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Electroencephalographic Study of Sleep Architecture in Infants at Four Months

Thesis presented by

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for the degree of

Doctor of Philosophy

University College of Cork

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2024

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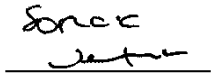
Declaration of Originality

This is to certify that the work I am submitting is my own and has not been submitted for another degree, either at University College Cork or elsewhere. All external references and sources are clearly acknowledged and identified within the contents. I have read and understood the regulations of University College Cork concerning plagiarism.

Team Contributions

All of the work conducted in this thesis was performed independently by the author, with the following exceptions:

- Dr Sean Mathieson and Dr Mick Carter from the INFANT Research Centre assisted in the acquisition of the term-born (ENRICH) 4-month electroencephalographies (EEGs).
- Sleep spindles, mean frequency, brain symmetry index (BSI), synchrony, and spectral power and EEG background quantitative EEG features of the visually identified spindles were calculated by Dr John O'Toole from the INFANT Research Centre.
- Chapter 2: Inter-rater agreement of spindle annotations of term-born infant EEGs between 2 experts (Dr Mary Anne Ryan, Soraia Ventura) was performed by Dr Lan Wei from University College Dublin School of Computer Science.
- Chapter 5: EEG recordings of moderate to late preterm (MLP) were acquired by Dr Mary Anne Ryan, from the INFANT Research Centre, in a separate study (SLEEPi).



(Soraia Ventura)

30/09/2024

Date

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Abstract

Early infancy is a period of high neuroplasticity. Infants spend a large portion of their time asleep, and sleep reflects and contributes to neurodevelopment changes. The study of the EEG features of infant sleep provides a unique window on brain maturation and offers a number of opportunities to benefit infant development, including measures of normal and of divergent development due to factors such as premature birth, enabling early intervention; and also, as outcome biomarkers for interventions to optimise development, such as routine massage.

Aims and Objectives:

This thesis aims to deepen our knowledge of infant sleep EEG characteristics at 4 months of age and their utility in assessing neurodevelopment. It is divided into four main objectives. 1) To explore the normative values of sleep macrostructure and sleep spindles of term-born infants. 2) Examine whether routine massage is associated with physiological changes in the sleep EEG. 3) To establish the ability of sleep EEG markers at 4 months to predict neurodevelopmental scores at 18 months. 4) To compare sleep biomarkers of moderate to late preterm-born infants at corrected age with their term-born peers.

Methodology:

The cohorts analysed include ex-term infants randomised into intervention (4-month daily parent-led massage routine) and non-intervention (care-as-normal) groups (BabySMART cohort) and a healthy moderate-to-late preterm group (SLEEPi cohort) recruited at Cork University Maternity Hospital study.

The 4-month multichannel video-EEG polysomnography consisted of 21 EEG electrodes following the 10-20 electrode placement system, and extra polygraphic channels. Griffiths Scales of Child Development, third edition (Griffiths III) assessments were performed at 4 and 18 months and sociodemographic questionnaires for BabySMART, at 2 weeks, 4 and 18 months.

Initial analysis of the sleep EEG included manual annotation of fronto-central sleep spindles (a feature of sleep microstructure) and sleep stages (sleep

macrostructure). Sleep macrostructure, spindle number, duration and density were calculated manually. Spindle power, frequency and synchrony were calculated with MATLAB R2020a. Brain Symmetry Index, Coherence and Spectral power of the background quantitative EEG features were calculated using the bespoke NEURAL software. Descriptive statistics and statistical analysis were calculated with IBM SPSS statistics 26.

Results:

1) I describe the normative values for sleep macrostructure and sleep spindles at 4 months. I found sex differences in spindle spectral power and BSI, and that spindle spectral power decreased from the 1st to the 2nd sleep cycle.

2) A 4-month routine of infant massage was associated with physiological changes reflected in sleep EEG features: higher EEG power, lower coherence and higher sleep spindle spectral power.

3) Sleep latencies, duration of non-rapid eye movement stage 3 of sleep (N3), spindle duration and synchrony, delta power, and coherence at 4 months were associated with neurodevelopment at 18 months.

4) The moderate-to-late preterm-born group showed changes in sleep parameters at 4-months corrected age compared to term peers and had a shorter N2 duration, higher delta power and higher frontal connectivity and lower connectivity between bilateral parietooccipital areas.

Conclusions:

We characterised normative data on sleep EEG biomarkers using the largest sample to date at four-months of age, providing a baseline for future studies of neurodevelopmental trajectory. Sleep spindle differences were observed between sexes and across sleep cycles, confirming and extending knowledge from older paediatric cohorts. I show for the first time that parent-led massage is associated with a more mature developmental pattern of the EEG of healthy term-born infants. Using a wide range of sleep features, I identified those relevant for the prediction of neurodevelopmental outcomes at 18 months. Lastly, I showed differences of sleep

spindles in moderate-to-late preterm infants at 4-months corrected age relative to term-born peers, denoting altered brain organisation.

Keywords:

EEG, polysomnography, sleep spindles, sleep macrostructure, brain connectivity, spectral power, infancy, prematurity, NREM, REM, brain maturation, sleep, infant massage, environmental enrichment.

Abbreviations Index

AASM	American Academy of Sleep Medicine
ADHD	Attention deficit and hyperactivity disorder
ARICD	Association for Research in Infant and Child Development
ASD	Autism spectrum disorder
AMPAR	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor
ATP	Adenosine triphosphate
BDNF	Brain-derived neurotrophic factor
BSI	Brain Symmetry Index
CT	C-tactile
DMN	Default Mode Network
ECG	Electrocardiogram
EEG	Electroencephalogram
EMG	Electromyogram
EOG	Electrooculogram
EPSP	Excitatory postsynaptic potentials
fMRI	Functional magnetic resonance imaging
GA	Gestational age
GABA	γ-aminobutyric acid
GD	General development
HP	High-pass filter
IAM	Individual adjustment method
IBI	Interburst Interval
I_h	Hyperpolarisation-activated cation current
I_{KCa}	Calcium-activated potassium conductance
IPSP	Inhibitory postsynaptic potentials
I_t	Low-threshold calcium conductance
KAR	Kainate receptor
LORETA	Low-resolution brain electromagnetic tomography
LP	Low-pass filter

LTD	Long-term depression
LTP	Long-term potentiation
MEG	Magnetoencephalogram
MLP	Moderate-to-late preterm
mRNA	Messager ribonucleic acid
NICU	Neonatal intensive care unit
NMDAR	N-methyl-D-aspartate receptor
NREM	Non-Rapid Eye Movement sleep
PMA	Post-menstrual age
PTP	Post-tetanic potentiation
qEEG	Quantitative electroencephalogram
REM	Rapid Eye Movement
SPW	Sharp wave
SPW-R	Sharp wave ripples
STD	Short-term depression
STP	Short-term potentiation

Aims and Outputs

This thesis aims to explore brain development and sleep in infancy. The thesis is divided into a literature review, 4 research chapters and, a summary and discussion. In the first chapter, I discuss the relevant literature on the topic and in the research chapters I aim to detail the sleep parameters in infancy and how they relate to neurodevelopment. I also aim to compare sleep physiology in environmentally enriched infant populations (routine massage) and MLP populations against term controls. Lastly, in the summary and discussion chapter, I provide this thesis rationale, the main findings discussion and future directions.

1) The first chapter consists of a theoretical introduction to neurodevelopment and sleep. Through a literature review, I explore how the sleep EEG in infancy is a potential biomarker for brain maturation, particularly how sleep spindles and slow waves are responsible for functions such as synaptic network homeostasis, learning and several cognitive abilities.

2) The second chapter consists of the investigation of the normative infant sleep data at 4 months of age. Results were presented online at 8th Congress of the European Academy of Paediatric Societies 2020 and was published in Sleep Journal: Ventura S, Mathieson SR, O'Toole JM, Livingstone V, Ryan M-A, Boylan GB. Electroencephalographic sleep macrostructure and sleep spindles in early infancy. Sleep. 2021;45(1). https://doi.org/10.1093/sleep/zsab262 (Appendix 1).

3) The third chapter consists of the sleep EEG analysis of a randomised controlled trial of routine parent-led massage in the first 4 months of age. This analysis showed massage-associated brain physiological changes in the full-term infant. Results were partially presented at New Horizons research conference in 2019 in Cork, Ireland and in full at the World Sleep Congress 2022 in Rome, Italy. This study was published in Developmental Medicine and Child Neurology: Ventura S, Mathieson SR, O'Sullivan MP, O'Toole JM, Livingstone V, Pressler RM, Boylan GB. Parent-led massage and sleep

EEG for term-born infants: A randomized controlled parallel-group study. Dev Med Child Neurol. 2023;00:1–13. https://doi.org/10.1111/dmcn.15565 (Appendix 2).

4) The fourth chapter describes the investigation of the ability of sleep parameters at 4 months to predict 18 months Griffiths' neurodevelopment assessment scores. This study showed an association between sleep spindles and slow wave activity and Griffiths' neurodevelopment assessment scores at 18 months. Results were presented at the World Sleep Congress 2022 in Rome, Italy. The resulting paper has been accepted to Pediatric Research pending revisions.

5) The fifth chapter is the comparative study of neurophysiological sleep features in moderate to late preterm-born 4-month (corrected age) and term-born peers and how the differing sleep features correlate with neurodevelopment at 18-months. This study showed that preterm sleep alterations extend to the moderate-to-late cohort when compared to term-born peers. Sleep differences between MLPs and term infants in coherence and spectral power, particularly in the delta range, are associated with development at 18 months. Results were presented at NEonatal Sleep Talks (NEST) 2023 in Cambridge, United Kingdom and at XXIV International Congress of Infant Studies (ICIS) 2024 in Glasgow, United Kingdom. The resulting paper is in preparation to be submitted to the Journal of Pediatrics.

6) The sixth chapter is the concluding chapter. I discuss the purpose of this work, briefly summarise and reflect on the outputs obtained on infant sleep and neurodevelopment through the different chapters. Strengths and limitations are discussed and I suggest future lines of investigation arising from this work.

Co-authored papers:

- Wei L, **Ventura S**, Lowery M, Ryan MA, Mathieson S, Boylan GB, *et al.* Random Forest-based Algorithm for Sleep Spindle Detection in Infant EEG. Annu Int Conf IEEE Engineering in Medicine & Biology Society. 2020;2020:58-61.

- Wei L, **Ventura S**, Mathieson SR, Boylan G, Lowery M, Mooney C. Spindle-AI: Sleep Spindle Number and Duration Estimation in Infant EEG. IEEE Transactions on Biomedical Engineering. 2022;69(1):465-74.

- Wei L, **Ventura S**, Ryan MA, Mathieson SR, Boylan GB, Lowery M, *et al.* Deep-spindle: An automated sleep spindle detection system for analysis of infant sleep spindles. Computers in Biology and Medicine. 2022;150:106096.

- Hogan R, Mathieson SR, Luca A, **Ventura S**, Griffin S, Boylan GB, O'Toole JM. Scaling convolutional neural networks achieves expert-level seizure detection in neonatal EEG. arXiv preprint arXiv:2405.09911. 2024

- Mathieson SR, **Ventura S**, O'Toole JM, Lawrence N, Boyd SG, Proietti J, Pressler RM, Boylan GB. Event-related potential testing of C-Tactile afferent fibres in infants and adults [submitted].

- O'Connor C, **Ventura S**, Proietti J, O'Sullivan MP, Boylan GB. Sleep and infant development in the first year of life [submitted].

Other participations

Presentations:

- Ventura, S; Mathieson, S. *Periventricular Leukomalacia - A Clinical Case*. Neonatal Brain Research Group (NBRG) series, at the INFANT Research Centre, UCC in Cork, Ireland, (25/03/2019);

- Ventura, S; Carter, M: Lenehan, S; Conway, G; Cronin, AM. *Baby Enrichment Research Study (ENRICH), project presentation*. Seminar Series at INFANT Research Centre, UCC in Cork, Ireland (07/09/2019);

- Ventura, S*. EEG 101*. NBRG series, at INFANT Research Centre, UCC, Cork, Ireland (15/11/2019);

- Ventura, S; Mathieson, S; O'Toole J; Livingstone, V; Boylan, G. *Sleep Microstructure Changes in Routinely Massaged Infants* [Poster]. New Horizons Research Conference, UCC, Cork, Ireland (5/12/2019);

- Ventura, S. *Thesis in 3: Sleep Microstructure Changes in Routinely Massaged Infants*. 5th annual INFANT Research Day at UCC Centre for Executive Education, Laps Quay, Cork, Ireland (12/12/2019);

- Ventura, S; Mathieson, S; O'Toole J; Livingstone, V; Boylan. *Qualitative and Quantitative Analysis of Diurnal Sleep of Infants at 4-5 Months* [Poster]. The 8th Congress of the European Academy of Paediatric Societies (EAPS) online (17/10/2020);

- Ventura, S. *Electroencephalographic Study of Sleep Architecture in Infants at Four Months: personal experience as a PhD student and overview of research developed*. Escola Superior Saúde, Porto, Portugal (2/12/2021);

- Ventura, S *Paediatric Sleep Staging using AASM 2.4 Guidelines - An Overview*. EEG meetings at INFANT Research Centre, UCC, Cork, Ireland (6/12/2021);

- Ventura, S; Mathieson, SR; O'Sullivan, M; O'Toole, JM; Livingstone, V; Pressler, RM, Boylan, GB. *Effect of a standardized massage routine on polysomnography in ex-term infants at 4 months of age: A Randomised Control Trial* [Poster]. World Sleep Congress 2022, Rome, Italy, (15/03/2022);

- Ventura, S; Mathieson, SR; O'Sullivan, M; O'Toole, JM; Livingstone, V; Pressler, RM, Boylan, GB. *Infant Sleep as a Marker of Neurodevelopment*. World Sleep Congress 2022, Rome, Italy (14/03/2022).

- Ventura, S; Ryan, MA; Mathieson, SR; O'Toole, JM; Livingstone, V; Boylan, GB. *The effect of moderate to late preterm birth on EEG sleep markers of development in early infancy*. NEonatal Sleep Talks (NEST) 2023, Cambridge, England (31/08/2023).

- Ventura, S; Ryan, MA; Mathieson, SR; O'Toole, JM; Livingstone, V; Boylan, GB. *The effect of moderate to late preterm birth on EEG sleep markers in early infancy*. XXIV International Congress of Infant Studies (ICIS) 2024, Glasgow, Scotland (10/07/2024).

Science outreach:

- *Tech Week Science Workshops* – Primary School Visit at Glasheen Girls School, Cork, Ireland *(25/04/2018);*

-*EEG demonstration to transition year students*, at the INFANT Research Centre, Cork, Ireland (14/05/2018);

-*Cork Carnival of Science* –presentation of INFANT Centre scope to the general public at the Fitzgerald Park, Cork, Ireland (23/06/2019);

-*Native Scientist Workshop* – program of science outreach directed to school-aged children in their native language, at the Cork City Library, Cork, Ireland (05/10/2019).

Chapter 1. Literature review

In chapter 1, I will discuss the relevant literature and provide a theoretical basis for the exploratory chapters. Brain development is at the centre of this thesis' purpose. The exploratory chapters of this thesis aim to characterise normative sleep features as surrogate markers of brain development, to compare these between different cohorts, and to study their correlations with neurodevelopmental assessments. As such, the initial section of this chapter (section 1.1.) is on brain development from neuroembryology to early life brain development. Prematurity is a disruption to intrauterine development. Consequently, the following section (section 1.2.) is a literature review on prematurity where I explore its definitions and statistical data. I also describe how prematurity is associated with an increased risk of mortality and morbidity, including neurodevelopmental issues. This section aligns with Chapter 5 on moderate to late preterm (MLP) infant sleep and neurodevelopment. Section 1.3. explores sensitive periods and environmental enrichment and how these two factors interact to improve neurodevelopmental outcomes. Within the environmental enrichment topic, I focus on affective touch and massage, central to Chapter 3 which describes a randomised controlled trial on infant massage. Section 1.4. is a broad introduction to sleep function theories, effects of sleep loss and the particular role of sleep in the maturing brain. The final section (section 1.5.) is on the electroencephalography (EEG). EEG history and neurophysiological basis are briefly discussed. The core topic in this section is sleep EEG and its association to development. Two sleep EEG transients, sleep spindles and delta waves, are extensively studied throughout the exploratory chapters. In section 1.5., I delve into the theoretical and empirical evidence regarding the sleep spindles and delta waves' association with neurodevelopment, learning and memory.

1.1. Brain development

Early brain development encompasses a series of complex processes involving gastrulation, neurulation and prosencephalic development, which will give rise to both subcortical and cortical functionally specialised regions. At the cellular level, progenitor cells proliferate, migrate, divide and differentiate, contributing to the formation of brain vesicles, the prosencephalon, the mesencephalon and the rhombencephalon that will further develop into the different central nervous system regions. A combination of different molecular signalling in proliferative areas will further determine patterns of differentiation and construct early cortical patterning. Neurons in the outer layer of the brain further divide and migrate to constitute the 6-layered cortex. Extending axons and dendrites establish synapses between neurons, some of which, after an initial overproduction, are subject to later activity-dependent pruning(1) (Figure 1.1). Early patterning is determined by genetics and by epigenetic modulation through maternal factors. However, after birth, activity and experience play a significant role in shaping development, as I will further discuss.

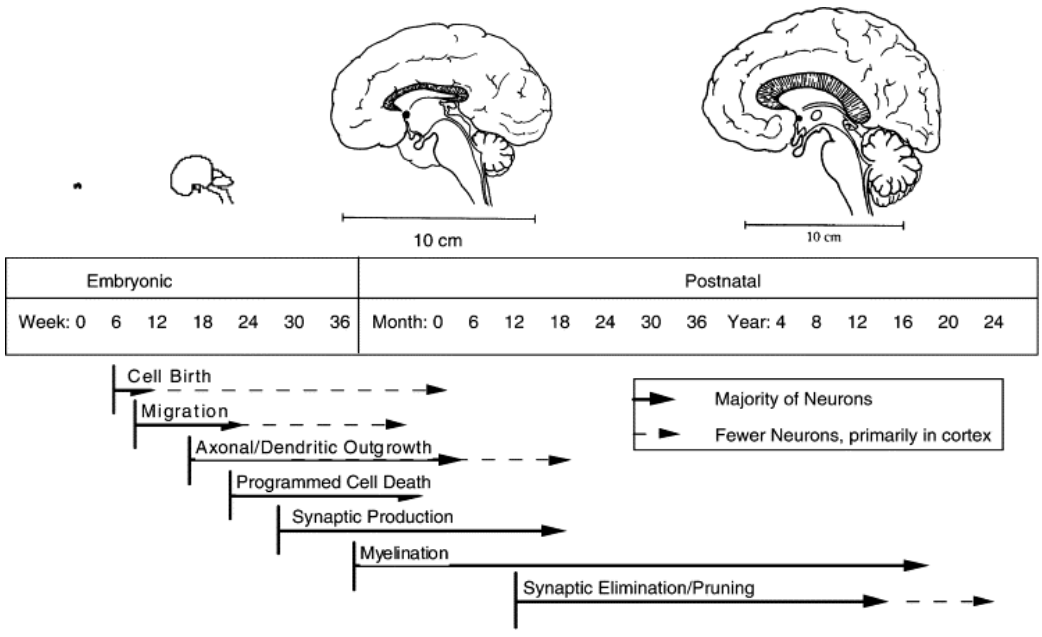


Figure 1.1 - Brain development
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Nervous system development starts with gastrulation during the embryonic period (Figure 1.2). Gastrulation is a phase of epiblast cellular migration through a structure on the longitudinal axis, named the *primitive streak*, and critical morphological alterations(1, 3). During this cell movement, cells are exposed to chemical signalling from adjacent tissue(1). The cell lineage is determined by where and when the epiblast cells pass through the primitive streak occurs (1, 3).

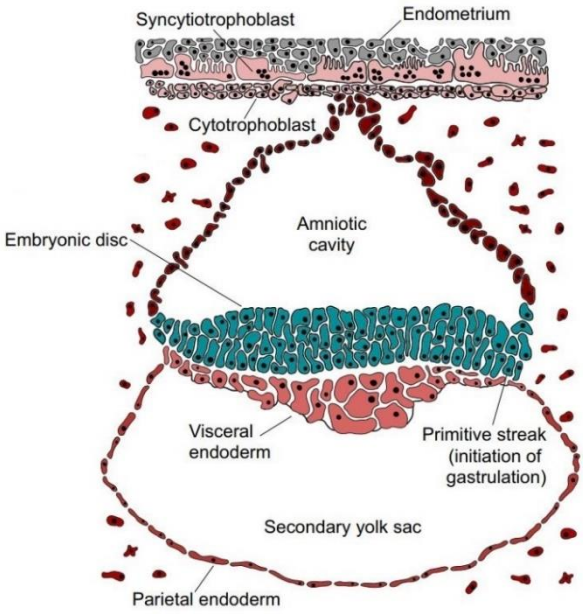


Figure 1.2 - Primate development at the onset of gastrulation.
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A portion of cells that do not migrate through the primary streak will give rise to the neural plate, a thick epithelial region containing neural progenitor cells displayed along the longitudinal axis and whose length correlates with the notochord length present ventrally. The notochord has inducive properties that will contribute to the development of the neural tube (5, 6).

During embryo folding, the neural plate evaginates, forming the neural groove and the neural crest cells displayed laterally on the neural plate fuse to form a neural tube (5, 6). This phase is termed primary neurulation. During this process, neural crest cells detach from the neural tube and will, in part, develop into neuronal cells of the peripheral nervous system (5, 6). Secondary neurulation begins as the caudal end of the neural tube (i.e., caudal neuropore) closes. Adjacent to the caudal end of the neural tube, a group of cells forms a cylinder that will undergo a process of cavitation, extending the neural tube caudally(5, 6). The neural tube will form the

central nervous system and, its lumen, the ventricular system. The neural progenitors will be located along the ventricular zone (adjacent to the lumen), and their differentiation will depend on where they are located in relation to the neural tube. Neurogenesis begins after a phase of intense neural progenitor cells proliferation(7). During neurogenesis, cells migrate from ventricular, and subventricular zones in the telencephalon region, divide and differentiate accordingly(6). Once neurons are established, they start the development of axons and dendrites connecting with other neurons(8). The first three bulges emerging from the neural tube are the primary vesicles: the prosencephalon, mesencephalon and rhombencephalon. With development, the prosencephalon can be subcategorised into the telencephalon and the diencephalon and, the rhombencephalon can be subcategorised into the metencephalon and the myelencephalon. Together with the mesencephalon, these now identifiable main structures are named *secondary vesicles*(5). With development, these bulges become increasingly more recognisable as specific brain structures from the cerebral hemispheres to the medulla oblongata, including the walls of the ventricular system(5, 9). A significant part of the production of neurons finishes by mid-gestation(8), from when microglial production from neural progenitor cells predominate(7). Neurogenesis persists after birth, although the time at which this process finishes is not fully understood(10, 11).

The foetal phase starts after the 9th gestational week until birth and encompasses a period of rapid general growth(12). In this phase, sulci and gyri are formed(8), body movements are detectable at the 7th gestational week, but as synapses are established, the repertoire of reflex movements expands. Also, the sensory systems begin to develop during the embryonic phase and evolve through the foetal stage. During gestation, maternal biologic inputs model the foetus' physiology and activity. However, upon premature birth, intrauterine circadian regulation of the foetus by maternal hormones (13), stimulation of foetal movement by maternal sympathetic nervous system inputs (14), and regulation of heart rate and heart rate variability according to mother's circadian rhythms cease(15). Axonal growth and important connectivity pathways are yet to develop in the foetus(16). Myelin production has started by the second trimester and continues postnatally(17) (Figure 1.3). Due to the overproduction of neural cells, neural apoptosis is necessary

and most of this process occurs before term-age. The number of cortical neurons reduces after 28-32 weeks of gestation at different rates in different brain regions, although cortical thickness increases(18). The subplate is a temporary cortical layer that receives ascending thalamic fibres and projects into the cortical plate connecting these two structures(19); its dissolution starts in the third trimester. This process will be complete only at 6 months postnatal age (PNA); however, interstitial white cells may be residual elements of the subplate(20). In recent years, a possible enabling role of the subplate region has been suggested for the development of sulci and gyri(21). For extreme preterms, birth occurs before reaching 28 weeks of gestation, making brain maturation particularly vulnerable for this population. As a result, at term-equivalent age, preterm infants have smaller brain volume, delayed gyrification and myelination(22) and aberrant connectivity(23). Prematurity will be further discussed in the next section 1.2.

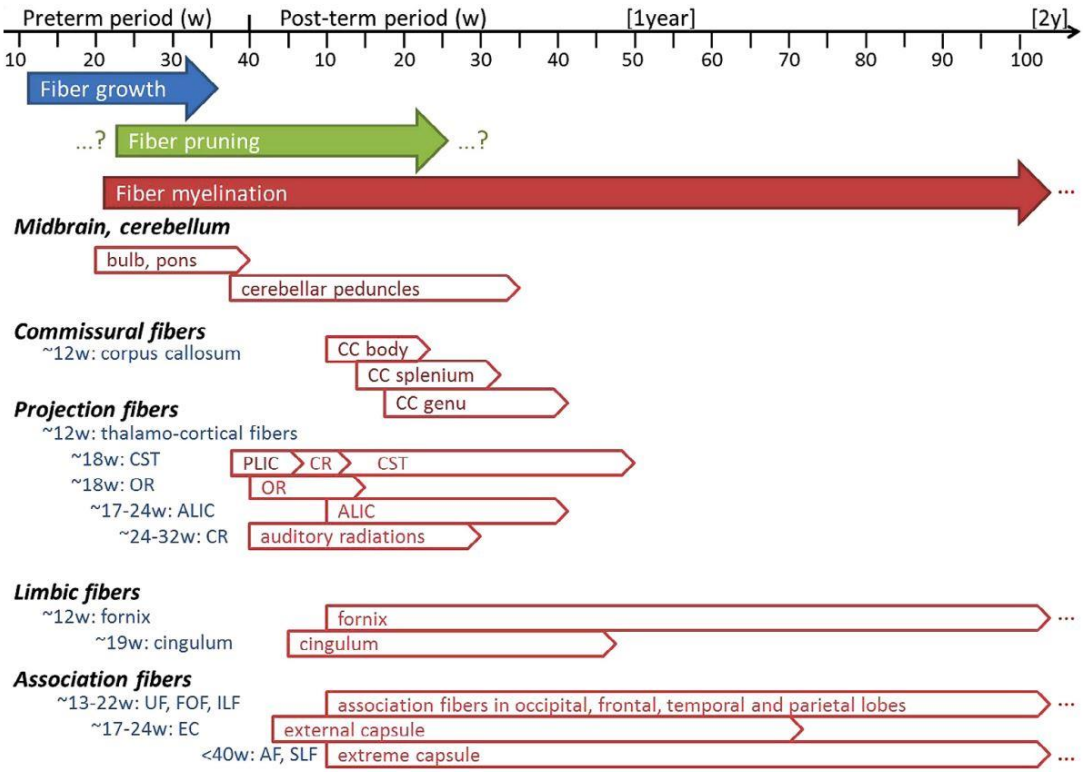


Figure 1.3 - Proposed timeline for the development of the axonal fibres.
White arrows correspond to myelination processes. AF, arcuate fasciculus; ALIC, anterior limb of the internal capsule; CC, corpus callosum; CG, cingulum; CR, corona radiate; CST, cortico-spinal tract; EC, external capsule; FOF, fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; PLIC, posterior limb of the internal capsule; OR, optic radiations; SLF, superior longitudinal fasciculus; STT, spino-thalamic tract; UF, uncinate fasciculus. Image reused from (24) with permission.

Endogenous stimuli play an essential role in refining topographic cortical mapping by feedback mechanisms, such as those described in 1.5.7.4 in which delta brushes, sleep spindles and spindle bursts (in the animal model) are proposed to contribute to sensorimotor mapping, or spontaneous bursts of activity in the retinal ganglion, which are proposed to contribute to the connectivity refinement between the retina and the lateral geniculate nucleus of the thalamus in the animal model (25).

Neurotrophins such as brain-derived neurotrophic factor (BDNF) regulate brain development, from neural stem cell survival and differentiation to neuronal maturation, synapse formation and refinement (26). BDNF versatility is partly associated with its multiple gene promoters and, therefore, multiple spliced BDNF messager ribonucleic acid (mRNA) variants (26). Neurotrophin expression and action depend on neuronal activity, which can be either triggered by endogenous or evoked from environmental stimuli (26). After birth, interactions with the environment will help shape further brain development. Anatomical connectivity is refined; while functional connectivity is largely reorganised, promoting efficient wider brain communication and shifting hubs, i.e. central clusters of communication, from primary to high-order brain regions (27). An immature brain presents a low structure organisation and overabundant synapses. Interactive specialisation theory proposes a model in which brain regions and networks become more responsive to particular stimuli or tasks through specific experience-dependent interactions(28).

Human brain development is far from being considered mature at the time of term birth. Brain growth, subplate dissolution, neural production and apoptosis, myelination, synapse formation and pruning will continue after term, and experience will intensely shape these processes in the new environment.

1.2. Prematurity

Prematurity refers to birth before 37 weeks GA. According to the World Health Organization classification, infants born prematurely can be divided into extremely preterm if born before 28 weeks, very preterm if between 28 and 32 weeks, and MLP if between 32 and 37 weeks GA. Premature birth may be spontaneous or induced for medical or non-medical reasons(29). Spontaneous premature birth has been associated with several risk factors such as age at pregnancy, multiple pregnancies, infection, cervical incompetence, poor maternal nutrition, lifestyle and underlying chronic medical diseases. Induced premature births include medical reasons such as preeclampsia(30). It is estimated that approximately 1/10 of births are premature, although this percentage varies with the region of the globe. In Europe, extreme, very and moderate to late preterm MLP births account for 5.1%, 10.9% and 84.5% of the total preterm births, respectively(31).

Unfortunately, these infants are more vulnerable. The lower the gestational age, the higher the risk for both mortality and morbidity(32). Preterm birth weight is also an essential factor affecting the risk of mortality and morbidity(33). Neonates can be categorised into three groups according to their weight at birth: small for gestational age, appropriately grown and large for gestational age. These categories are defined as weighing below the 10^{th} percentile, between the 10^{th} and 90^{th}, and above the 90^{th} percentile, respectively. Worldwide, complications from preterm birth are the primary cause of child mortality (from 2000 to 2019, complications from preterm birth accounted for 17.7% of deaths in children under five years old)(34). Access to more effective antenatal and neonatal care has reduced mortality numbers. With increasing survival rates, the focus is now also on reducing morbidity(32). Incidence of factors like respiratory distress, jaundice, intraventricular haemorrhage, necrotising enterocolitis, bronchopulmonary dysplasia is inversely associated with gestational age at birth(35). Long-term comorbidities include neuromotor outcomes such as cerebral palsy and auditory, visual, cognitive and behavioural impairments(32).

Late preterms is a subgroup of MLPs, born the closest to term age, between 34 and 36^{+7} weeks. Complications are more infrequent in late preterm than in other preterms groups, however this group still presents with higher mortality and morbidity rates than term infants. Late preterms are three times more likely to be admitted to the neonatal intensive care unit (NICU) than term-born infants(36, 37). The gap between late preterm and term-born children is still present in early childhood; Late preterm groups have a higher incidence of poorer childhood physical health and well-being, and language and communication development, higher risk of repeating a year at kindergarten/school and higher incidence of attention deficit and hyperactivity disorder (ADHD) in comparison with term-born children(38).

Complications in preterm-born children may be associated with the primary causes of premature birth(37), treatment side-effects(32) and early exposure to the extrauterine environment as immature organs, including the brain, are forced to adapt and function in a very different environment(32, 37, 39). Preterm birth has been associated with decreased glutamatergic neurogenesis, possibly due to exposure to higher oxygen concentrations outside the uterus, which are known to affect neuronal differentiation(40). Early exposure to extrauterine stimuli may also affect size, type and laminar distribution of synapses(41). As for EEG, preterm newborns show an immature background and transients (see section 1.5.4.) In physiological conditions, these immature patterns tend to disappear as the preterm newborn ages(42), however some EEG differences to term-born groups may still be present at least up to teenage years. In 1.5.4.1 I will discuss how the evolution of EEG patterns may contrast in preterm and term-born children.

Several environmental factors help reduce the developmental vulnerability of preterm infants. Adequate nutrition is an important immunological and neuroprotective factor(43, 44). Breast milk composition differs between mothers of preterm-born and term-born neonates, adapting to the needs of the neonate. Breast milk is not only a source of nutrition and prebiotics but also of probiotics and bioactive molecules, such as microRNA, that regulate gene expression(44) and hormones that promote preterm growth(45) and may increase nocturnal sleep length and efficiency(46). Sleep contributes to immunity(47), is thought to maintain

synaptic homeostasis(48), and is fundamental for good neurodevelopment(49, 50). Sleep in premature infants, especially those admitted to Neonatal Intensive Care Unit (NICU) may be less than ideal(51), and its protection must be promoted(52). For example, the existence of quiet times and of a mild-intensity cycling light before hospital discharge may promote more effective entrainment of the natural sleep/wake cycles(53). Frequent exposure to stress in early life also has the potential to impact brain maturation(54). Medical procedures on preterm infants may induce stress and pain, with potential risk for negative epigenetic alterations, such as methylation of the serotonin transporter gene(55). Painful medical procedures are also associated with limited brain growth and affect connectivity that is not mitigated by oral glucose administration(56). The number of skin-breaking procedures that preterm infants are exposed to during the NICU stay is associated with lower scores on personal-social scales on Griffiths' neurodevelopmental assessments at 1 year-old(55). Conversely, an environmental enrichment may decrease stress levels in neonates(57), and procedures like massage appear to have a positive effect on brain maturation(58, 59). Benefits of affective touch and massage for premature infants will be further discussed in the section 1.3.1. I will discuss the importance of environmental enrichment and sleep for infant neurodevelopment in section 1.3 and 1.4.1.1, respectively.

1.3. Sensitive periods and environmental enrichment

As discussed in section 1.1, the brain is not mature at birth, with some developmental processes extending to adulthood(60). In neurobiological sciences, sensitive periods correspond to developmental periods marked by an increased potential for learning a skill or trait if presented with a specific stimulus of correct intensity and at a preferential timeframe(61-63). The timeframe for sensitive periods varies for particular traits and skills as brain maturation proceeds. The concept of 'sensitive periods' differs from the older concept of 'critical periods', from which it derives, as it is less centred on the timing/age and more focused on the underlying biological processes themselves, opening up opportunities for rehabilitation(63). Depending on their nature, stimuli may result in better or worse development outcomes. For example, early exposure to stress is related to harmful long-term effects in different domains of an individual's life(64). In contrast, time-investment of parents on young children has the opposite effect(65).

Thus, the environment surrounding a growing child can have a profound long-term impact. Concepts of enriched and impoverished environments come from standardised experimental studies, initially on animals, aiming to observe the effects of different conditions (i.e. deprived or enhanced social and/or physical conditions) on brain maturation during sensitive periods(66). Some of the first experiments involved rhesus monkeys reared in social isolation and looked at the effects on behaviour and learning(67), other studies compared problem-solving abilities, brain weight and cholinesterase levels between rats in a enriched environment (raised in large cages with mazes, toys and other rats) and those in an impoverished environment (small cages with lower stimulation and in social isolation)(68). Epigenetic changes caused by different experiences modulate brain development [see for example (69, 70)]. According to Sale (66) environmental manipulations can be categorised according to stage of application: prenatal (e.g. gestational maternal physical exercise), early postnatal (i.e. during infancy, e.g. kangaroo care) and late postnatal (i.e. after infancy, e.g. socialisation with peers) (Figure 1.4). Prenatal and early postnatal enrichment may directly affect the maturing brain, e.g. acceleration

of neural progenitor proliferation, differentiation, and apoptosis. Although there is a greater potential for improved outcome by applying an environmental enrichment in early life, the adult brain may also benefit from an environmental enrichment through structural and functional neuroplastic changes and enhanced vascularisation(66).

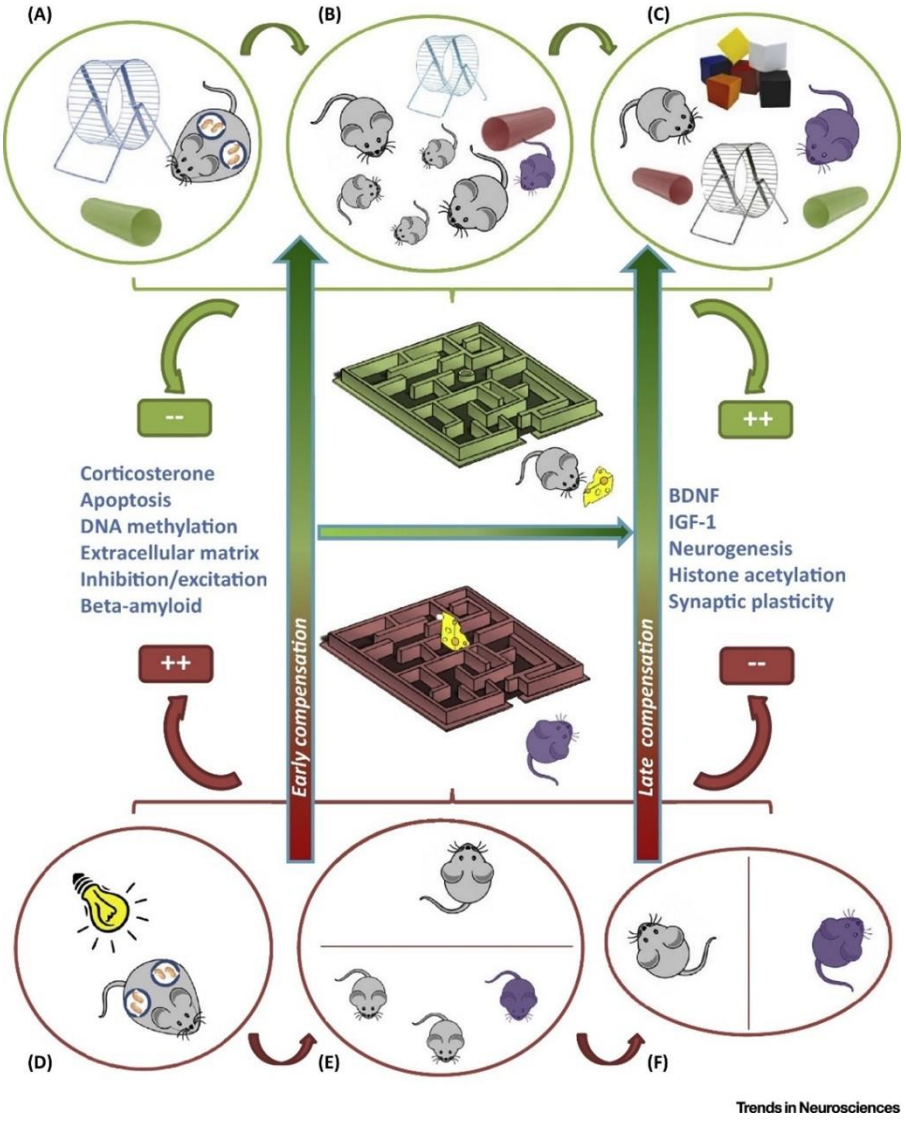


Figure 1.4 - Enriched and impoverishment environments at different developmental stages. Enriched, circled in green, and impoverishment environments, circled in red during prenatal (A and D), early postnatal (B and E) and late postnatal phase (C and F), resulting from presence or absence of cognitive, social and physical stimuli and proposed underlying biological mechanisms that shape brain maturation and plasticity. IGF, Insulin-like growth factor 1. Image reused from (66) with permission.

