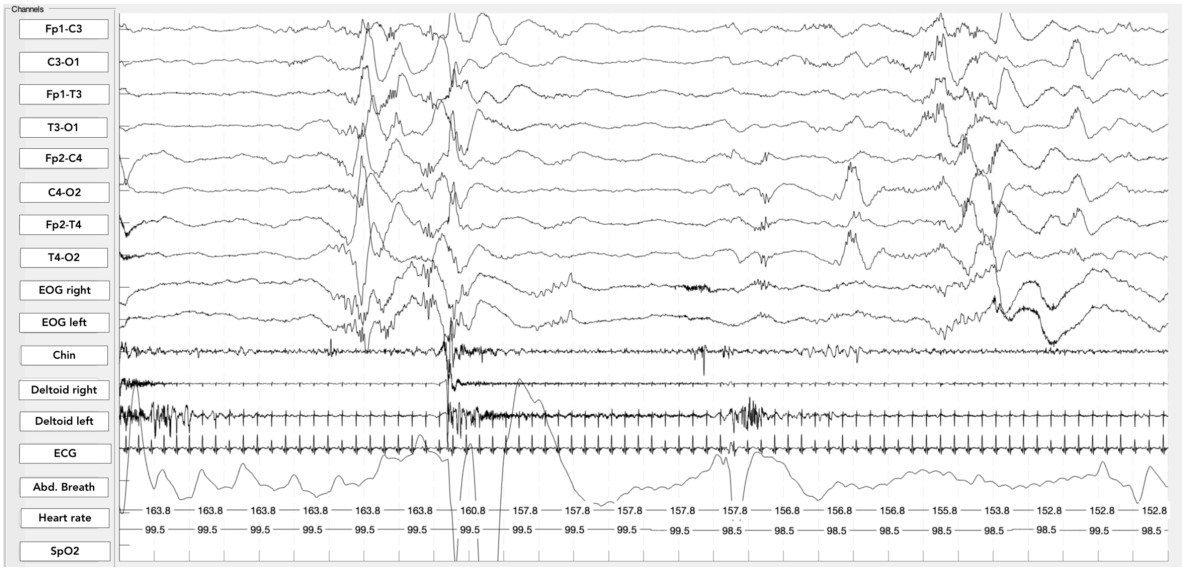
Figure 4. Example of quiet sleep in a very preterm infant recorded at 32.5 weeks PMA



Legend. Note the discontinuity of the traces and the alternance of burst of high amplitude waves with low-voltage highly sporadic activity in presence of chin tone, regular breath and heart rate small variability.

Transitional sleep (TS) was scored when polygraphic patterns were equally attributable at both SOAS/AS and QS (Grigg-Damberger, 2016) (*Figure 5*).

Figure 5. Example of transitional sleep in a very preterm infant recorded at 32.5 weeks PMA



Legend. note the discontinuos EEG pattern associated to chin tone suggest QS stage, but irregular breath and heart rate and muscle activation (deltoids), and some eye movements are in favour of AS.

Acquired polysomnographic data were transformed to EDF (European Data Format) to be imported into MatLab (MatLab 9.10, The Matworks Inc., Natick, MA, USA). Scoring data were then performed and stored in a software (PSG Lab) linked to the traces, developed in the laboratory of Institute of Molecular Bioimaging and Physiology, CNR, Genoa, Italy, which facilitates quantitative EEG analysis (Nobili et al., 2011).

Spectral EEG analysis

The above mentioned served for spectral EEG analysis. Fast Fourier Transform (Welch method) was applied. EEG signals were filtered at high-frequency filter of 70 Hz, low-frequency filter of 0.5 Hz. Artifacts (excessive movement, ventilatory machines artifacts, and instrumental noise) were removed. Inter burst low amplitude activity present in QS and TS was also removed. The bipolar Fp2-C4, T4-O2, Fp1-C3 and T3-O1 channels were chosen used for the analysis to catch differences among anterior and posterior regions in the two hemispheres, in the hypothesis that differences in brain maturation, that show a posterior-anterior gradient (Brody et al., 1987; Dubois et al., 2008), could be observed. The best 10 minutes of each sleep recording were divided into an equal number of epochs (10 steps) at a rate of 1 minute.

Mean amplitude values were obtained from each sleep stage. Five frequency bands were obtained [slow delta (δ), 0–2 Hz, delta (δ), 2–4 Hz; theta (θ), 4–8 Hz; alpha (α), 8–12 Hz; and beta (β), 12.0–24 Hz] and expressed as mV/min. Total power over the entire frequency range of interest (0.5–30 Hz) was also generated. Power spectra expressed in terms of absolute values (over total spectral power. To analyse possible differences in brain gradient

maturation, a Δ was calculated among posterior and anterior relative powers for one of two hemispheres.

Neurological and neurobehavioral assessment at 34 +/- 2 weeks PMA at TEA

Infants were assessed midway between feedings in a quiet environment, at the presence of their caregiver, by a trained examiner (SU) at 34 +/- 2 weeks PMA and at TEA, blinded to medical information at the moment of the evaluation.

The behavioural and neurological assessments (preformed in their complete version) have several items that are administered in both examinations, such as coding of neurological reflexes, observation of movements, tone patterns and head control. Hence, a standard protocol of assessment was developed and a standard a procedure of assessment was performed. The standard administration protocol commenced with the behavioral evaluation and then continued with the neurological and visual assessments. Repeated items were performed once and served for both the scales.