1.3.1. Affective touch

C low-threshold mechanoreceptors (C-LTMR) have been associated with affective touch. In human studies, an equivalent term for these receptors is C-tactile afferents (CT). Due to a lack of functional differences, Schirmer and McGlone propose using the term C-LTMR regardless of being human or non-human(71). In this thesis, I will also use the denomination of C-LTMR for non-human and human fibres. These refer to unmyelinated fibres that respond to low pressure and are particularly sensitive to slow caress of hairy skin(72). They respond optimally at a stroking speed of 3cm/s at temperatures close to skin temperature; these conditions correspond to higher levels of perceived pleasantness(73). Thus, C-TLMR can signal affective-related touches such as caress, grooming or massage. In non-human primate behavioural studies, brief touch and prolonged touch interactions play different social roles. The first type is generally employed to indicate amicable intent and reduce the risk of conflict with unfamiliar individuals or after an interpersonal clash. Grooming, a prolonged form of contact, is in many aspects comparable to human massage and, has the important function of establishing and maintaining bonds in friendly relationships (74).

The affective touch stimulus is detected by C-LTMR and is thought to travel through the anterolateral spinal tract, but not exclusively(75). Stimuli like pain that also travel through the anterolateral spinal tract reach the thalamus, and from there, projections can be distributed to the prefrontal cortex, anterior cingulate cortex, insular cortex, somatosensory cortex, striatum, and the amygdala(71, 76). However, as noted by Marshall *et al.*(77), anterolateral cordotomy in humans impairs pain and itch sensations but does not affect the subjective feeling of pleasantness to affective touch. In his review on processing and transmission of affective touch, Marshall argues that pleasant touch sensation may be obtained from convergent information from several LTMRs and ascending pathways(78). C-LTMR can be co-activated with fast fibres in response to brushing stimuli. While the latter, predictably, elicits an early response in the somatosensory area, C-LTMR-related potentials show a frontal

ultra-late potential during Event-Related Potential (ERP) testing that is sensitive to stimulation speed(72).

Beyond the sensory role, C-LTMR mediates pain and may mediate stress. Pain modulation is achieved through two pathways: by releasing neuromodulators at the spinal cord and expressing proteins that mediate pain endogenously. In males, C-LTMR expressed proteins are associated with anti-inflammatory properties and temperature perception(75). Maternal touch also induces temporary cardiorespiratory changes in the infant, particularly when the touch consists of stroking(79) and, results in stress resilience as measured by stable cortisol levels and vagal tone, which is not observed in the non-touched infants. In the latter study, stress was induced by maternal still-face, an experimental paradigm in which mothers maintain a neutral face while also refraining from communicating with their child (80).

Although the C-LTMR is generally associated with pleasant touch, Bohic and Abraira propose that social touch is experienced through a combination of receptors and converging signals at different scales. For example, MrgprB4+, a class of unmyelinated sensory fibres, which unlike C-LTMR are not detected through von Frey filaments (calibrated plastic filaments used to test mechanical nociception), also respond to brush strokes in hairy skin (81), Ab-field LTMR fibres respond to light strokes over large areas of hairy skin, and slowly-adapting Merkle cells respond to pressure [reviewed in(75)]. An oscillating deep-pressure stimulus is shown to produce similar affective and calming effects to brushing(82). All of the mentioned receptors are likely involved during a massage. Integration of the pleasant stimulus is complex and may depend on cues from other senses, such as vision and hearing(83) and top-down control mechanisms that modulate its perception(75). In addition, other contextual factors, i.e. setting, intra- and interpersonal aspects between the participants, expectations and perceived intentions, are likely to be at play for the conscious feeling of pleasant touch(84). In fact, the social aspect cannot be extricated from the mechanical act of affective touch. Who is performing the social touch matters(85). In caregiver-infant dyads, affective touch is proposed to act as a mechanism of social conditioning, representing also an opportunity to establish and deepen attachment and feeling of security(69, 86, 87).

Autonomic and behavioural responses to affective touch may develop before term age(88). Preterm-born neonates stroked at C-LTMR optimal velocity (3cm/s over 10cm) show a reduction of the heart rate and increased oxygen saturation(89). Tuulari *et al.* showed that term-born neonates and infants up to 36 days already show activations from gentle skin stroking (3cm/s) in the postcentral gyrus (related to tactile processing) and posterior insular cortex (related to socio-affective processing) on the functional magnetic resonance imaging (fMRI) (90). Other brain areas may develop responses to social touch later in infancy. Miguel *et al.* showed posterior temporal (related to socially relevant stimuli processing) haemodynamic activations in response to 8cm/s brush strokes at 12 months but not at 7 months(91). Mediation of these responses by C-LTMR in neonates and infants using neurophysiological recording of event related is yet to be proven.

Infant caregiving touch can take the form of skin-to-skin contact, massage, stroking and breastfeeding(69). Baby massage and similar forms of infant caregiving can be considered an environmental enrichment with two stimuli components: tactile stimulation and social bonding (59) and produce epigenetic changes during neurodevelopment(69). Caregiving touch alters glucocorticoid receptor gene expression, regulates oxytocin receptor genes, and genes related to metabolism, immunity and, neurologic and psychiatric disorders(69, 86). By increasing the expression of glucocorticoid receptors on the hippocampus and medial prefrontal cortex and, by reducing its expression and increasing benzodiazepine receptor expression in the amygdala, neonatal touch may downregulate hypothalamic–pituitary–adrenal axis activity, potentially modulating stress responses(92).

Another proposed pathway for stress reduction through affective touch is the hypothalamic downregulation of brainstem noradrenergic neurons which increases parasympathetic tone, enhancing immune function through oxytocin and microbiota modulation and is protective of physical and mental health(86). Of note, maternal care in rodents induces differential neuroplastic alterations within the hippocampus. In low maternal licking and grooming pups, long-term potentiation is enhanced in the ventral but decreased in the dorsal hippocampus. The ventral hippocampus, which synapses with the amygdala and the hypothalamus, shows enhanced excitability in adulthood, due to altered intrinsic action potential properties and voltage-gated

sodium-channel upregulation, allowing calcium influx on the postsynaptic neuron. These excitatory properties observed on the ventral part of the hippocampus may therefore be associated with impaired psychosocial development(93). Neonatal handling is also associated with additional changes in the amygdala's functional and anatomical alterations(92).

Interactions between parents and infants promote transitory behavioural and neural activity coupling (86). Children exposed to a higher proportion of affective touch tend to be more socially oriented(86, 88). Plastic changes, such as regulation of oxytocin, dopamine and opioid receptors in brain areas related to social processing and motivation, are modulated and may explain secure attachment and increased social competencies induced by affectionate touch(86). Beyond touch, maternal attention in the form of non-physical interactions (e.g. vocalisations and playful face expressions) to their infants are associated with higher performance IQ (requiring fluid reasoning and visuospatial skills) and verbal IQ of their offspring at 4.5 and 7 years-old(94).

1.3.1.1. Massage benefits: evidence from infant massage studies

Similarly to other forms of affective touch, infant massage is not only physical but also a social event in the sense that it classically involves an interaction between the person applying massage and the infant (95). This is an aspect highlighted by the International Association of Infant Massage during their massage training. Infant massage also trains parents to be more responsive to their children’s cues by remaining vigilant and to adapt practice across the session as needed. As mentioned earlier, affective touch does not occur in isolation from the perceived context during which it is administered(84). White-Traut *et al.* have shown that a successful reduction of stress hormones through infant massage depends on other concurrent social engagements such as eye contact and vocalisations. Salivary cortisol levels decreased in the group whose massage also included social engagement(57). Whereas massage alone, i.e. while intentionally ignoring the infant by not establishing eye contact nor engaging in vocal interactions, is a driver for increased cortisol levels in the infant(57). The person who is interacting with the infant is also

relevant. Infants' heart rate decreased more effectively when the stroking was perceived to be done by the caregiver(85).

Mother-infant massage dyads benefit both the infant and the mother. For example, mothers perceived improved sleep aspects for themselves and their infants after a bedtime routine massage with body lotion(96). After performing a short session with their preterm babies, mothers reported decreased state of anxiety and, levels of the self-reported state of dysphoria decreased if massaging as well as when just observing their child being massaged(97). Although there were no statistical differences in cortisol levels between groups, mothers routinely massaging their infants show more vigour and less depressive symptoms assessed with the Japanese edition of *Profile of Mood States* than non-massaging mothers (98). In general, routine massage is associated with an improvement in parent's emotions and attitudes after parenthood. Some aspects include higher maternal self-confidence, parenting satisfaction over time and the participation of both parents in baby care(99). Infant massage is also an opportunity for parents to connect with their child(100). Parents' physical and mental well-being enhances the quality of care provided and emotional availability for a new baby, which may ultimately contribute to the infant's development(101).

Infants of mothers with depression may display hormonal and behavioural symptoms related to their mother's condition and are at greater risk of physical and mental complications later in life(102). Frontal asymmetry on EEG, a marker positively related to depressive symptoms in adult studies, is significantly reduced in 1-month old infants during and after massage(103). With continued massage, these infants show more balanced baseline levels of stress hormones, increased serotonin, and better expressions of mood than their non-massaged peers(104).

Similarly, insulin-like growth factor 1 and insulin levels are higher in routinely massaged preterm infants (59, 105). As a consequence(105), studies report higher weight gains(59, 105-107) and growth(107, 108) in massaged infants. Another possible factor regarding the weight gain is the increased of vagal tone in moderate-pressure massage but not in light pressure. Increased vagal tone decreases gastric movements measured by the electrogastrogram, allowing more time for nutrient absorption(105, 109). Another explanation discussed suggested that the calming

effects of massage leads to less energy expenditure and, consequently, more weight gain(110). Families allocated to the group that used lotion to massage tended to be more consistent with the massage routine than the dry-massage group; observations are probably related to comfort, or lack of it, in applying the massage (96). The choice of emollient may also play a role in growth. Agarwal *et al.* studied the effect of different massage oils in term-born infants starting a 1 month routine massage at 6-weeks. Sesame oil had a positive effect on the growth rate. Other vegetable and mineral oils did not show statistical differences, despite showing a tendency towards greater growth than the non-massage controls. The sesame oil group also experienced increased vessel diameter and blood flow measured at the femoral artery(108). Also, a 14-day routine massage with sunflower oil resulted in greater weight gains compared to dry-massage on low birth weight preterm-born infants (111). A similar observation was noted both in term and preterm-born infants after a month of massage with coconut oil compared to dry-massage(112). In summary, use of emollients increase massage protocol adherence, and specific oils may increase infants' weigh due to their contribution to thermoregulation, lessen water loss and transcutaneous absorption of fatty acids(111).

Preterm infant massage is also associated with also improvement of the immune response through enhanced cytotoxicity of natural killer cells (113). It is unsurprising therefore that in a very preterm cohort undertaking skin-to-skin care, an additional 4/day maternal massage routine lowered the rates of late-neonatal sepsis and increased nearly twofold the likelihood of the infant being discharged from hospital earlier than their counterparts that were exclusively given skin-to-skin care (114). In particular, for sepsis, the application of emollient again may play a role. Khatun *et al*. argue that coconut oil may improve skin condition and have anti-inflammatory and antimicrobial effect, resulting in lower rates of sepsis compared to dry-massage peers(112).

In addition, poor motor performances in very preterm infants with very low birth weight were ameliorated following a 4-week moderate pressure massage routine compared to light (sham) pressure massage(115). Another study found that preterm infant massage routine is associated with a higher display of balanced tonus

and earlier mixed postures of limbs extension and flexion, more coordinated movements, more hand-to-face movements and of postural support(116).

Effects of massage on preterm infants may extend to sleep and awake behaviours and EEG. Baby massage is shown to increase the predisposition to sleep longer in the period after application, though not affecting the total amount of time spent sleeping in a day (108). Also routine massage with lotion improves newborn sleep quality, as perceived by parents, in terms of lower difficulty falling asleep, lower number of awakenings, and longer nighttime sleep (96). Routinely massaged preterm infants show a greater duration of active alertness than non-massaged infants(116). The amount of pressure applied during massage seems to effect sleep and awake behaviours in preterms; comparing before and during a massage routine, a moderate pressure group had a lower decrease in percentage of deep sleep and lower increase of active sleep than those receiving light pressure massage, based on behavioural observation. The moderate pressure group of preterms also lowered their heart rate and showed less increases in fussing and crying than those receiving light pressure massage. Again, results suggest more effective relaxation during moderate-pressure massage than light-pressure(110). Routinely massaged preterms also show a higher propensity for more regular breathing patterns(116). Negative associations between massage and sleep have also been described. For example, in a study on NICU-based preterm infants who received massage on alternate days, Yates *et al.* showed firstly no difference in actigraphic assessments of sleep efficiency, oxygen saturation and heart rate after a massage session compared with the no-massage day and, secondly, that there were also fewer infants sleeping after massage than at the corresponding time of a no-massage day. However, the NICU setting in which the study was carried out may represent a less-than-ideal context for sleep(117). In a study based on mother's perception of infants sleep after a bedtime routine with massage, mothers reported less number of awakenings, improved sleep quality and infant mood in the morning, but no change in other aspects such as on sleep latency and durations of sleep and awakenings(100).

A routine of 15 minute-massage, 3 times daily for 10 days, resulted in higher spectral EEG power in low-risk preterms compared with a no-massage control group. By the end of the massage routine, controls had widespread reduction of delta and

beta activity over the central areas compared to the massaged group, which tended to maintain the same levels as at the start(58). As suggested by the authors of the study, massaged preterms showed spectral power levels more comparable to term peers at term age(58, 118). Similar massage conditions were applied during another study by Guzzetta *et al*. investigating the effect of massage on the interburst interval (IBI) in preterm neonates during quiet sleep. Those who had massage showed a median reduction of 7s of IBI duration compared with a 2.8s reduction from the controls after the completion of the massage protocol(59). These results may be indicative of an accelerated brain maturation in the intervention group as quiet sleep EEG background becomes continuous with development.

Khatun *et al.* compared motor and mental developmental using the Developmental Assessment Scale for Indian Infants (DASII) on groups massaged either using coconut oil or dry-massage during the first month of life. Massage with coconut oil was associated with better motor outcomes at 6 months and mental outcomes from 6 to 24 months. On subgroup analysis according to term and preterm-born status, motor development was only marginally statistically significant between the term infant groups. Conversely, mental developmental differences were observed in preterms, favouring massage with coconut oil. The authors hypothesise that massage with coconut oil may have contributed to cognitive outcomes beyond the effects of affective touch due to the transcutaneous absorption of essential fatty acids and, to the emollient properties in reducing neuroinflammation and oxidative stress(112) that may be increased in preterm infants(119). Contrasting results were obtained by a study employing light massage and kinesthetic stimulation for 10 days on preterm infants. In the latter study, no evidence of massage benefits on Dubowitz neurobehavioural assessments (evaluating motor and behaviour characteristics) nor developmental quotients (linguistic, clinical adaptative and full scale) at 3 months was found(107).

To conclude, routine infant massage may potentially facilitate brain maturation, particularly in preterm infants; however, more studies are required to determine if massage benefits brain maturation in healthy term-born infants (95).

1.4. Sleep

Sleep is a vital behaviour controlled by the interplay between the circadian (also known as process C) and homeostatic sleep drive (also known as process S)(120). Rather than a homogeneous entity, sleep presents several stages with distinct behavioural and physiological characteristics which are reflected in the electrophysiology of the brain. Up to 2 months post-term sleep can be divided into active, quiet and indeterminate sleep. After that age, sleep is composed of rapid eye movement (REM) and non-rapid eye movement (NREM) sleep stages. Within NREM, sleep can be subdivided into NREM stages 1, 2 and 3, i.e. N1, N2 and N3 respectively(121). Sleep has been suggested to subserve several functions; beyond partial energy preservation by reduction of the metabolic rate and activity limitation(122), sleep plays a vital role in preventing oxidative stress(123) and enhancing the immune response(47), regulating gene expression(124), contributes to brain maturation(125), neuronal homeostasis(48), neuroplasticity(49, 50, 126) and promotes learning and memory consolidation(127). Sleep function theories can be grouped into energy conservation, restorative functions, and Information Processing (128) which can be interconnected and will be discussed next.

Energy Conservation describes the role of sleep on energy preservation. Sleep pressure increases with wakefulness. In animal models, experimental exhaustion of adenosine triphosphate (ATP) stores in specific brain regions like the basal forebrain (area situated at the inferior surface of the telencephalon(129)) results in sleep inducement(130). This is supported by the fact that species with higher metabolic rates and lower weight, the latter generally associated with lower percentages of body fat, demonstrate the need for more extended periods of sleep. From this point of view, the role of sleep is to reduce the energetic costs during this phase of inactivity[reviewed in(122)]. An argument against this theory is the fact that although brain ATP levels increase during NREM, the ATP level differences are still minimal on cortical excitatory cells compared to the waking state. However, during REM, ATP levels decrease significantly. The authors of this study suggest that changes observed in REM may be due to increased anaerobic glucose metabolism, which is a less

efficient mechanism, and possibly due to blockage of ATP synthesis during oxidative phosphorylation (131). Overall, these observations suggest that energy saving *per se* may not be the determinant factor of why we sleep.

Restorative Theory proposes that sleep supports the homeostasis of the organism. Metabolic restorative processes are favoured during the sleep period which occurs in association with prolonged fasting(120). An early paper on this theory related levels of available ATP to metabolic processes of synthesis and degradation. Higher ATP levels were related to more anabolic processes, fundamental for life maintenance, and less catabolism. Sleep would constitute a period of lower energy demands and thus favourable for synthesis. In addition, sleep is associated with greater release of anabolism-promoting hormones such as growth hormone (132). In keeping with this homeostatic function of sleep, sleep acts as an antioxidant system as subjects have shown higher oxidative stress levels when sleep-deprived (123). The Energy Allocation theory proposed in 2014 by Schmidt, contends that lower vigilance, immobility and decreased thermoregulation during sleep allows redirection of energy expenditure to "biological investment", meaning functions such as protein synthesis, cellular repair and waste clearance [but see Miao *et al.*, for a recent contrasting study that demonstrated lower clearance during anaesthesia and sleep, particularly during high delta power periods, compared to wake periods, at least for small molecular weight dyes in the mouse(133)], enhanced immune function and neuroplastic changes. In this way, this theory integrates the concept of the Sleep Homeostatic Hypothesis that I will discuss next (134).

Information Processing theory is linked to the ability to learn and retain information and is connected with the concept of plasticity; there are two main theories about the role of sleep, as an instrument of direct memory consolidation (the active systems consolidation model) or of homeostatic synaptic elimination (Synaptic Homeostatic Hypothesis). The Synaptic Homeostatic Hypothesis argues that synaptic potentiation occurs during wakefulness when the individual is consciously experiencing the surrounding environment, taking in information from an array of stimuli. This activity results in raised energy consumption levels and cellular stress. Sleep is then required as a counterbalance system. During sleep, the majority of synapses are downscaled (i.e., weakened) while protecting a selection of

synapses, the stronger synapses. This will lead to the recovery of cellular and synaptic homeostasis and improve subsequent level of alertness, learning during waking and memory function (48). The active systems consolidation model argues that memory consolidation also benefits from lower external stimuli input. In the presence of other sleep elements, recent memory traces stored in the hippocampus are reactivated and consolidated to long-term storage in the cortex (135). This process is further described in the section 1.5.7.4 *Learning and long-term memory formation*. Although these two theories are very distinct, it is possible that, in their essence, they are complementary instead of conflicting, explaining both a reduction of less relevant synapses and the consolidation of newly acquired memories taking place in a state with less interfering input of external stimuli. Some conciliatory papers have been put forward. For example, it was proposed that morphological and functionally distinct delta wave types are responsible for either weakening or strengthening synapses (136). Also, the *priming theory* suggests that during sleep, synapses and networks are weakened or strengthened dependent upon previous "tagging", which occurs during the waking experience depending on their activation history. In this theory, overall weakening and strengthening occur approximately at the same rate (137).

Although the role of REM and NREM sleep is not yet entirely clear, specific functions have been attributed to both. REM (49, 50) and NREM (138) appear responsible for synaptic maintenance and downscaling. Declarative memory from the hippocampus (i.e. explicit memory of facts and episodes, *know-what*) and possibly procedural memory (i.e. implicit memory associated to some degree with motor skills*, know-how*) may be consolidated during NREM sleep; temporary memory traces are coordinated with other NREM transients (the sleep spindles and slow oscillations), repetitively reactivated, and transferred to the neocortex, as further discussed in 1.5.7.4 *Learning and long-term memory formation* (135, 139). Active sleep (a precursor of REM sleep) is thought to contribute to sensorimotor mapping in the neonatal period (125, 140); while subsequent sensorimotor development may be attributed to features of NREM, namely sleep spindles(141). This topic is further explored in the section 1.5.7.4 *Spindle activity and Brain*

development. Both REM and NREM are associated with specific roles in visual learning (142) and visual synaptic remodelling during critical periods (143) as well as emotional processing (144). Specific sleep oscillations occurring during NREM sleep like slow waves (transients predominantly of N3) and sleep spindles (transients present in N2 and N3) provide the neurobiological basis for some of the properties attributed to NREM sleep. I will discuss in detail the properties of sleep spindles and slow waves in sections 1.5.7 and 1.5.8.

1.4.1. Effects of Sleep and Sleep Loss

Some of the roles of sleep may be indicated by the effects of sleep deprivation. Sleep deprivation affects multiple aspects of the organism, from potential alteration of gut microbiota composition(145), increased risk for the development of metabolic and cardiometabolic diseases(146), affective disorders (147), and neurological diseases due to deficient neurotoxic clearance, increased neuroinflammation and oxidative stress, and altered metabolism and gene expression(148). Inadequate sleep also reduces cognitive performance(149) and affects memory consolidation(150).

Studies on adult animal models have shown an inhibitory effect of region-specific neurogenesis associated with increased cortisol levels in longer (72h) sleep deprivation but not on shorter [12h (151) and 24h (151, 152)] periods. Upon sleep fragmentation, the expression of genes plasticity modulators decreases, potentially affected differentially by the amount of NREM or REM sleep loss (153), reducing the number of mature neurons by the 4^{th} and 7^{th} day of experimental sleep fragmentation (126). After sleep measures and cortisol levels return to baseline, a temporary excess of neuronal production follows this inhibitory effect on the granule cell layer. This excess production may assist the recovery of normal cognitive function (152). Glial cell production is not affected by sleep fragmentation (126). Sustained complete sleep deprivation in humans is rare. As these are animal studies in experimental conditions, the observations of these studies may not translate entirely

to human's physiology in a normal setting of sleep deprivation, but they provide hints on how and why sleep is associated with neurogenesis.

A way to approach the effects of acute sleep loss in humans is through graph theory models. Functional connectivity can be assessed by mathematical models of graph theory extracted from data obtained from techniques like EEG or fMRI. A systematic review (154) on the effects of sleep loss using graph theory revealed that 24 hours of acute sleep deprivation or even sleep disruption during the night is enough to affect human brain connectivity. The studies reviewed are methodologically different; however, an important conclusion is that sleep deprivation affects different systems heterogeneously, with some changes possibly reflecting impairments to normal functioning while others reflecting compensatory mechanisms. Alterations of the default mode network (DMN), frontoparietal, attention, sensory and memory network connectivities may influence behaviour and cognition in result of sleep deprivation (154). For example, Ben Simon *et al.* found that sleep deprivation is associated with decreased modularity (i.e. property describing highly intraconnected areas) on the limbic, DMN, salience and executive networks on fMRI, which in turn are associated with decreased cognitive performance, negative mood (155) and increased processing on neutral emotional distractors (155, 156). While Liu *et al.* found increased small-worldness (a measure of network integration efficiency) particularly on occipital, sensorimotor and frontoparietal networks on fMRI after sleep deprivation, which is interpreted as a compensatory mechanism; the latter authors also observed that higher levels of neuroticism are associated with minor changes on this measure after sleep deprivation (157).

Experimental studies have shown that sleep deprivation affects executive functioning. Adequate total sleep time is associated with executive memory and verbal fluency in young adults but not in older adults. Sleep continuity also relates to different aspects of executive functioning varying across the lifetime (158). Microstructure variables of sleep may also relate to executive function; such that, those whose slow waves were the most enhanced during the up-phase through targeted auditory stimuli, had better verbal fluency and working memory (159).

Sleep following a learning task is essential for efficient learning. As demonstrated by Stickgold *et al*. (150), adult participants who slept the night after a visual discrimination learning task had performance improvements the following day, contrasting with sleep-deprived participants who showed no significant improvements. During the following days, all participants were allowed to sleep. However, performance improved only in the group that slept after learning, showing that the night of sleep following learning may be irreplaceable(150). Regarding procedural learning, this can rely both on sleep and wakeful consolidation. For fine motor sequence training (finger tapping), musicians are not as dependent on sleep as non-musicians. This may suggest a wakeful consolidation of information running under specific circumstances (160). In fact, sequential motor learning relies on both spatial and motor processes and two neural pathways are used; the hippocampal cortex and striatal cortex. The hippocampal pathway is mainly related to improving the spatial aspect in motor sequences and is dependent on sleep. In turn, the striatal pathway is primarily associated with preserving motor memory; and although it benefits from sleep, it is not totally dependent on it (161, 162).The effectiveness of declarative memory consolidation may also depend on the presence of sleep (163, 164) and sleep duration (163). Moreover, adequate sleep length allows declarative information to be retained according to the importance attributed(165). In the same way, emotional memory may also be facilitated during sleep; However, the research is not yet conclusive, as discussed in a metanalysis by Lipinska *et al.* (166).

In summary, brain functional connectivity is altered with sleep deprivation, however, not all brain regions are affected equally, and some alterations may indicate compensatory mechanisms. Studies in animals have shown that neurogenesis is affected after extended periods of sleep deprivation. Gene expression of plasticity modulators are affected by sleep deprivation although evidence points to different contributions of NREM and REM in different modulators. As a result, neurogenesis is suppressed upon sleep loss but, after sleep parameters and cortisol levels return to baseline, an increased production of neurons occurs as a compensatory mechanism. Human sleep loss affects executive functioning and verbal fluency, procedural (potentially through consolidation of the spatial aspects),

declarative and, potentially, emotionally-related memory. As demonstrated in Stickgold *et al*., (150) sleep deprivation after a learning task may constitute a lost opportunity for effective learning, that cannot be replaced with sleep over subsequent days.

1.4.1.1. Importance of Sleep in a Maturing Brain

Sleep occupies a significant portion of an individual's life. However, sleep time is the longest during early life, occupying over two-thirds of the day at term and gradually decreasing to a quarter in older age(167, 168). This predominance of sleep during infancy occurs during a period of intense brain development.

Effects of infant sleep quality can be observable in an infant's mood(169). Lower quality of infant sleep also increases the risk for greater parental sleep fragmentation, stress and dysphoric mood, which, in turn, may compromise the quality of parental care(170). But the benefits of infant sleep quality extend beyond infant's mood and possible disruption on quality of parental care, as sleep is key to cognitive processes during brain development (171). As in adults, paediatric sleep is considered the preferential state for memory consolidation and cellular and synaptic homeostasis. In infants, sleep assumes a crucial role in a period of increased environment exploration and learning. In animal models, active and REM sleep selectively prune or strengthen newly formed dendritic spines, while protecting selective brain areas from neuronal apoptosis(49, 50). A period of poor sleep quality during the vulnerable window of brain development is also associated with long-term neuroinflammatory changes(172). The sleep homeostasis hypothesis advocates that synaptic downscaling occurring during sleep is essential for brain efficiency. There is still no clear understanding of the specific roles of NREM and REM. Two of the potential mechanisms include the activation of the proteins Arc and Homer1a. Arc is hypothesised to weaken underused novelty-induced synapses selectively through endocytosis of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPARs). Once the Arc is accumulated on the dendritic shafts or spines, it may be activated during NREM slow wave up-states. Similarly, activation of Homer1a, a protein that slows calcium release from the intracellular stores and is associated with

downscaling of glutamate receptors, is achieved with NREM slow wave up-states and possibly REM depolarised states(138). Studies on NREM slow oscillations on synaptic homeostasis and learning are further discussed in 1.5.8.1 (see also 1.5.7.4 for sleep spindles contribution in plasticity, learning and memory). Considering that the infant brain contains a substantial amount of unmyelinated fibres and a far higher number of synapses compared to adults, many of them superfluous, the downscaling is even more necessary(48). An efficient brain allows more learning, fundamental during maturation as it involves several critical windows. Twitches present in REM sleep and, possibly NREM sleep spindles play a role in somatosensory brain mapping and motor learning (125, 140, 141). The duration of infant recognition for novel words(173) or faces(174) is briefly extended if napping follows the stimuli exposure and may promote the association between an object and its name(175). This has also been shown in older children, for whom sleep improves cognitive memory performance. A study on 16-month-old children suggested that a sleeping opportunity between training and memory testing improves vocabulary recalling in 16-month-old children (176). and performance on logic tasks in 6-12-year-old children(177). Furthermore, Wilhelm *et al*. observed that children scored better results in paradigms exploring explicit learning (this is, a type of learning that makes use of declarative memory) when compared to adults and hypothesised that differences in the proportion of slow wave sleep between the age groups might contribute to the differences observed in cognitive performance (178). In a neonatal animal model experiment, the effects of poor sleep quality are also translated into long-lasting impairment of novel object recognitions due to less competent exploration and performance on working memory-related tasks(172). Naturalistic observations of sleep quality in early life also shown to impact human development in the long run. Actigraphy assessments on 12 months infants suggested an association between, sleep efficiency and night wakings (but not sleep duration) with attention and behaviour control, executive function, hyperactivity, and externalising behaviour problems at 3 to 4 years old. Sleep at 3 to 4 years old was not associated with the same-age outcomes(179). From 3 to 24 months, shorter sleep duration measured through a parent questionnaire, particularly nighttime data, is associated to lower cognitive and language scores at 2 years. Children with persistent sleep disordered breathing,

a condition that affects sleep architecture, was also showed lower language scores at 2 years. However, in this study it is not possible to disentangle the effect of otitis, which this group has increased risk and that can also affect language development(180). In line with earlier findings, duration of consecutive sleep (based on maternal perception) at 6, 18 and 30 months was associated with later, but not same-age, language outcomes at 18, 30 and 60 months. Significant observations were mostly on consecutive daytime sleep and daytime to nighttime ratios; these associations were negatively correlated to language outcomes. Together, these two studies point to a preferential nighttime sleep consolidation for language acquisition(181). A review showed that consistent poor sleep quality during maturation is associated with higher risk for internalising and externalising behavioural issues and a predisposition to risk-taking behaviours in adolescence and adulthood(182). The correlation between sleep aspects and later but not same-age outcomes may indicate a causal relationship of sleep on these developmental outcomes.

Taken together, due to the effect of sleep on synaptic regulation, and the potential reciprocal communication between hippocampus and neocortex for memory consolidation and plasticity in infancy, infant's chronic sleep loss may affect memory consolidation, delay sensorimotor maturation and language acquisition and compromise the elimination of less efficient or even aberrant synapses, carrying risks for behavioural and cognitive development(183).

1.5. EEG

EEG provides a dynamic window to important cortical processes occurring during awake and sleep. Next, I will briefly discuss some basic considerations of EEG and the origins of this technology.

1.5.1. Introduction to EEG

The first studies on the effect of electrical currents on organisms were conducted by Luigi Galvani back in the 18th century. He realised that applying electricity to frogs' dissected legs or exposed spinal cord produced a muscle contraction in response. His contemporary and compatriot, Alessandro Volta raised the concept that a biological tissue could work as an electrical conductor. It was also during that and the next century that the first studies on electrotechnology were developed [reviewed in(184)].

The first known electrical brain activity observed was performed using a galvanometer. This feat was thanks to Richard Caton who, in 1875, reported currents using an electrode on the cortical surface referenced to another electrode on the skull. He also described the dramatic activity changes in mammals during sleep, anoxia, anaesthesia and death [reviewed in (185, 186)]. Between 1913-1914, Napoleon Cybulski recorded, possibly the first, electrical brain changes during seizures induced in a dog. Cybulski also contributed to the notion of action and resting potentials [reviewed in (187)].

It was Hans Berger who performed, in secrecy, the first human EEG recordings on patients with damaged skulls in a quest to investigate "psychic energy". His first paper on human EEG was published five years later, in 1929. Berger reported several phenomena such as alpha rhythm and sleep spindles and described EEG in several pathological conditions affecting the brain. He also coined the term *Elektrenkephalogram* [reviewed in (186, 188)]. He observed seizures and associated interictal spikes to a higher likelihood of seizures in an individual [reviewed in (189)].

Potential fluctuations of the electroencephalogram were converted to a continuous ink written paper trace with a device designed by Jan Tönnies, while Adrian and Matthews advanced the EEG by developing the differential amplifier [reviewed in (189)]. In 1935, the Grass Model I was used for the first time to record three-channel EEG on paper rolls. This was the same year that probably the first EEG conference took place organised by Alfred Lee Loomis, another pioneer of EEG, in his lab in New York [reviewed in (189)]. There have been many scientists contributing to the knowledge and the technology we have available today; these early experiments and developments enabled the flourishing of new advances and diversification into different areas of neurophysiology.

1.5.2. Neurophysiological basis of EEG

Most of the fluctuating activity present on the EEG is from the varying summed electrical charge at the cortical surface, as a result of ionic currents generated from multiple excitatory and inhibitory postsynaptic potentials (EPSPs and IPSPs, respectively) [reviewed in (190)].

The minimum cortex area involved in generating these signals to be detectable by EEG is 6 cm^2 [reviewed in (191)]. This does not mean that the action potentials per se, as a synchronised inter-neuronal event, do not cause fluctuations on the EEG, as is the case of epileptic discharges and some sleep phenomena [reviewed in (190)]. The bulk of the electrical activity that is read on the EEG is generated due to pyramidal neurons from layers III-V. Pyramidal neurons are dipoles, i.e. they may present positive and negative charges over a short distance which generates transmembrane ionic currents as a result of synaptic inputs [reviewed in (191)]. Excitatory impulse leads to a more negative net extracellular charge; the reverse is true for an inhibitory impulse. Synaptic inputs at different levels of the pyramidal cells influence voltage polarity at the cortical surface (Figure 1.5). The interstitial fluid and also glial cells, due to their electrical properties, are thought to be the main conductors of this activity to the scalp, contributing this way to the signal detection on scalp EEG [reviewed in(192)].

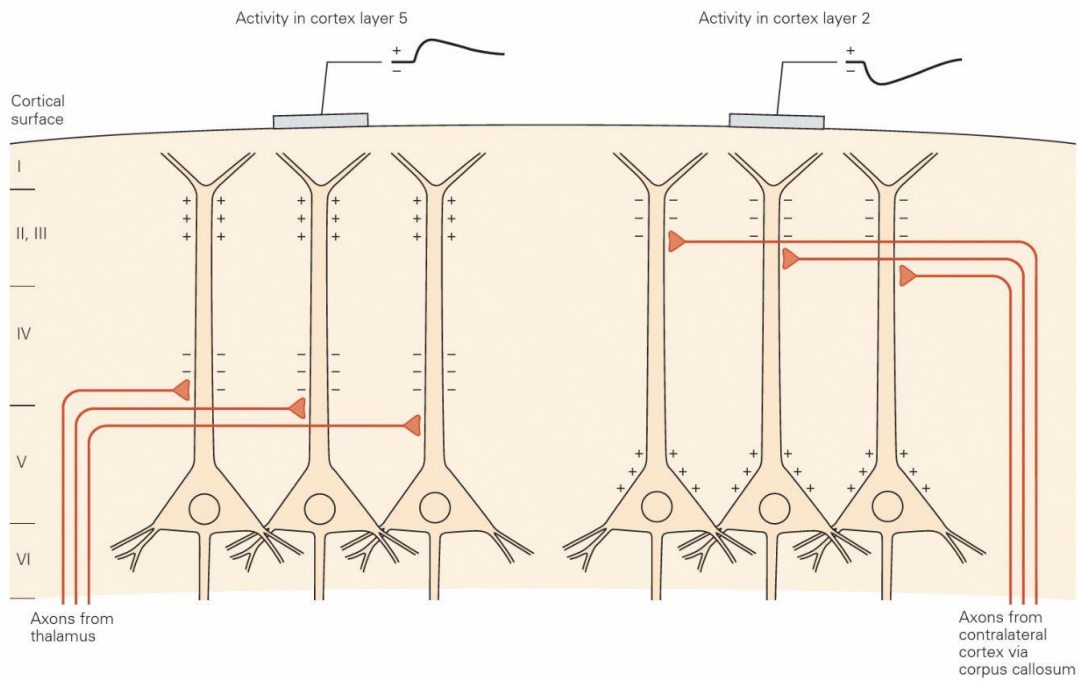


Figure 1.5 - Location of synaptic inputs and polarity of surface potentials. Image reused from (193) with permission.

To acquire these signals, electrodes are placed on the scalp according to a common protocol. The most used EEG electrode placement is the International 10-20 System (Figure 1.6), in which the measurements rely on computations of distances of 10 and 20% from certain landmarks on the head. The use of a measurement system ensures that electrodes are placed accurately relative to underlying cortical brain areas and also that these relative positions are maintained with repeat recording in the same or different institutions(192).

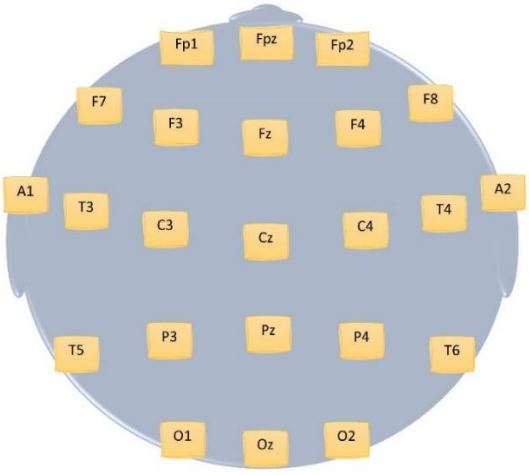


Figure 1.6 - 10-20 International System of electrode placement.