Neonatal Behavioural Assessment Scale (NBAS)

Neonatal Behavioural Assessment Scale (NBAS) developed and revised by Brazelton TA (Brazelton, 1984) was chosen for assessing their behavioural pattern. It is a well-known instrument that allow the evaluation of behaviour maturation among neonates, being also a good screening tool for predicting cognitive performance later in life (Canals et al., 2011). The full version of NBAS consists of 28 behavioural items, 18 reflex items, and 7 supplementary items (infant's alertness, infant's cost of attention, examiner's facilitation, infant's general irritability, infant's robustness and endurance, infant's regulation capability, and examiner's emotional response), these last ones describing more qualitative aspects of

the infant's assessment, to catch stress signs. The scale was scored adopting the method ideated Lester and Brazelton in 1982, where higher scores correspond to better performances (Lester & Brazelton, 1982). In this method, behavioural clusters are identified from the first section of the examination: habituation (which assesses the competence in responding and returning to an asleep state after a awakening stimulus), orientation (which assesses the engagement to stimuli and the quality of overall alertness), motor performance (which assesses the motor maturation), range of state (which describes infant's states, including arousal and calmness, and their lability), regulation of state (which assesses infant's skill to regulate his or her state in the face of increasing levels of stimuli) and autonomic nervous system stability (which assesses signals of autonomic stress related to homeostatic adjustments).

Hammersmith Neonatal Neurological Examination (HNNE)

The HNNE is a neurological evaluation developed for assessing term and preterm infants during the neonatal period. It includes six domains of assessments (categorized in items following a five-point Likert scale): tone, tone patterns, reflexes, spontaneous movements, abnormal neurological signs, and behaviour. The tool has been therefore interpreted on the basis of the optimality score developed by Dubowitz and Mercuri in 1998, which gives an overall optimality score of maximum 34 points (Dubowitz et al., 1998).

The HNNE has been traditionally validated for infants assessed at term or at TEA (Mercuri et al., 2003) and predict motor outcome (Ricci, Romeo, et al., 2008), by the way in recent years this tool has been adopted for studying preterm infants at earlier PMA, showing high predictive values (Howard et al., 2023). In this work, although normative data have been provided for early assessment (Spittle et al., 2016), we referred to the optimality score

developed by Dubowitz at al. in 1998, for the 37-38 weeks PMA, as previous studies did (Howard et al., 2023). HNNE has been chosen primarily because it is a reliable measure of neurological outcome among preterm infants, but also because of is correlation to cognitive outcome (Huf et al., 2023).

Neonatal assessment of visual function (by Ricci et al., 2008)

The neonatal visual assessment battery was developed by Ricci et al. in 2008 includes 9 items assessing ocular movements (spontaneous behaviour and in response to a target), the ability to fix on and follow a black/white target (horizontally, vertically and in an arc), reaction to a colored target, the ability to discriminate black and white stripes of increasing spatial frequency and the ability to keep attention on a target moved slowly away from the infant (Ricci, Cesarini, et al., 2008). The individual items are summed to compose a global score for the neonatal visual assessment. Lower scores indicate better visual performance (Ricci et al., 2008).

This battery was chosen since it has been designed as a quick feasible instrument that can be administered as early as 31 weeks of PMA, when most preterm infants manage to complete the test (Ricci et al., 2010, p. 201).

Follow-up at 6 months of CA

Neurodevelopmental assessment at 6 months of CA was performed using the Griffiths Mental Development Scales – III edition (GMDS) (Green et al., 2020), in the Italian n their Italian adaptation (Lanfranchi et al., 2019). The tool assesses the following domains: Learning fundations (which gives information on cognitive skills and precursors of executive funcioning), Language and Communication (which gives information on receptive and

expressive language), Eye and Hand Coordination (which gives information on fine motor skills), Personal-Social–Emotional (which gives information on social interaction and emotional regulation), and Gross Motor Skills (which gives information on gross motor funcioning). Obtained scores are converted to standardized development quotients, DQ, (mean range of 100 +/- 15, 10^{th}-90^{th} centile).

Statistical Analysis and Interpretation

Statistical analyses were performed by MatLab (MatLab 9.10, The Matworks Inc., Natick, MA, USA). All the tests were two-sided and statistical significance was set at a p value ≤ 0.05 with a 99% confidence interval. Borderline significance (p value > 0.05 and ≤ 0.08) will be reported.

The Shapiro–Wilk test was performed to evaluate the distribution of continuous variables, and outliers were checked by visual assessment of distribution graphs.

Continuous variables are reported in terms of either means and standard deviations or medians and minimum-maximum values. Categorical variables are reported in terms of absolute frequencies and percentages.

Fisher Exact or Mann-U Whitney test were considered appropriate to analyse differences among variables. Associations were calculated with Spearman correlation test.

For having an exploratory view on sleep/wakefulness distribution among the sample, we divided the sleep hours in day time (08:00-20:00) and night time (20:00-08:00), as previous studies did (Giganti et al., 2007). Wilcoxon rank-sum test was adopted for determining significant differences among these two times.

RESULTS

We enrolled 28 subjects born VLBW. Of them, we conducted preliminary exploratory analysis on a sample of 10. Variables showed a skewed non-normal distribution.

Sample features

Table 1 reports prenatal, intrapartum, and neonatal features and the prevalence of brain lesions detected at bran MRI at TEA of the sample. Moderate/severe brain lesion among the sample was 1 severe IVH (grade IV). Mild brain lesions were 2 posterior PML and IVH grade I-II. Distribution of described categories and of brain lesions resemble the one observed in large sample presented in our previous studies (Malova et al., 2023; Uccella et al., 2023), so it has been considered to be representative of our casistics.