These electrodes allow the electrical impulses that reach the scalp, after passing through several anatomical structures, to be detected for subsequent amplification by an amplifier. The signal arising from the differential amplifier represents the difference of potential between the active (also known as lead 1) and the "reference" recording electrode (also known as lead 2), which is common to all other channels. The analogue signal is also filtered, sampled (commonly at ~250Hz) and digitalised.

The introduction of the digital EEG machine allowed for settings such as amplitude, frequency filters, timebase and montages to be altered not only during acquisition but also during review. The final product is a multichannel graphical representation of the differences of potential between brain regions with very high temporal acuity (Figure 1.7).

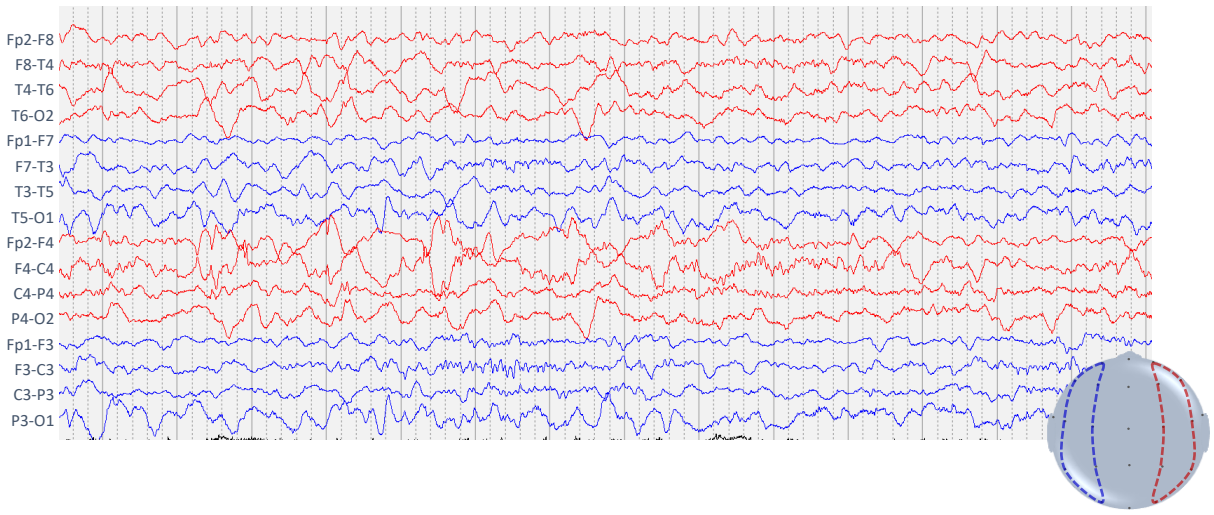


Figure 1.7 - Example of an EEG.
Double-banana montage, timebase=30mm.s^{-1}, sensitivity=150µV.cm^{-1}, high-pass filter (HP)=0.5Hz, low-pass filter (LP)=70Hz

A montage describes the electrodes that make up each EEG channel trace and how those channels are organised on the screen. The raw EEG signal montage corresponds to a referential montage (e.g. Figure 1.8). However, the EEG can be remontaged whereby the difference in potential between any pairs of electrodes of choice can be displayed. A new montage can be calculated based on the difference between two channels' instantaneous values with a common reference. For

example, the raw EEG channels Fp1-ref and F7-ref can be remontaged into Fp1-F7 because (Fp1-ref) – (F7-ref) = Fp1-F7. Montages can be either bipolar or referential. Bipolar montages are characterised by a chain of channels that, with exception of the electrodes situated at the extremes of the row, an input that serves as a reference in a channel, functions as an active on the next. Within the bipolar montages, these chains can be organised into transversal, i.e. left-to-right, longitudinal, i.e. anterior-to-posterior (e.g. double-banana montage as in Figure 1.7) arrangements, or a mix of both. The referential montages can be subgrouped based on whether a designated common reference electrode (e.g. Cz as in Figure 1.8) or group of electrodes (e.g. average of all signals) will be the common reference across the channels.

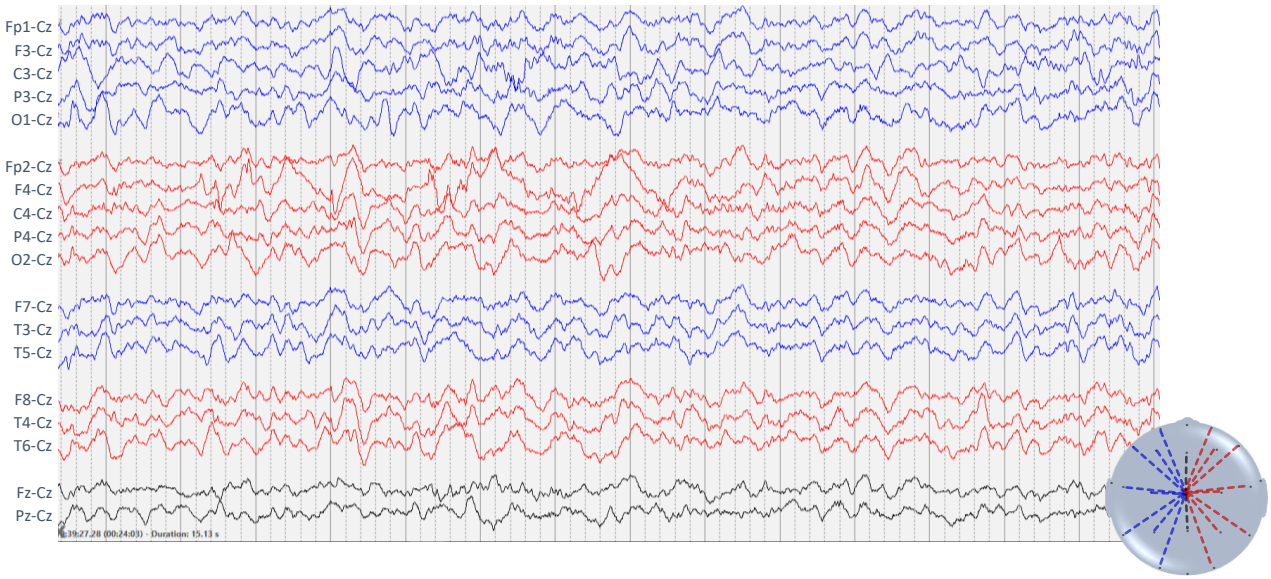
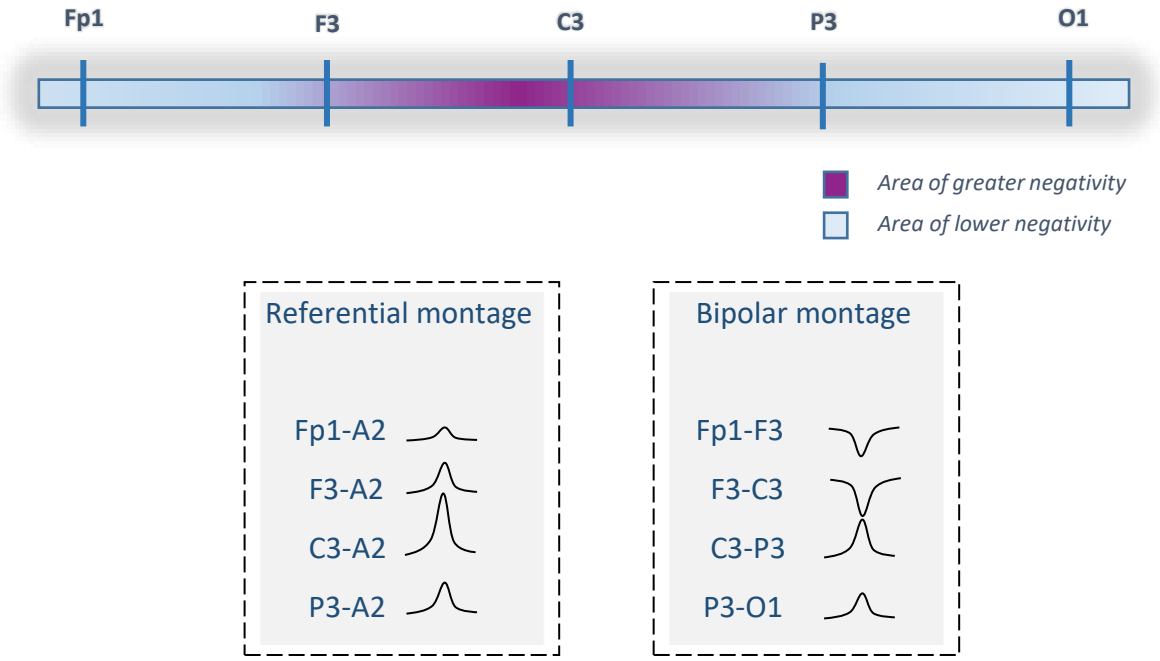


Figure 1.8 - Referential montage.
Same epoch as Figure 1.7, here on a referential montage to Cz. Timebase =30mm.s-1, sensitivity=150µV.cm^{-1}, HP=0.5Hz, LP=70Hz.

The selected montage influences how an electrical field at any given time will be represented (see Figure 1.9). An upward channel deflection reflects a lower amplitude input from the active electrode in relation to the reference. Similarly, the amplitude of the input on the active electrode exceeds the one on the reference electrode results in a downward deflection on the respective EEG channel. As shown in Figure 1.9, in a referential montage, the channel using the electrode nearest to the potential of maximal negativity (in this case, C3) presents the major deflection. The further away a channel is from the source of that potential, the smaller the deflection

is. Meanwhile, the bipolar montage will display what is commonly referred to as "phase reversal" (in this case between F3-C3 and C3-P3). This means, the signal's polarity is opposite in the channels that include the electrode nearest to the potential of maximal negativity as active and reference respectively. So, taking the example of Figure 1.9 and *A* meaning amplitude of the signal, we have that: A_{F3}-A_{C3}>0, so the difference of electrical charges is positive and by consequence deflection will be



downward; while A_{C3}-A_{P3}<0, the difference of electrical charges is negative and therefore display an upward deflection.

Figure 1.9 - Polarity of a potential.
Example of a potential with maximal negativity over the left central area (C3) and scheme on how it is depicted in the referential and bipolar montages.

Both referential and bipolar montages present advantages and disadvantages as discussed by Cooper *et al*. in *Techniques in Clinical Neurophysiology*(194). Starting with a common referential montage, a problem with this type of montage is that channels will display different interelectrode distances. As interelectrode distance is a determinant of signal amplitude (electrodes that are close together are more likely to be recording similar activity from the same generator which is largely cancelled out by the differential amplifier which only amplifies the difference in signal), meaning that channels with electrodes at increasing distances from the reference will show increasing amplitudes, resulting in amplitude differences due to distance

which is not ideal. The choice of position for the reference electrode is also fundamental for straightforward interpretation. As such, topography of expected artefactual (e.g., muscle activity, eye blinks) and non-artefactual signal (e.g., vertex sharp waves, epileptogenic focus) should be considered when determining the reference electrode as all channels will be affected by artefact in the reference electrode. The common average montage can be an alternative to the common referential montage. Instead of a single reference electrode, the reference value is calculated from the averages of all electrodes' instantaneous values, negating the reference distance problem. The amplitudes of the deflections tend to be lower than in the first montage as the value is partially subtracted from itself. This montage also has the disadvantage that high amplitude artifacts can contaminate all channels. Bipolar channels may overcome some of these issues, and the principal criticism concerns the localisation of some events. For example, two parallel chains of channels in which one chain has an electrode over the area of maximal negativity, and the other is parallel to the event. The further away chain may also exhibit a phase reversal; however, this localisation issue can be resolved with a perpendicular chain of electrodes. Less pronounced deflections may also go under-detected in channels with small interelectrode distances as in the bipolar montages. Another important criticism is the under-detection of suppressions that are very localised to an electrode area. To conclude, there is no ideal montage; an experienced EEG reviewer will consider the pitfalls of all montages when reviewing and adapt the montage in display to the needs.

EEG is a complex, time-varying, multichannel graphic. While traditional EEG analysis relies on the visual observation of the EEG and often a descriptive assessment of the EEG pattern, research often requires more direct quantitative measures of the EEG. To respond to this need, quantitative EEG (qEEG) toolboxes such as NEURAL for MATLAB were developed(195). NEURAL measures EEG magnitude, spectral distribution, continuity and connectivity, which are not readily accessible upon EEG visual interpretation. EEG magnitude contains measures of range-EEG (rEEG) which quantifies peak-to-peak amplitude. Spectral power measures the amount of activity for different frequencies in a segment of EEG. The

most common technique used to derive a spectral frequency metric from a time series such as EEG is Fast Fourier Transformation. Spectral distribution informs the spectrum's shape and includes features such as spectral flatness that measures its entropy, absolute spectral power and relative spectral power, which is the normalised value for the total spectral power. BSI measures the symmetry between hemispheres. Coherence is a connectivity measure calculated by matching frequency components between signals. A full description of these features can be found in O'Toole *et al.*(195).

1.5.3. Polysomnography

Polysomnography is the study of sleep using polygraphic techniques. Chin electromyogram (EMG), electrooculogram (EOG), and EEG channels are fundamental for correct polysomnography sleep scoring; other measurements such as electrocardiogram (ECG) and respiratory movement sensors are generally included. Polysomnography is considered the gold standard for sleep staging by providing measures and a graphic of the various sleep stages over a period of sleep. Sleep staging analysis is based on classifying sleep stage using an epoch-by-epoch approach. Recommended epoch length depends on the scoring criteria in use and can range from 20 to 60 seconds in paediatric sleep studies(196). Several polysomnographic-based scoring criteria have been proposed for infants and children which target slightly different age groups, these include the Anders manual, Hoppenbrouwers'; Guilleminault and Souquet's; Crowell's; and Scholle and Shäufer's sleep scoring criteria(197). The American Academy of Sleep Medicine (AASM) proposed integrating the paediatric scoring criteria into their already well-accepted Manual for the Scoring of Sleep and Associated Events, originally designed for adults. Paediatric scoring criteria proposed by the AASM was extended to include infants below 2 months of age in 2015 (197).

1.5.4. Maturational evolution of sleep patterns

As the infant ages, daily sleep diminishes from 17-18 hours at the newborn stage, to an average of 15 hours at the age of one(168). Sleep fragmentation throughout the 24h-cycle is characteristic of infancy. Sleep tends to be consolidated towards one main period of sleep at night-time, as circadian control of sleep develops during infancy(198, 199). Although the suprachiasmatic nucleus is already formed, functionally prepared for photic entrainment and manifesting cyclic circadian activity at birth, neonates do not produce melatonin and conform to an ultradian sleep cycle with bouts of sleep typically lasting 50 to 60 minutes spread across a 24-hour period(200), interspersed with periods of waking. In the first few months with the onset of melatonin production, the establishment of circadian fluctuations of cortisol concentration and other hormones and factors, infants increasingly follow a circadian sleep pattern with the longest period of sleep occurring during darkness(53). By one year of age the longest sleep period typically last approximately 7 hours (201). Figure 1.10 represents an infant's sleep pattern and illustrates changes occurring within the first 6 months of age.

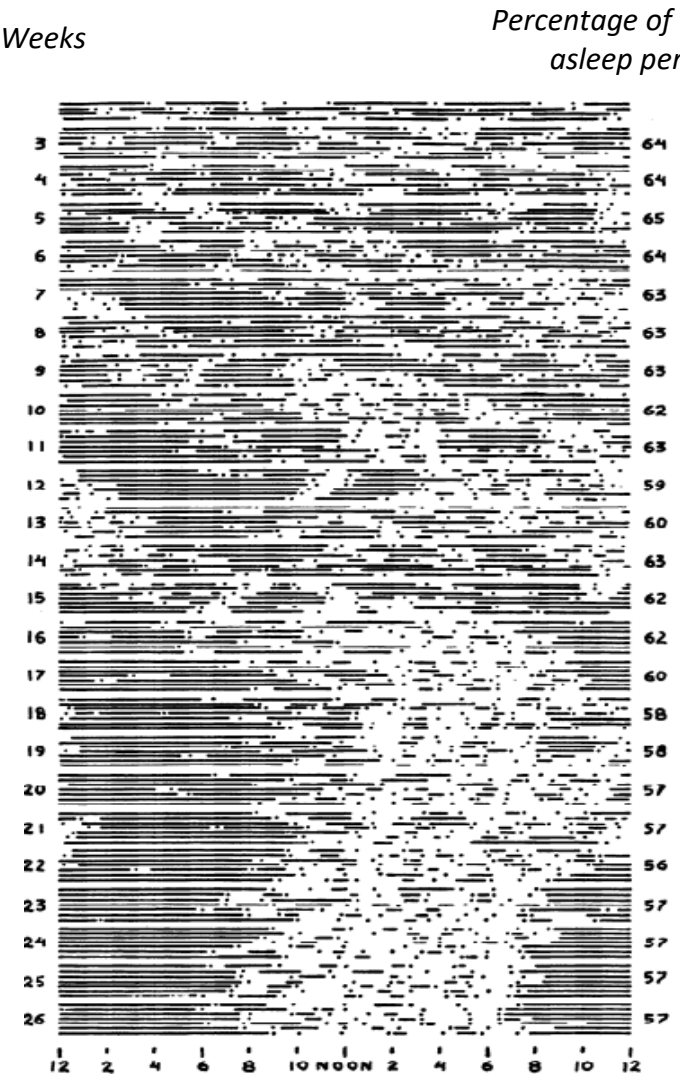


Figure 1.10 - Sleeping patterns of a single infant from 11 days old until 6 months of age. Each row corresponds to a day. Numbers on the left correspond to percentage of time asleep each week. Lines are periods of sleep; blank spaces are waking hours and dots correspond to feeding times. Image reused from (198) with permission.

Not only sleeping patterns but also sleep features are considered evolving entities which develop and differentiate from early in the antenatal period. This progress is particularly noted in the first months (Figure 1.11). Regular sleep maturation is an important indicator of brain development (168, 202, 203).

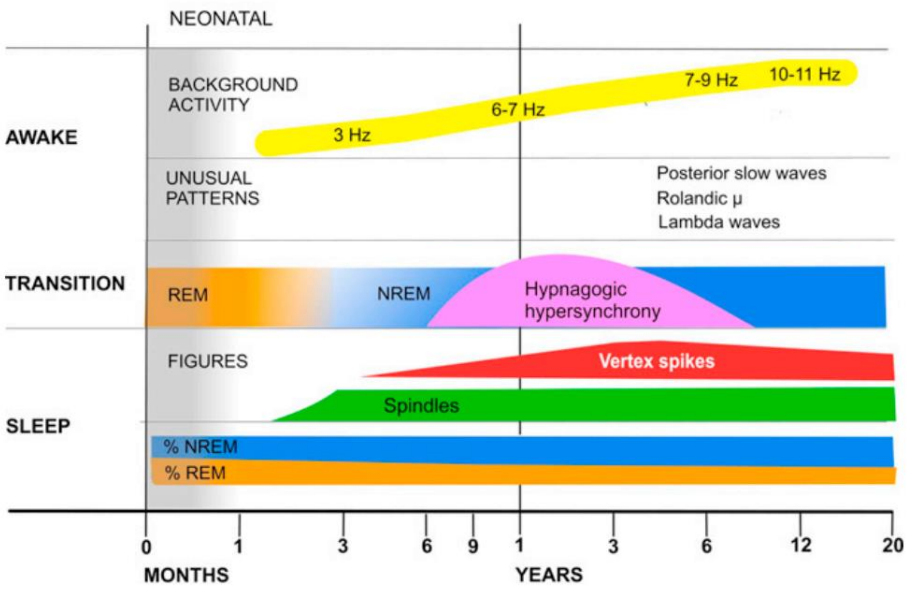


Figure 1.11 - Expected EEG features according to age.
Image reproduced with permission from Eisenmann M, Kaminska A, Moutard ML, Soufflet C, Plouin P. Normal EEG in childhood: From neonates to adolescents(204). Neurophysiologie Clinique/Clinical Neurophysiology. 2013;43(1):35-65 Copyright © 2013 Elsevier Masson SAS. All rights reserved.

Polysomnographic studies can provide insight into sleep maturation. Features such as delta brushes and *encoches frontales* are normal transients that may only be seen during the neonatal period(42). EEGs in premature infants up to 29 weeks of gestational age (GA) typically show a pattern called *tracé discontinu*, with bursts of activity interrupted by intervals of attenuated EEG (interbursts). Bursts become longer and interbursts become shorter with increasing GA (203). From 31-32 weeks GA, sleep cycling develops into clearly differentiated active and quiet sleep. Active sleep presents a nearly continuous EEG and rapid eye movements while, quiet sleep corresponds to a period of greater physical quiescence and discontinuous EEG background(205). By the age of 36 weeks GA, active sleep shows two different continuous patterns, the *mixed* frequency activity (see Figure 1.12) and *low voltage irregular*. Quiet sleep is primarily characterised by an EEG of alternating higher amplitude bursts and lower amplitude activity (termed *tracé alternant, see* Figure 1.13) that, closer to term age, is preceded by a shorter period of continuous high amplitude slow wave pattern, *High Voltage Slow* (HSV) (168, 202, 203, 205, 206).

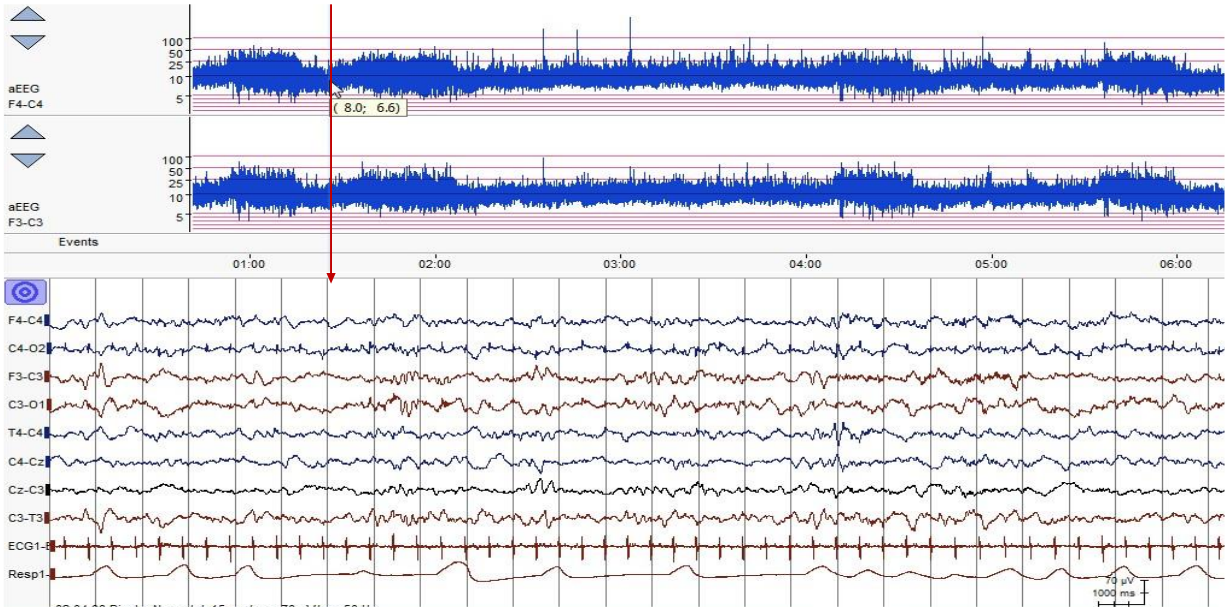


Figure 1.12 - Active sleep of a term infant.
Gain=70µV/mm; Timebase=15mm/s. Courtesy of Prof. Geraldine Boylan, INFANT reseach centre.

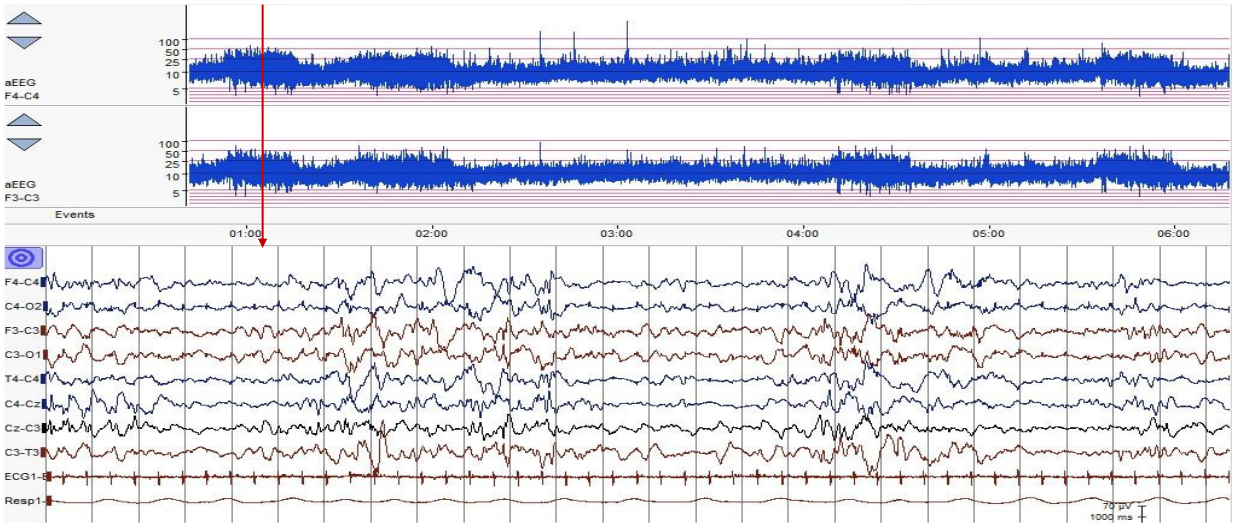


Figure 1.13 - Quiet sleep of a term infant.
Gain=70µV/mm; Timebase=15mm/s. Courtesy of Prof. Geraldine Boylan, INFANT reseach centre.

Around the 44th week GA, there is a shift in the onset of sleep from active to quiet (Figure 1.11) (168). *Tracé alternant* pattern is replaced by an HVS (196). Active and quiet sleep are generally assumed as the precursors of REM (rapid eye movement) sleep and NREM (non-rapid eye movement), respectively. However, the Paediatric Task Force(196) argues that there is no evidence that quiet sleep is a precursor of NREM, especially before the emergence of NREM sleep hallmarks. REM/NREM terminology is recommended after two months after term. A further

distinction between NREM sleep stages may occur as early as 4 months after term in the presence sleep spindles and slow wave activity(196). NREM sleep is then subdivided into stages N1 (Figure 1.14), N2 (Figure 1.15), collectively termed light sleep and N3 (Figure 1.16), also called slow wave sleep (corresponding to deep sleep) (168, 206). N1 marks the beginning of light sleep, where the EEG activity is of low amplitude and mixed frequency, with vertex sharp waves appearing as the infant matures. N2 onset is indicated by the appearance of sleep spindles and/or K complexes. Finally, N3 is the stage of deep sleep where the EEG is predominantly populated by high-amplitude slow rhythms. REM (Figure 1.17) EEG is marked by low amplitude mixed frequencies, sawtooth waves accompanying the characteristic rapid eye movements; and irregular breathing and heart rate (121).

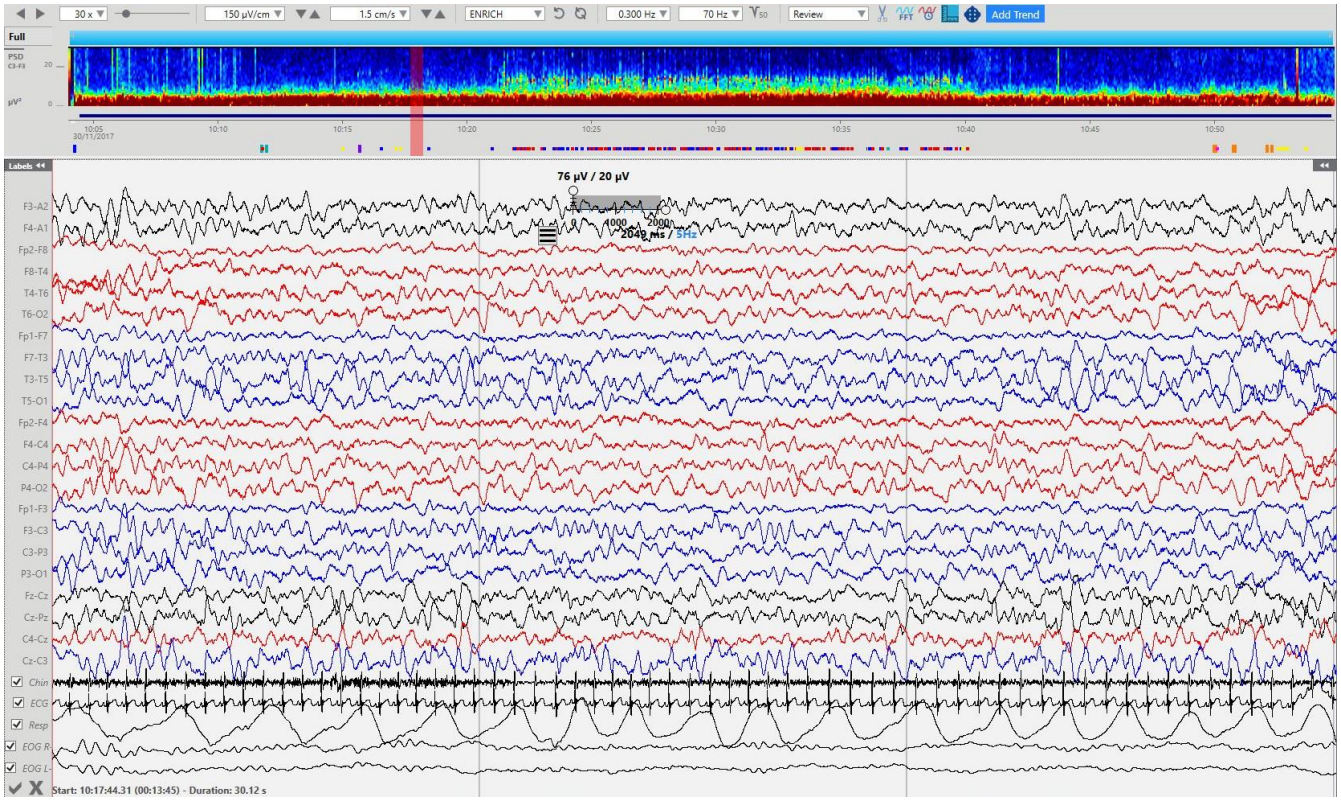


Figure 1.14 - N1 sleep stage of a term-born 4-month old.
EEG shows N1 characteristic relative low amplitude, mixed frequencies. EOG shows slow eye movements and intrusion of frontal activity. Incipient vertex sharp waves are present in the central regions. Gridlines represent 10 s-intervals. Power spectrum density (PSD) window on top of the EEG represent spectral power of frequencies from 0.5 to 30 Hz (y axis) across the approximately 1 h of recording (x axis). Red colours represent the highest spectral power for a particular frequency and time on the spectrum; colours close to dark blue represent the lower spectral power. Image reused from (207) under CC-BY 4.0

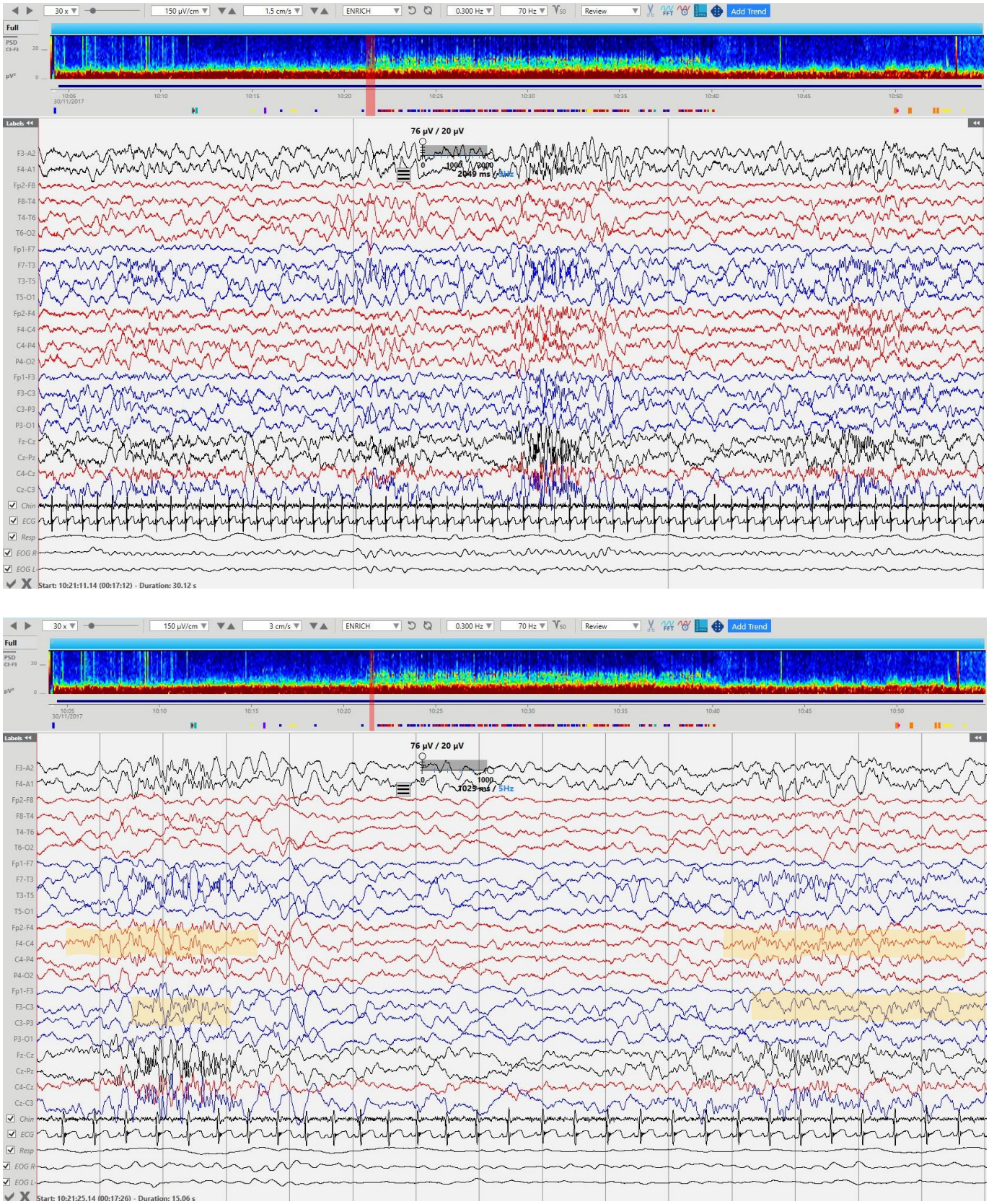


Figure 1.15 - N2 sleep stage of a term-born 4-month old.
Sleep spindles marked the onset of this epoch. Top EEG: timebase = 1.5 cm/s, gridlines correspond to 10 s intervals. Bottom EEG: detail of the previous page to elucidate spindles, timebase = 3cm/s, gridlines correspond to 1 s intervals. Right and left fronto-central sleep spindles in yellow. Image reused from (207) under CC-BY 4.0

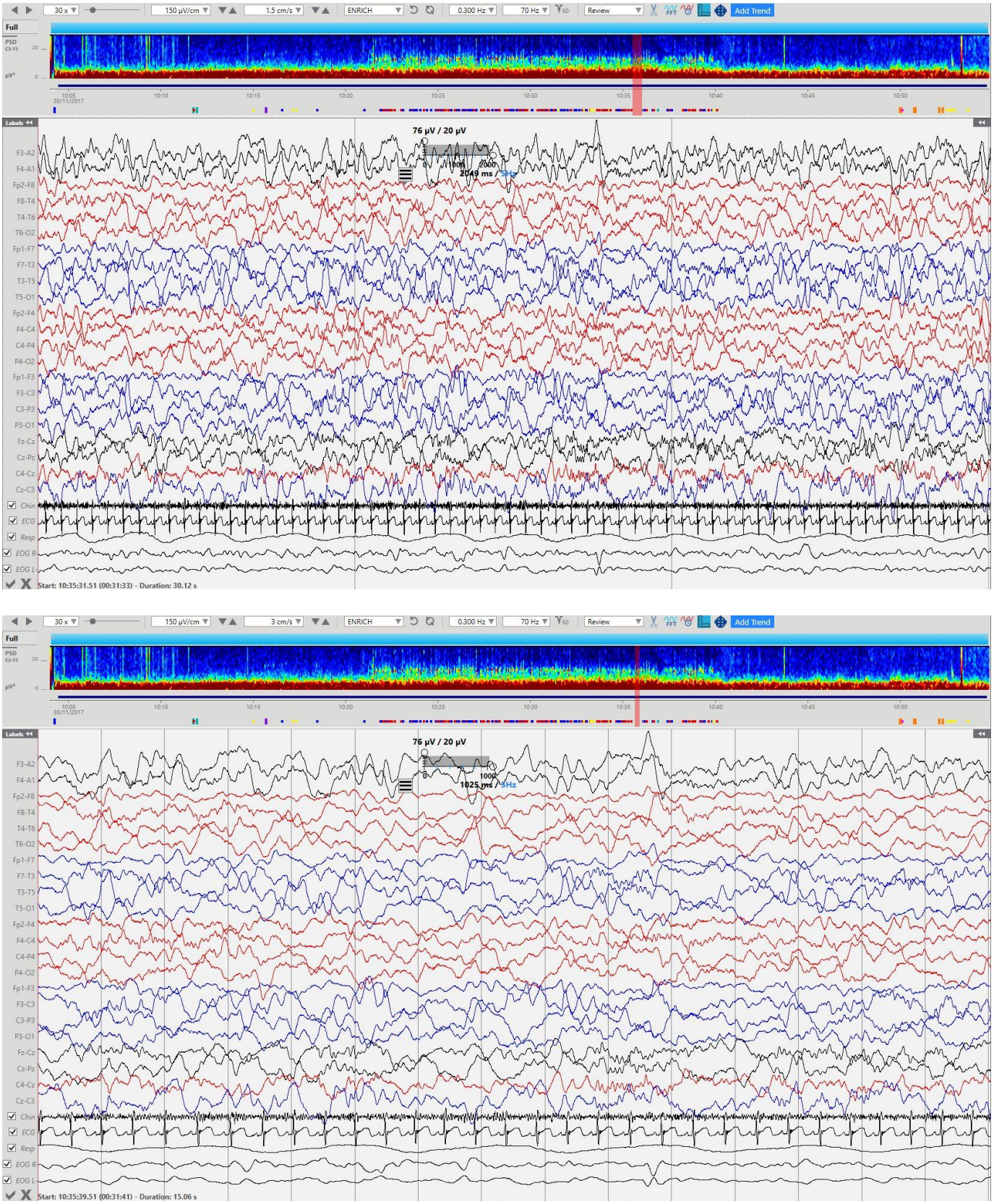


Figure 1.16 - N3 sleep stage of a term-born 4-month old.
High amplitude slow waves are a hallmark of this stage (intrusions of frontal high amplitude activity present on the EOG channels). Top EEG: timebase =1.5cm/s, gridlines denote 10 s intervals. Bottom EEG: detail of the previous page, timebase = 3cm/s, gridlines denote 1 s intervals. Image reused from (207) under CC-BY 4.0

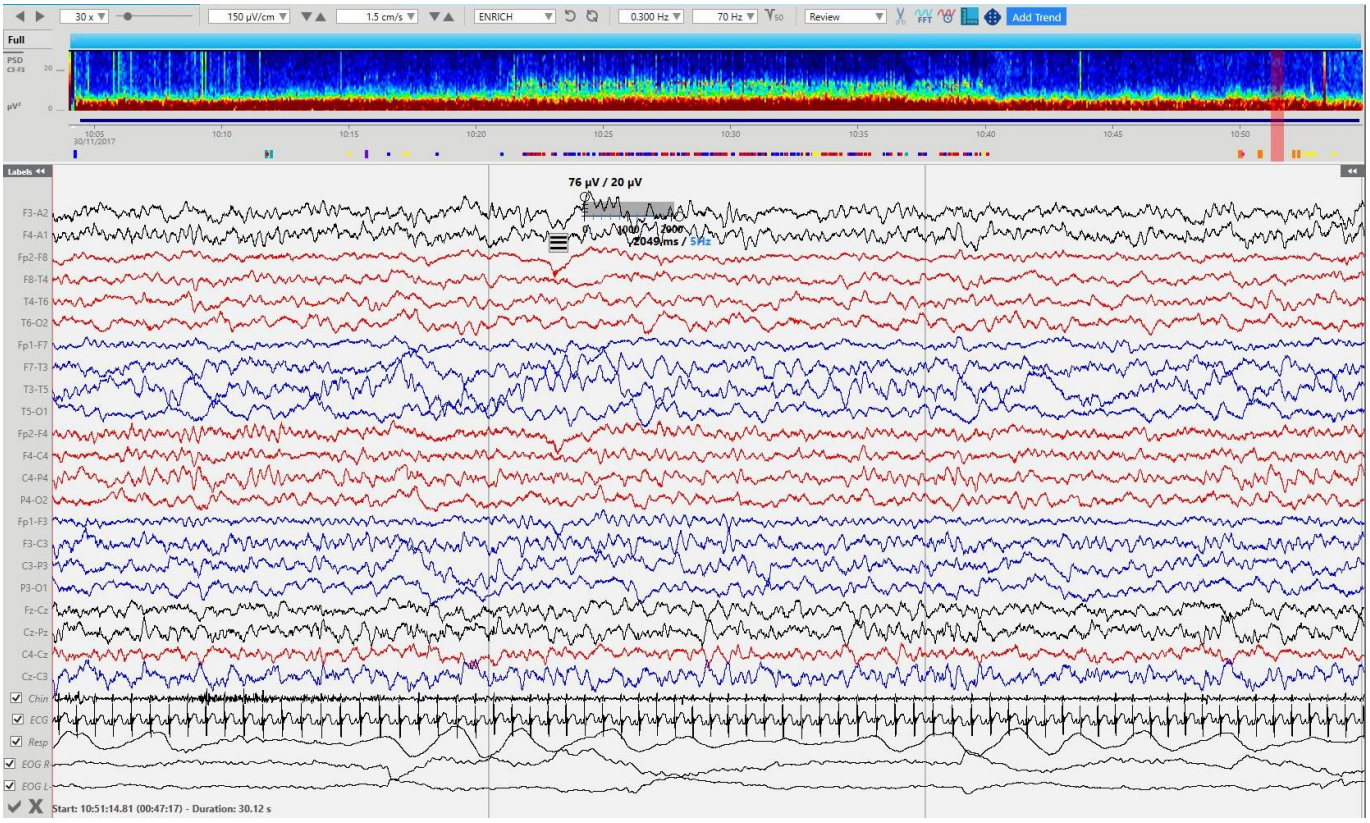


Figure 1.17 - REM sleep stage of a term-born 4-months old.
Sleep stage marked by relatively low amplitude activities, accompanying conjugate rapid eye movements, irregular respiration rate and lower chin EMG. Image reused from (207) under CC-BY 4.0

The EEG of a 4-months infant in physiological conditions is expected to show entirely continuous EEG activity and emerging sleep phenomena. Vertex sharp waves are transients characteristic of stage N1 sleep. The earliest signs of these sleep transients were described by the chronological age of 3-4 weeks (208, 209). However, at the 6th-month post-term, they still appear sparsely. As the infant ages, vertex sharp waves become more frequent and undergo alterations in morphology, becoming clear at the age of 16 months [reviewed in (168, 196)]. At 4 months vertex sharp waves are still developing, being poorly defined and difficult to distinguish from the background activity of similar frequency(196). This factor creates a significant challenge to identifying them reliably and makes the validity of subsequent quantification questionable. K complexes are waveforms of stage N2, but are rare and poorly formed phenomena at 4 months. They are reported in the literature to emerge after 5-6 months of age; K complexes morphology changes significantly after first appearing and assume a mature morphology by 18 months of age(196). In contrast, sleep spindles at 4 months (Figure 1.18) are already frequent, well-defined

and relatively high-frequency components of stage N2 sleep that also appear in N3; these features are thought to be involved in basic maturation processes and memory consolidation. Slow waves are also present, although more prevalent over the posterior areas at 4 months. Slow waves are also associated with memory consolidation, homeostatic regulation and myelination. The factors mentioned above make sleep spindles and slow waves ideal candidates for the study. I will discuss more in depth these microstructure elements of the sleep EEG in sections 1.5.5 to 1.5.8.