Table 1. Prenatal, intrapartum, and neonatal features of the investigated sample

	Total (n 10)
Multiple gestation, *n (%)*	4 (40%)
Monochorial twins, *n* (%)	3 (30%)
IUGR, *n (%)*	5 (50%)
Maternal hypertension, *n (%)*	3 (30%)
Gestational diabetes, *n (%)*	1 (10%)
Absent/incomplete antenatal steroid course, *n (%)*	2 (20%)
pPROM, *n (%)*	1 (10%)
Birth by caesarean section, *n (%)*	7 (70%)
Gestational age (weeks), *mean (sds)*	30.5 (3.2)
Birth weight (grams), *mean (sds)*	1300.0 (344.7)
Apgar at 1st minute, *mean (sds)*	6.4 (2.1)
Apgar at 5th minute, *mean (sds)*	8.0 (1.3)
Intubation during first 72 h, *n (%)*	4 (40%)
Pneumothorax during first 72 h, *n (%)*	2 (20%)

HFO during first 72 h, *n (%)*	0 (0%)
MV > 14 days, *n (%)*	1 (10%)
Multiple surfactant doses, *n (%)*	2 (20%)
EOS, *n (%)*	1 (10%)
LOS, *n (%)*	1 (10%)
NEC, *n (%)*	1 (10%)
Surgery, *n (%)*	1 (10%)
BPD, *n (%)*	2 (20%)
ROP (stage III-IV), *n (%)*	1 (10%)
Mild brain lesions	3 (30%)
Moderate/severe brain lesions	1 (10%)

Legend. EOS, early-onset sepsis; GA, gestational age; IUGR, intrauterine growth restriction; HFO, high-frequency oscillations; MV, mechanical ventilation; NEC, necrotizing enterocolitis; EOS, early-onset sepsis; LOS, late-onset sepsis; pPROM, premature prolonged rupture of membranes; ROP, retinopathy of prematurity

Table 2 summarises the scores obtained at the neurobehavioral assessments at 34 weeks PMA, at TEA and at 6 months of CA. The sample showed features similar to the ones reported by literature (Canals et al., 2011; Mercuri et al., 2003; Ricci et al., 2010).

Table 2. Longitudinal Neuobehavioral assesments of the studied sample

Evaluation at 34 weeks PMA	*median (min-max)*
HNNE total score	28 (1.50-30)
Visual assessment score	3.5 (2-11)
NBAS assessment	
Habituation	6.8 (4.3-8.0)
Orientation	7.4 (2.8-8)
State Organization	5.5 (3.3-6.3)
State Regulation	6.6 (3.8-7.5)
Autonomic Organization	5.2 (3.7-6.3)
Motor Organization	5.1 (4.4-6.5)
Supplementary Items	7.1 (4.6-7.4)
	4.0 (1-13)
Evaluation at 34 weeks PMA total score *median (min-max)*	
HNNE total score	32.0 (13.5-33.5)
Visual assessment score	1.5 (0-8)
NBAS assessment	
Habituation	7.3(4.3-7.8)

Orientation	7.8 (3.3-8.1)
State Organization	4.6 (3.3-7.8)
State Regulation	6.5 (4.3-8.0)
Autonomic Organization	7.0 (4.0-8.0)
Motor Organization	6.8 (4.4-7.5)
Supplementary Items	7.5 (4.6-8.3)
	0.5 (0-15)
Evaluation at 6 months CA	
Griffiths Mental Development Scales III	
Total Developmental Quotient	117.5 (55-128)
Learning foundations	126.5 (56-138)
Language and Communication	105 (40-120)
Eye and Hand Coordination	110 (61-133)
Personal-Social–Emotional	114 (67-128)
Gross Motor Skills	113.5 (54-118)

Legend. HNNE, Hammersmith Neonatal Neurological Examination; NBAS, Newborn Behavioral Assessment Scale. Note that lower scores at visual assessment and higher scores at HNNE, NBAS and Griffiths-III correspond to better performances.

In *Table 3* are summarised polysomnographic values obtained by the 24-hours video-PSG scoring. Our sample respect data already present in literature (André et al., 2010; Curzi-Dascalova et al., 1993, 1993).

Table 3. Distribution of behavioral stages

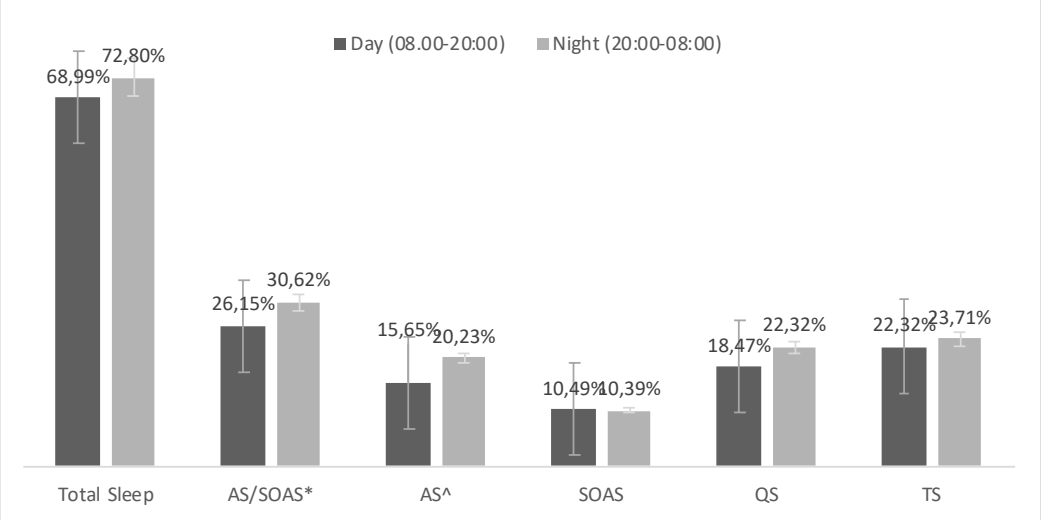
	mean ± sds (%)
Total Sleep time	**1020.8 ± 70.3 (70.8)**
Total Active Sleep (SOAS+AS)	**408.7 ± 121.8 (40.1)**
Sleep Onset Active Sleep	150.4 ± 68.11 (14.7)
Active Sleep	258.3 ± 90.9 (25.4)
Quiet Sleep	**280.8 ± 63.27 (27.5)**
Transitional Sleep	**331.3 ± 141.78 (32.4)**