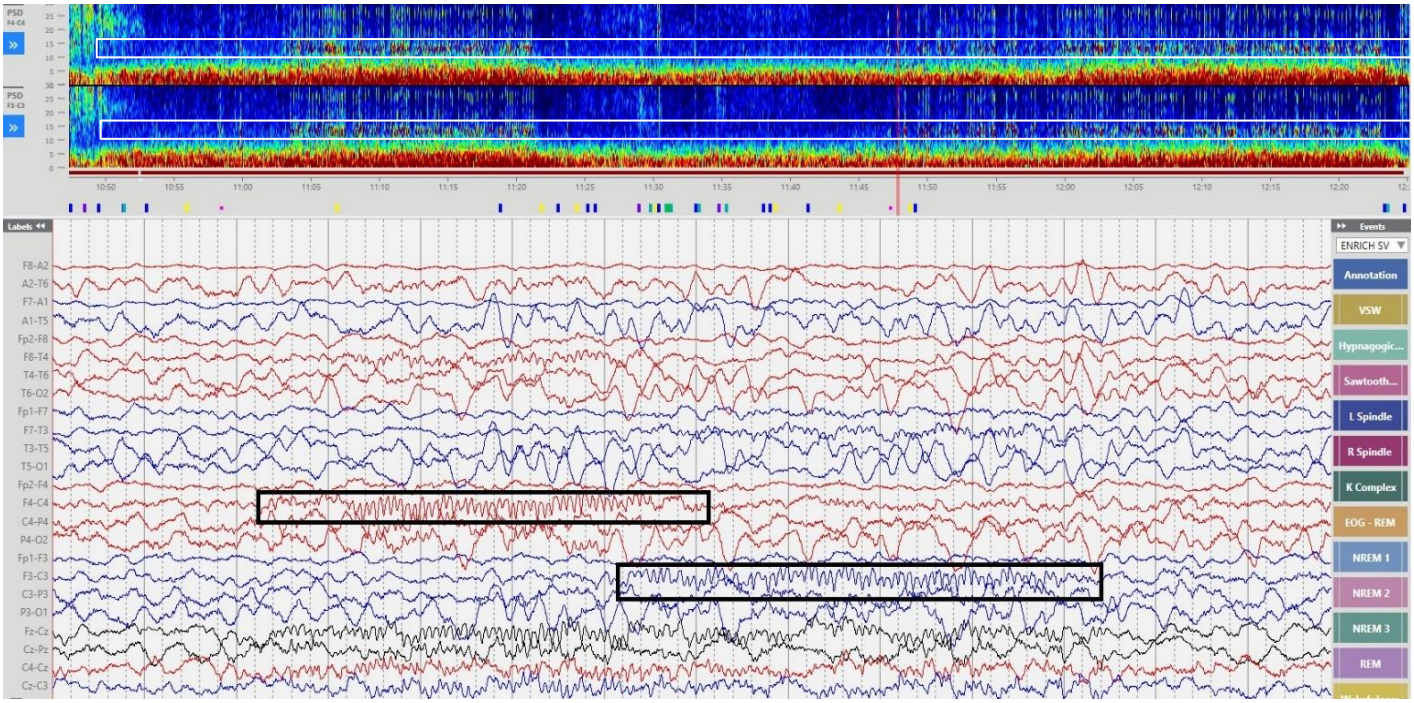


Figure 1.18 - Sleep spindles of a term-born 4 months old.
Upper two windows: spectral power density graphs of the right and left fronto-central channels; red dots of ~13Hz (white boxes) indicate occurrence of sleep spindles across the first two sleep cycles of this infant. Lower window: Appearance of sleep spindles on EEG a 4-months old, black boxes indicate right and left fronto-central sleep spindles. Gain=150µV/mm; Filters: high-pass=0,3Hz, low-pass=70Hz; Timebase=30mm/s.

1.5.4.1. Maturation of sleep in the preterm-born

In the preterm some sleep aspects may reach maturation at different timepoints compared to term-born children, while other aspects occur at similar corrected ages. Ellingson and Peters found in a daytime study that the timing of the shift to quiet sleep onset and the percentages of active and quiet sleep during the first year at corrected age did not differ between infants born between 30-33 weeks

GA and age-matched term-born peers (210). For other observations, such as the dissolution of tracé alternant and appearance of the first sleep spindles, the same group of preterm infants appears to be ahead at corrected age(210). Guyer *et al.* corroborated the observations of no differences in the length of total sleep duration or active or quiet sleep, either at term age or at three months corrected age, compared to term-born peers in their daytime study on very preterm-born infants (211). As for nocturnal sleep, Scher *et al.* found that age-matched preterms born <32 weeks GA had longer quiet sleep segments and longer, less disturbed sleep than the term-born neonates(212). Ryan *et al.* found that within MLP sleep at 36 weeks GA, total sleep time and quiet and indeterminate sleep time and percentage was not significantly different with increasing gestational age at birth; active sleep percentage and duration, however, tended to be greater for those born at later gestational ages. As decreased active sleep is associated with increased maturation, this study may indicate that some sleep-associated maturational processes may be more advanced in those MLPs with greater chronological age(213). Comparing nighttime sleep two days after birth for both MLP and term infants, Guyon *et al.* showed that, in contrast with the previous studies, preterms have shorter, less efficient sleep (i.e., lower ratio of total sleep time per total time recorded). Preterms also showed a lower arousal index with lower arousals in active sleep and higher in quiet sleep than term-born infants. These differences persisted in nighttime sleep at 6 months. The authors highlighted that lower number of arousals, particularly in active sleep/REM, are a risk for sudden infant death syndrome. In this study, group differences in quiet and active sleep percentages were significant not at birth but at 6 months for both night and day sleep. The latter could be consistent with forced *acceleration of maturation* on the preterm groups; the authors speculate that differences could be due to stress caused by earlier birth in a phase of higher brain vulnerability and/or early adaptation to environmental conditions after birth. Alternatively, NREM percentage may be increased in preterms due to sleep pressure arising from a higher number of arousals during this sleep stage(214).

A quantitative EEG study by O'Toole *et al.* followed the first 72 hours of extreme and very preterm-born infants showed that the evolution of several qEEG parameters during the hours and days after birth progress more rapidly than during

the corresponding period of intra-uterine life; this information was obtained when comparing with age-matched peers immediately after birth(215). Results may indicate birth recovery and postnatal adaptation (215), and/or postnatal maturational processes caused by exposure to extrauterine stimuli(216). Guyer *et al.* reported lower average spectral power in frequencies >5Hz in the preterm-born group at term-equivalent age but showed no statistical difference from the term group at three months corrected age. However, also at three months, an occipital-to-central shift of the 5-9Hz frequencies was more accentuated in term infants. The authors interpret these later outcomes as a sign of EEG maturity of the term infants(211).

In very preterm-born groups, spectral power differences to term-born peers persist at 13 years of age, at least in the awake resting state. The authors of that study also identified different EEG profiles associated with the likelihood of high/low IQ, academic and motor performance. Very preterm-born adolescents were likely to present an EEG profile (higher spectral power in delta and lower theta, alpha and beta) that was associated with lower IQ, academic and motor performance (217). However, the risk of cognitive and behavioural adverse outcomes associated with prematurity is not exclusive to extreme and very preterms(38); further studies on EEG alterations in healthy MLP are needed(214).

To conclude, although there is variation in the studies' methodology, some sleep parameters are intriguingly more developed in premature-born infants. Several authors have suggested that observations may be due to longer time of birth recovery in age-matched analysis of neonates at term(215) or part of a process of adjustment to the stress from premature birth (214). It may also vary with environment in which the infants are introduced postnatally(214). Adolescents born preterm also show differences in their awake EEG(217). However, these are no longer considered more mature, in fact, these are associated with lower cognitive abilities. It is possible that as the child grows, the difference of weeks of extra-uterine experience becomes less significant and the immaturity at birth along with socioeconomic status(38) becomes the major determinants of cognitive maturation.

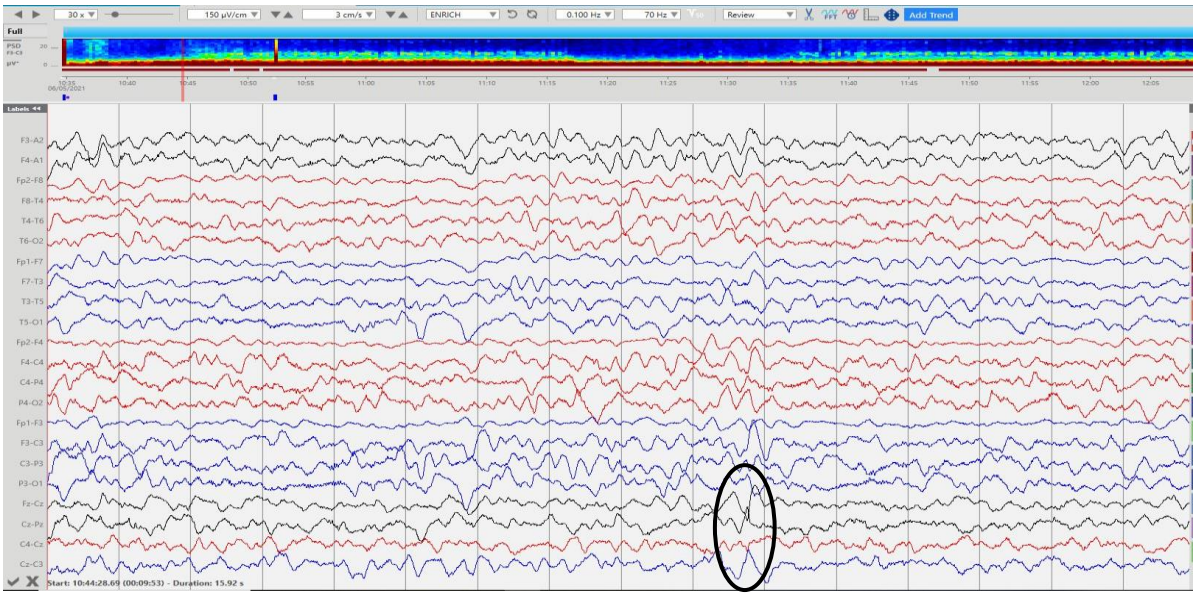
1.5.5. Vertex sharp waves

Vertex sharp waves are negative waves maximal over central areas of the brain (vertex) with a duration <0.5 second(196) (Figure 1.19A). Analysing their occurrence with EEG and fMRI techniques simultaneously, two main areas are activated, the bilateral primary sensorimotor cortex, particularly the medial precentral gyrus and the medial occipital cortex(218). Stern *et al.* suggest the sensorimotor cortex may be the generator of the waveform as it also corresponds to the region of maximal amplitude of the vertex sharp wave, which may subserve brief multimodal sensory experiences, such as somatosensory and visual (218).

1.5.6. K-complexes

K-complexes (Figure 1.19B) are N2 transients. Visually they are frequently biphasic with an initial negative sharp wave trailed by a positive waveform and are maximal over the frontal regions lasting more than 0.5 second(168, 219). There is evidence that K-complexes are a result of internal or external (auditory/tactile) stimuli (168, 220, 221) and have been observed to suppress cortical activity, which may serve as a mechanism to maintain sleep after minor sensory stimulation (222). Ioannides *et al.* suggest a more intricate role for K-complexes, in which some stimuli processing occurs following a K-complex event which may determine either an increment of the level of arousal or maintenance of sleep (223). A diverse set of areas are involved during K complexes including the thalamus and several cortical regions including the insula (see Figure 1.20) (224).

A)



B)

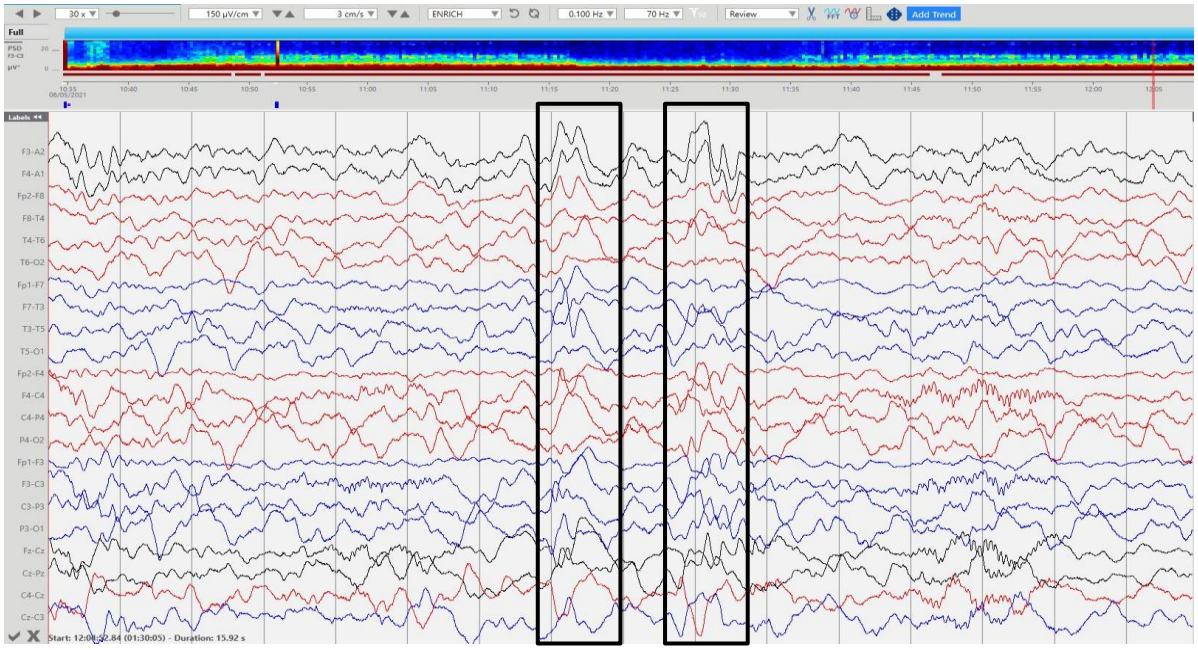


Figure 1.19 - Vertex sharp waves and K complexes in an older infant.
Infant age: 7 months and 18 days. A) Examples of vertex sharp waves and B) K complexes. Gain=150µV/mm; Filters: high-pass=0,1Hz, low-pass=70Hz; Timebase=30mm/s. Courtesy of Dr Cathal O'Connor from the INFANT reseach centre.

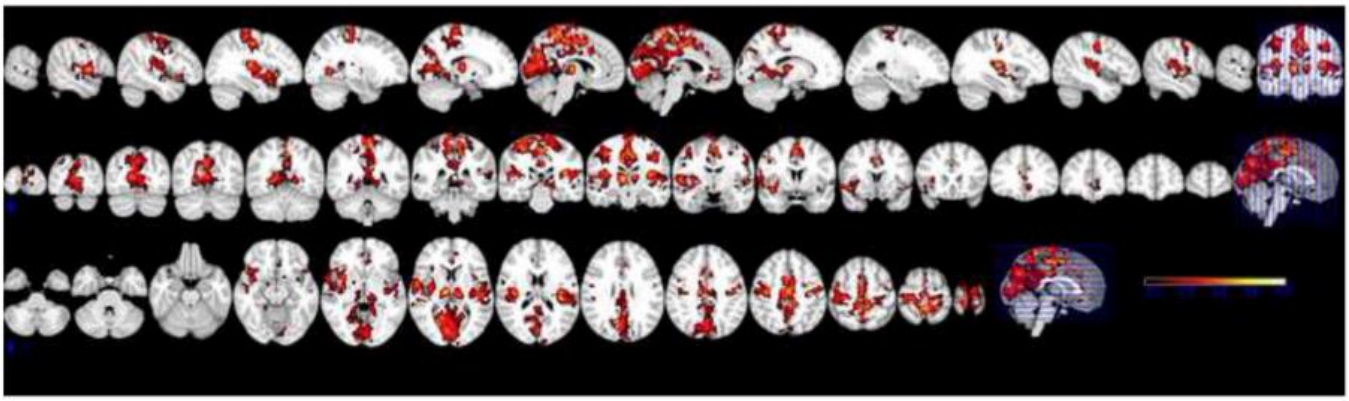


Figure 1.20 - Areas of increased signal during K complexes.
Image from fMRI on the adult brain. Reused from (224) with permission.

1.5.7. Sleep spindles

It is during stages N2 and N3 that sleep spindles may be found in healthy subjects after approximately 46 weeks (post-menstrual age) (206). They consist of roughly 11-16Hz rhythmic bursts present maximally either at frontal and centro-parietal areas (121, 225) (Figure 1.18). Sleep spindles evolve during the first months of age in frequency, amplitude, occurrence, duration, synchrony and symmetry (226). This sleep feature is generated from thalamic and cortical loops in conjunction with intra-thalamic circuits, particularly between the reticular nucleus of the thalamus and the thalamocortical neurons (Figure 1.21) (220, 227, 228). In addition, their excitability is subject to modulation at the level of the superior olivary complex of the lower ventral brainstem and inferior colliculus of the upper dorsal brainstem, nuclei known to be involved in the auditory information transmission to the cortex (229).

During both sleep spindles and synchronised slow waves of deep sleep, intra- and extracellular recordings of thalamic neurons have shown bursts of action potentials superimposed on a long-duration calcium spike after hyperpolarisation (Figure 1.22A and B). This occurs as a result of ionic exchanges within the cell membrane, which I will discuss in more detail in 1.5.7.1. These action potentials that behave in a cyclic manner lead to a gradual depolarisation of the cell. Once the depolarisation reaches a certain level, the previous activity gives way to sustained

tonic potentials, characteristic of waking and REM sleep [reviewed in (230)]. Despite tonic potentials predominating during waking, bursts may still occur triggered by events such as visual stimuli. Switching between modes is thought to play an important role in attention mechanisms – "wake-up call" theory (231, 232). I will discuss the burst mode sensory transmission gating function during sleep spindles in the section 1.5.7.4 *Maintenance of sleep*. Interestingly, it appears that the same pattern of burst activity during the oscillatory mode is present in some types of epilepsy, particularly during absence seizures. This is suggestive of a link between this pattern of activity and attenuated levels of responsiveness [reviewed in (230)].

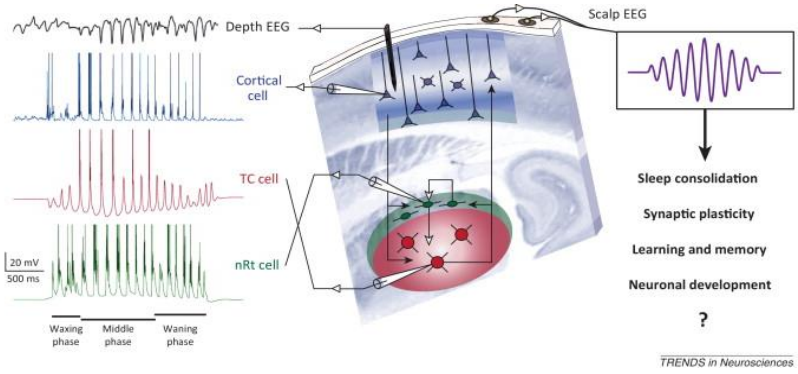


Figure 1.21 - Cortical, thalamocortical and reticular nucleus intracellular activities and resulting depth and scalp EEG traces during a sleep spindle.
Image from (233) with EEG and intracellular recordings originally from (234); all reused with permission.

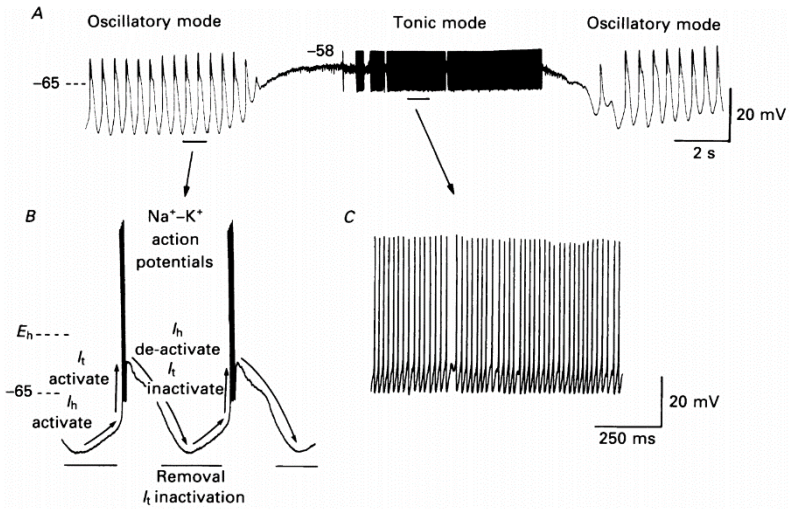


Figure 1.22 - Neuronal firing of a cat thalamus, recordings from the Lateral Geniculate Nucleus.
(A) Oscillatory and tonic bursting mode. Tonic mode was achieved through intracellular injection of current. Magnified view of (B) the oscillatory cycles and respective ionic events and of (C) the tonic cycles. Ih - Hyperpolarisation-Activated Cation Current; It - Low-threshold Calcium Conductance. Image reused from (235) with permission.

These oscillations may result in two types of sleep spindles recorded at the surface: slow and fast sleep spindles with distinct topographic characteristics, which were first described by Gibbs *et al.* (236), according to (237). Zeitlhofer *et al.* grouped spindles according to frequency: from 11.5Hz to 14Hz (slow spindles) and from 14Hz to 16Hz (fast spindles) in adults (225), but some level of variance is noted across different authors. This is because typical slow and fast sleep spindles differ at the individual level (238) and intra-individual: these two peaks are also age-dependent (239). In children as young as 4 to 8 years old, it is already possible to distinguish two spectral power peaks on the sleep spindle frequency spectrum, in a proportion of 24 out of 28 children, with both peaks below 13Hz (239). For this reason, Bódizs *et al.* developed the Individual Adjustment Method (IAM), which defines the frequency boundaries of slow and fast sleep spindles for each subject through a set of biological signal processing approaches (240, 241).

As mentioned, different frequency bands of sleep spindles appear to be location-dependent: the slower frequencies of sleep spindles are predominant over the frontal areas, whereas the faster frequencies are in the centroparietal regions (225, 236, 238, 240, 242-246). Andrillon *et al*. noted that the virtual boundary between slow and fast sleep spindles locates between pre-supplementary and supplementary motor areas. However, despite the predominance of fast spindles, it is possible to observe both types of sleep spindles in centroparietal regions; In contrast, frontal areas are limited to slow spindles(246). Computational models have indicated that, in comparison to slow spindles, high-frequency spindles tend to occur more rhythmically, and when there is a superimposition of the two types of sleep spindles, high-frequency spindles are frequently the very first component of the complex (242). On fMRI, both types of sleep spindles are associated with the activation of the lateral and posterior regions of the thalamus bilaterally, paralimbic areas and both superior temporal gyrus (Figure 1.23). In addition, there is augmented activity in the superior frontal gyrus during slow spindles and, supplementary motor, sensorimotor, mid-cingulate areas and the left hippocampus during fast spindles (247). The two types of sleep spindles also tend to interact differently with slow

oscillations, as discussed in the section 1.5.7.3, with possible implications for memory consolidation (245).

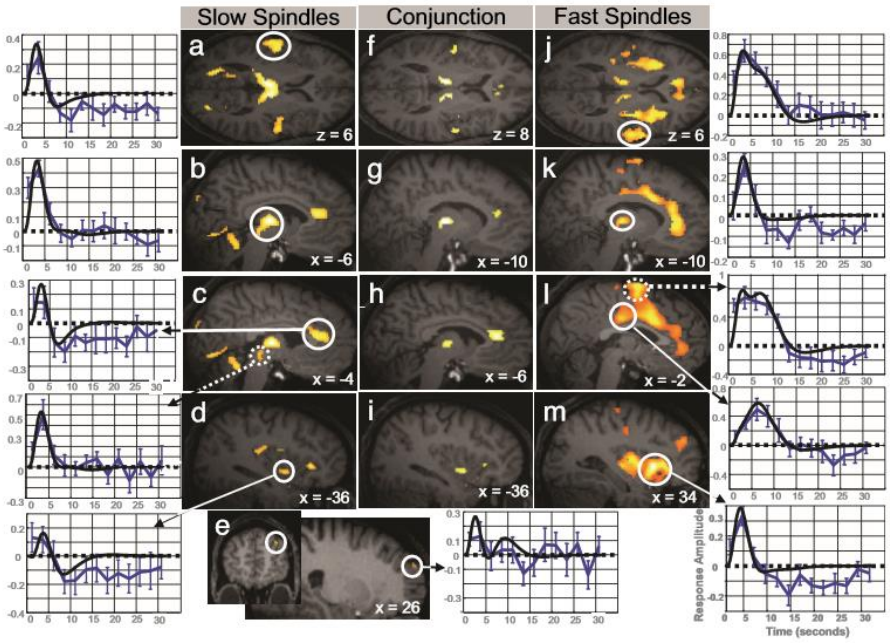


Figure 1.23 - fMRI activity during slow and fast spindles and their common activity.
Spindles identified based on automatic detection algorithm; band-pass filters: 11-13Hz for slow spindles and 13-15Hz for fast spindles. Image reused from (247), copyright 2007 National Academy of Sciences.

1.5.7.1. Mechanisms of sleep spindles genesis

The reticular nucleus of the thalamus, populated by GABAergic (inhibitory) neurons, is key to the generation of sleep spindles. After isolating the reticular nucleus from the rest of the thalamus, it is possible to record spindle-like oscillations from the anterior part of the nucleus, whereas spindle activity is not present in the remainder of the thalamus lacking the reticular nucleus (227, 248).

Sleep spindle generation depends on the membrane polarisation level (249). For the reticular nucleus and other relay thalamic nucleus (ventrobasal) cells, membrane potential must be between -60 and -65mV to de-inactivate the *calcium-dependent T current* or *Low-threshold Calcium Conductance* (I_t) and, consequently, to transition from tonic to bursting mode (250-252).

Low amplitude spontaneous excitatory potentials trigger an AMPA-mediated response of a limited number of pyramidal layers to the TC and reticular nucleus(253-255). In this hyperpolarised environment, a depolarising potential arising from

synchronous and sustained cortical activity or the inhibition of the reticular nucleus hyperpolarising current can generate a cascade of events that leads to cycles of bursts (256), similar in many aspects to events represented in Figure 1.22B [reviewed in (230, 257)]. Indeed, reticular neurons can generate these bursts by themselves, as observed by Bazhenov *et al.* (258).

Activation of low-threshold Calcium Conductance is voltage-dependent and depends mainly on $Ca_V3.3$ but also $Ca_V3.2$ channels (259-261). The entrance of calcium facilitates *Calcium-activated potassium conductance* (I_{KCa}). As a result, I_{KCa} further increases the activation rate of the I_t channels (251). This induces a fast depolarisation of the cell membrane on the reticular nucleus causing bursts of action potentials, resulting from exchanges of sodium and potassium along with this slow calcium spike (235). The fast action potentials will display an *accelerando-ritardando* pattern characteristic of the reticular nucleus' cells. As the name of the pattern indicates, it refers to acceleration and subsequent deceleration of the rhythm of discharges of the spikes during the burst (262).

Depolarisation of the reticular nucleus generates an inhibitory postsynaptic potential on the TC cells through γ-aminobutyric acid (GABA) action, both $GABA_A$ and $GABA_B$ (263). This temporarily inhibits the excitatory synapses from TC to the reticular nucleus. As the reticular nucleus depolarisation phase ends and hyperpolarisation-activated cation current (I_h) re-starts on the TC cells, a *post-inhibition rebound* led by I_t activation generates a long calcium spike, during which, fast action potentials take place (Figure 1.22B) [reviewed in (230)]. As a result, TC cells excite more cortical neurons and their collaterals excite cells from the reticular nucleus and synchronises activity, causing another burst on the reticular nucleus, closing the cycle. At each cycle, more cells are included(256, 263) The cortical input, at this stage, plays a vital role in synchronising the spindle oscillations (264).

The spindle waning phase is the result of the conjunction of factors: membrane bistability of 1/5 of the reticular neurons, meaning the response to stimuli is dependent on the membrane potential at rest in which the cell is found at that moment; thalamocortical cells innervated by these neurons exhibit lower rates of

rebound discharges [reviewed in (257)]; a progressive hyperpolarisation-activated cation current leads to slowly depolarises TC cells, inhibiting another calcium spike from being generated and desynchronising network activity (265). Increasing calcium spike latencies cause thalamic and cortical activity to phase out (254). Reticular neurons also show inhibitory properties amongst themselves, by reducing the number of reticular neurons in burst mode, this intra-reticular inhibition may weaken thalamocortical responses with consequent desynchronisation of the oscillations(266, 267).

Despite this well-accepted theory on the generators of sleep spindles, questions have arisen from the fact that certain ion channels of the thalamus are specific or affect slow and fast spindles in different ways (268). This factor, amongst others such as the dissimilarities in the relationship of the slow and fast sleep spindles with slow oscillations discussed in 1.5.7.3, led Timofeev and Chauvette to contest this one-fits-all theory of the generating mechanisms for both slow and fast spindles [see (269)].

1.5.7.2. Pathways to the different types of spindles

Interestingly, sleep spindles can reach either the upper (layer 1 and partially layer 2), the middle cortical layers (mostly layer 4), or both with a delay of tens of milliseconds on the upper laminar layers. While activity from the supragranular neurons may generate spindles detected in the granular and supragranular layer, neurons on the infragranular layer exclusively give origin to the spindles detected on the granular areas. (270). This variety of cortical sources of sleep spindles reinforces the theory of the two different pathways that give rise to matrix and core spindles, which I will be describing next (Figure 1.24).

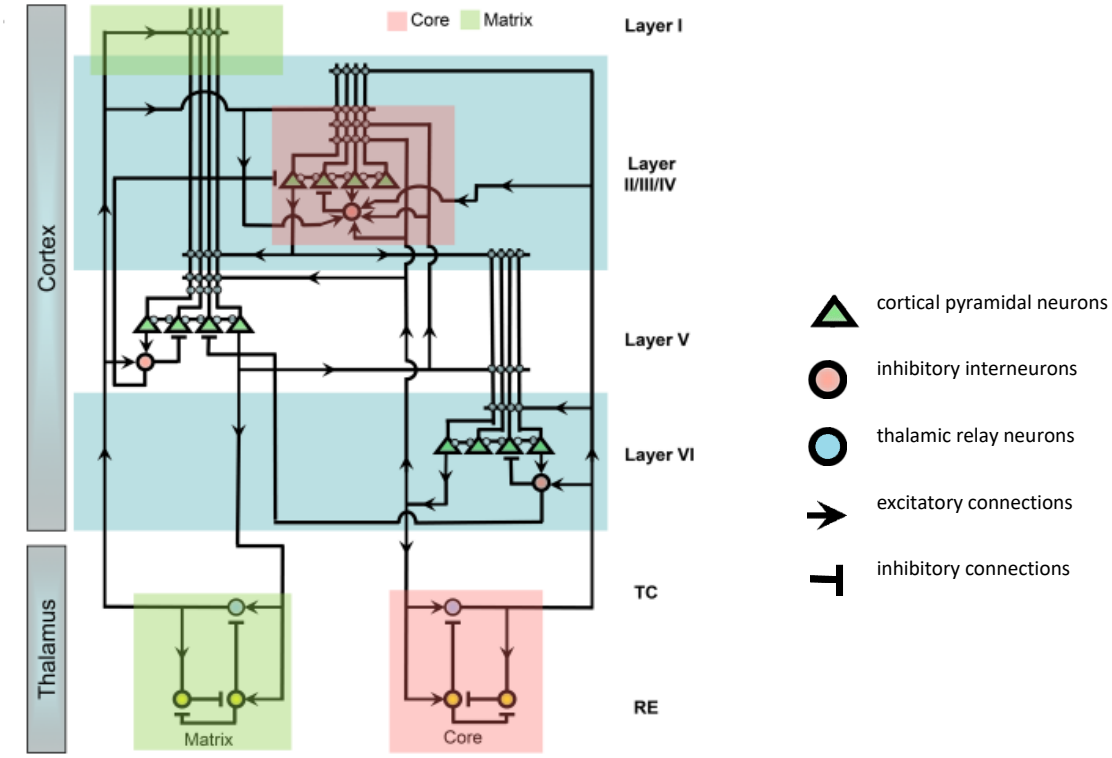


Figure 1.24 - Model of matrix and core pathways.
Note that it is possible for sleep spindles originally from the core system to spread to the matrix system. Imaged reused from (255) under CC BY 4.0.

The concept of Core and Matrix sleep spindles had its origin in (271). Here, Jones suggests two major pathways for sleep spindles from the thalamus to the cortex. The core cells are parvalbumin-positive and are located in specific thalamic nuclei, which project into limited areas of the middle layers of the cortex. Calbindin-positive cells from the entire thalamus form the matrix of cells that projects to extensive areas of the upper layers of the cortex (271).

Spindle features show differences when detected on EEG or magnetoencephalography (MEG) techniques, particularly synchrony (higher in EEG spindles) and duration and density, i.e. number of spindles per minute, (higher in MEG spindles) (255, 272). As EEG is more sensitive to matrix spindles (due to the superficiality of the cortex layers in which they occur and the orientation of the neuron as a dipole), led Khrishan *et al.* to hypothesise that the variances observed correspond to typical differences in either matrix and core spindles (255). Computational models indicate that matrix spindles have a cortical and thalamic spread ten times higher than core spindles and that both can simultaneously occur,

noting that individual incidence tends to happen more frequently on core spindles (255).