Legend. Data are expressed in mean minutes and percentages are referred to the whole registration (24 hours) for the Total Sleep time and to total sleep time for the different behavioural states. SOAS stands for Sleep Onset Active Sleep and AS stands for Active Sleep.

VLBW infants show different trends in sleep stages distribution during the 24 hours

In the examined sample a trend of less sleep quantity during daytime hours (08:00-20:00) than in night-time (20:00-08:00) was observed (*Figure 6*). This trend affects all types of states examined and has statistical significance for AS/SOAS ($p = 0.05$) and borderline significance for AS ($p = 0.069$).

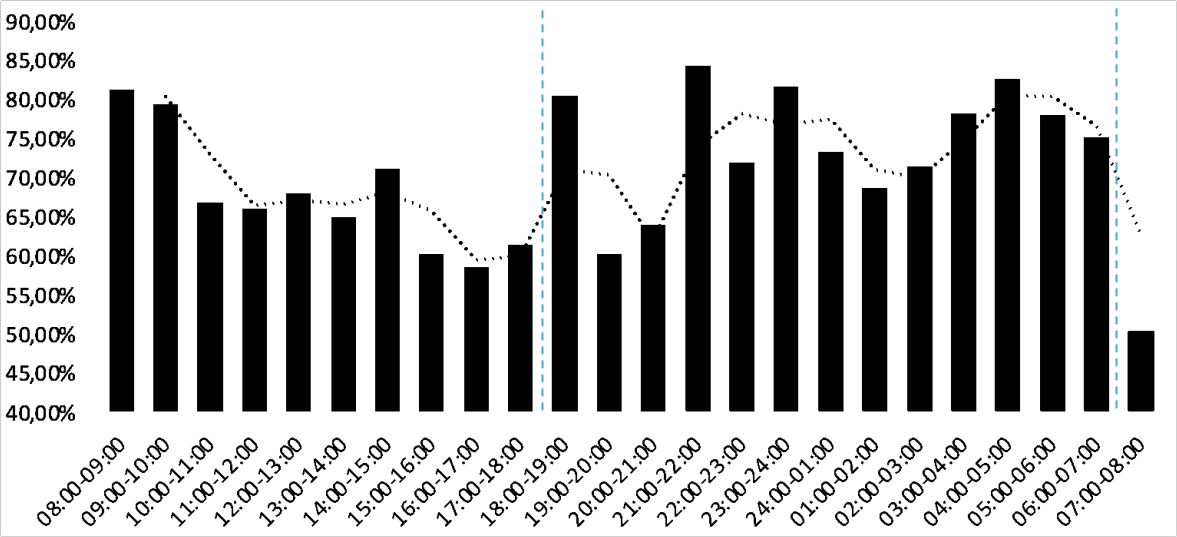
Figure 6. Average distribution of sleep states among day and night times.



Legend. * *p value* = 0.05; ^*p value* = 0.69. Differences among continuos variables have been calculated with Wilcoxon rank-sum test.
AS, Active Sleep; QS, Quiet Sleep; SOAS, Sleep Onset Active Sleep; TS Transitional Sleep.

When looking at the average distribution of the hourly amount of sleep during the 24 hours, a biphasic distribution is intuitable, with a prevalence of sleep in the evening and early morning periods (18:00-07:00) (*Figure 7)*.

Figure 7. Average distribution per-hour of total sleep time during the 24 hours.



Sleep states correlate with neurobehavioral state at 35 weeks PMA

In the sample examined, a strong correlation of sleep states set was seen with neurological, neurobehavioral and visual skills at 35 weeks of PMA (*Table* 4). Higher total sleep times were associated with HNNE scores and the NBAS at all domains. Quiet sleep and TS showed strong positive correlations, while SOAS and AS had a strong negative correlation to the outcomes assessed.

Table 4. Correlation among sleep distributions and neurobehavioral assessments performed at 35 weeks PMA.