Between slow and fast spindles (differentiated by IAM) and between local and global sleep spindles, the amplitude distribution in the different cortical layers is approximately similar. However, the same study found that the different spindles diverged as they tended to aggregate local cortical activities at different points of the spindle negative slope. The latter finding may underlie the dynamics of the different functions attributed to sleep spindles. Amplitude distribution across the cortical layers and point of co-occurrence of local cortical activities was variable within each participant, different areas of the microelectrode placement and very limited number of individuals may explain this variability (273).

Low-resolution brain electromagnetic tomography (LORETA) is a technique that integrates high time resolution and computations of the electrical source location. Based on the cortical distribution of the fast and slow sleep spindles, Anderer *et al.* (274), using LORETA, argue in favour of different thalamic nuclei producing slow and fast spindles. Slow spindles (<13Hz) are present predominantly in Brodmann areas 9 and 10 in the prefrontal source and are thus hypothesised to be generated in the anterior dorsomedial thalamic nucleus. Faster spindles (>13Hz) predominantly localise over Brodmann area 7, at the precuneus, and are therefore hypothesised to be generated from either the lateroposterior, posterior laterodorsal and rostral intralaminar centrolateral thalamic nuclei (274).

Further studies will be needed to draw more definite conclusions on whether the core and matrix pathways play a role in determining the frequency of the sleep spindles recorded on the scalp.

1.5.7.3. Slow oscillations and slow wave associations with sleep spindles

Delta waves are present in the infant EEG (Figure 1.25) and may co-occur with sleep spindles. Delta waves present frequencies up to 4.5Hz recorded on scalp EEG and reflect slow oscillation activity(275). The interactions between sleep spindles and slow oscillation are considered as the basis for memory consolidation, as discussed in 1.5.7.4 *Learning and long-term memory formation*.

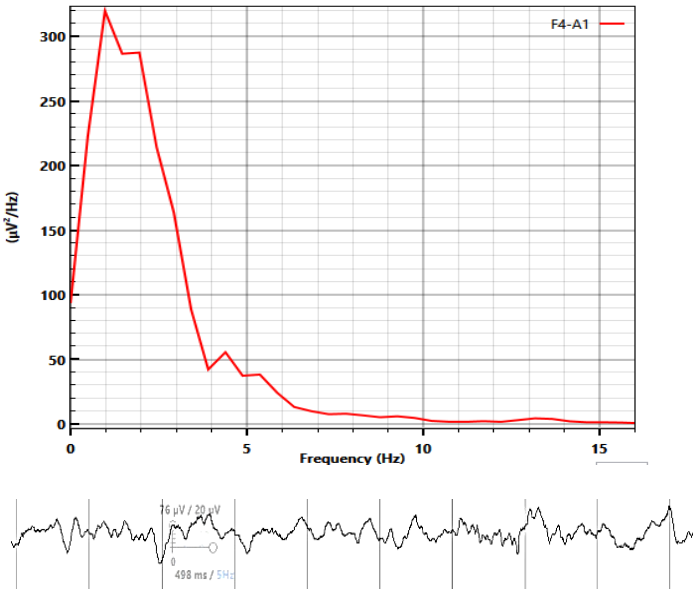


Figure 1.25 - Delta rhythms in a 4-month infant sleep. EEG activity during N3 over right frontal to left auricular channel. Top image: graph of the spectral power distribution of the EEG during N3 segment. The peak spectral power is observed for frequencies below 4Hz. Low image: corresponding EEG segment.

Slow oscillation is classically defined within the frequency range of ≤1Hz, which occur during NREM sleep and, in particular, with more intensity during deep sleep [(276) and reviewed in (277)]. Each slow wave that constitutes slow oscillations can occur either in trains or singularly (245),and alternates from up-states (also known as active states) and down-states (or silent states, caused by general cessation of neuronal firing), provoking strong depolarisation and hyperpolarisation within the brain. These slow oscillations have the potential to reach all cortical layers and to allow synchronisation of a wide range of brain rhythms, including sleep spindles and cortical delta waves (276, 278). The historic assumptions of their autonomous cortical generation have been recently challenged and it is possible that both thalamus and cortex contribute to this oscillation(279). At the cellular level sleep spindles and thalamic delta waves are under mutually exclusive mechanisms with thalamic delta waves requiring a higher degree of hyperpolarisation than sleep spindles.

Not all sleep spindles are associated with scalp EEG slow waves (280). In fact, adult studies have shown an inverse relationship between these two features once

the density and frequency of sleep spindles significantly decrease during deep sleep, which is the sleep stage when the levels of slow waves are at their highest (246), although this may not be translated into infant recordings (226). Positive and negative half-waves influence the drive for sleep spindle generation (280). When coinciding, these two rhythms (sleep spindles and slow waves that constitute the slow oscillation) can regulate themselves mutually (245): fast spindles (>12Hz) tend to occur in an earlier phase of a slow wave when compared to slow spindles(<12Hz) (a difference of approximately 500ms). Consequently, the onset of a fast spindle tends to be at the depolarising phase of the slow wave, and slow spindles tend to start at the peak of depolarisation of the slow wave potential (245, 246). When forming a complex with a slow wave, sleep spindle power will vary according to the position of that slow wave in a slow oscillation; this variation also depends on whether spindles are slow or fast. There is an increased probability of having a preceding slow wave in a train associated with a relatively high-power fast sleep spindle. In contrast, a high-power slow sleep spindle tends to override a slow wave embedded in a slow oscillation (245). With prior learning experience, up-state positivity of the slow wave embedded in the train and fast spindle amplitudes were enhanced. Slow spindles showed less consistent results with prior learning (245). Conversely, the exact timing of coupling of fast spindles on the slow oscillation is associated with memory performance, whose properties deteriorate with ageing (281). Associations between spindles and slow wave activity in infants (46-63 weeks conceptional age) were confirmed by a MEG study over the right central areas. Preceding sleep spindle periods, slow wave activity increases, decreasing after the sleep spindle period ends. These central spindles are recurrent at approximately 0.1Hz and tend to occur just after the onset of the depolarising phase similar to the fast spindle profile observed in adults (282).

1.5.7.4. Sleep spindle function

Maintenance of sleep

Most sensory pathways relay via the thalamus; additionally, the thalamus also has a role in the higher-order processing of olfactory stimuli, therefore developing a regulatory function on stimuli processing(283). It is believed that sleep spindle generation mechanisms are involved in maintaining sleep and have a gatekeeping function controlling stimuli inputs. To test this, rats were genetically modified to increase power in the sleep spindle range during NREM, associated with prolonged burst discharge mode on the reticular nucleus. This contributed to more extended periods of sustained NREM sleep, and the chance of waking up upon auditory stimuli during NREM sleep decreased (284). Studies using fMRI and EEG simultaneously have shown the human brain to be much more responsive to auditory stimuli during wakefulness and NREM sleep without sleep spindles than during a sleep spindle event (285). The thalamic sensory information relay cells enter into burst mode upon small excitatory inputs on a relatively hyperpolarised environment characteristic of NREM sleep. Burst mode is known for lower firing rates which may lead to less sensitivity and lower levels of retransmission of endogenous spontaneous activity [(286), reviewed in (287)]. Subsequently, a study that compared reactivity to different frequencies of auditory stimulus between wakefulness, REM and slow wave sleep found that, in contrast to REM and wakefulness, slow wave sleep is more selective in response to different pitches (286). From these studies, I can conclude that the previously discussed properties present in burst mode lead to consequent selection and alteration of sensory input transmission.

Neuroplasticity

It has been proposed that sleep spindles may play an important role in neuroplasticity. In this section, I will briefly discuss neuroplasticity and explain how sleep spindles are associated with it.

Plastic alterations that a neuron might undergo may be grouped into four types: short-term potentiation (STP), long-term potentiation (LTP), short-term

depression (STD) and long-term depression (LTD). The first two mechanisms result in enhanced postsynaptic response due to increased sensitivity after synapse conditioning, although a presynaptic component response such as increased neurotransmitter release could also contribute(288, 289). In contrast, depression processes reduce synaptic efficacy after neuronal stimulation. Potentiation changes are thought to be associated with the entry of calcium into the presynaptic cell, which promotes more neurotransmitter vesicles to be released, and activating a cascade of events that will lead to postsynaptic functional changes [reviewed in (288-290)].

Short-term potentiation may last for a few minutes depending on the subjacent mechanisms; these can be facilitation, augmentation or post-tetanic potentiation (PTP). The complete reversal of STP changes might take up to 40 minutes [reviewed in (291)]. Although STP may appear like a distinct entity from LTP, according to Bliss *et al.*, the STP is simply a type of LTP(291). Short-term plasticity is thought to be involved in mechanisms of filtering and control of information transmitted, detecting recurrent stimuli and their frequency in case of potentiation [reviewed in (289)].

LTP is traditionally associated with plastic changes that endure more than half an hour and may occur as early as the first week of a newborn's life (292). Processes to achieve LTP vary within different synapses, N-methyl-D-aspartate receptor (NMDAR) dependent LTP has been the most extensively studied and is the most prevalent LTP mechanism in the brain [reviewed in (288, 291)]. Postsynaptic NMDAR is generally blocked by magnesium and thus, does not respond to glutamate biding released from the presynaptic neuron. To remove magnesium blockage, postsynaptic membrane must depolarise. One way to depolarise the membrane is through temporal summation of AMPA-mediated EPSP, but other factors such as neuromodulators may also contribute. Once magnesium blockage is removed and glutamate is bound to NMDAR, this receptor allows an influx of calcium into the postsynaptic neuron which activates calcium/calmodulin-dependent protein kinase II (CaMKII), but other factors such as protein kinases C (PKC) and A (PKC) may also be involved in the LTP generation. This process follows Hebbian rule in which repeated

firing by the presynaptic neuron to the postsynaptic can lead to increased synaptic efficacy [reviewed in (288, 291)]. The processes mentioned above appear to have a role in certain aspects of learning, such as odour recognition, fear-conditioning learning and environment-exploration behaviour [(293) and reviewed in (291, 294)].

In contrast to LTP, LTD is characterised by enduring weakening of synaptic strength. It is mainly mediated by NMDARs and metabotropic glutamate receptors (mGluRs) but can also be induced by AMPARs, kainite receptors (KARs) as well as muscarinic receptors. LTDs can lead to the elimination of some AMPARs. If the process is continued, the synapse will have no persisting AMPAR, increasing the likelihood of being pruned. This process is fundamental for brain maturation. Experimental studies have shown that LTD processes intervene in learning and memory, adaptation to new environments, and extinction of familiar fear-inducing situations. Additionally, LTD facilitates the establishment of eye dominance upon monocular deprivation and plays a role in the development of some mental illnesses and drug-addition [reviewed in (295)].

There is growing evidence that sleep spindles are involved in brain plasticity. Steriade *et al.* were one of the first groups to hypothesise plastic properties of sleep spindles (278). *In vitro* experiments using slices of rat cortical pyramidal cells (stimulating layers II-III and recording over layer V) demonstrated that conditioning protocol using a simulating spindle pattern that was previously recorded intracellularly *in vivo*, may be inductive of STP and LTP. Sleep spindles conditioning followed Hebbian rules. Applying the conditioning protocol in just one of the cells (only reaching statistical significance in the presynaptic neuron) resulted in LTD. LTP is independent of the amplitude of post-conditioning EPSP and of GABAergic inhibition. Blocking certain receptors before conditioning showed that sleep spindle-induced STP is NMDA receptor-dependent, whereas LTP is L-type calcium channel-receptor dependent. Other patterns of stimulation were studied. While stimulation mimicking spindle burst patterns recorded intracellularly were able to produce both STP and LTP and tonic stimulation at 10Hz could only produce STP, a mirrored or shuffled version of the recorded spindle pattern did not produced an effective

potentiation pointing to the specificity of the patterns needed for generating potentiation (296). Other studies were performed on rats *in vivo.* Repeated bursts at intervals of 10Hz on the corpus callosum of rats can either lead to LTD or LTP on the layer V of the sensorimotor cortex. Potentiation and depression depend on the protocol applied, and those plastic changes persist for weeks (297, 298).

Learning and long-term memory formation

Given the neuroplastic properties of spindles, it is not surprising that there is an association between the ability to learn different tasks and sleep spindle properties, which has been extensively demonstrated. For example, in empirical studies on mammalian animal models, after visual pattern stimulation (adult mice) and ocular dominance tasks (young cats), depth electrodes show simultaneous firing between neurons of the primary visual cortex and spindle oscillations on TC neurons during sleep. This synchrony can be compatible with both adaptive plastic changes associated with alterations of primary visual neurons' pattern orientation preference or with a more effective ocular dominance switch (299, 300). Empirical studies in humans also have shown the association of sleep spindles and learning and memory activities, including motor sequences (301, 302), visuospatial (303) and spatial orientation (304, 305), and verbal, including learning word-pairs (175, 245, 306-308), as I will discuss next. The sleep spindles' role in hippocampal-dependent memory consolidation is hypothesised to result from a transitory synchronisation provided by slow oscillation [but see Schreiner *et al.* (309) for discussion on potential role of the thalamus to synchronise these transients]. The hippocampus has long been posited to encode episodic memory and spatial aspects of procedural memory (162, 310). During NREM sleep, sharp-wave ripples (SPW-R), hippocampal complexes containing compact neuronal representations of memories, are reactivated and repeatedly synchronised with sleep spindle troughs, which in turn are also modulated by slow oscillation (Figure 1.26) (311). These complexes are transferred from the hippocampus, where information is temporarily stored, to neocortical locations. The repetitive nature of this process throughout sleep and the sleep spindle neuroplastic properties are considered crucial factors for long-term memory consolidation (312, 313).

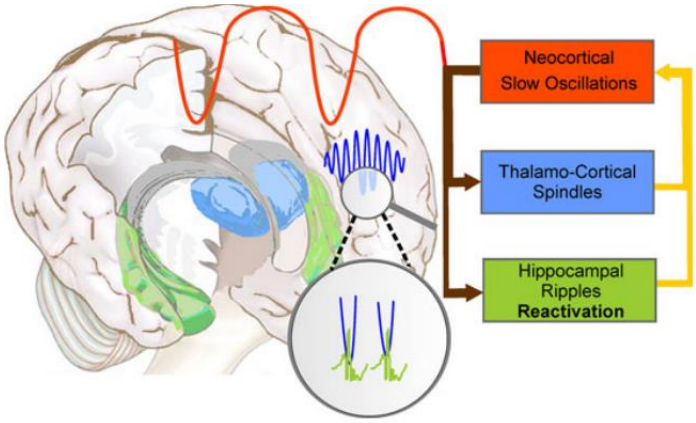


Figure 1.26 - Representation of the interactions between different transients occurring during NREM sleep and leading to neocortical storing of memory traces.
Image reused from.(135) with permission.

The sharp waves (SPWs) are high amplitude local field potentials that present a wide range of firing patterns corresponding to different encoded memory traces. Ripples are transients of very high frequency accompanying the SPW thought to act as a mechanism of local electrical homeostasis in response to the CA3 disinhibition. Together, these two features form the SPW-R complexes [reviewed in (314)]. Using hippocampal implanted electrodes in the rat model, SPW were demonstrated to be present from postnatal day 4, being the first organised hippocampal pattern (315), while the accompanying ripples emerge from postnatal day 14 (316). Of note, although challenging to pinpoint exact age equivalences between other mammals and humans, the rat brain maturation at birth is delayed compared to humans and the first rat neonatal days of brain development are thought to correspond to the human last trimester and rapidly maturing afterwards (317). Consequently, sharp wave ripples may be present in humans at term age, while ripples are predicted to emerge on 3.5-4 month old infants according to model proposed by Workman *et al.* (318). As argued by Mason and Spencer, immaturity of CA3 connectivity may not preclude active systems of memory consolidation in infancy. In fact, memory consolidation has been empirically proven to be associated with sleep spindles in infancy. As hypothesed by these authors, the need for frequent bouts of sleep may be in place to compensate for the fact that hippocampus connectivity is still developing(319).

It is theorised that memory representations are coded into those pre-existing distinct SPW-Rs patterns. Once coded, SPW-Rs allow information recall and a combination of them can used as a context for other executive centres to generate predictions and plans based on previous experiences [reviewed in (314, 320)]. These SPW-Rs are reactivated during certain times during wakefulness (mainly rest) and NREM sleep by disinhibition of a group of cells on the *cornu ammonis* 3 (CA3) [reviewed in (314)]. CA3 is a region of high interconnectivity on the hippocampus, receiving direct and indirect input from multiple sensory modalities converging on the entorhinal cortex. CA3 inter and intra-connectivity and neuroplastic properties have been linked to rapid encoding and retrieval of spatial and sensory temporary associative memory. The recurrent collaterals of the CA3 region are well suited to a putative function as an auto-associative network, both capable of associating different sensory aspects into a composite memory and are also capable of retrieving the whole memory with a partial input, a process known as pattern completion [reviewed in (321)]. CA3, in turn, stimulates neighbour areas, including *cornu ammonis* 1 (CA1). As a result of this process, the excited CA1 produces SPW-Rs[reviewed in (314)]. During NREM sleep, slow oscillation promotes a transient co-activation of SPW-R and sleep spindles, which can be retransmitted to neocortical structures for long-term storage. While spindles detected in the parahippocampal region are presented with no lag to parietal spindles, parahippocampal detected spindles and ripples precede frontal spindles (313). A recent review by Peyrache and Seibt proposes that the time lag observed between SPW-R and frontal spindles may favour a mechanism in which hippocampo-cortical network reactivation through SPW-Rs provides a synaptic tag, is followed by frontal sleep spindles whose plastic properties are then necessary for hippocampal-dependent memory stabilisation(139). Plasticity may be mediated via mechanisms that include the repeated influx of calcium on cortical layer V pyramidal dendrites which is decoupled from the soma during spindles(322, 323). Upon repetitive stimulation postsynaptic cell calcium influx in the dendritic spine activates protein kinases that ultimately increase conductance and incorporation of more AMPARs; voltage-gated calcium channels may also be involved in NMDAR-independent LTP processes. Similarly, calcium may be involved in the LTD processes through NMDAR dependent and

independent processes that culminate in the removal of postsynaptic AMPARs and, through the release of retrograde messengers, inhibit presynaptic voltage-gated calcium channels affecting neurotransmitter release [reviewed in (324)]. The repeated retransmissions allow neuroplastic changes (312, 313).

Sleep spindles have been shown to contribute to the refinement of somatosensory mapping and procedural learning. This factor and the SPW-Rs-related theory discussed above is incompatible with the traditional view that procedural memory consolidation is dependent on striatal pathways, and not hippocampal pathways [(325), and reviewed in (326)]. However, relatively recent findings showed the co-activation of both hippocampus and striatum during sleep spindles (224, 327), allowing striatal and hippocampal systems of memory consolidation interaction during sleep for procedural memory (328, 329).

In the model proposed by Albouy *et al.*, hippocampal and striatum systems weight changes inversely during initial learning; striatal influence may be dampened during a first stage of spatial encoding favouring flexible representations, in which hippocampus system is the most influential, and gradually increases for a automatised, concrete motor consolidation(328). Procedural memory may be categorised into simple (like a motor sequence task) and more complex learning that require a novel cognitive approach (like learning how to solve the Tower of Hanoi puzzle – Figure 1.27). Van Den Berg *et al.* proposes that N2 sleep and sleep spindles, in particular, may play a part in the consolidation of simple procedural tasks when the rule is learned explicitly. Whereas in complex tasks, stage N2 and sleep spindles perfect the performance of a skill already assimilated [reviewed in (329)]. Although the hippocampal contribution appears to be the most reliant on sleep, both striatal and hippocampal consolidation/refinement of memories may be enhanced during sleep (162). This way, these two structures seem to maintain a cooperative function

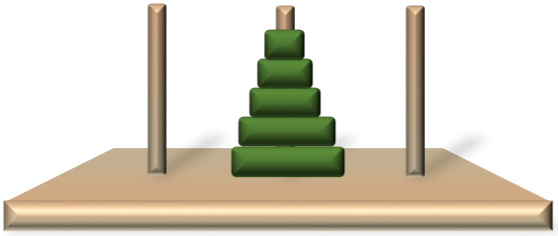


Figure 1.27 - Tower of Hanoi, a complex procedural task where a strategy is required to solve the puzzle correctly.

between themselves during sleep [(330) and reviewed in (328)]. Consequently, we cannot exclude a possible role of the SPW-Rs on procedural memory consolidation.

Next, I will discuss the empirical evidence of the association of sleep spindles and learning and memory tasks and how sleep spindles properties can be modulated by learning experiences.

Sleep spindles associations to learning and memory and: empirical evidence in humans

I have discussed the neuroplastic properties of sleep spindles and presented possible models to explain the role of sleep spindles in learning and memory consolidation. This section will present evidence from several human studies on how sleep spindles are associated with different learning tasks.

Visual and Spatial perception

Visual learning ability is important for infants to allow facial recognition and to navigate their surroundings as they become more mobile. Three months-old infant that napped showed preference to a novel cartoon face than the previously presented cartoon face. Sleep spindle density of that nap was correlated with shorter required time for cartoon habituation (assessed as when infant becomes uninterested in the image) (174). An adult study had similar results on facial recognition. In adults, facial recognition was found to be correlated to duration of NREM sleep after being presented the faces. However, correlation to sleep spindle number did not reveal associations of both statistical and clinical significance(306). Protocols, participants' age and sleep spindle features of interest differed between both studies, more studies will be necessary to determine a possible sleep spindle for facial memory consolidation.

Adult studies complement the knowledge on the role of spindles regarding other aspects related to visual memory. For example, testing visuospatial memory, the number of sleep spindles over the mid-parietal area between being presented with a specific image, the *Rey-Osterrieth Complex Figure*, and recalling moments, predicted recall performance (303). Also, city maze orientation exercises on

computer were associated with a rise in the density of sleep spindles during the N2 stage on the night of sleep after the task (304). Simpler mazes in which it is possible explore the map thoroughly and repetitively and to construct a cognitive map of the maze contributed to extended periods of stage N2 and higher spindle density when compared with complex mazes (304). Another study showed a weak to moderate correlation between fast sleep spindle spectral power over the bilateral frontopolar and left anterior temporal locations and navigation learning efficiency. They also demonstrated that it was possible to boost sleep spindle spectral power after virtual reality city scenario. This was possible by timing an auditory stimulous relevant to the task to occur on the transition from a cortical hyperpolarised state (downstate of the slow oscillation) to a cortical depolarised state (upstate of slow oscillations)(305).

Verbal

Changes in sleep spindle features also appear to be associated with language acquisition and verbal memory encoding. Six to 8-month infants were repeatedly presented with “pseudo-category names” and images of “pseudo-objects” dyads, a control group was presented with inconsistently grouped dyads that did not allow memory processes to occur. All infants were tested after a nap or, in the wake group, approximately 42 minutes after learning. In the memory retrieval phase, the intervention group was presented with old pairings and novel objects whose category name corresponded to old pseudo-objects of similar appearance; the control group again, was presented with inconsistent pairings. Infants who slept longer and had longer N2 had an adult-like event-related response (N400), associated with long-term lexical memory formation, in response to novel words in the correct category. Short sleepers with short N2 presented a late negativity, also observable in younger ages in response to novel words in the expected categories, indicating some level of perceptual associative memory. The awake group did not show significant differences between intervention and control responses. Moreover, N400 potential was associated with sleep spindle magnitude between those with a longer N2, and with the number of spindles both in short and longer N2 sleepers, indicating spindles’ involvement in semantic generalisation(175).

Some studies exploring the association of sleep spindles and verbal learning and memory were based on older paediatric populations. The following articles are examples.

Hoedlmoser *et al.* (308) showed that in children from 8 to 11-years old word-pair memory performances were worse after a night of sleep, independently of having increased/decreased values of spindle activity (i.e., mean amplitude multiplied by mean duration of sleep spindles; boundary between slow and fast spindles established at 13Hz) after word-pairs learning compared with baseline testing. However, those with increased spindle activity in the experimental night performed higher on the word-pair recall absolute scores after sleep. The authors also noted a non-significant positive association between spindle activity variation and the performance variation between word-pair recall exercises before and after learning(308).

Of note, no spindle peak above 13Hz, “fast spindles”, were present. The absence of a clear peak in this study may arise from a methodological choice of the frequency boundary for slow and fast spindles distinction. Hoedlmoser *et al.* (308), in the present article, considered all spectral peaks on the sleep spindle range <13Hz as being slow sleep spindles. This was the criteria previously used by some of the same co-authors in (331) to study slow and fast sleep spindles on learning memory and IQ in adults. As shown by Ujma *et al.* in (239), 24/28 children between 4 and 8 years old showed two unambiguous spectral power peaks below 13Hz, likely representing slow and fast sleep spindles. Although there was no denominated “fast spindle” in (308), these spindles might *in fact* be present as spindle spectral peaks, in this paediatric population, appear to be situated below 13Hz.

The same dataset was used to study slow (<13Hz) and fast (>13Hz) sleep spindles associations to word-pairing memory performance. In general terms, the children whose memory retention deteriorated after a night of sleep also registered an increased density of the frontal and central slow spindles from a baseline night sleep to the sleep after learning. At the same time, these individuals showed no significant changes in fast spindle density in frontal and central locations. No alterations of note on spindle density were present in children who best maintained

the memory for the word-pairs after a night of sleep. The study was repeated when participants reach 14 to 18 years old. Those who forgot more word-pairs after a night of sleep again had increased central slow spindle activity, whereas memory maintainers increased central fast spindles. Regarding frontal spindles, there were no significant changes in this age group. The same study suggested an association between the increment of the fast spindle density from pre-puberty to puberty and increased memory retention. Thus, fast spindles density enhancement may be necessary for the verbal memory consolidation (307).

A similar role of sleep spindles is observed in adults. For example, number of sleep spindles over the midline and left fronto-central areas (i.e. Fz, Cz, F3, C3) occurring in stage N2 after a face-name association learning, predicted performance of name retrival on the next day (306). As discussed in section 1.5.7.3, there is an association between some sleep spindles and slow oscillations. Mölle *et al*., focused on the behaviour of this relationship after paired-words learning. Although the number of slow oscillations *per se* did not change with prior word-pairs learning, the number of slow waves occurring in runs was enhanced. Prior learning was also correlated with increments of fast spindle activity, particularly during the negative peak of the first slow oscillation of a train of slow oscillations, which occurred alongside increased up-state positivity of slow oscillations located at the middle of the run (245).

Motor

As discussed in section 1.5.7.4 *Learning and long-term memory formation*, sleep spindles have the potential to facilitate aspects of procedural memory. Literature on sleep spindles being associated with motor memory in infants is scarce. Indirect evidence shows that after demonstration of a 3-step action with a puppet and successful reproduction of the steps, 6- and 12-months infants retained and reproduced more of these actions after a 4- and 24-hours interval if they were allowed to nap shortly after demonstration. An additional trial on 12 months infants showed that they could also adapt practices by reproducing the steps on a novel puppet. However, sleep spindles were not studied in these experiments. Also, more

complex procedural memory can be improved by sleep. Novice walkers avoid to crawl; Berger and Scher used a tunnel obstacle that could only be passed through by crawling to test complex procedural memory. Thirteen months-old novice walkers learned implicitly how to overcome the obstacle.. Following an interval, these infants achieved the aim more rapidly and with fewer attempts if they napped in that interval. Again, sleep spindle associations were not investigated in this study(332).

More direct evidence of the role of sleep spindles in procedural memory is provided by adult studies. One technique to test it is using motor sequential learning (MSL), in which the ability to memorise a succession of movements like finger-tapping in a particular order is tested.

MSL learning compared to a non-specific motor control task, in young adults increased the number and duration of the sleep spindles in subsequent sleep. An increase in spectral power at 13Hz during N2 and N3 was also noted on the night of sleep after MSL. However, no statistical associations were noted between spindles and the performance improvements observed after a night of sleep(301). Fogel *et al.* found similar results when evaluating the whole bandwidth of spindles but, positive associations were obtained when restricting analysis to fast spindles on parameters of density, amplitude, and duration. These associations varied with age groups (younger and older adults). Although the authors do not have a definite explanation for this age dependency, the differences might be correlated to changes in the volume of grey matter that accompanies the ageing process. This study showed an association between the volume of grey matter structures from the cerebellum (particularly in fast sleep spindles), the hippocampus, the cingulate and the parietal grey matter and the duration of spindles during a nap. There was also an association between the volume of the supplementary motor area and slow spindle amplitude and duration (302).

Intelligence and cognitive abilities

Sleep spindles have been associated with intelligence and cognitive abilities in paediatric and adult studies. Jaramillo *et al.* studied the density of automatically detected sleep spindles above 13.5Hz during early night sleep of 32 6-month old infants and its association to parent reported Ages and Stages Questionnaire at 3, 6, 12 and 24 months. The aspects of Ages and Stages Questionnaire analysed were personal-social, motor and general development. Spindle density and mean spindle frequency were positively associated with gross motor skills at 24 months but not earlier, after correcting for age and sex. Spindle density was also associated with general scores at 12 months(333). In a different overnight study with 36 8-months old, Satomaa *et al.* indirectly analysed sleep spindles by analysing sigma frequency's power. The authors found topographically-specific associations of overnight power in sigma frequencies and cognitive, expressive and receptive language and fine motor scores, assessed with Bayley-III after approximately 3 weeks on average(334).

As discussed in section 1.5.7.3, sleep spindles can be associated with slow waves. Jaramillo *et al.* found evidence of slow wave and sleep spindle coupling, particularly over occipital regions, an area of sleep spindle scarcity; as the occipital area a region of early maturation, the authors suggest this finding may reflect regional brain development. No associations of slow wave and sleep spindle coupling to scores of Ages and Stages Questionnaires were found(333). It is also important to iterate that in adults, this slow wave and fast sleep spindles coupling over the left frontopolar areas is associated with abstract reasoning(240).

The cross-sectional study of Page *et al.* focused on the cognitive abilities of 27 infants (12 males) ranging from 12 months to 30 months old. The skills tested were communication, socialisation and motor functions through Vineland Adaptive Behaviour Scales and fine motor and visual functions through Mullen Early Scales of Learning; testing was performed around the same time as the sleep EEG recording. Regarding sleep spindle analysis, the authors used an automatic detection method during stages N2 and N3 of a nap. No associations were found between the studied age-corrected cognitive abilities scores and sleep spindles in this age group. Interestingly, there was a positive correlation between the maternal academic level

and sleep spindles' power (335). The small inclusion numbers of participants associated with a large age range during a highly dynamic brain maturational stage may have precluded results. Alternatively, it is possible, as observed in study by *Jaramillo et al.* or even *Satomaa et al.* that during early infancy sleep spindles predict future and not present developmental features.

Ujma *et al.* (239) focused on the distinction between trait and state sleep spindle parameters, meaning the stable mostly genetically determined and the variable portion of these features, and their relation to cognitive ability. The study was done in 28 children (15 girls) from 4 to 8 years of age who took Raven's Coloured Progressive Matrices. Through proceeding or not to the correction for the effect of age on the correlation between sleep spindles and test scores, the authors claimed that it is possible to evaluate both the mental age (state) and the individual trait. The sleep spindle parameters studied were density, duration and amplitude (given by the envelope of the EEG signal) for slow and fast sleep spindles (according to IAM). Analysing boys and girls separately, the authors confirmed the presence of sex differences in sleep parameters as markers of cognitive ability in this age group similarly to adults (336, 337). The correlations between sleep spindles and intelligence depended on the frequency range studied, i.e. slow or fast sleep spindles. The results also varied according to the night of recording, with a modest correlation between 1st night of polysomnographic recordings against the 2nd or 3rd. Considering all studied nights and after age correction, only girls showed a positive association of the scores obtained with the slow sleep spindle amplitudes over right mid-temporal areas. In boys, the associations observed after age correction during the first night (slow spindles density nearly across all regions) were not observed on the second studied night, consequently, these observations were hypothesised to be attributable to state rather than a trait (239).

Various studies have examined sleep spindle parameters as markers of intelligence using intelligence scales in school aged children; Gruber *et al.* (338) included 29 children of both sexes between 7-11 years old; Chatburn *et al.* (339) included 27 girls and boys with a mean age of 8 years-old; Hoedlmoser *et al.* (308)

included 59 children of both sexes from 8 to 11 years; and Geiger *et al.* (340) included 14 children ageing between 9 and 12 years-old of both sexes for two nights. In all but Chatburn *et al.* study children's cognitive abilities were measured with the Wechsler Intelligence Scale for Children-IV. Geiger *et al.* (340) also included a full-scale Intelligence Quotient (IQ) score and Assessment of Attention (KITAP). In the second study, Chatburn *et al.* (339), children were assessed with the Stanford Binet Intelligence scale and the neuropsychological development assessment (NEPSY) tool. It was noted that slower sleep spindles during the night were associated with high scores in particular aspects of intelligence: working memory (negative association to overall sleep spindle frequency) (338, 339), perceptual reasoning, verbal comprehension (both with negative associations to overall spindle frequency) (338), planning (negative association to overall spindle frequency) and sensorimotor integration (negative association to overall spindle frequency but also to number and density of fast spindles) (339); No association was noted for processing speed in Gruber *et al.* study (338). No association was noted between density, duration and amplitude of all sleep spindles and any of the subscales of the Welsher Intelligence Scale for Children – IV in Gruber *et al.* study (338), but in Chatburn *et al.* (339), despite number and density of fast spindle negative association to sensorimotor integration, narrative memory was positively associated with fast spindle density. Hoedlmoser *et al.* found a positive association when evaluating spindle activity as the mean amplitude multiplied by mean duration of sleep spindles and general cognitive abilities(308). Evaluating spectral power during stages N2 and N3, Geiger *et al.* found a positive association between general IQ and spectral power on values ranging from 10 to 13.25Hz and values greater than 15.5Hz. Fluid IQ was positively associated with power values greater than 13Hz and, working memory was positively correlated with higher power values on faster frequencies above sleep spindles typical range (>16Hz). This group also found that the mean peak frequency of spindles during N2 was inversely associated with IQ (340). These last two studies curiously contrast with the results from Chatburn *et al.*(339) in which many cognitive abilities were associated with sleep spindles but where no correlation was noted between overall IQ test and this sleep transient. The authors of the later article point out the distinct

methodological approaches used in the studies to justify the difference in results(339).

Hahn *et al.* (307) studied the cognitive abilities of children from 8 to 11 years old and again when they reached 14 to 18 years of age. The adolescents who scored better in the adult version of the Wechsler Intelligence Score were the ones who had higher increments of frontal slow sleep spindle density from childhood to adolescence(307). A study by Bódizs *et al.* (341) on late adolescents also showed a dichotomy between sexes and sleep spindle parameters as an indicator for fluid intelligence, this is, ability to reason given a novel context, and was assessed with the Raven Progressive Matrices Test. Female adolescents with higher IQ scores showed a higher amplitude of fast sleep spindles (>13Hz) over the fronto-central regions, as seen in adult women (336), and also higher fast spindle density (maximally correlated over frontal regions). Males, by contrast, showed a positive correlation between fluid IQ scores and fast sleep spindle frequency. For Bódizs *et al.*, the sex-differences may be due to different timings and courses of brain maturation for the two sexes and use of different cognitive strategies(341).

Similarly, adult studies corroborate with most observations of association between sleep spindle properties and cognitive abilities. Although sleep spindles are associated with verbal memory as observed in the previous section, verbal IQ (which includes aspects such as comprehension, vocabulary and detection of meaning similarities) is not associated with spindle number nor sigma power(342), and is in keeping with the observations in school-age children(338). However, within higher broad cognitive ability, performance IQ level was associated with and 14-16Hz sigma power and the number of central spindles. Within Performance IQ, the subtest of Picture Completion had the highest correlation with the number of spindles, in Fogel *et al.*(342). The authors speculate that a highly active or highly efficient thalamocortical system is required for higher performance IQ which in turn translates into higher number of spindles and sigma power(342). Adults with high scores abstract reasoning or higher memory performance tended to have higher sleep spindle activity (as a result of multiplication of sleep spindles mean duration and

amplitude) and spectral power over central areas but no statistically significant associations were found for number of spindles in Schabus *et al.*(331). There was also a post-training effect in which sleep spindle activity, but not spindle power nor number, increased on the night after declarative or procedural memory tasks. Analysing all participants, the authors found a positive correlation between scores of cognitive abilities and fast spindles activity over left central areas but not slow(331). The density of fast spindles over the right frontal and frontopolar areas were also positively associated with abstract reasoning. Similarly to previously mentioned study, there were no significant associations between slow spindles and abstract reasoning, in this case for the density parameter (240). As in children, adults also show sex differences on the association between sleep spindles and non-verbal intelligence: in women, higher amplitudes of fast spindles over anterior and central regions and the duration of slow spindles were directly associated with non-verbal intelligence; In men, intelligence was negatively correlated to fast spindle density, and no significant correlations to slow sleep spindles were observed(336). A possible exception for slow spindles correlation to intelligence in the male group is a positive association between the duration of the slow sleep spindles across bilateral frontal, central and parietal regions and intelligence scores exclusive to daytime nap analysis(337).

As it can be appreciated through this section, although data indicate an association between sleep spindles and cognitive abilities, results may vary within studies. It is speculated that some differences found between children and adults may be attributable to intrinsic brain developmental processes (194). Different studies correlate intelligence to different sleep spindle parameters and topographies. Those asymmetries may be explained by the fact that the tests and the subscales used varied, and consequently, they assessed heterogeneous aspects of intelligence. Another possible factor may be differences in the parameters used to measure sleep spindles and the montages applied; additionally, some studies rely on relatively small samples. Finally, as we have seen, there are sex differences in the way sleep spindles reflect intelligence levels. The sex distribution is inconsistent from

study to study, which may also determine the incongruencies stated. Despite this, throughout the studies, there is a nearly unanimous association between fast/faster spindles and the intelligence trait in adults, while studies in paediatric cohorts vary. As I will discuss in section 1.5.7.5 *[Sleep spindle parameters throughout] Ageing*, the two spectral peaks corresponding to fast and slow sleep spindles emerge by the first year(343) and boundary frequencies are lower in children [see (239) and (336)]. This factor may also contribute to confound slow and fast sleep spindles dichotomic association to cognitive ability or brain development in paediatric studies. In a meta-analysis that included paediatric and adult studies, Ujma *et al.* found modest associations between sleep spindles and intelligence. However, the authors highlighted the different methodologies employed, generally small samples (median sample size of the studies correlating spindles and intelligence is of 24 participants), some publication bias (344), lack of spindle detection method validity that may lead to divergent results within the field and advised caution when interpreting results from individual intelligence subscales as these share some common neurological substrates (345).

Spindle activity and Brain development

In the section 1.5.7.4 *Verbal [learning and memory]*, I discussed how sleep spindles might be involved in language acquisition. Other types of spindle-like oscillations have been related to brain maturation and sensorimotor development, and these may give a clue to other possible functions of early-stage sleep spindles as well. I will first discuss what is known about one such spindle-like functional role on brain maturation, and particularly the sensorimotor mapping, and then present more recent evidence on sleep spindles sensorimotor refinement.