ρ	**Total Sleep time**	**AS/SOAS**	**SOAS**	**AS**	**QS**	**TS**
HNNE total score	**0.681***	-0.527	**-0.707***	-0.359	0.563	**0.651***
Visual assessment score	-0.125	**0.751***	0.551	**0.751***	-0.426	**-0.701***
NBAS						
Habituation	**0.718***	**-0.635***	-0.431	**-0.707***	0.216	**0.695***
Orientation	**0.852***	**-0.755***	-0.671*	**-0.731***	**.743***	0.503
State Organization	**0.871***	**-0.732***	-0.659*	**-0.732***	**.878****	0.415
State Regulation	**0.643***	**-0.731***	-0.455	**-0.802****	0.311	**0.719***

Autonomic Organization	**0,845****	**-0.647***	-0.335	**-0.766***	0.467	0.591
Motor Organization	**0.892***	**-0.695***	**-0.743***	**-0.647***	**0.850****	0.419
Supplementary Items	**0.683***	**-0.732***	-0.537	**-0.683***	**0.823****	**0.927***
Reflexes	**0.621***	**0.695***	0.874**	0.383	-0.431	**-0.647***

Legend. Correlation at the Spearman test * significance at the 0.05 level (2-tailed); ** significance at the 0.01 level (2-tailed). SOAS stands for Sleep Onset Active Sleep and AS stands for Active Sleep. Note that lower scores at visual assessment and higher scores at HNNE and NBAS correspond to better performances.

When looking at possible associations between amount of sleep during the night, the sample showed positive significant correlations ($\rho > 0.8$; $p < 0.05$) among total sleep time and TS with NBAS scales of Orientation and state organization, while AS and SOAS showed negative significant moderate ($-0.6 < \rho < -0.8$; $p < 0.05$) correlations with the same scale.

Observed sleep states distributions have an influence on neurobehavior at TEA

Observed associations at 35 weeks PMA, persisted with the same trend of significance, also at TEA. Of noted difference, Total Sleep Time correlated with Autonomic Organization at TEA (ρ 0.878, $p < 0.01$).

Observed sleep states distributions have an impact on neurodevelopment at 6 months CA

At 6 months of CA, an influence of sleep states organization on total neurodevelopment, learning and fine motor skills is visible (*Table 5*).

Table 5. Correlation among sleep distributions and neurodevelopmental assessments performed at 6 months CA.

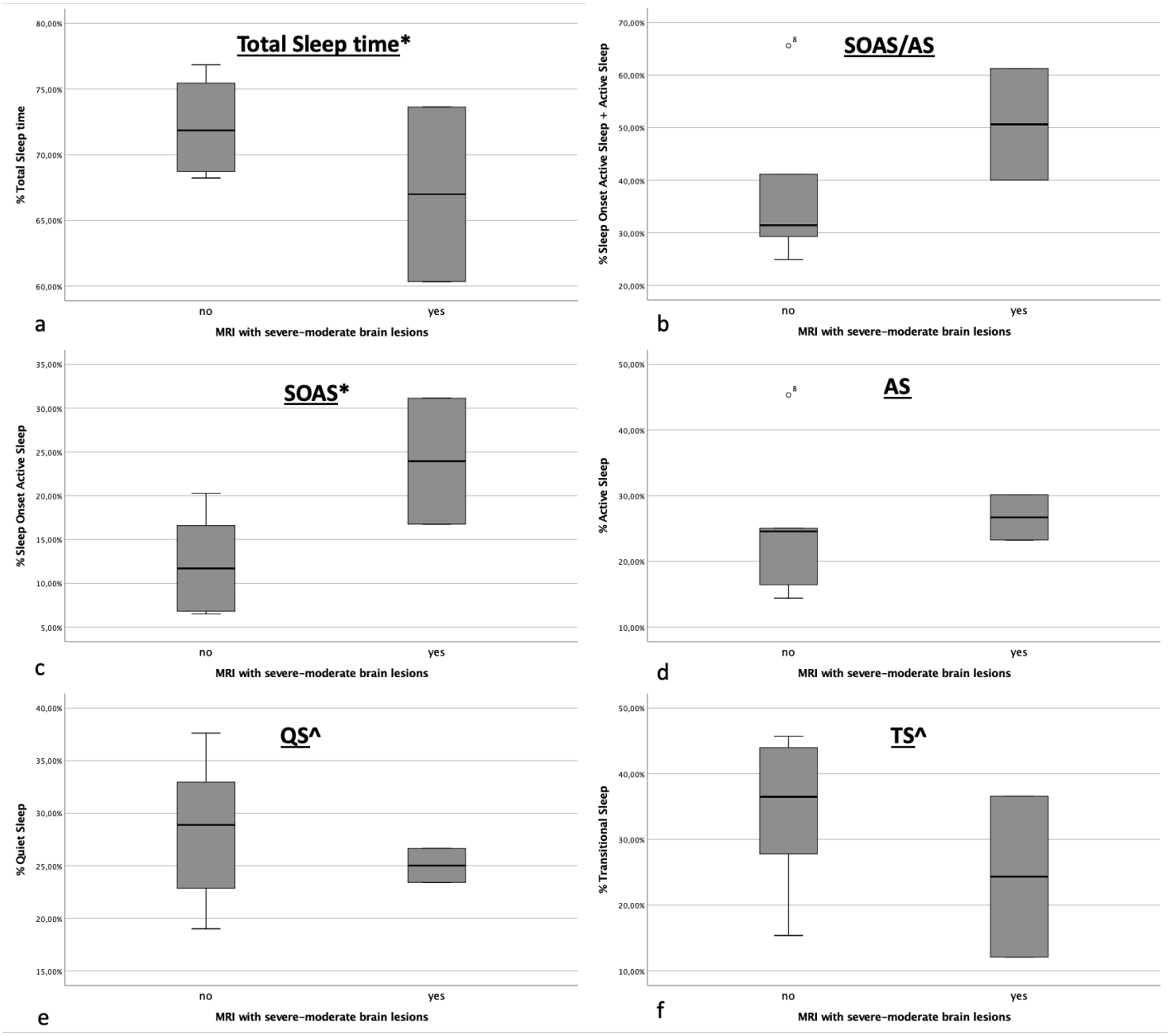
Griffiths Mental Development Scales - III	Total Sleep time	AS/SOAS	SOAS	AS	QS	TS
Total Developmental Quotient	**0.852****	**-0.905****	**-0.929****	-0.299	**0.881****	**0.848****
Learning foundations	**0.838****	-0.587	**-0.850****	0.06	0.771	**0.886****
Language and Communication	0.491	-0.431	-0.599	-0.199	0.707	0.299
Eye and Hand Coordination	**0.747***	-0.659	-0.635	-0.265	0.563	**0.743***
Personal-Social–Emotional	0.395	-0.623	-0.695	-0.343	0.802*	0.347
Gross Motor Skills	0.238	-0.619	-0.667	-0.18	0.833*	0.167