Delta brush is a transient associated with prematurity and can be present from 24 weeks post-menstrual age (PMA), are most prevalent around 32 weeks GA and wane towards term. It consists of a train of fast activity at 12-20Hz overlying a delta wave [reviewed in (346)], which has been implicated in the sensorimotor mapping.

There is evidence that delta brushes may be involved in establishing neural connections [reviewed in (347)]. Likewise, animal models have been studied for the

function of a similar spindle-like transient: the neonatal spindle burst, present in some species such as rodents. Neonatal spindle bursts are considered the animal analogue of delta brushes (348). These have been associated with the construction of a spatial representation of the somatosensory maps in the early stages of life through mechanisms of feedback: in pup rats, spontaneous movements are immediately followed by a spindle burst in the contralateral primary somatosensory area; the same occurs after external stimuli. Despite the majority of the delta bursts being elicited by sensory stimulation, these transients also occur spontaneously. This is evidenced by the fact that after a spinal cord section in animal models, some delta bursts persisted, though lower in number (140). Tactile stimulation causes not only spindle bursts on the primary somatosensory area but also the primary motor area. Spindle bursts in the primary visual area also seem to play a role in the maturation of this region and removal of the retina will severely decrease the number of spindle bursts. Spindle bursts also play a role in synchronisation at various scales, from within small restricted areas of the brain up to inter-hemispheric coordination [reviewed in (349)]. Furthermore, there is some evidence that early synaptic activity, including spontaneous activity, may influence targeting of the axonal projections and/or functionality of the neurons (350-352); spindle bursts are also thought to prevent useful synapses being pruned by promoting the release of BDNF [reviewed in (349)].Human delta brushes seem to play a similar role in the maturation of somatosensory (353), visual (354) and auditory cortex (355). Although sleep spindles and delta brushes are not the same entities, they share striking similarities. Clawson *et al.* discussed the possibility of delta brushes being *functional* predecessors of sleep spindles, pointing out the similarities: Despite delta brushes initially occurring in any stage of sleep or awake, as the preterm ages, they become present only during sleep. Delta brushes consist of two nested oscillatory activities likely resulting from interactions between the thalamus, cortical plate and the subplate, the latter being a transitory structure that contributes to the communication between thalamic and cortical areas and whose dissolution begins before term [reviewed in (356)]; these EEG transients disappear approximately at 38-42 weeks GA [reviewed in (42)]. Spindles in turn, originate in the thalamus and are synchronised by the cortex and start to appear after the first month after term. Both sleep spindles and delta brushes

contain relatively fast oscillations (209) with shared targets to somatosensory cortices following somatosensory stimulation. Finally, both are implied in neural plasticity [reviewed in (357)].

Sleep spindles have been shown to be associated with motor learning in adults as discussed in the section 1.5.7.4 *Motor* [learning and memory]. It is thus possible that sleep spindles play a role in brain maturation, namely in the refinement of the sensorimotor mapping beyond the time of delta brushes extinction. Corroborating with this idea, a recent study on human infants has shown a temporal correlation between quiet sleep/NREM twitches on the contralateral arm or lower limbs and sleep spindles in the corresponding sensorimotor area. In early infancy sleep spindles precede the onset of the twitches but at later stages of infancy, these two events co-occur. Altogether, this study suggests that spindles may contribute to the refinement of the brain functional mapping(141).

1.5.7.5. Variety within sleep spindles and associated factors

Sleep spindles exhibit a considerable degree of variation in morphology and density. These variations are due to several phenomena, some physiological, others pathological, and the effects may be transient or more constant. Here I will discuss some of the factors that lead to those changes.

Genetic factors

Despite the fact that there is a degree of fluctuation of sleep spindle power between individuals due to multiple factors, which will be discussed later, this measure tends to be fairly constant at an individual level. Spectral power of frequencies in the sleep spindle range were studied in different conditions, such as partial sleep deprivation and recovery. Participants tended to show a degree of stability in the range of sleep spindle spectral power at the individual level. The information points to the presence of distinctive features of EEG power in the sleep spindle range for each person, which was proposed as an *electroencephalographic fingerprint* (238). This also suggests that some sleep spindle characteristics are partly genetically determined, which is also observed in twin studies. Spectral power of the

spindle frequency band is associated within mono- and dizygotic twins(243). Monozygotic twins show a stronger association; the values obtained from them are similar to intra-individual association values from their previous study (238, 243). The heritability of the spectral power in monozygotic twins was estimated at 95.6% (243). Spindle density, particularly density of fast spindles, amplitude, duration and frequency are also heritable, the two first characteristics being the most impacted by genetics (358). The differences in spindle frequency spectral power, density, amplitude and duration observed between afro-descendants and caucasians may contribute to corroborating the influence of genetic factors. However, socio-demographic factors may partially explain the latter results(358).

Circadian rhythm and homeostatic pressure

Despite the genetic component, sleep spindle parameters may also be influenced through the circadian rhythm and homeostatic pressure.

In adults, within each NREM sleep, spindle frequencies(359) density, and sigma spectral power (360) drop within the middle phase at the peak of slow wave activity, revealing an inverse relationship between spindles and slow waves. Density showed a U-shape profile, particularly within fast spindles (244). In contrast, in terms of frequency, fast spindles tend to be similar between N2 and N3 averages of the whole night spectral, while slow sleep spindles tend to be slower during N3 (a slow wave rich sleep state) than in N2. This may indicate functional differences between these two types of spindles(245). These U-shape patterns were not observed in infants in this study, at least for sigma spectral power(361); but comparing N2 with N3, Louis *et al.* (226) faster spindle frequencies predominated in N2 and, slower spindles in N3 at 1.5 months and described trends of longer duration during N2 compared to N3 at 1.5 and 3 months, similar to that observed in adults (360), and lower asynchrony in N2. This observations in infants may indicate that the inverse relationship observed between sleep spindles and presence of slow waves described in adults may be partially present in early infancy.

In studies analysing spindles across the night, reduction of sleep pressure and circadian rhythm exert influence of spindles. Observations in infants showed at a

trend level that the percentage of lower frequency spindles (10-12Hz) at the end of the night depended on the age, increasing in infants at 1.5 months and decreasing at 6 months, similar to adults; while higher frequency spindles (12-14 Hz) start decreasing from the middle of the night in all age groups up to 6 months(226). Throughout the lifetime, topographical frequency evolution is partly sex and age-dependent(362, 363). Slow and fast spindle frequencies in children of 4-10 years old and older participants tendentially decrease during the initial stage of the night, returning to initial frequency values afterwards. Exceptions are observed for slow spindles in girls, whose frequencies tend to be more constant across the night, and in older adults. The degree of the mid-night frequency decrement is attenuated with age. Children's spindle frequency nadir occurs between 1am and 2am, independent of bedtime. This may indicate a predominant role of the circadian rhythm as opposed to homeostatic pressure in the modulation of spindle frequency across the night (362).

Louis *et al*. found no spindle amplitude differences in infants across the night in the infant population (226). Longitudinally analysing sigma range spectral power during NREM of 11 infants at 2 weeks, 2, 6 and 9 months, Jenni *et al.* found statistically lower values during the second than the third NREM period at 6 months, and a trend at 9 months(361). These observations contrast with adult studies whose sigma power, and amplitude increased across each NREM period in Djik *et al.*(360), while no spindle amplitude variation across NREM episodes in adults is observed in Martin *et al.* (363). The differences observed between infants and between adults may be explained by maturational aspects, different methodologies and a very small pool used in some of the studies.

Overnight spindle duration remained stable except for an initial drop during the first hours of the night for the 1.5-month infants at trend level (226). In adults, the durations of slow and fast spindles behave differently. Slow sleep spindles shorten significantly from the first to the third cycle, and on the fourth cycle duration rises abruptly both in the frontal and parietal regions. Fast spindles duration increases over the parietal areas (241).

No significant density trajectories were noted in infants across NREM periods (226). In adults, sleep spindle density evolution over the night is also dependent on age and topography, tending to increase in posterior areas, and decreasing at the beginning of the night and increasing at the end over frontal regions in young adults; While in the elderly population, density decreases(363). Age differences are also patent when analysing slow and fast spindles separately. The graphics below (Figure 1.28) summarises the findings. In addition, densities are affected by whether N2 occurred between the transition from wakefulness, N1 or REM to N3 (more dense sleep spindles) or the inverse route (sparser sleep spindles) (358).

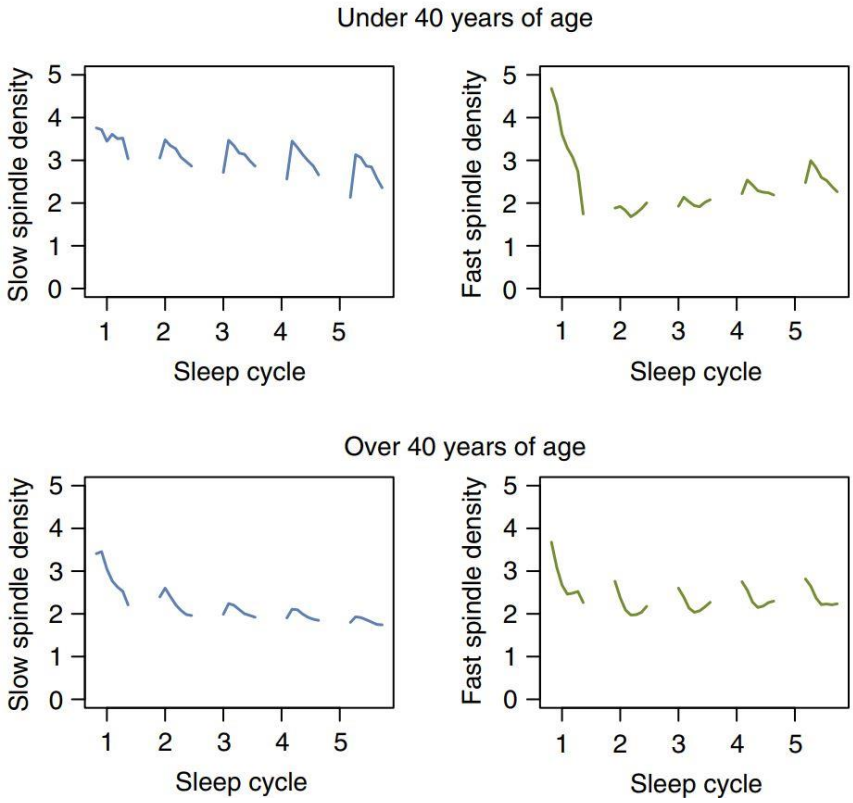


Figure 1.28 - Evolution of sleep spindle densities during N2 sleep within and across the different sleep cycles. Image reused from (358) under CC-BY 4.0.

Despite limited information on paediatric studies, adult studies highlight the differences in spindle parameters recorded during day and night sleep studies. Slow and fast sleep spindles present lower density and higher frequency during daytime naps compared to nighttime, fast spindles are longer and of lower amplitude(337, 362). These and spindle changes across the night as observed above may both be explained by reduction of sleep pressure (homeostatic drive) and the circadian

rhythm. To understand the role of both, it is important to test sleep in manipulated settings.

The influence of the circadian rhythm on spindle parameters are mediated at least in part by melatonin, produced by the pineal gland in response to lower light levels. In normal conditions, plasma levels of melatonin peak between 3 to 4 am (364). To ascertain the effect of chronobiology, adults participated in an experimental sleep setting with cycles of scheduled 75 minute naps and 150 minutes wake periods in controlled light conditions. Naps during periods of the day with concomitant high levels of melatonin showed higher spectral power in the lower sleep spindle frequency range and, lower spectral power in the higher sleep spindle frequency range. High melatonin is associated with lower frequency and higher amplitude over Fz and Pz, higher density and duration over Pz, and lower duration in Fz(365). Consequently, the timing of the scheduled nap influence sleep spindle parameters.

Sleep homeostatic drive as a possible influence for sleep spindle modulation may be tested by using protocols of sleep deprivation in adults. In recovery sleep after 40 hours of sleep deprivation, sleep spindle parameters of sigma spectral power, spindle density and amplitude decreased in comparison with baseline recordings. This is particularly seen in early sleep. No changes are observed in terms of frequency. (360). Fragmented sleep disruption affecting primarily deep sleep also reduces sleep spindle density. This effect is exclusive to the first sleep cycle of the overnight sleep, as the subsequent sleep cycles showed no statistical differences from the baseline(366). A separate analysis of fast and slow spindles showed that the power of the slower sleep spindles increased during sleep recovery in comparison with the baseline recordings. Inversely, the fast spindles spectral power decreased (243). The results seen here may be the effect of increased conditions for delta oscillations which, as we have seen in 1.5.7.3 and in this section, are roughly inverse to sleep spindles, particularly within fast spindles.

In conclusion, within NREM cycles, sleep spindle parameters demonstrate a roughly inverse relationship with the presence of slow waves and this may be partially in place in infancy. Patterns also evolve across the night of sleep. Some contrasting results between studies were observed; age, sex interactions and different methodologies may account for some variability between studies. Studies on variability of spindles across the night in infants are sparse and our present knowledge on this topic is largely due to the work of Louis *et al*. who followed up the nocturnal sleep of 12 infants up to 6 months. The low number of infants included may have critically affected statistical results. The study showed an age-dependent trajectory of the percentage of slower 10-12Hz frequencies across the night, increasing at 1.5 and decreasing at 6 months and, of faster 12-14Hz spindles decreasing from the middle of the night. While amplitude, duration and density remained mostly stable across the night. At 4.5 months, asymmetry increased, and asynchrony remained stable throughout the night(226), though these were only non-significant trends. Studies where sleep is experimentally manipulated indicate that across-sleep spindle parameters may be both determined by homeostatic sleep drive and also circadian rhythm, as it becomes established in infancy.

Ageing

Spectral power analysis of sleep spindle-related frequencies during NREM offers an indirect method to observe changes in sleep spindles as the frequencies tend to stand out from the rest of the EEG background. Coinciding with the emergence of the first sleep spindles, full-term born infants at three months of age exhibit high spectral power over the central areas in frequencies close to the sleep spindle range(211). Novelli *et al.* described a progressive increment of the alpha power of frontal:occipital ratio, particularly around 11.5Hz, of individuals up to 48 months of age (367). This frequency range includes sleep spindle frequencies seen in childhood(239, 343) and may correspond to slow sleep spindles, particularly after the first year(343). This anteroposterior gradient was also confirmed in the midline regions of slow spindles (<13Hz) in infants from 0-48mo. (343). Together, these studies point to an initial central maximum and a dynamic shift towards more anterior locations during the first years of life.

A study by Hughes was one of the first studies involving infants' sleep spindles; it was cross-sectional on a small sample of 16 infants born at term, studied at varying ages, from 10 to 52 weeks old. This study had some weaknesses, such as using different montages (both referential and bipolar) but no mention of the channels used, no indication if the sleep analysed was diurnal or nocturnal, and each child was the only representative of their own age group. However, this paper includes one of the youngest populations in which sleep spindles have been studied (368). In this study, they noted that the longest sleep spindles were seen in the 13-week-old child (a mean of 6.2s), which then shorten rapidly until the age of 23 weeks, followed by a more gradual decrease until the eldest child of the study, reaching a mean duration of 1.7s. In contrast, inter-spindle intervals achieved a roughly opposite behaviour to the sleep spindle duration. At its lowest point, at 13 weeks, the duration between spindles was 3.4s and reached approximately 7.0s at 52 weeks. The author also studied synchrony, synchronous spindles being defined here as the percentage of bilateral sleep spindles where the interval between both onsets is less than 400ms. They concluded that despite fluctuations across ageing, there was a general tendency for synchrony to increase. Values were 10% at 10 weeks and approximately 30% at 52 weeks. Asynchrony (unilateral spindles), in turn, was higher (65%) in the youngest child and about 30% at 52 weeks (368).

Louis *et al.* studied the development of several features of sleep spindles, following-up infants from the age of 1.5 months, every 1.5 months up to the age of 6 months (226). Probably due to the small sample size (12 infants), few differences between ages in the features analysed were reported as statistically significant. This study is one of the few longitudinal studies showing the trends of overnight sleep spindle parameters in a young paediatric cohort, minimising interindividual variability. Table 1.1 refers to the specific values obtained at the age of 4.5 months.

Table 1.1 - Sleep spindles parameters expected at the age of 4.5 months.

Parameters	Stage N2	Stage N3
Density	4.1 ± 0.6	4.0 ± 0.8
Duration	3.3 ± 0.3	2.3 ± 0.5
Frequencies		
10-12Hz	15%	18%
12-14Hz	55%	81%
14-15Hz	2.5%	0.2%
Amplitudes		
10-25µV	27%	24%
25-50µV	59%	64%
50-75µV	13%	11%
Asymmetry	13.7%	10.7%
Asynchrony	25.2%	30.9%

Calculations based on approximately 2000 sleep spindles from 10 individuals. Information taken from varying channels according to highest amplitude presented for each individual sleep spindle. Adapted from (226).

They concluded that there was a rapid increase in spindle density (Figure 1.29A) during stages N2 and N3 as well as an increase in duration (Figure 1.29B) from 1.5 to 3 months of age. In N2, mean (SD) density rose from 1.7 (0.5) spindles per minute to 3.3 (0.9), and duration from 2.8 (1.5)s to 3.3 (0.7)s. In deep sleep, density rose from 2.1 (0.9) to 2.8 (0.7) and duration from 2.1s (0.9) to 2.8 (0.7)s.

Spindle density tended to increase until the end of the study, whereas their duration tended to decrease after 3 months. The density rose to 3.9 (1.3) and 4.2 (0.9) in N2 and N3, respectively, up to 6 months of age; duration decreased to 2.0 (0.3) and 2.1 (0.4) in N2 and N3, respectively, at 6 months (226).

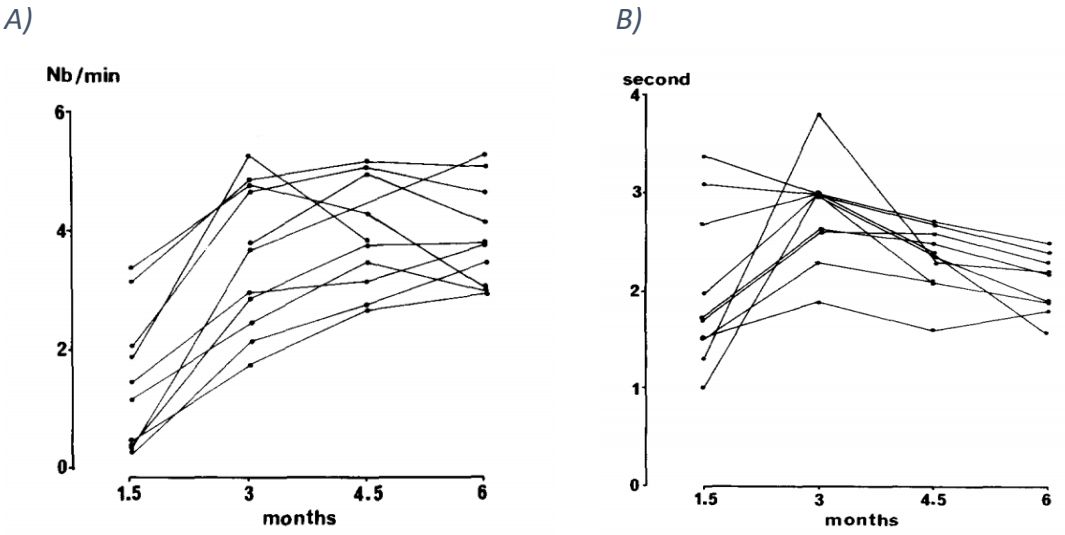


Figure 1.29 - Evolution of sleep spindles parameters during N2 and N3 for each individual across their age. A) density, units given in the number of sleep spindles per minute; B) duration, units given in seconds. Image reused from (226) with permission.

The frequency content of the spindles was also age-dependent. At the age of 1.5 months, frequencies grouped from 10-12Hz and 12-14Hz were present in similar amounts. However, as the infant matured, 10-12Hz tended to give way to faster 12-14Hz frequencies. 14-15Hz spindles first appeared predominantly at 4.5 months (see Figure 1.30A) (226).

Regarding amplitudes, spindles were grouped into amplitude bands; lower amplitudes (up to 25µV) tended to become less and less frequent in comparison with higher amplitudes which started appearing more often as the infant aged (see Figure 1.30B) (226).

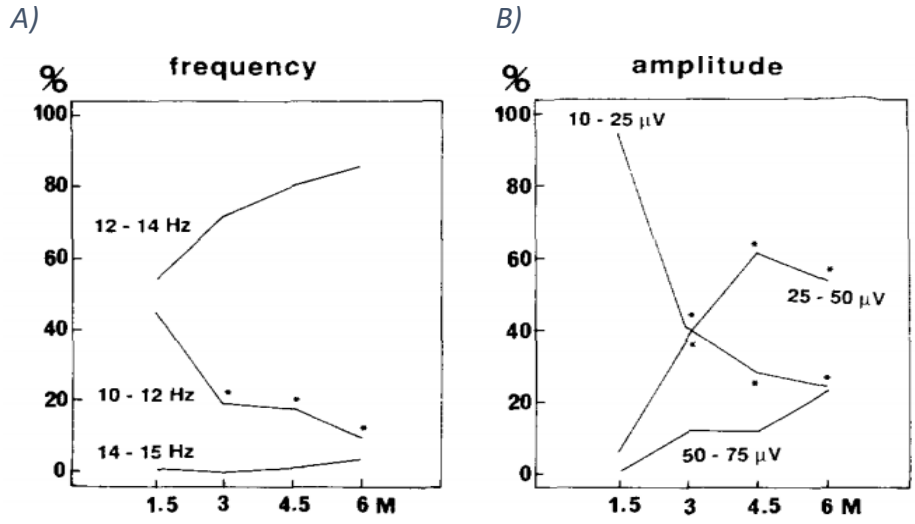


Figure 1.30 - Percentage of sleep spindles grouped for A) frequency and B) amplitude across age. Dots represent statistically significant differences from 1.5 months data point. Image reused from (226) with permission.

In this study, two interhemispherical sleep spindles were considered asynchronous whenever there was an interval between their mid-points greater than two seconds; the choice of this method was not justified in the article and appeared to be arbitrary. Asynchrony showed a sharp decrease from 1.5 to 3 months, then a steady increase to 6 months (Figure 1.31A). This result on asynchrony is at odds with the results of the study discussed earlier from Hughes (368). Different definitions of asynchrony and synchrony, methodologies (e.g. possible differences in the electrodes used; longitudinal vs cross-sectional studies) and both being based on small cohorts may explain the difference in results (226). Asymmetry was defined by Louis *et al.* as amplitude differences at least twice the contralateral sleep spindle. Asymmetry increased from 1.5 months, reaches its peak at 4.5 months and then reduces again (Figure 1.31B)(226).

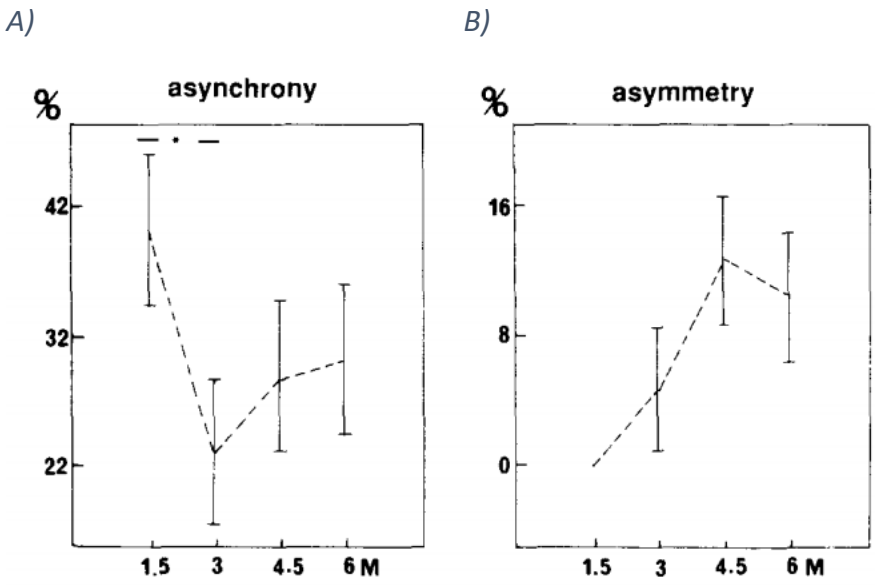


Figure 1.31 - Percentage of A) asynchrony and B) asymmetry across age.
The dot represents a statistically significant difference. Image reused from (226) with permission.

D'Atri *et al.* studied several sleep spindles properties of 39 infants grouped according to age (youngest group: 0-3mo.; eldest group: 25-48mo.)(343). The team observed that the highest mean frequency of the overall sleep spindles in the frequency band 11 to 15 Hz was seen in children grouped from 4 to 12 months-old

over frontal, central and parietal areas. In comparison, total range spindle density showed no statistical variance from age alone. Instead, there was a significant interaction between spindle density between age and electrode position (see Figure 1.32). This study also analysed fast and slow sleep spindles separately (13-15Hz and 11-13Hz). From the first year, fast spindles decreased, while slow spindles, emerging at this stage, increased with age and plateaued in the second year (343). Over Fz, there is a positive linear correlation between age and slow spindle density, while fast spindle density describes a non-linear evolution over the same area.

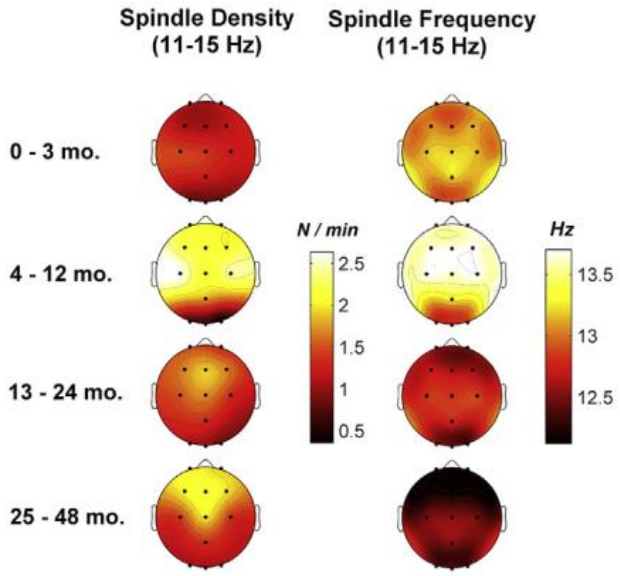


Figure 1.32 - Paediatric topographical maps of the sleep spindles distribution. Left column: of mean spindle density; right column: mean spindle frequency distribution up to the age of four years old. Image reused from (343) with permission.

Scholle *et al.* carried out a study on frontal sleep spindles. Considering the changes in spindles across sleep and with circadian rhythms, the authors analysed only the spindles from the second half of the night. This study included a considerable number of 120 participants, aged between 3 months and 16 years old. Participants were divided into 12 groups of 10, according to their age. The longest interspindle intervals were recorded between groups averaging 1.1 to 2.3 years old, these were statistically different to those for 4-year-olds and older groups but not to younger groups. The shorter sleep spindles were noted around 1.7 to 3 years old. There was an initial high level of interindividual variability that decreased and peaked again at 6.1 years of age. After an initial drop from infancy to the lowest point at 1.7 to 2.3

years of age, sleep spindles density had a pronounced increase starting from around 4 years old (369).

McClain *et al.* conducted a longitudinal study of sleep spindles on 8 children of both sexes at the ages of 2, 3 and 5 years old. The EEG acquisition and analysis used bilateral central and occipital derivations (no frontal derivations included), and sleep spindles were detected with an algorithm (370). This algorithm was previously validated on a young adult population with a precision of ~0.73 and ~0.36 and recall of ~0.69 and ~0.75 for experts 1 and 2, respectively (expert 1 identified spindles restricted to the information from 11-15Hz pass-band filtered C3 signal, while expert 2 complied to AASM rules and minimum duration was fixed at 0.5s)(244). Four parameters were analysed: frequency, duration, density and Integrated Spindle Amplitude, a value derived from the sum of all sleep spindles amplitudes (370). There were no significant differences in any of the parameters from 2 to 3 years of age. The following results are with respect to left central and occipital areas, respectively, from 2 to 5 years of age, similar trajectories are seen over the right side (370). It was concluded that duration significantly increased both in central and occipital derivations from 2 to 5 years from mean (SD) 0.85 (0.13)µV to 1.11 (0.21)µV and 0.64 (0.05) µV to 0.94 (0.22)µV. Frequency, on the other hand, decreased with age from 13.10 (0.20)Hz to 12.77 (0.25)Hz and 13.35 (0.37)Hz to 12.80 (0.26)Hz. Integrated Spindle Amplitude also increased from 805.5(156.4)µV to 1253(358.2)µV and 436.4(86.0)µV to 815.5(228.5)µV. Density was the only studied parameter that did not show statistically significant changes across age(370).

From childhood, both frontal and centroparietal spindle frequencies increase (237, 371), while duration(371) and spectral power decrease (237). Amplitude decreases rapidly reaching puberty(371) and density results are conflicting(307, 371). Through ageing in adulthood, fast sleep spindles, but not slow spindles, density and duration decrease significantly on later sleep cycles. (241). Although spindle density decreases with age, it is more marked in men. Spindle frequencies decrease as women age and the opposite is true for men. Duration and amplitude decrease up to

middle-age in both sexes. Observations may reflect processes of neurodegeneration and sex specificities(363).

As observed in this section, studies employed different methodologies and may analyse different spindle parameters. It is challenging to have a linear picture of all parameters' evolution across time. However, it is clear that sleep spindle parameters change with age, with the greatest changes occurring during the first years of life.

Table 1.2. summarises the studies in this section. Associations between spindle parameters of density, duration and amplitudes and timing of major brain maturation events are suggested in Figure 1.33. Aspects like thalamocortical and intracortical network connectivity (255) and axonal space (372) have been associated with some spindle properties that evolve with age.

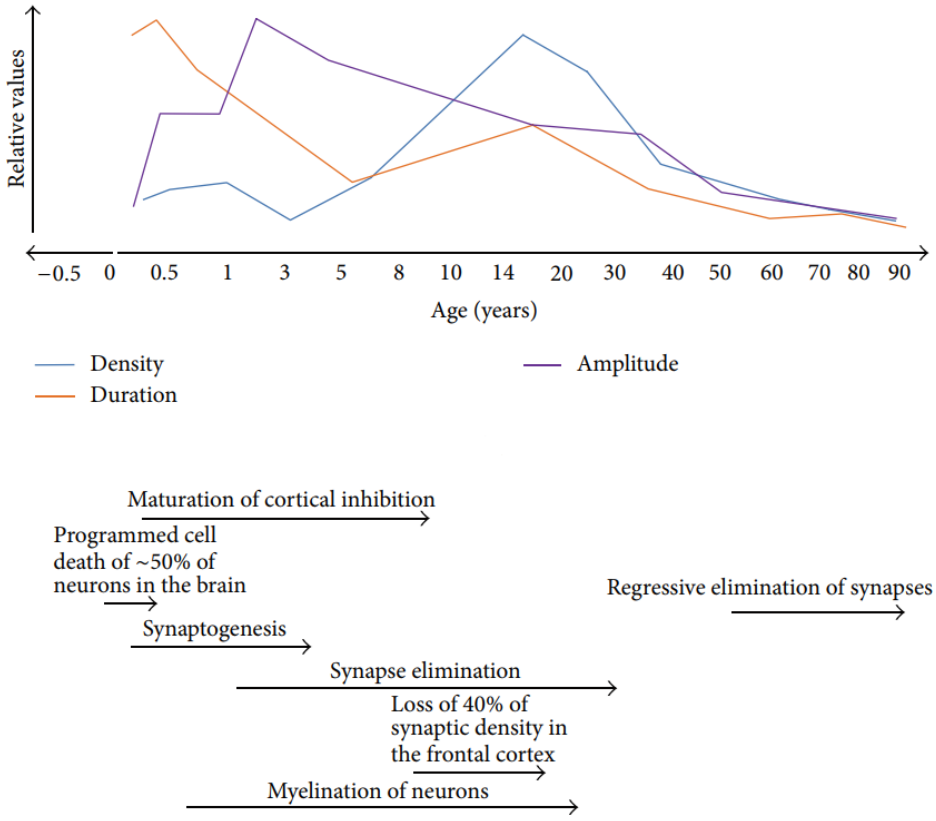


Figure 1.33 - Graphic representation of the evolution of sleep spindles density, duration, and amplitude with ageing and correspondent neuronal events below.
Image reused from (357) under CC-BY 4.0

Table 1.2 - Summary of sleep spindle trends across different stages of life.

	Reference	Number of participants	Population age	Brain regions analysed	Recording time	Duration	Density	Asynchrony	Asymmetry	Frequency	Magnitude
Infants	Hughes, 1996	16 (10 males)	10 to 52 weeks old full-term gestation	varying	ø	↗ until 13 wo, then ↘	↗ until 13 wo, ↘ until 23wo and stabilise (using 1/interspindle pauses as a proxy measure)	non-linear ↘ from 10 to 52wo	ø	ø	ø
	Louis *et al.*, 1992	12 (6 males)	1.5 to 6 mo	Bilateral prefrontal, central, temporal and occipital. Midline central and parietal	Overnight	↘ after 3mo (trend)	↗up to 3 mo, then ≈	↘ from 1.5 mo to 3mo, then ≈	↗ from 3mo to 6 mo (trend)	10-12Hz↘; >12Hz ↗ (trend)	10-25µV ↘; 25-50µV ↗; 50-75µV↗ (trend)
Infants and Toddlers	D'Atri *et al*., 2018	39 (25 males)	0 to 48mo	Bilateral prefrontal; Midline and bilateral frontal, central, parietal and occipital	Overnight	ø	↗ up to 4-12mo, then ↘	ø	ø	Whole spindle range:↗up to 4-12 mo, then↘↘ 11-13 Hz: ↗; 13-15Hz: ↗up to 4-12mo, then↘	ø
Infants, Toddlers, Children and Adolescents	Sholle *et al.*, 2007	120 (64 males)	3 mo to 16 yo	Bilateral frontal	Overnight	↗ from 1.1 to 11yo	↘from 9.6mo to 1.7-2.3yo, then ↗	ø	ø	ø	ø
Children	McClain *et al.*, 2016	8 (3 males)	2, 3 and 5 yo	Bilateral central and occipital	Overnight	↗from 2 & 3yo to 5yo	≈	ø	ø	↘from 2 & 3yo to 5yo	↗from 2 & 3yo to 5yo
Children, adolescents and young adults	Shinomiya *et al.*, 1999	32 (17 males)	4 to 24 yo	Midline and bilateral frontal; Midline centroparietal	Day nap	ø	ø	ø	ø	Frontal: ↗ (?trend); Centroparietal : ↗	Frontal: ↘ (?trend); Centroparietal : ↘
Older children and adolescents	Hahn *et al.*, 2018	34 (12 males)	8-11yo; 14-18yo	Midline and bilateral frontal, central and parietal	Overnight	ø	Overall ↗; Slow spindles: ↘ (except ≈ in frontal areas); Fast spindles: ↗	ø	ø	ø	ø
Young and middle-age adults	Bódzis *et al*., 2009	46 (29 males)	17 to 55 yo	Bilateral frontal and parietal	Overnight	Slow spindles: ≈; Fast spindles: ↘ (measurements during the 3rd and 4th cycles)	Slow spindles: ≈; Fast spindles: ↘ (measurements during the 3rd and 4th cycles)	ø	ø	ø	≈ (exception: ↘ for fast spindles over F3 during 2nd sleep cycle)
Young adults, middle-age and elderly	Martin *et al.*, 2013	114 (60 males)	20-30 yo; 41-59 yo; 60-73 yo	Bilateral prefrontal, frontal, central, parietal and occipital	Overnight	↘ from young to middle-age (++parietal) , then ≈	♂: ↘; ♀: ↘ from young to middle age, then ≈	ø	ø	♂: ↗ young and middle-age to elder; ♀: ↘ young and middle-age to elder.	Young ↘ until middle-age (++F3), then ≈

ø	No information available
↗	Values increased with ageing
↘	Values decreased with ageing
≈	Values mantained with ageing
wo	weeks old
mo	months old
yo	years old

1.5.7.6. Sleep spindles in pathologies

There is a different array of pathological conditions in which sleep spindles are altered. This also applies to conditions observed in paediatric populations like infant iron-deficiency anaemia which is associated with lower density and frequency of sleep spindles. The sleep of people with Williams syndrome (which occurs due to genetic deletions in chromosome 7) shows increased synchrony of several frequency bands, including fast sigma and beta activity during NREM sleep. Those two bands include sleep spindle frequency (373). Gibbs and Gibbs described abnormally high amplitude sleep spindles (200-400µV), the Extreme Spindles, which can be present in children with intellectual disability (374). Extreme Spindles or total/near absence of spindles may be present in cerebral palsy(375). Children and adolescents with childhood onset schizophrenia present with a general decrease in sleep spindle activity, similar to observations in adult studies but more topographicaly spread. Their sleep showed lower spindle amplitude, duration, density and frequency, NREM absolute and relative sigma spectral power, NREM absolute sigma coherence, and higher relative sigma coherence, in comparison with age-matched healthy participants, and were not associated with medication dose(376). These alterations may have a genetic component as observed in adult studies(377). Furthermore, most of these features were associated with positive symptoms, including hallucinations, and worse global functioning(376).

Children with ADHD and comorbid autism spectrum disorder (ASD) showed increased 12Hz relative power during N2 compared to typically developing children (frontal areas) and children with only ADHD (right frontal and temporal areas). Relative power at 12Hz tended to be lower in typically developing children than children with only ADHD over frontal regions, however differences were not statistically significant(378). A study on spectral power from delta to gamma preceding, during and following sleep spindle events in children with and without a diagnosis of ADHD demonstrated significant changes. Most of the significant spectral differences coincided with the period following spindles, and within the frequency

ranges, delta power had the most widespread statistical differences, with lower power in children diagnosed with ADHD (379) which may indicate different network activation following spindles. In a 2020 review on sleep parameters in neurodevelopmental disorders (ADHD, ASD without intelectual disability and developmental dyslexia) in paediatric populations, *Gorgoni et al.* pointed to the need for homogeneity in the methods, consistent results as well as need for replication studies in this population (380).