Legend. Correlation at the Spearman test * significance at the 0.05 level (2-tailed); ** significance at the 0.01 level (2-tailed). AS stands for Active Sleep, QS, Quiet Sleep; SOAS, Sleep Onset Active Sleep; TS, Transitional Sleep.

Moderate/severe brain lesions impair sleep quantity and sleep distribution

Infants carrying moderate/severe brain lesions showed lower Total Sleep time (66.9% ± 7.39 vs 72.2% ± 3.52, $p = 0.047$) accompanied by a higher prevalence of SOAS (23.9% ± 10.2 vs 12.26% ± 5.5 $p = 0.048$). A trend of shorter duration of both QS and TS was observed (*Figure 8*). Infants carrying minor brain lesions showed a similar trend, although not significant.

Figure 8. Distributions of sleep stages among VLBW carrying or not moderate-severe brain lesions.

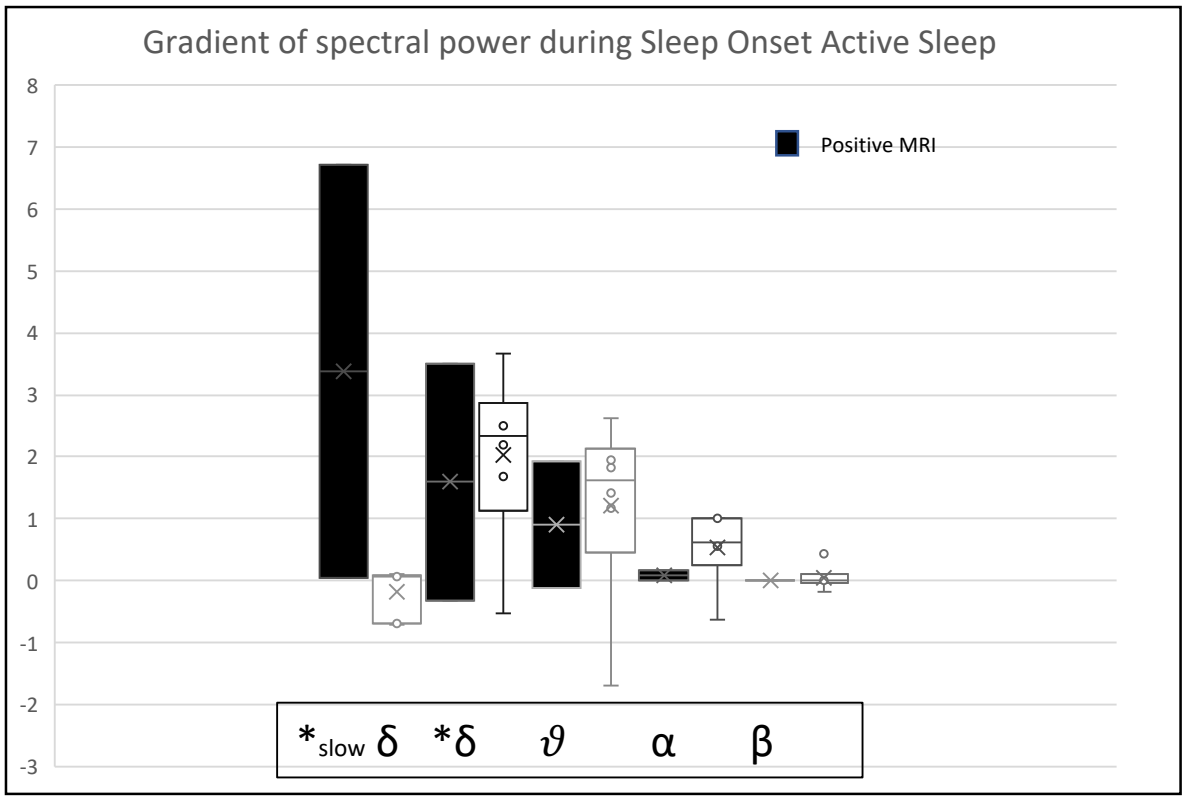


Legend. * *p value* < 0.05; ^*p value* < 0.8. Differences among variables have been calculated with Mann-U Whitney test.
AS, Active Sleep; QS, Quiet Sleep; SOAS, Sleep Onset Active Sleep; TS Transitional Sleep.
Each sleep stage is here expressed relative values (over the total sleep time in minutes).

Severe-moderate lesions are associated to posterior slow-waves activity during SOAS

Spectral analyses revealed that VLBW infants carrying moderate-severe brain lesions showed positive gradient of slow waves (slow δ and δ) in SOAS (respectively: Δ slow δ SOAS in subjects with severe-moderate brain lesions 3.3 ± 4.6 vs -1.2 ± 0.1, $p < 0.01$; Δ δ SOAS in subjects with severe-moderate brain lesions 1.6 ± 2.7 vs Δ δ SOAS of subjects with no lesion 2.1 ± 0.6, $p < 0.05$). *Figure 9* illustrates gradients powers for SOAS.

Figure 9. Distributions Δ spectra divided for VLBW carrying or not severe-moderate brain lesions.



Legend. Δ was calculated starting from mean spectra over total spectral power. Posterior and anterior derivations were then confronted. *p* calculated with Mann-U Whitney test.

DISCUSSION

This pilot exploratory study served for setting first steps in advancing further analyses for understanding the intrinsic meaning of sleep behavioural stages among very low birth weight (VLBW) infants.

In the examined sample preliminary observations on 24-hour videoPSG recordings the total amount of sleep and of transitional sleep (TS) significantly correlated with neurobehavioral assessment at 34 weeks of postmenstrual age (PMA), at term equivalent age (TEA) and with neurodevelopment at 6 months of corrected age (CA) in a sample of 10 VLBW infants (*Tables 8-9*). Infants carrying moderate-severe brain lesions showed lower total sleep time and higher amount of total active sleep (which includes both sleep onset active sleep, SOAS, and active sleep, AS), with a general trend to lower prevalence of both quiet sleep (QS) and transitional sleep (TS). It was also possible observing a trend of different sleep distribution across the day, with a prevalence of sleep (compared to wakefulness) in the evening and early morning periods (18:00-07:00) and higher prevalence during daytime hours (08:00-20:00) of active sleep (both SOAS and AS) when compared to night-time (20:00-08:00) (*Figure 7*).

Sleep is a fascinating window that allows both understanding of physiological mechanisms of neuronal processes and pathological bases of the diseases, thus allowing target strategies of intervention. The importance sleep in participating in developmental processes is largely exemplified by the large amount spent in sleeping behaviours by foetuses, newborns and infants (Roffwarg et al., 1966).

Both nREM (non-Rapid Eye Movement) and REM sleep have been linked to processes of neuronal plasticity and therefore cognition, memory reconsolidation, and emotional

regulation (de Vivo et al., 2017; Li et al., 2017). Sleep loss can be detrimental, and has been linked to a systemic proinflammatory state and to brain microglial activation in absence of other neuroinflammatory processes (Bellesi et al., 2018), which, at early ages, may be the base for neurodevelopmental disorders.

Premature birth is a source of sleep perturbation in terms of functional connectivity and this is related to neurodevelopmental outcome later in childhood (Tokariev et al., 2019).

Although scientific evidence is emerging regarding sleep impairment among very preterm infants (Bennet et al., 2018; Dereymaeker et al., 2017; Gogou et al., 2019; Uchitel et al., 2022), the role of preterms' sleep architecture in determining their well-known adverse neurological-neurobehavioral features and neurodevelopmental outcome has yet been unexplored. Old studies coming from behavioural observation or PSG recording during the 24 hours, described sleep distribution in smaller samples of preterm infants, and no correlation with neurological state and neurobehavioral outcome was provided (Biagioni et al., 2005; Curzi-Dascalova et al., 1993). Our prospective and ongoing study aims to fill this gap.

Results of our preliminary observations, show that total sleep time was a constant metrics of positive correlation with neurological, neurobehavioral and neurodevelopmental states. Reduced total sleep time is indeed a common finding among children suffering from neurodevelopmental disorders such as Autism Spectrum Disorders (Morgan et al., 2020), who also can present nREM sleep slow wave activity alterations of NREM sleep, disorders in initiating and mantaining sleep (Miano et al., 2007). Total sleep time has been also designated as a predictor of late-in-childhood developmental outcome for critically ill

newborns (Shellhaas et al., 2017). It could become a feasible screening tool for very preterm infants' wellbeing and future neurodevelopment.

From this set of data, active sleep at the start of sleep time (SOAS) and active sleep after quiet sleep (AS) were associated to more immature performances at neurological and neurobehavioral assessment while quiet sleep (QS) and transitional sleep (TS) correlated with more advanced performances at 34 weeks PMA and TEA, and also at 6 months of CA. It can be postulated that in very preterm infants active sleep, especially in the form of SOAS, may be related to maturational processes that are likely to disappear during foetal and neonatal life, thus representing a marker of immaturity if present at this stage of development. Active sleep, which equals to the activity of deep brain structures, is related to neuronal plasticity and to development of the sensory-motor processes, that are pillar to further cognition development, and follows, in animal models, fixed of deployment (Blumberg et al., 2020, 2022; Del Rio-Bermudez & Blumberg, 2018). Thus, it might be that active sleep, especially SOAS, may be a marker of immaturity of brain processes in very preterm infants. On the other hand, quiet sleep has been instead related to long-range cortical connectivity in preterm infants at term of equivalent age (Tokariev et al., 2019) brain maturation, and postnatal cerebral maturation (Cailleau et al., 2020), and to memory consolidation later in life (Friedrich et al., 2020), being thus a possible marker for better brain organization if assessed early in very preterm infants.

In this context, the role for transitional sleep should be clarified, but it may be that it might mark the transition to a "more mature" state of brain organization. In fact, sparse literature postulated the maturational role played by transitional sleep among very preterm infants (de Weerd & van den Bossche, 2003).

It was also possible observing the clinical early proof that active sleep (both at the start of the sleep time, and after QS) may be associated to early visual performances at 34 weeks PMA and at TEA. Literature has already postulate the structural and functional link between visual cortex and AS/REM (Tokariev et al., 2019), to the point of saying that REMs can be considered "ocular twitches" through witch AS reorganise brain visual networks (Blumberg et al., 2022).