The diagnosis of neuropsychiatric disorders is primarily based on phenotype presentation. This phenotype presentation is variable within the same syndrome and frequently results from multiple mutations. The non-consistency of EEG results, as discussed above (380), might also be explained by the fact that syndromes are polygenic and variable. Electrophysiological studies are likely underpowered, considering the hundreds of different genetic variants found in a single disorder(381). Endophenotypes are individual or a collection of behavioural traits associated with known genetic alterations which may be observed in different syndromes(382). Krol *et al.* propose the study of indicators of neural circuit endophenotypes as factors intermediating complex genetic and several network-level processes, resulting in (behavioural) endophenotypes, narrowing the complexity in this assessment(381). For example, certain at-risk genes are highly expressed over the reticular nucleus of the thalamus. In ASD those genes are PTCHD1 and CHD2 and in schizophrenia, Cacna1i and Grm3. For example, the reticular nucleus participates in attention processes, and PTCHD1 selective deletion in thalamic reticular nucleus was shown to be related to hyperactivity, sleep dysregulation but also attention deficits. In terms of electrophysiology, knockout of this gene leads to a reduced bursting of thalamic reticular nucleus neurons from which sleep spindle generation depend. Sleep spindles may serve to inform thalamic function and may be an interesting biomarker for more mechanism-focused diagnosis when combined with other assessments (381).

Myatchin and Lagae compared sleep spindles amongst treated and untreated children with primary generalised spike-and-wave discharges and controls (no epilepsy diagnosis). In agreement with observations reported in 1.5.7.5, spindle

frequency increased from the 1st to the last N2, but only in controls. Neither treated and untreated children with primary generalised spike-and-wave had significant changes in this domain. In the 1st N2, the mean frequency was significantly higher in untreated children than in controls and no significant differences were observed in treated children and the other two groups. Spindle density was not significantly different between control and treated groups, but was lower in the untreated group. (383).

Sleep spindle generator mechanisms may be misused during absence-type seizures present in idiopathic generalised epilepsies, giving rise to ~3Hz spike-and-wave observed on EEG. The mechanisms are not fully understood, but some explanations may be predicated on decreased intra-reticular nucleus inhibition that leads to hypersynchronisation and genetic mutations that increase the excitability of the reticular nucleus cells (384). To demonstrate empirically that spike-and-wave discharges are not simply aberrant sleep spindles, Kozák *et al.* used local field potentials and single-unit recordings in a rat model. Long-Evans rats are known for the spontaneous spike-and-wave discharges accompanied by absence-like behaviour which respond to drug treatment. As expected, there were state-dependent differences in the occurrence of these waveforms, with spindles tending to occur during periods consistent with NREM sleep, and spike-and-wave discharges in periods consistent with inactive wake periods. The authors also showed different trajectories from juvenile to mature animals of these two transients in parameters such as spindles and spike-and-wave density, duration, and total time spent in the two transients. Also, the effects of ethosuximide, an antiepileptic drug (whose main action targets T-type calcium channels) differ between the transients after an initial period of inhibition of spindles and sharp-wave-discharges, sleep spindle number increased above baseline while sharp-wave-discharges remained suppressed(385). Contributing to understanding the causal mechanisms of spike-and-wave discharges (385), Kozák *et al.* showed that although some cells presented synchronous activity, most cortical cells are silent during the spike-and-wave discharges. Decreased inhibitory interneurons activity as the rat matures may be due to weak thalamocortical feedback or altered rhythmicity leading to an excitation/inhibition

imbalance, accompanying the increased probability for the focal spike-and-wave discharges to develop into generalised seizures (385).

Apart from these pathologies, sleep spindles may be altered in a subtype of primary insomnia (386), Parkinson's (387), senile dementia (388, 389), Alzheimer's Disease, Amnesic Mild Cognitive Impairment (390) and are associated with episodic memory (391).

1.5.8. Slow activity

As mentioned in 1.5.7.3, scalp EEG slow activity is present in infants and reflects the underlying slow oscillation. This slow oscillation consists of waves of hyperpolarisation (down-states) and depolarisation (up-states) fluctuating at 1Hz or lower (392), with the ability to nest other rhythms and potentially participate in memory and synaptic homeostatic processes. The role of slow oscillation is discussed below. In contrast with earlier studies on the topic (see for example (393)), Timofeev *et al.* argues that slow oscillation can not be assessed directly with scalp EEG, but only from more invasive neurophysiological techniques (275), in part due to the blurred boundaries of slow oscillation rhythms and delta activity on EEG, as will be discussed below (394). As a result, paediatric knowledge of relating slow oscillations are based on extrapolation from in silico, animal and adult surgical candidates' studies or by the indirect study of scalp slow waves.

1.5.8.1. Cortical Slow Rhythms

Wilson and Groves initially characterised slow oscillation in striatal recordings using animal models, the presence in cortical and thalamic regions was later demonstrated by Steriade [reviewed in (395)]. Slow oscillations may occur in natural sleep and under anaesthesia. New evidence suggests that thalamic activity may trigger the neocortical up-states, regulate its synchronisation and be responsible for the propagation across the cortex (309, 396, 397). Slow oscillation is also subject to modulation by astrocytes (398). During slow oscillation pyramidal and local-circuit

inhibitory neurons participate with excitatory and inhibitory inputs (392) during the depolarising states. In contrast, hyperpolarised states are characterised by virtual synaptic silence caused by synaptic disfacilitation (399, 400). These oscillations provide alternating cycles of strong depolarisation and hyperpolarisation which impose a rhythm to other oscillations, namely other delta oscillations, sleep spindles and fast spontaneous oscillations leading to their synchronisation, [reviewed in (277)], i.e. rhythms become nested. In this process, pyramidal cells at resting membrane potential may produce cortical delta oscillations (predominantly at 3 to 4 Hz) after the inducement of up-states[reviewed in (401)]. Slow waves are predominantly local phenomena, particularly at the end of the night sleep but may also be global, tending to propagate along the anterior-to-posterior axis and to the hippocampus, stimulating ripple initiation during depolarising states (402). Nesting rhythms through means of alternating depolarisation and hyperpolarisation oscillations provide an output comparable to syntax organisation and is necessary for efficient neuronal communication (403).

Sleep pressure effects and possible role on sleep homeostasis

In analysis of human sleep using EEG spectral power, slower and faster slow waves showed different trajectories with variation of sleep pressure, supporting the concept of two separate slow wave entities(393, 404).

The sleep homeostatic hypothesis developed by Tononi and Cirelli proposes that synaptic strength is experience-dependent and develops during wakefulness. Maintaining unnecessary synapses is costly in terms of energy and resources. Sleep, as a state of relative disconnection from external inputs, presents as the ideal “offline” period for net synapse downscaling in which global synaptic strength is reduced. Tononi and Cirelli have suggested that slow wave activity is associated with synaptic strength as this activity reflects sleep pressure and previous learning activity and is allied to synchronised waves of cellular hyperpolarisation and depolarisation possibly contributing to the downscaling processes(405).

To investigate the associations between slow wave activity and synaptic strength, Esser *et al.* in an *in-silico* study simulated a large model of thalamic and cortical regions and their connections(406). By reducing cortico-cortico synaptic

strength, the calculated local field potentials had a reduced number of high amplitude slow waves (0.5-4Hz), decreased slow wave slope and increased multipeak slow waves. The changes were explained by the fact that lower synaptic strength leads to lower amplitude membrane potential oscillations, decreasing the probability of firing and to lower synchronisation between individual cells (reducing amplitude) and fragmentation into several neuronal populations (increasing number of peaks in a wave); lower synaptic strength also lowers the rate of a cell switching from a depolarised to a hyperpolarised phase and vice-versa (resulting in changes in slope) (406). The findings of the calculated local field potentials in this *in silico* model were corroborated with animal studies where synaptic strength varied due to changes in homeostatic pressure over sleep time and upon recovery after sleep deprivation(407). Furthermore, slow wave changes were predominantly associated with sleep homeostatic pressure rather than circadian rhythms(407). A shift of the peak of slow waves relative power towards lower frequencies (from 1.5Hz to 0.75Hz) occurs across sleep due to reduction of the slope steepness(407).

Using the typical slow wave range studied in polysomnographic studies(121, 408), i.e. 0.5-2Hz, a scalp EEG human study(394) across a night of sleep showed slow wave changes (spectral power, slope, multipeak morphology and differential incidence of higher versus lower amplitude slow waves) from early to late sleep, compatible with the previous observations based on calculated and animal local field potentials, which are topographically dependent, particularly accentuated over frontal and temporal regions with the lowest changes around fronto-central midline region and occipital areas(394) Similar conclusions were demonstrated in the inverse scenario of *increased* homeostatic pressure due to prolonged sleep deprivation in human EEG. Riedner *et al.* (394) question the distinctiveness between slow oscillation and delta activity on EEG and their differentiated response to homeostatic pressure: as observed in (407) and (394) there is a decrease of the number of high amplitude slow waves with decreasing homeostatic pressure that is reflected in the spectral power; the spectral power of the slow oscillation may not reflect this amplitude decrease because, as the steepness of the slopes decreases, there is a switch of slow wave power peak towards lower frequencies(394). EEG boundaries between delta waves and slow oscillation may also be blurred as faster delta activity may result from

multipeaks from local neural population clusters desynchronisation while undergoing a slow oscillation(406) or occur as a summation of two slow oscillations originating from distinct brain regions(394).

Considering the evidence from these studies, it is possible to hypothesise that slow wave activity decreases with decreasing sleep pressure due to reducing synaptic strength(394, 406, 407). Slow wave power is intimately correlated to its slope (r=0.97-0.98)(394), which is consequently, a measure of synaptic strength. This is because synaptic strength determines the synchrony of neural populations and the rate of individual cortical neurons switching between hyperpolarised and depolarised states, which influence the steepness of the slope(406). These three studies also corroborate the sleep homeostatic hypothesis as they provide evidence of slow activity reflecting synaptic strength, which increases in response to prolonged wakefulness but decreases during sleep, conceivably as a mechanism for maintaining network homeostasis.

Neuroplastic changes and learning

As discussed in 1.5.7.3, the intense cortical hyperpolarising-depolarising sequences of slow oscillation play a role in stimulating and temporally aggregating different sleep transients. Thus, slow oscillation may not only be implied in processes of sleep homeostasis, as discussed above but may also be a facilitator of long-term memory consolidation. Learning tasks have been shown to result in a local increase of slow wave activity. Learning of a visuomotor task not only drives an increase in slow wave activity during the following sleep over right parietal areas (responsible for converging proprioceptive and visual stimuli for spatial attention), but also these localised increases are also positively associated with better performance following sleep(409). In contrast, restriction of a synaptic network activation with limb immobilisation, reduces slow wave activity in the respective somatosensory area(410). Those studies demonstrate empirically the association between synaptic network stimulation/understimulation, localised changes of sleep slow wave activity and subsequent performance. Across ageing, as the medial prefrontal cortex atrophies, slow wave-spindle coupling degrades. This age-related synchronisation

deterioration is speculated to be the substrate for compromised hippocampal-dependent consolidation observed in later life(411).

Maturation

In section 1.5.8.1 I described the mechanisms imputed to slow waves association with synaptic homeostasis and synaptic strength. The ability of downregulating global synaptic strength is of extreme importance in infancy due to the number of superfluous and unmyelinated synapses which represent an increased energetic cost (171). We also observed from the previous section that slow waves may underlie learning and memory processes. Next, I will discuss how slow waves evolve during maturation.

Satomaa *et al.* showed that at 8 months, overnight slow wave activity up to 1.75Hz and up to 4Hz displayed a progressively lower topographical gradient from occipital to central and to frontal regions. Both slow wave frequency bands in different topographies were positively associated with several Bayley-III subscales including language and fine motor skills assessed after approximately 3 weeks (334).

In a cohort ranging from newborns to 48 months of age, Novelli *et al.* showed that overall slow wave activity during NREM sleep (spectral power of the frequency band of 0.5-4.5Hz) peaked at 4 to 12 months. In a topographical analysis, slow wave activity predominates over the posterior regions in infancy. The authors noted a trend from 25-48 months; slow wave activity started shifting towards more anterior locations, although increments of the frontal to occipital ratios between age groups were not yet statistically significant (367). Page *et al.* showed that as children age from 12 to 30 months old, significant increases in NREM slow wave activity (spectral power of the frequency band of 0.5-2Hz), occur, particularly in the anterior and right temporal regions (335). On different regions that generally included occipital areas, slow wave activity was positively associated with fine motor skills, but in contrast with Satomaa *et al.* in this age group, slow waves were also negatively correlated with expressive language and with social skills development.

Analysing age-overlapping cohorts from 6- to 18-year-olds, Feinberg and Campbell noted that NREM central delta activity (1-4Hz) increased up to 8 years,

declining afterwards, with the fastest decline occurring at 13 years of age. Age-related reduction of spectral power was 2.5 times higher from ages 12-16.5 than 8-12 years; a similar trajectory was observed at frontal locations but not on occipital areas. The authors hypothesise that decreasing slow wave power may denote higher synaptic pruning rather than proliferation which is not observed in occipital regions as the majority of maturational pruning is completed in younger ages(412).

Studying NREM slow waves at 1-4.5Hz in a cohort from 2.4 to 19.4 years also confirmed the peak of slow waves in children aged 8-11 years, with the greatest differences observed in the first hour of NREM. Like the previously discussed studies in this section, Kurth *et al.* found slow waves to predominate over occipital areas in younger groups and shifted anteriorly with ageing. The frontal to occipital slow wave ratio showed a linear growth that was stable across sleep duration(413).

In a cohort from 2.4 to 8 year old children, this frontal-to-occipital ratio of slow waves during the first hour of NREM) was shown to be predictive of whole brain myelin content 3.5 years later at follow-up, but was not however associated with global cortical morphologic maturation (414). Also, the predominant location of slow waves (1-4.5Hz) predicted the development of skills obtained during maturation, such as complex motor skills, approximately 3.7 years in advance(415). However, Beaugrand *et al.* studying topographical predominance of the slow waves at 6 months observed no association between previous (at 3 months), current, and later (12 and 24 months) Age and Stages Questionnaire general, personal social and gross motor subscales scores. The authors point out that factors like low sample size and possibly lack of sensitivity of the EEG marker used and, of the parents questionnaire used in a healthy full-term born infants population may have affected the results(416).

Earlier studies in adults have shown a relative independency between low delta measures of power and coherence (417). Kurth *et al*. studied overnight coherence in bilateral central and occipital areas in a paediatric cohort longitudinally from 2.5 to 6 years old. Across the night, low delta (0.8-2Hz) intrahemispherical coherence decreased and interhemispherical coherence increased. The authors hypothesised that the later findings may be related to plastic changes associated with

network maturation. As for maturational changes, low delta intrahemispherical coherence increased with maturation with significant differences between 2.5-3 and 5.5-6-year old age groups. Considering age interactions with across-night dynamics, right intrahemispherical coherence decreases across the night were more accentuated in the older group in a broader delta range of 0.8-4.6Hz but not on low delta (0.8–2 Hz). The interhemispherical decreases across sleep are speculated to be due to age-related decreases in integration of main association fibres due to reducing synaptic strength (418). In young adults, no 1-2Hz inter- and intrahemispherical coherence differences were observed across different NREM cycles(417). However, it is impossible to compare the coherence results directly across sleep duration between children and adults due to the choice of different EEG channels for analysis.

Schoch *et al.* (419) analysed the dynamics of high amplitude slow wave (0.5-4.5Hz) propagation in three cohorts: pre-schoolers, children of school age, and adolescents. Pre-schoolers were the only age group in this study that showed a statistical decrease in the distance of propagation between the early and the late part of sleep; Results should be treated with caution however as the study was potentially underpowered with only 9-11 participants per group. The number of electrodes involved in each slow wave decreases across sleep for all age groups, conforming to the dynamics observed in adults using scalp EEG and intracranial recordings showing that slow waves become increasingly more local towards the end of sleep(402). According to the authors, differences between age group dynamics across sleep may be related to increased myelin content in the corpus callosum or a higher degree of downscaling required in younger groups. Speed of propagation showed an interaction between age group and period of the night. Whole-night mean propagation distance and speed increased from pre-schoolers to adolescents and to school-age children, respectively, possibly attributable to network refinement and increased myelination across age(419).

Although slow waves originate predominantly in anterior regions in adults (369) and adolescents, in pre- and school-age children, the cortical origin is centro-parietal, as demonstrated by Timofeev *et al.* (275). This observation parallels the slow

wave activity dominance shift along the posterior-to-anterior axis observed as children age and may be related to progressive cortical maturation.

Fattinger *et al.* analysed the overnight dynamics of slow wave slopes (1-4Hz) in a longitudinal study on infants from 2 to 9 months of age(420). A later study by Jaramillo *et al.* complemented the first by studying slow wave (0.5-4Hz) slope dynamics from childhood to adulthood (8-29years)(421). Overall, slopes tended to increase with age during infancy (comparing mean overnight C3-A2 slow waves, of the same amplitude of 55µV, chosen due to high incidence in all infancy age groups)(420) and decrease from childhood to adulthood (comparing the first hour of NREM) (421). Overnight slope decreases became more pronounced as the infant matures(420) and less pronounced with age from childhood to adulthood(421). Examining the topographical distribution during early sleep in infancy, the authors found that 55µV slow wave slopes were the highest at occipital regions, a region of intense synaptogenesis during this phase of life (420); while from childhood to adulthood, there was a shift of the maximal slope from central to frontal electrodes in the highest amplitude slow waves (no topographical differences across age were observed regarding smaller amplitude waves) (421). This data is in accordance with the posterior-to-anterior maturation pattern observed for slow wave activity. The later study also analysed topographical interactions of the overnight slope decreases across ageing, finding that the minor overnight changes were in central areas for higher amplitude slow waves and, occipital areas for lower amplitude slow waves. Overall, in the model described by Esser *et al.*(406), previously discussed, this increase of the slow wave slope may be associated with increased synaptic strength and increased need for homeostatic regulation across maturation. According to Fattinger *et al.*, the contrasting topographical evolutions across age between maximal slope in early sleep and maximal slope overnight may suggest a biased downscaling during brain maturation in which areas of synaptic strengthening during waking experience may not necessarily correspond to the areas to be downscaled during sleep, preserving basic connections such as somatosensory learning(420).

Chapter 2. Electroencephalographic sleep macrostructure and sleep spindles in early infancy

2.1. Introduction

The process of neurodevelopment during the first months of age is dynamic with changes in sleep macrostructure and sleep spindles reflecting some of these alterations. These features may provide early biomarkers for development. Sleep spindles, in particular, show a unique individual fingerprint (226) and are affected by different neurologic pathologies. The normative values for the 4 to 5-month age group may offer a benchmark with which to assess a potentially abnormal developmental trajectory.

Some of the most important sleep waveforms recorded on EEG are sleep spindles. These transients, defined by the American Academy of Sleep Medicine as runs of 11-16Hz oscillatory activity occurring over central locations during N2 and N3 stages of sleep (121), arise from the thalamus (248) during a state of relative hyperpolarisation (249). Spindle properties are dependent on intra-thalamic interactions, particularly between the reticular nucleus and thalamocortical neurons, and amongst thalamic and cortical structures (230, 254, 257, 422). In humans, the first spindles can be detected on surface EEG as early as 6 to 9 weeks postnatal age (PNA) (226).

Since early analysis by Gibbs and Gibbs (236), it has been extensively demonstrated that there are two spindle frequencies: slow spindles, prevalent over the frontal areas, and fast sleep spindles, prevalent over the central/centroparietal areas, in children (237, 239, 343) and adults (225, 238, 240, 242, 246). The two types of spindles correspond to different underlying dynamics (242, 245, 246), cortical and subcortical activations (247) and may reflect specific processes of learning and cognitive ability (240, 245, 246, 308, 331, 423). Different studies have used different cut-off frequency points for the two types of sleep spindles, and this inconsistency may be related to interindividual differences (241).

Sleep spindle features are partially dependent on genetic factors (243), including sex (241, 363). However, several environmental factors can influence sleep spindle parameters, including circadian rhythm and sleep pressure (241, 337, 358-360, 363); activities where learning might be involved (301, 303, 304, 306, 307, 424); cognitive abilities; and pathological conditions such as Alzheimer's (391), Parkinson's (425), ADHD (426), ASD (380), schizophrenia (427, 428) and some cases of cerebral palsy (375). A possible explanation linking some manifestations of neurological diseases to alterations in spindle parameters may reside in the fact that several 'at-risk' genes for these pathologies are highly expressed in the thalamic reticular nucleus (381), which is integral to spindle generation.

With ageing, there is an early shift of the maximal spindle-related power towards anterior locations (367), and evolution of several individual sleep spindle parameters such as density, frequency, duration and amplitude (343, 363, 369). The most marked changes occur during infancy but continue to a lesser extent until adulthood, accompanying major brain maturation processes, further to this, sleep spindles might present properties that actively contribute to some synaptic development (296) (for review see (357)).

In the paediatric population, slower and fast sleep spindles appear to play different roles in the varying factors that compose intelligence (239, 338-340). However, no association was observed in a cohort from 12 to 30 months in a series of tests that included socialisation, hand-and-eye coordination and other motor functions (335). Similar to the adult population (429), there has been no evidence that sleep spindles in children are related to verbal cognitive ability with the exception of narrative memory (338, 339). Also, like adult studies, there seem to be sex differences regarding these associations in populations as young as 4 to 8 years old (239). It has also been demonstrated that there are reciprocal interactions between sleep spindle characteristics such as number (301, 303, 306), density (302, 304, 307), spectral power (301, 424), amplitude (302), duration (302) and relationship with slow waves (245), and the ability to learning different tasks.

At term, the proportion of time spent in active sleep is at its highest (430) and sleep tends to initiate with this sleep stage. The shift to quiet sleep onset occurs after

the 44th week of postmenstrual age (168, 205). During infancy, latency to REM increases and becomes more dependent on duration of the previous waking period (431), whilst the proportion of active to quiet sleep and, subsequently, REM:NREM sleep continues to decrease (167, 430). In infancy, REM:NREM proportions from daytime naps maturate earlier, relative to nocturnal proportions (432). From 4 months of age, NREM sleep can be further classified into stage 1 (N1), stage 2, (N2) and stage 3 (N3), as the stages become increasingly differentiated (196).

Sleep is theorised to endorse homeostatic balance at the cellular level (433) and to prevent neuroinflammation (48, 171, 172). Sleep reshapes the maturing brain (49, 50); in a period of life with less efficient neural networks, sleep in thought to contribute to a general energy saving by reducing the number of redundant synapses, and enhancing strategic synapses, thus promoting learning and memory consolidation (48). It allows somatosensory and motor development (125, 140), prolongs stimuli recognition in infants (175, 434, 435) and contributes to mood regulation (169). Poor quality of sleep during development is associated with higher risk for the development of psychopathological symptoms(436) and lower cognitive outcomes later in life (172).

In this chapter, I analyse the macrostructure of sleep, as well as sleep spindles of ex-term infants at 4 to 5 months of age, possible influences of sex, period of the day the nap occurs, and differences from first to second sleep cycle. The sleep parameter trends are also analysed across gestational, postmenstrual, and postnatal age.

2.2. Methods

This study was ethically approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals (Appendix 3). The guardians of the participants were approached for consent in the postnatal ward at Cork University Maternity Hospital between 2017 and 2018 (Appendix 4). The criteria for inclusion were being healthy, singleton and born after 37 weeks of gestational age.

Infants were enrolled into the BabySMART study (Study of Massage Therapy, Sleep And neurodevelopMenT, registered identifier NCT03381027, https://clinicaltrials.gov/search?term=NCT03381027), a randomised controlled study investigating the impact of an intervention (infant massage) on sleep and neurodevelopment in early infancy. EEG analysis was performed blinded to group allocation. Only the data from the non-intervention group was used in this analysis. Infants participated in a sleep EEG recording at approximately 4-5 months of age (Figure 2.1). Appointments occurred during the day, either during the morning or the afternoon, generally between 9.30 and 12.00am, and 1.30 and 4.00pm respectively. The soundproof room was at a comfortable temperature according to parent's judgement and lights dimmed; guardians could opt to let the infant sleep on their lap, the buggy or in a cot. During EEG electrode placement, feeding was encouraged to distract the infants from the procedure and soothe them.



Figure 2.1 - INFANT Centre sleep lab

2.2.1. EEG recording

Video-EEGs were recorded using a Lifelines (Lifelines Neuro, UK) EEG acquisition system. Electrode placement followed the international 10-20 system and

included FP2, FP1, F8, F7, F4, F3, Fz, A2, A1, T4, T3, C4, C3, Cz, T8, T7, P4, P3, Pz, O2, O1, reference and ground. Disposable cup EEG electrodes were used and impedances were maintained below 10 kΩ. Additional polygraphy, including ECG, EOG, submental EMG and respiration, was recorded. The EEG was sampled at 500 Hz. The EEG was recorded for the sleep duration and finished after the baby fully woke and was no longer able to return to sleep. A clinical physiologist remained in the room during the recording to promptly address any technical issues that might arise and to flag any incidental EEG findings.

2.2.2. EEG analysis

The post-acquisition EEG analysis of sleep macrostructure and sleep spindles was performed by an experienced clinical physiologist (SV). EEG was reviewed using standard high- and low-pass filters of 0.5 and 70Hz respectively.

Sleep staging was performed according to the AASM guidelines version 2.4, in 30-second epochs and annotated by SV using sleep staging software (Nicolet, Natus, USA). Sleep spindles present over F4-C4 and F3-C3 EEG channels were visually identified post-acquisition on Stratus EEG (Kvikna, Iceland) software and were marked on the EEG with duration annotations by SV, populating an events list that could be exported as a text file for further analysis. The spindle parameters analysed were the number of spindles, density (number of sleep spindles per minute of NREM), duration (s), median sleep spindle spectral power (μV^2), mean spindle frequency (Hz), brain symmetry index (437) (a measure of divergence of inter-hemispheric power), and synchrony (%). This data was segregated by sleep cycles. Spectral power and mean frequency were calculated from power spectral density (PSD) estimates of each spindle. These measures used a periodogram PSD. Brain symmetry index was calculated over a 30 second epoch with 75% overlap; non-spindle activity in this epoch was ignored. This measure used a Welch PSD with a 0.5 second Hamming window with 75% overlap. All PSD estimates were limited to the range 9 to 16 Hz and spindles of <0.5 seconds in duration were ignored. The median values of number of spindles, duration, median spindle spectral power and mean spindle frequency were calculated as a summary measure per infant. Synchrony, a

measure of coincidental contralateral sleep spindles, was calculated as the percentage overlap between spindles in the left and right hemispheres. Duration of spindles was obtained from the sleep spindles individual annotations and density was calculated based on the number of sleep spindles and the duration of NREM sleep during each sleep cycle. Mean frequency and synchrony were calculated using MATLAB (The MathWorks Inc, Natick, Massachusetts, version 9.8.0 R2020a); brain symmetry index and spectral power of the spindles were calculated using NEURAL software (a neonatal EEG feature set in MATLAB, version 0.4.3) (195).

Griffiths Scales of Child Development, third edition (Griffiths III) is a neurodevelopmental assessment tool, designed to be administered to children from birth to 5 years and 11 months (438). Infants with an overall Griffith's developmental quotient (GDQ) of 85 or lower at 18 months were considered to have developmental delay and were excluded from this study. Infants were also excluded if their GDQ score was not available at 18 months, a sleep recording was not obtained or EEG abnormalities were found. Further exclusions were applied depending on the amount of sleep data recorded. As there were some infants that fell asleep before the recording initiated, those infants were excluded for calculations of spindle number and density due to the potential for missing data. Only infants with a complete first sleep cycle were considered for the macrostructure analyses. Here, complete sleep cycle meant that infants only fell asleep after the onset of the polysomnography and had both NREM and REM sleep, with a requirement for N2 and/or N3 during NREM state being present. There were exceptional cases where REM sleep occurred before meeting criteria for N2; to avoid joining the norm with the exception on the measures of latency to REM, these infants were excluded from these specific variable analyses. For comparison between first and second cycle, the previous rules still applied on the micro- and macrostructure; additionally, for microstructure analysis, infants were included if they had NREM recorded for both sleep cycles; and for macrostructure, infants were included if they had at least two complete sleep cycles.

2.2.3. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (version 26.0, IBM Corp., Armonk, NY, USA). Continuous variables were described using mean and standard deviation (SD) or median and interquartile range (IQR) and categorical variables were described using frequency and percentage. Relationships between continuous variables were initially investigated using Pearson's correlation coefficient.

Univariable and multivariable linear regression was used to investigate factors associated with sleep features. The factors investigated were age (gestational and postnatal age), sex, and period of the day. Some sleep features were log-transformed prior to the regression analyses to meet Pearson's Correlation Coefficient assumptions of data normally distributed: spindle spectral power and brain symmetry index and macrostructural metrics of time in REM, first cycle duration, latency to sleep, i.e. time from the period of lights off at the start of EEG to onset of sleep, and to REM variables. The regression coefficients (B) and their corresponding 95% CIs were back-transformed and presented in the original units.

Differences in sleep parameters between the first and second sleep cycle were investigated using the Wilcoxon signed-rank test. All tests were two-sided and a p-value <0.05 was considered statistically significant. Sleep spindles annotations analysed in this study were compared with annotations of a second-rater using the Kappa statistic.

2.3. Results

Ninety-eight infants had an EEG recording at 4 to 5 months of age. Of those, two were excluded, as one did not fall asleep and the other had an abnormal EEG. Four infants were further excluded once they did not have a Griffith's-III neurodevelopmental assessment at 18 months and one infant whose GDQ score was equal or lower than 85 (Figure 2.2). Table 2.1 describes the demographic information of the 91 infants included in this study.

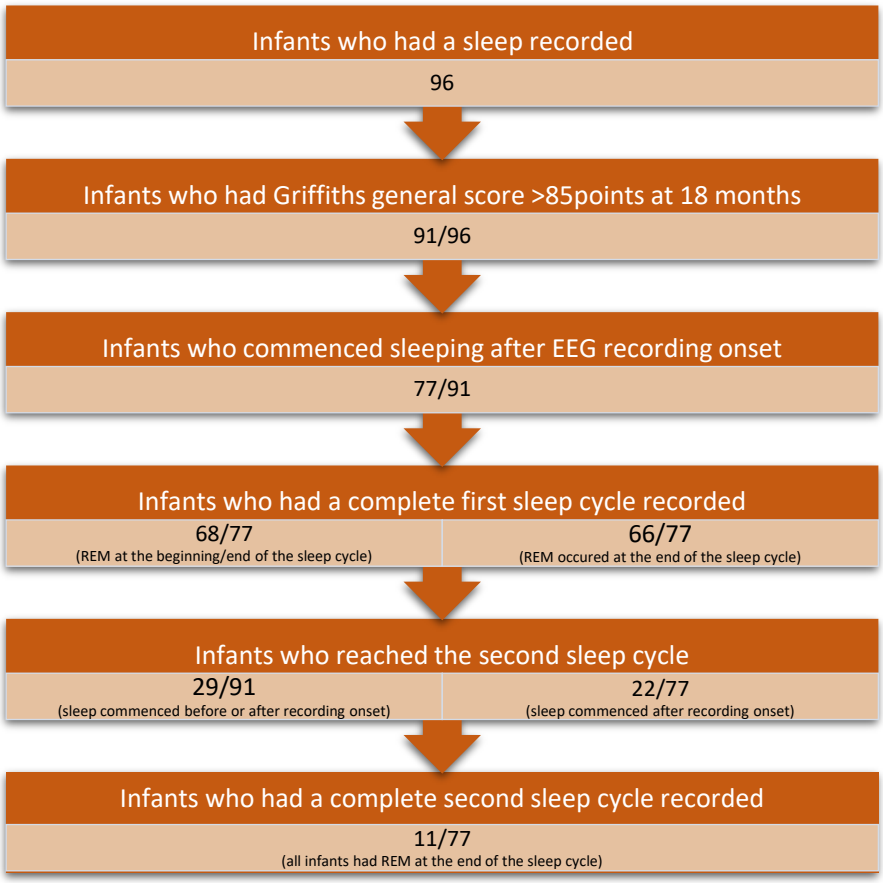


Figure 2.2 - Flow diagram detailing numbers of participants in analysis.

Table 2.1 - Demographics.

Characteristics	Mean (SD)[a] (n=91)	
Sex – female, n (%)	37	(40.7)
Weight at birth (kg)	3.57	(0.46)
Gestational age (weeks)	39.8	(1.2)
PNA at the appointment (weeks)	19.5	(1.3)
PMA at the appointment (weeks)	59.3	(1.8)
Timing of the nap – afternoon, n (%)	49	(53.8)
Mother's ethnicity, n (%)		
White Irish	82	(90.1)
Non-Irish White	6	(6.6)
Asian	2	(2.2)
Latin-American	1	(1.1)
Father's ethnicity, n (%)		
White Irish	82	(90.1)
Non-Irish White	6	(6.6)
Asian	2	(2.2)
Arabic	1	(1.1)

[a]Unless otherwise stated; PNA = postnatal age; PMA = postmenstrual age. gestational age is referred as the time from mothers' last menstrual cycle to delivery, postnatal age as age at the occasion of the EEG recording counted from birth and postmenstrual age as the sum of both(439).

2.3.1. Analysis of diurnal sleep macrostructure

A median (IQR) total recording time of 65.0 (49.5-87.5) minutes was obtained for ninety-one infants in the study; 42 recordings (46.2%) occurred during the morning time. In total, 14 infants fell asleep during electrode application. The total nap duration for those who fell asleep after the recording started had a median (IQR) of 49.0 (37.8 – 72.0) minutes (n=77). Of those 77 infants, 68 had a complete first sleep cycle recorded. Twenty-nine infants out of the total 91 reached the second sleep cycle. When including infants that fell asleep after the recording started (n=77), only 22/77 (28,6%) (Figure 2.2) reached at least the 2nd sleep cycle; 5/77 (7.8%) achieved the 3rd cycle. One infant out of 77 reached the 4th sleep cycle. Two infants out of 77 (2.6%) reached REM stage before meeting the criteria for N2.

Table 2.2 represents the duration of sleep stages during the first complete cycle and latencies to sleep and REM (n=68).

Table 2.2 - Duration of sleep stages on the 1st cycle.

Sleep Stages	Median (IQR) (n=68)			
	Time (minutes)		Time (% from cycle duration)	
Latency to sleep	8.5	(4.0-14.0)	-	-
Cycle duration	42.8	(37.0 – 51.4)	-	-
NREM	35.5	(30.1-40.5)	82.6	(72.3-90.5)
N1	8.0	(4.5-13.4)	18.8	(13.3-28.0)
N2	4.5	(2.5-6.5)	10.9	(6.0-16.5)
N3	20.3	(16.5-25.4)	46.0	(38.3-59.7)
REM	6.5	(3.6-11.5)	17.4	(9.5-27.7)
Latency to REM[a]	36.0	30.5-41.1	-	-

[a] n=66, infants who meet criteria for staging REM before N2 were excluded.

Of the 96 infants with a clinically normal EEG and with sleep recorded, 5 were excluded from the study due to lacking or having a low score on Griffiths and, 15 were excluded from macrostructure analysis due to falling asleep before the recording onset. As an indicator of sample size calculation for future studies, ignoring the mentioned exclusions, 72/96 infants (75.0%) reached a complete sleep cycle (N1 may not have been recorded). Two further infants had REM before reaching N2 and N3 out of the 81 whose sleep onset was recorded (2.5%).

2.3.2. Analysis of diurnal sleep spindles

To calculate interobserver agreement for detection of sleep spindles, the first sleep cycles of 65/96 infants were annotated by two raters and compared. Kappa scores for spindle identification were 0.82 and 0.80 for right and left fronto-central spindles respectively, which confirm that sleep spindles are discrete and readily identifiable waveforms. 50.3% of the sleep spindles were right-sided. From the 91 participants, a total of 29,947 sleep spindles were recorded. For the first cycle, 21,867 spindles were recorded. Table 2.3 provides the characterisation of the sleep spindles during the first sleep cycle across infants. The distribution of sleep spindle frequencies (Hz) is shown in Figure 2.3.

Table 2.3 - Analysis of sleep spindle features during the first sleep cycle.

Sleep spindle Parameters	Median (IQR) (n=91)
Frequency (Hz)	13.0 (12.8-13.3)
Duration (sec)	2.9 (2.6-3.6)
Spectral Power (μV^2)	7.8 (4.7-11.4)
Brain Symmetry Index	0.20 (0.16-0.29)
Synchrony (%)	59.5 (53.2-63.8)
Number[a]	241.0 (193.0-286.5)
Density[a] (spindles/min)	6.6 (5.7-8.0)

[a]Infants who fell asleep before EEG started were excluded (n=77).

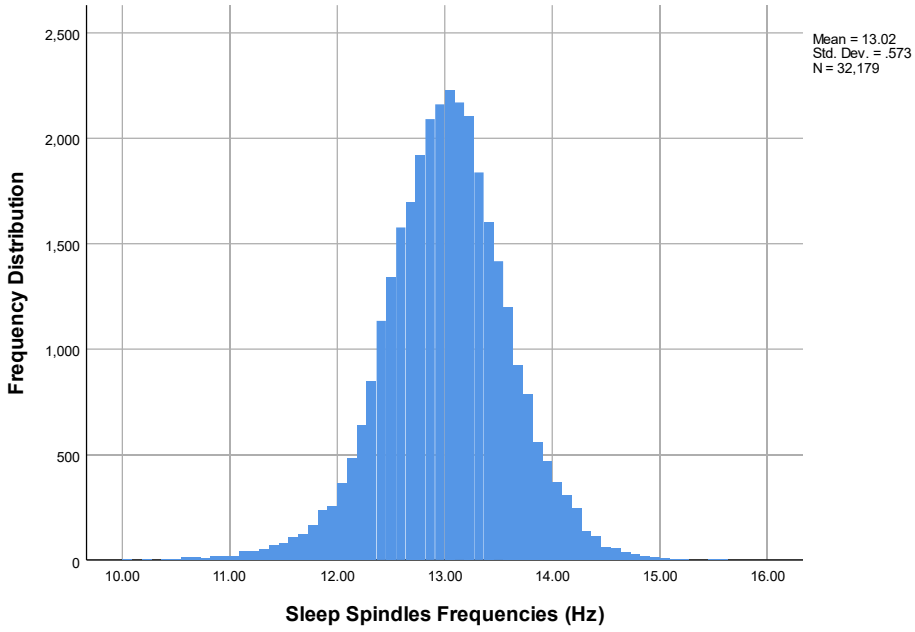


Figure 2.3 - Distribution of sleep spindle frequencies (Hz) from all sleep cycles recorded.

2.3.3. Sleep parameter regression analysis

Results from the univariable and multivariable linear regression analyses between sleep parameters at 4 to 5 months during the first sleep cycle and GA, PNA, sex and timing of the nap are presented in Table 2.4. In the univariable analysis, GA was negatively correlated with synchrony [B(95% CI): -1.307(-2.583 to –0.031), p=0.045] but this relationship was no longer statistically significant in multivariable

analysis [B(95% CI): -1.216(-2.547 to 0.115), p=0.073]. Likewise, PMA and spindle duration were negatively correlated [B(95% CI): -0.086(-0.158 to -0.015), p=0.018] in the univariate analysis, losing statistical significance when controlling for sex and timing of the nap (results not included). Sleep spindle spectral power and brain symmetry index differed by sex in the univariable analyses with significantly higher power and lower brain symmetry index in females (p=0.022 and p=0.010, respectively). These differences remained statistically significant in the multivariable analyses (p=0.032 and p=0.011, respectively). Comparisons of sleep parameters by timing of the nap (morning or afternoon) demonstrated lower spindle duration during afternoons in the unadjusted analysis (p=0.034) but the difference was not significant in the adjusted analysis (p=0.115). No significant relationships between the factors and the macrostructure outcomes were found in the univariable or multivariable analyses (Table 2.5).