Of note, the Eye-Hand coordination domain of Griffiths at 6 months CA correlated negatively with amount of active sleep. This could be related to the fact that the scale assesses more complex brain functioning, which involves not only vision but also fine motor coordination and learning abilities, which is are related to cortical brain areas, brainstem and cerebellum (Miall et al., 2001; Rizzo et al., 2020).

Moreover, infants carrying moderate-severe brain lesions (in this sample: 1 IVH grade III and 1 grade IV, frequent lesions of prematurity (Ramenghi, 2015)) showed lower Total Sleep time, higher prevalence of SOAS, and shorter duration of both QS and TS. The increment of SOAS/AS, although surprising, may be attribute to the ontogenetic role that it plays across development. AS/REM sleep is pillar for pruning and scaling synapses, acquisition of sensory-motor, visual, and adaptive behavioural skills. It might be postulated that exactly SOAS might be the designated to the process of post-injury synaptic repair and remodelling (Blumberg et al., 2020, 2022) after IVH. However, the significance of active sleep at sleep onset has to be clarified, as well as the appropriateness of using the term SOAS to distinguish it from active sleep after QS.

Indeed, at a preliminary quantitative analysis, they also showed a gradient for higher power of posterior slow activity during SOAS from the posterior cerebral regions. These observations may be also related to the pathophysiology of germinal matrix-intraventricular haemorrhages, that are associated with microstructural alterations caused by inflammatory processes starting from the blood remaining on the ependymal surface (Ballabh, 2014) located diffusely in the white matter, but mostly posteriorly (Tortora et al., 2018).

In the examined sample, we also observed different trend in sleep distributions across the 24 hours, with sleep time being more present during night hours. Although it is possible that nursing and medical procedures are likely to be performed more during day time thus disturbing preterm neonates' sleep, presence of circadian rhythms among newborns and very preterm infants is debated since cortisol secretion seems show a two-phase pattern (Spangler, 1991) and melatonin production starts from the 6 week of life, but is delayed in premature infants. (Kennaway et al., 1992). Of interest, earlier melatonin production start is associate with better neurodevelopment (Tauman et al., 2002).

The group of Pisa showed interesting clues regarding the distribution of sleep patterns and yawns across 24 hours. Biagioni et al, assessed 11 neonates (of them 8 preterm infants, 5 early and 3 late preterms referred having a normal neurological outcome) and observed, from the electroencephalographic traces, a different distribution of sleep patterns among day and night (with a higher prevalence for the equivalent of QS during the day.

To the best of the present knowledge, this was the first study investigating the short and mid-term outcome of sleep pattern among VLBW. However, these data are only preliminary observations, where the small sample size did not allow for analyses of possible

interrelationships of other prenatal/neonatal variables on Sleep patterns of VLBW. They need to be furtherly corroborated by larger datasets and more accurate and complex metrics. For instance, a targeted metric phase synchrony analysis is being developed and will be tested on a larger dataset constructed on the basis of the present illustrated in this work.

CONCLUSIONS

Although the limited sample size and the exploratory nature of this study, data clearly show that very preterm infants present impaired sleep distribution across the 24 hours that is linked to neurological neurobehavioral set at early stages and at mid-term. Brain lesions worsen this condition. Claim for more accurate measure of sleep care to be intended as early intervention for very preterm infants is mandatory.

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PhD PROGECT RELATED RESEARCH ACTIVITY

1. Potenzieri, A; Uccella, S; Deborah Preiti, Matteo Pisoni, Silvia Rosati, Chiara Lavarello, Martina Bartolucci, Doriana Debellis, Federico Catalano, Andrea Petretto, Lino Nobili, Tommaso Fellin, Valter Tucci, Luca Antonio Ramenghi, Annalisa Savardi1, Laura Cancedda. Early IGF-1 receptor inhibition in mice mimics preterm human brain disorders and reveals a new therapeutic target – *under submission on Sci Trasl Med*

2. Andreato, A; Uccella S The possible role of the superior sagittal sinus in regulating cerebrospinal fluid dynamics among preterm infants. A case report and a review of the literature – *under review on Neuropediatrics*

3. **Uccella, S**; Cordani, R; Salfi, F; Gorgoni, M; Scarpelli, S; Gemignani, A; Geoffroy, PA; De Gennaro, L; Palagini, L; Ferrara, M.; De Gennaro L; Nobili L. Sleep Deprivation and Insomnia in Adolescence: Implications for Mental Health,Brain Sciences,13,4,569,2023,MDPI

4. Uccella, S; Parodi, A; Calevo, MG; Nobili, L; Tortora, D; Severino, MS; Andreato, C; Eu-Brain Neonatal Group; Rossi A; Ramenghi LA; Influence of isolated low-grade intracranial haemorrhages on the neurodevelopmental outcome of infants born very low birthweight,Developmental Medicine & Child Neurology,,,,2023,Wiley Online Library

5. Malova, M; Parodi, A; Severino, MS; Tortora, D; Calevo, MG; Traggiai, C; Massirio, P; Minghetti, D; Uccella, S; Preiti, D, Neurodevelopmental Outcome at 3 Years of Age in Very Low Birth Weight Infants According to Brain Development and Lesions.,Current Pediatric Reviews,,,,2023,

6. Wong, N HC; Cross, S; Zavaleta-Ramírez, P… Uccella, S et al.,Self-harm in children and adolescents who presented at emergency units during the COVID-19 pandemic: an international retrospective cohort study,Journal of the American Academy of Child & Adolescent Psychiatry,,,,2023,Elsevier

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