To study sleep macrostructure across the first two cycles, further exclusion was made to only include infants who had two complete sleep cycles (n = 11). No statistically significant differences were observed in sleep macrostructure parameters between cycles (Table 2.6). Changes in sleep spindle parameters with expected reduction of sleep pressure from the first to second cycle were investigated using the data of 29 infants who reached a second sleep cycle. Sleep spindle spectral power decreased significantly on the second cycle (p<0.001, Table 2.6).

Table 2.4 - Univariable and multivariable linear regression analysis of the sleep spindle features on gestational, postmenstrual and postnatal age, sex and timing of the nap.

Dependent Variable	Independent Variable		Univariable analysis (n=91)				Multivariable analysis (n=91)		
			r/median (IQR)	B	95% CI	p-value[a]	B	95% CI	p-value[b]
Frequency (Hz)	GA		0.024	0.007	(-0.053 to 0.066)	0.825	-0.004	(-0.066 to 0.058)	0.906
	PMA		0.066	0.013	(-0.028 to 0.054)	0.534			
	PNA		0.068	0.018	(-0.038 to 0.074)	0.520	0.010	(-0.048 to 0.068)	0.723
	Sex	Female (ref)	13.1 (12.8 to 13.3)						
		Male	13.0 (12.8 to 13.2)	-0.051	(-0.197 to 0.096)	0.494	-0.041	(-0.191 to 0.110)	0.593
	Timing of the nap	Morning (ref)	13.0 (12.7 to 13.2)						
		Afternoon	13.0 (12.9 to 13.3)	0.101	(-0.042 to 0.244)	0.165	0.098	(-0.053 to 0.249)	0.202
Duration (s)	GA		-0.169	-0.086	(-0.192 to 0.020)	0.109	-0.064	(-0.171 to 0.043)	0.240
	PMA		-0.246*	-0.086	(-0.158 to -0.015)	0.018*			
	PNA		-0.179	-0.086	(-0.185 to 0.014)	0.090	-0.068	(-0.168 to 0.033)	0.185
	Sex	Female (ref)	2.7 (2.6 to 3.5)						
		Male	3.1 (2.6 to 3.6)	0.158	(-0.104 to 0.421)	0.233	0.109	(-0.152 to 0.370)	0.409
	Timing of the nap	Morning (ref)	3.2 (2.7 to 3.7)						
		Afternoon	2.8 (2.6 to 3.4)	-0.276	(-0.530 to -0.022)	0.034*	-0.210	(-0.473 to 0.052)	0.115
Spectral Power (μV^2)[c]	GA		0.168	1.088	(0.980 to 1.206)	0.112	1.079	(0.970 to 1.198)	0.158
	PMA		0.167	1.059	(0.986 to 1.137)	0.113			
	PNA		0.072	1.034	(0.937 to 1.141)	0.500	1.010	(0.915 to 1.115)	0.839
	Sex	Female (ref)	8.9 (5.9-12.2)						
		Male	6.4 (4.3-10.3)	0.746	(0.581 to 0.958)	0.022*	0.755	(0.584 to 0.976)	0.032*
	Timing of the nap	Morning (ref)	7.2 (4.2-10.3)						
		Afternoon	8.2 (5.0-11.6)	1.119	(0.868 to 1.442)	0.380	1.052	(0.813 to 1.361)	0.697
BSI[c]	GA		0.090	1.029	(0.962 to 1.102)	0.396	1.021	(0.954 to 1.093)	0.545
	PMA		0.017	1.004	(0.958 to 1.051)	0.875			
	PNA		-0.062	0.981	(0.920 to 1.046)	0.561	0.993	(0.931 to 1.058)	0.822
	Sex	Female (ref)	0.18 (0.16-0.24)						
		Male	0.24 (0.17-0.32)	1.239	(1.054 to 1.456)	0.010*	1.242	(1.052 to 1.465)	0.011*
	Timing of the nap	Morning (ref)	0.19 (0.16-0.27)						
		Afternoon	0.22 (0.17-0.30)	1.108	(0.941 to 1.305)	0.216	1.108	(0.939 to 1.309)	0.222
Synchrony (%)	GA		-0.211*	-1.307*	(-2.583 to -0.031)	0.045*	-1.216	(-2.547 to 0.115)	0.073
	PMA		-0.144	-0.613	(-1.500 to 0.273)	0.173			
	PNA		0.001	0.004	(-1.223 to 1.231)	0.994	-0.067	(-1.313 to 1.178)	0.915
	Sex	Female (ref)	60.4 (53.4 to 64.6)						
		Male	59.1 (53.0 to 63.6)	-1.386	(-4.585 to 1.813)	0.392	-1.547	(-4.785 to 1.690)	0.345
	Timing of the nap	Morning (ref)	61.4 (54.6 to 65.0)						
		Afternoon	58.2 (52.2 to 63.7)	-1.724	(-4.868 to 1.420)	0.279	-1.047	(-4.301 to 2.206)	0.524

Number[d]	GA		0.095	5.787	(-8.203 to 19.777)	0.413	3.410	(-11.072 to 17.892)	0.640
	PMA		-0.050	-2.180	(-12.103 to 7.743)	0.663			
	PNA		-0.157	-9.078	(-22.204 to 4.048)	0.172	-9.648	(-23.303 to 4.007)	0.163
	Sex	Female (ref)	237.5 (179.0 to 305.8)						
		Male	245.0 (208.0 to 285.0)	-0.200	(-34.134 to 33.734)	0.991	-4.750	(-39.596 to 30.095)	0.787
	Timing of the nap	Morning (ref)	230.5 (192.8 to 287.3)						
		Afternoon	253.0 (200.0 to 287.0)	18.152	(-14.911 to 51.214)	0.278	17.448	(-16.737 to 51.632)	0.312
Density (spindles/min)[d]	GA		0.015	0.021	(-0.307 to 0.348)	0.900	0.006	(-0.340 to 0.351)	0.974
	PMA		-0.009	-0.009	(-0.240 to 0.223)	0.940			
	PNA		-0.025	-0.034	(-0.344 to 0.276)	0.826	-0.048	(-0.373 to 0.278)	0.771
	Sex	Female (ref)	6.9 (5.3 to 8.8)						
		Male	6.6 (5.8 to 7.9)	-0.117	(-0.908 to 0.674)	0.769	-0.142	(-0.972 to 0.689)	0.735
	Timing of the nap	Morning (ref)	6.7 (5.3 to 8.0)						
		Afternoon	6.6 (5.8 to 8.1)	0.081	(-0.696 to 0.857)	0.837	0.082	(-0.733 to 0.897)	0.842

Pearson Correlation Coefficient (r); Reference variable (ref); Females n=37; males n=54; morning n=42; afternoon n=49, unless stated otherwise. [a]from Simple Regression Analysis. [b]from Multiple Regression Analysis. [c]log-transformed prior to analysis and then back-transformed. [d]Infants who fell asleep before EEG were excluded (n total= 77; females: n=30; males: n=47; morning: n=34; afternoon: n=43). p<0.05 marked with an asterisk (*).

Table 2.5 - Univariable and multivariable linear regression analysis of the sleep macrostructure features on gestational, postmenstrual and postnatal age, sex and timing of the nap.

Dependent Variable	Independent Variable		Univariable analysis (n=68)				Multivariable analysis (n=68)		
			r/median (IQR)	B	95% CI	p-value[a]	B	95% CI	p-value[b]
Time in NREM (min)[c]	GA		0.029	1.006	(0.955 to 1.060)	0.814	1.003	(0.951 to 1.059)	0.905
	PMA		-0.095	0.986	(0.951 to 1.022)	0.443			
	PNA		-0.166	0.965	(0.917 to 1.016)	0.177	0.966	(0.915 to 1.019)	0.197
	Sex	Female (ref)	36.5 (26.0-40.5)						
		Male	34.5 (30.5-42.8)	1.041	(0.916 to 1.184)	0.533	1.025	(0.899 to 1.170)	0.707
	Timing of the nap	Morning (ref)	34.0 (29.5-39.5)						
		Afternoon	36.0 (30.2-43.0)	1.051	(0.928 to 1.192)	0.429	1.055	(0.926 to 1.201)	0.414
Time in REM (min)	GA		-0.030	-0.157	(-1.436 to 1.122)	0.807	-0.181	(-1.522 to 1.161)	0.789
	PMA		0.055	0.199	(-0.695 to 1.094)	0.658			
	PNA		0.109	0.571	(-0.710 to 1.852)	0.377	0.531	(-0.804 to 1.866)	0.430
	Sex	Female (ref)	7.5 (4.4 to 13.6)						
		Male	6.2 (2.9 to 11.5)	-0.870	(-4.039 to 2.299)	0.585	-0.674	(-3.976 to 2.628)	0.685
	Timing of the nap	Morning (ref)	7.5 (4.5 to 11.5)						
		Afternoon	6.0 (3.5 to 11.5)	-0.218	(-3.317 to 2.881)	0.889	-0.198	(-3.436 to 3.041)	0.903
%REM	GA		-0.014	-0.118	(-2.196 to 1.960)	0.910	-0.053	(-2.193 to 2.087)	0.961
	PMA		0.104	0.617	(-0.830 to 2.064)	0.397			
	PNA		0.164	1.397	(-0.667 to 3.461)	0.181	1.284	(-0.847 to 3.414)	0.233
	Sex	Female (ref)	17.7 (10.7 to 29.1)						
		Male	17.2 (7.5 to 25.8)	-2.899	(-8.008 to 2.209)	0.261	-2.315	(-7.583 to 2.954)	0.383
	Timing of the nap	Morning (ref)	18.0 (10.7 to 28.0)						
		Afternoon	17.1 (7.9 to 26.9)	-1.970	(-6.979 to 3.039)	0.435	-2.120	(-7.288 to 3.047)	0.415
Cycle duration (min)[c]	GA		0.021	1.004	(0.953 to 1.058)	0.866	1.002	(0.949 to 1.059)	0.937
	PMA		-0.042	0.994	(0.958 to 1.031)	0.735			
	PNA		-0.081	0.983	(0.932 to 1.036)	0.511	0.982	(0.930 to 1.037)	0.506
	Sex	Female (ref)	44.0 (35.1-52.4)						
		Male	41.8 (37.4-51.1)	1.007	(0.884 to 1.146)	0.920	0.999	(0.872 to 1.143)	0.983
	Timing of the nap	Morning (ref)	43.5 (36.5-51.0)						
		Afternoon	42.5 (37.5-51.8)	1.028	(0.906 to 1.166)	0.663	1.030	(0.902 to 1.176)	0.660
Latency to Sleep (min)[c]	GA		0.071	1.062	(0.863 to 1.306)	0.566	1.016	(0.821 to 1.258)	0.880
	PMA		0.094	1.057	(0.915 to 1.221)	0.448			
	PNA		0.063	1.055	(0.857 to 1.300)	0.608	1.028	(0.831 to 1.272)	0.795
	Sex	Female (ref)	8.8 (3.5-23.9)						
		Male	7.5 (4.7-13.6)	0.800	(0.479 to 1.334)	0.387	0.810	(0.479 to 1.372)	0.428
	Timing of the nap	Morning (ref)	7.0 (4.0-12.0)						
		Afternoon	9.0 (3.5-23.2)	1.470	(0.897 to 2.406)	0.124	1.453	(0.867 to 2.434)	0.153

Latency to REM (min)[c,d]	GA		0.133	1.032	(0.973 to 1.095)	0.289	1.026	(0.965 to 1.092)	0.404
	PMA		-0.010	0.998	(0.958 to 1.041)	0.936			
	PNA		-0.147	0.966	(0.910 to 1.024)	0.239	0.963	(0.907 to 1.024)	0.222
	Sex	Female (ref)	36.8 (28.9-42.0)						
		Male	35.0 (30.6-40.8)	1.010	(0.871 to 1.170)	0.898	1.000	(0.860 to 1.161)	0.996
	Timing of the nap	Morning (ref)	35.0 (29.0-40.0)						
		Afternoon	36.5 (30.5-43.5)	1.069	(0.927 to 1.234)	0.353	1.060	(0.913 to 1.231)	0.436

Pearson Correlation Coefficient (r); Reference variable (ref); Females n=26; males n=42; morning n=31; afternoon n=37. [a]from Simple Regression Analysis. [b]from Multiple Regression Analysis. [c]log-transformed prior to analysis and then back-transformed. [d]Infants who did not have a complete sleep cycle recorded or meet criteria for staging REM before N2 were excluded (n total=66; females: n=26; males: n=40, morning: n=31; afternoon: n=35).

Table 2.6 - Comparison of macrostructure and spindle parameters' values from 1st to 2nd sleep cycles.

	Median (IQR)				p-value[a]
	1st cycle		2nd cycle		
Macrostructure Analysis (n=11)[b]					
Time in NREM (min)	28	(20.0-36.0)	30	(27.5-37.0)	0.285
Time in REM (min)	10.5	(4.5-14.5)	7.5	(3.5-12.5)	0.533
%REM	29.7	(13.8-33.0)	16.7	(11.6-29.4)	0.213
Cycle duration (min)	37	(29.0-43.0)	40.5	(34.5-45.0)	0.929
Latency to REM (min)	28.5	(21.5-36)	31	(27.5-37.5)	0.213
Sleep spindle Analysis (n=29)					
Frequency (Hz)	13	(12.8-13.2)	13	(12.8-13.3)	0.689
Duration (s)	2.9	(2.6-3.6)	2.9	(2.7-3.4)	0.496
Spectral power (μV^2)	8.2	(4.9-14.5)	6.4	(4.4-12.2)	<0.001*
Brain Symmetry Index	0.18	(0.16-0.26)	0.19	(0.16-0.26)	0.627
Synchrony (%)	58.2	(52.6-61.6)	57.2	(52.6-61.6)	0.358
Number[c]	227	(174.2-246.5)	237.5	(170.0-302.5)	0.200
Density[c] (spindles/min)	6.4	(5.4-7.6)	7.6	(6.1-9.2)	0.082

[a] From Wilcoxon test for paired samples. [b] Infants who did not have two complete sleep cycles recorded were excluded.[c]n=22 due to additional exclusion of infants who fell asleep during electrode placement. p<0.05 marked with an asterisk (*).

2.4. Discussion

Sleep macrostructure and sleep spindle parameters were described in the largest cohort to date of infants from 4 to 5 months of age. Sleep spindle spectral power decreased from the first to the second sleep cycle. From multivariable analysis it is noted that two spindle features differed by sex: spindle spectral power and symmetry; There were no significant correlations between spindle features and time of recording (am/pm), gestational and chronological age. No association were observed between sleep macrostructure analysis and sex, time of recording (am/pm), gestational and chronological age. This data serves as baseline normative values, which can be used in future studies to compare with infants at risk of abnormal neurodevelopment.

2.4.1. Normative values for sleep macrostructure and sleep spindles at 4 to 5 months and their maturational evolution

Around the 44th week of PMA, sleep onset starts shifting from REM to NREM sleep (168). In this study, only two infants reached REM sleep before reaching N2. As reviewed by Weerd and Bosshe (440), this is in keeping with other studies that did note a complete REM onset of sleep in some infants of this age group. Schulz *et al*. hypothesised that increased latency to REM in infancy is dependent on through the variation of the body temperature with the circadian rhythm and reduction of sleep fragmentation (431).

In the present study, the median (IQR) sleep latency was 8.5 (4.0–14.0) min [mean (SD) of 12.7 (15.8) min]. This latency is shorter in comparison with studies based on parent perception (441, 442). The present results for sleep latency are similar to other studies when compared using the mean instead of the median (I opted to use the median as sleep latency data has a skewed distribution). Considering the high standard deviation of the latency to sleep in the discussed papers and of the present study, it is clear that there is a high variance in sleep latencies for this age group which may further explain the different results. Additionally, differences in sleep latency may be explained by the use of polysomnographic recordings in the present analysis which provide a more accurate and objective measure of sleep onset, with all recordings being carried out in a temperature controlled, low noise sleep lab. At the same time, for my research feeding was encouraged to improve the infant experience and tolerability during electrode placement but it may have had a soothing effect that increased the infants' predisposition to fall asleep, even before turning off lights and suspension of other stimuli. In fact, 14 infants fell asleep during the lights-on period while electrodes were being placed.

To date, the majority of studies seeking to characterise the evolution of sleep spindle parameters during the first year of life have included only small cohorts of infants. Nevertheless, these pioneer studies allow insight into the trends and values of different sleep spindle parameters during this neurodevelopmentally rich phase of life. During the first 6 months, the increasingly dominant sleep spindle frequencies range from 12.0 to 14.0Hz (226). This finding is in accordance with the present results, in which the median sleep spindle frequency was 13.0Hz with a narrow interquartile range of 12.8 to 13.3Hz, suggesting it is well

conserved across infants. In contrast, spindles of slower rhythms (<12Hz) become significantly less common across the same period (226). Particularly after the first year, and at least, until reaching the 4-year mark, slow spindles reverse this tendency and increase in density (343). It is widely accepted that two individual types of sleep spindles develop during childhood; fast spindles over more centroparietal areas and slow spindles over more anterior areas, that have different dynamics (247, 443) and subserve different functions (see for example (302, 424)). In the present study, the fronto-central channels were chosen to manually annotate the sleep spindles due to their predominance in this area on visual inspection of the EEG. However, if two sleep spindle types with distinct frequencies were present in these EEGs, it would be expected some level of intrusion of fast sleep spindles through central electrodes and these two distinct frequency peaks on these channels would be detectable. However, the histogram of the distribution of mean frequencies of each spindle (Figure 2.3), does not show obvious evidence of a second peak. The infants' young age may explain the absence of a clear double peak and thus, its presence later in life may constitute a marker of neurodevelopment. This finding is consistent with previous observations which suggest that the two types of sleep spindles do not co-exist before the 1st year of life (335, 343). Nevertheless, it remains a possibility that the two potential peaks were concealed by intra-individual differences, driven by plotting N2 and N3 spindles simultaneously, and inter-individual differences in frequency (238, 443) and further investigation on this matter may be required.

In the first four years, sleep spindle density is highest between 4 to 12 months (343). Sleep spindle synchrony and asynchrony values fluctuate during the first year with the highest sleep spindle asynchrony in earlier infancy (226, 368). Symmetry is expected to be lowest between 4 to 5 months of age (226). I identified two previous studies addressing spindle synchrony and one on spindle symmetry in infants (226, 368). However, in the present study, I used different methods to calculate symmetry and synchrony to these studies and my results may not be directly comparable. In the former studies, spindles were considered asymmetrical if one of the spindles were twice the amplitude of the contralateral side and were considered asynchronous if two contralateral spindle mid-points were 2 seconds apart. I used the Brain Symmetry Index which has been used before in adults (444) and I consider it to be a more accurate metric of spindle symmetry than the binary system used previously in infants. I also considered the percentage of coincidental contralateral spindles less arbitrary than the 2-second rule for the synchrony calculation. Along with asymmetry, from infancy to

approximately two years of age, spindle duration was reported to reach its maximum between 3 and 5 months of age, decreasing thereafter (226, 368, 369). In keeping with these findings, my thesis showed a weak but statistically significant association between spindle duration and PMA with a decrease in the short period of time from 55+1 to 63 weeks PMA (Table 2.4), for each week of PMA, there is a decrease of 0.09s in the spindle duration (p=0.018). However, after controlling for sex and timing of recording, (results not included) correlations lost statistical significance.

I also found that interhemispheric synchrony of sleep spindles was weakly correlated with gestational age (Table 2.4) with synchrony decreasing 1.31% for each increment of a week on the GA at birth (Table 2.4). However, this correlation was lost after correcting for postnatal age, sex and time of recording.

2.4.2. Relationship between sex and sleep parameters

From simple linear regression analysis, spectral power was 25.4% lower (p=0.022) and BSI 23.9% (p=0.010) higher in males. These values changed to 24.5% (p=0.032) lower and 24.2% (p=0.011) higher in males respectively after controlling for GA, PNA, sex and timing of the nap (Table 2.4). Sex differences in some sleep spindle parameters have previously been directly (358) and indirectly observed, both before (239) and after puberty (341) in older cohorts. Fast spindle density is higher in female cohorts (358) and may be partly related to menstrual cycle (445). Also, the evolution of sleep spindle frequency and density across ageing is different for both sexes (363). To my knowledge, this is the first time that sex differences have been reported in sleep spindle power and spindle symmetry in infancy. These differences at this young age may be driven by different hormonal (446), and brain connectivity profiles (447). In light of these findings, future studies should consider sex differences when studying sleep spindle power and symmetry properties in infants. No sex differences were found in the sleep macrostructure parameters.

2.4.3. Analysis of the relationship between sleep pressure and sleep time, and sleep features

I compared the sleep macrostructure and sleep spindle parameters between the morning and the afternoon recordings to determine if the time of recordings and circadian rhythm influences sleep patterns during daytime. Results from univariable analysis indicated that spindle duration was 0.28 seconds shorter in the afternoon (p=0.034) but, after correcting for sex, gestational and postnatal age, the correlation was no longer significant (Table 2.4). Also, no differences were found in sleep macrostructure (Table 2.5). As expected, the proportions of REM/NREM diurnal sleep in this chapter were smaller than those reported during night sleep, although the duration of a typical sleep cycle is similar (430). This nocturnal/diurnal contrast in REM and NREM sleep proportions has been described previously not only in adults but also in infants by Louis *et al.* (432) and might be at least partially related to the oscillation of body temperature across the day (448) (for a review (449)).

Similarly, although some macrostructural sleep elements are expected to change across sleep (449), no changes from the first to second sleep cycle were observed in this study which might be due to the narrow sleep time-frame of the analysis (Table 2.6). Adult studies have shown sleep spindles to be sensitive to reductions in sleep pressure through consecutive sleep cycles (241, 358, 363) and increases following sleep deprivation (243, 360, 366). In my thesis (Table 2.6), one sleep spindle parameter, spectral power, decreased in response to a decrease in sleep pressure from the first to the second NREM period. This contrasts with adult studies that found an increase in the spindle related spectral power concurrent with a decrease in delta activity across sleep cycles (360, 450). There is scarcity of studies investigating spindle spectral power across consecutive sleep cycles in infants. Louis *et al*. showed no significant changes in amplitude across cycles in the 12 infants studied(226) and *Jenni et al.* reported trend of alternating sigma power across cycles; However, statistically significant differences were only between the second and third cycles in 11 6-months infants(451). Hence, this is an area that warrants further research in infancy.

2.4.4. Strengths

I used polysomnography in this study, which is considered the gold-standard for sleep studies; additionally, good spatial coverage was assured by the use of 21 active electrodes. Recording in the sleep laboratory allowed for a standardised environment and control for factors such as light, noise and temperature. A clinical physiologist was present at all times during the recording to address any technical problems.

All infants had a Griffiths III assessment performed by certified clinical neuropsychologist/ paediatricians at 18-months, ensuring that the infants included in this study had no neurodevelopmental impairment.

Spindles were identified as discrete features and selected for analysis manually by an experienced clinical physiologist which would be considered the gold standard for spindle detection and allowed correct detection of the spindle segments, as opposed to spectral power analysis of sigma frequencies or more rudimentary spindle-detection algorithms. High agreement scores were obtained when comparing a subset of these EEGs with the markings from a research nurse experienced in EEG.

The present study included a large cohort of infants and described the sleep macrostructure at 4 to 5 months of age and is the biggest infant cohort reported to date describing sleep spindles and this will allow comparison with futures studies on maturation and neurodevelopment.

This chapter resulted in the detailed description of the sleep spindles using parameters which generally are not studied together: frequency, duration, spectral power, brain symmetry index, synchrony, number and density, permitting a broader picture of the several elements that describe sleep spindles. To my knowledge, this was also the first study to report sex differences in sleep spindle parameters in infancy and future studies relating sleep spindles to neurodevelopmental pathologies will have to take account of those differences.

Spindles result from complex interactions between the thalamus and the cortex; they depend on the numerical extent of cortical neurons involved as well as the cortical layers involved and pathways (255). Surface EEG, in turn, is only able to detect post-synaptic potentials from the most superficial region of the cortex. Detection of potentials is also

dependent on the position of the neurons, as biological dipoles, on the cortical gyri, and the number of synchronised firing neurons (452). As a result, only a fraction of the spindle oscillations generated are indirectly detectable by this technique. Nevertheless, the study of spindles with EEG is an accessible, real-time, non-invasive technique with promising results reflecting cognitive abilities (239, 240, 308, 331, 341) and neurologic conditions (380, 391, 425-428).

2.4.5. Limitations

There are a number of limitations to my study. First, the fact that some of the infants fell asleep during the trip to the sleep lab was not controlled; effects of a short nap during the car journey may have affected sleep pressure; as seen in Table 2.6, sleep spindle power change from the first to the second cycle. For this reason, a possible reduction of sleep pressure resulting from a nap during the trip to the INFANT centre sleep lab may have affected sleep spindle power.

Although sleep spindle identification kappa scores were high between two raters, sleep staging was only performed by one expert rater and this may constitute a limitation to this analysis.

Despite being the biggest cohort of infants reported to date which provides a detailed analysis of sleep spindles, the number of infants may still be a limitation for group comparisons by sex and time of the day and particularly for comparisons between the first and second sleep cycle. Given the sample size, only medium/large effect sizes could be detected.

It was only possible to record one polysomnography per infant. Due to this, there was no previous infant adaptation to the laboratory. Results from first recording sessions may differ from results from other consecutive sessions (196). Regarding sleep spindles, it is accepted that, based on the EEG spectra, they are fairly constant and represent an individual signature (238). In a study of twelve adolescents, not all parameters presented good intra-individual reliability with one single recording; those parameters were slow spindle density and slow spindle duration (453). There is no study so far on the intra-individual reliability of sleep spindles in infants. However, considering that only slow sleep spindles showed lower

intra-individual reliability in adolescents (453) and that these type of spindles tend to appear towards toddlerhood (343), I do not believe this had a major impact on the present sleep spindle analysis.

To conclude, this chapter characterises normative values for daytime sleep macrostructure and sleep spindles from 4 to 5 months of age. Spindle spectral power decreased from the first to the second sleep cycles. Sex differences in the sleep spindle spectral power and symmetry were the only independent variables studied from the 1st sleep cycle that remained statistically significant after controlling for gestational and postnatal age at the recording, and time of nap. For this reason, sex differences should be taken into consideration in future studies in this age group. These values may provide a benchmark for future studies focusing on early recognition of risk of atypical neurodevelopmental trajectory.

Chapter 3. Parent-led massage and sleep EEG for term-born infants

A randomised controlled parallel-group study

3.1. Introduction

Early infancy is a period of intense learning and development including the processes of neural proliferation, differentiation, migration, myelination, and circuit formation(8). During this crucial time, we may expect to see the most marked effects of environment-dependent enrichment. Environmental enrichment occurs when stimuli are introduced into the environment, within the appropriate timeframe, positively affecting development through epigenetics and neural plasticity (66). Infant massage can be considered an environmental enrichment as it involves tactile stimulation and social bonding (59). Studies suggest that infant massage may counterbalance some negative outcomes resulting from adverse events in early life (454), and accelerate preterm neurodevelopment (58, 59, 455), although some changes fade over the second half of infancy (59). However, much remains unknown about the possible benefits of infant massage on the neurodevelopment of term-born, low-risk infants.

A considerable part of infant life is spent in sleep, with term-born infants sleeping more than two-thirds of the day. Important sleep transitions related to maturation occur during infancy. For most infants, by 4 months of age, sleep onset no longer starts in REM but in NREM and sleep stages become more mature, with three different NREM stages being readily identifiable. Sleep spindles are well established by 4 months of age as a result of thalamic and cortical interactions and their features reflect processes of ageing, memory consolidation, and cognitive skills (456). In addition, other sleep EEG features such as EEG power spectrum characteristics also evolve with ageing, particularly in early life, and these changes are partly associated with neurodevelopment (413).

The primary aim of this study was to explore the impact of massage on infant neurodevelopment using EEG assessment of sleep parameters such as sleep macrostructure, sleep spindles, and qEEG.

3.2. Method

3.2.1. Participants

Neonates were recruited post-delivery at Cork University Maternity Hospital, Cork, Ireland, during 2017 to 2018 to the BabySMART study (Study of Massage Therapy, Sleep And neurodevelopMenT, registered identifier NCT03381027), a randomised controlled parallel-group trial to evaluate the impact of massage intervention on infant neurodevelopment. The study was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Cork, Ireland. Inclusion criteria were gestational age greater than or equal to 37 weeks and singleton birth; exclusion criteria included admission to a neonatal intensive care unit, and suspected congenital or metabolic anomalies. Written informed consent was obtained from all parents or guardians. Infants were randomly assigned (1:1) to the intervention (daily massage) group or the control (routine care) group. Block randomisation (with varying block sizes of two, four, and six) was used to allocate infants to each group. The randomisation list was generated by a biostatistician using the 'ralloc' procedure in Stata. Sequentially numbered, opaque, sealed envelopes ensured concealment. Owing to the nature of the intervention, the recruiting research personnel, clinical team, and families of the infants were aware of group allocation. EEG recordings, neurodevelopmental assessments, and statistical analysis were performed blinded to group allocation.

3.2.2. Massage

A massage training session (Figure 3.1) of about 45 minutes at the 2-week appointment and optional review classes during the study were provided to parents in the intervention group by a physiotherapist, certified by the International Association of Infant Massage (massage instructions booklet in Appendix 5). From 2 weeks of age until the 4-month appointment, parents were asked to perform a massage routine three times daily for at least 15 minutes on at least two body areas and to keep a massage diary. The parents were given 'Johnson's® Head-to-Toe®" extra moisturizing baby cream' and parents were encouraged to

use it during the infant massage but could use other products if they preferred. The control group were requested to use standard care throughout the study.



Figure 3.1 - INFANT Centre premises allocated to teaching the massage protocol to the intervention groups.

3.2.3. Assessments

The EEG recording technique has been described in detail in Ventura *et al.* (207) Briefly, at 4 months, EEGs were recorded using the Lifelines EEG (Lifelines Neuro, UK) acquisition system. An EEG was recorded using disposable electrodes placed according the 10–20 system at positions Fp1, Fp2, Fz, F3, F4, F7, F8, Cz, C3, C4, T3, T4, Pz, P3, P4, T5, T6, O1, O2, A1 and A2, plus reference, and ground with impedances less than 10kΩ. Extra channels included chin electromyography, electrooculogram, electrocardiogram, and a movement sensor to monitor respiration. The recording started as soon as all the electrodes were applied and ended when the infant was fully awake. During acquisition, quality of the recording was monitored using 24 bipolar EEG channels in a modified double-banana montage and 5 extra-EEG channels. The appointments coincided with their normal diurnal nap time to increase the likelihood of sleep and were performed in the INFANT Research Centre sleep laboratory to reduce the influence of external stimuli.

Post-acquisition, sleep staging was performed according to American Academy of Sleep Medicine 2.4 definitions and rules (121) using a sleep staging application (Nicolet, Natus,

USA); sleep spindles were annotated over left and right fronto-central regions with StratusEEG (Kvikna, Iceland) software. Both sleep stages and sleep spindles were identified manually by SV. All analyses were restricted to the first sleep cycle to avoid potential effects of decreasing sleep pressure on sleep features in subsequent sleep cycles. Parameters in the analysis included sleep spindles, sleep macrostructure derived from the sleep staging data such as sleep stages duration and latencies, and qEEG features, a set of objective biomarkers that capture key characteristics of the background EEG pattern.

Infants who fell asleep during electrode placement were excluded from sleep spindle number and density calculations because of a potential for missing spindles. Analysis of macrostructure features, except total sleep time, and qEEG was only performed for infants who had a complete first sleep cycle recorded. Within infants with a complete sleep cycle, those who had REM sleep before reaching criteria for staging N2 were excluded from the latency to REM analysis.

Spindle number and duration were calculated for each infant. Spindle density was defined as the number of sleep spindles per minute of NREM sleep. Mean frequency, brain symmetry index (437), synchrony, and spectral power of sleep spindles were calculated using MATLAB (version R2020a, The MathWorks Inc., Natick, MA, USA). Spindle brain symmetry index was determined per epoch of 30 seconds using a sliding window with 75% overlap and an additional 9Hz to 16Hz passband filter to reduce the background activity present during a sleep spindle event. Synchrony calculation was based on the percentage of coincidental contralateral sleep spindles (207).

Owing to the complexity of the EEG it was not possible to fully represent all EEGs by a finite set of features (457). Instead, I characterised salient attributes of this complex, time-varying, multi-dimensional signal by generating multiple features that captured different overarching characteristics of the EEG. These features, generated using an open-source MATLAB toolbox (NEURAL version 0.4.3) (195), were divided into four groups: EEG magnitude, spectral distribution, continuity, and connectivity. Each group contained multiple features, described as follows.

The EEG magnitude group included features of spectral power and rEEG. Spectral power quantified EEG power in different frequency bands and rEEG quantified peak-to-peak amplitude. Four features were extracted from the rEEG: the standard deviation (SD), median, and the 5th and 95th centiles of the rEEG; these last two are known as the lower (5th) and

upper (95th) margins. The spectral distribution group captures frequency characteristics of the EEG which are independent of amplitude and power. This group included fractal dimension, an estimate of the slope of the power spectrum; spectral flatness, a measure of disorder (entropy) of the spectra; and spectral difference, a measure of change of the spectra over time. The continuity group captured deviations from a continuous EEG activity pattern. Features in this group included the skewness and kurtosis of the EEG and the rEEG asymmetry measure. The last group included measures of interhemispherical connectivity: brain symmetry index, a measure to quantify differences in spectral power across the hemispheres; and interhemispherical coherence, a measure of coupling calculated across bilateral electrode pairs.

Where applicable, features were further subdivided into a standard set of frequency bands: delta 1, 0.5Hz to 2Hz; delta 2, 2Hz to 4Hz; theta, 4Hz to 8Hz; alpha, 8Hz to 12Hz; sigma, 12Hz to 15Hz; beta, 15Hz to 30Hz; and gamma, 30Hz to 45Hz. Fractal dimension was calculated across the full bandwidth (0.5–45Hz) and rEEG was pre-filtered to the 1Hz to 20Hz bandwidth, as detailed elsewhere (195). All features were calculated for the different sleep stages. qEEG and spindle features estimated on short-time segments were summarised by a median value over time.

Infant neurodevelopmental outcome was assessed by a trained psychologist at 4 months and 18 months of age respectively using the Griffiths Scales of Child Development, Third Edition. The Griffiths Scales of Child Development, Third Edition measures overall general development (GD) and five neurodevelopmental areas: foundations of learning (A), language and communication (B), eye and hand coordination (C), personal–social–emotional (D), and gross motor abilities (E), with lower scores indicating poorer neurodevelopment. Sociodemographic questionnaires were given to the parents to complete at 2-week, 4-month, and 18-month appointments (data of the 18-month questionnaire not shown). At 4 months mothers also completed the Maternal Postnatal Attachment Scale, a 19-item questionnaire with possible scores ranging from 19 to 95, the latter score indicating the greatest maternal attachment (458) and Edinburgh Postnatal Depression Scale, a 10-item questionnaire with possible scores ranging from 0 to 30, the latter score indicating greatest likelihood of depression (459).

3.2.4. Statistical analysis

For statistical analysis I used IBM SPSS Statistics (version 26.0, IBM Corp., Armonk, NY, USA). Continuous variables were described using median and interquartile range (IQR), and categorical variables using frequency and percentage. For comparisons between groups, a Mann–Whitney *U* test was used for continuous variables and a χ^2 test (with continuity correction for 2 × 2 tables) or Fisher's exact test (in the case of small expected counts) for categorical variables. All tests were two-sided and $p < 0.05$ was considered statistically significant.

The subgroup analysis consisted of the comparison between regularly massaged infants in the intervention group and never-massaged infants in the control group. Participants in the intervention group were considered to massage regularly if the infant received at least two massage sessions, that lasted at least 5 minutes, over at least 4 days within each week for at least 10 weeks from week 4 to the end of week 16. Never-massaged control infants were identified on the basis of questionnaire responses from the parents.

I did not adjust for multiple comparisons in the analysis owing to the exploratory nature of this study and because reducing the probability of type I error would increase the probability of type II error (460, 461).

An a priori sample size calculation indicated that a sample of 150 infants (75 per group) was necessary for an independent-samples *t*-test to detect a difference of 6% in mean active sleep between the intervention and control groups, assuming a mean of 52% in the control group, a standard deviation of 13% (462), a power of 80%, a level of significance of 0.05, and a two-tailed test.

3.3. Results

3.3.1. Demographics

A total of 408 newborn infants were consented to the study and randomised to intervention and control groups (n = 204 per group). At 4 months of age, 84 infants remained in the intervention group and 98 in the control group. Three infants were excluded from the

analysis: two females, one from each group, who did not sleep during the EEG, and a male infant in the control group who had a grossly abnormal EEG and was referred for further clinical assessment. Hence, 179 infants were included in the main analysis (83 from the intervention and 96 from the control group) (Supplementary Figure 1 – Appendix 6). Demographic variables were similar between groups (Table 3.1). To assess the risk of bias, I compared demographic characteristics provided at registration between (1) infants included (n = 179) and those excluded from the analyses (n = 229) and (2) within those excluded, infants allocated to intervention (n = 121) and control (n = 108) groups (Supplementary Tables 1 and 2 – Appendix 7). Maternal age was significantly lower in the excluded group than in the included group (mean 32 years 4 months [SD 5 years 3 months] vs 34 years 11 months [3 years 12 months], $p < 0.001$). No other statistically significant differences were observed. For the subgroup analyses, 33 regularly massaged infants in the intervention group and 57 never-massaged control infants were included (Supplementary Figure 2 – Appendix 6). By 18 months, six infants were lost to follow-up. Hence, for the comparison of neurodevelopmental outcome at 18 months, 173 infants were included in the main analysis (81 from the intervention and 92 from the control group) and 89 infants were included in the subgroup analysis (33 from the intervention and 56 from the control group).

3.3.2. Sleep analyses

For those who had sleep onset recorded (n = 156), median (IQR) total sleep time was 49.8 minutes (39.1–71.4) with no significant differences between groups; the intervention group slept 50.5 minutes (40.0–68.0), n = 75; the control group 49.0 minutes (37.8–72.5), n = 81 (p = 0.76). Similarly, in the subgroup analysis, there were no statistically significant differences (p = 0.68) between total sleep time of regularly massaged intervention (50.0 minutes [42.6–62.4], n = 32) and never-massaged control infants (50.0 minutes [37.0–72.0], n = 47).

There were no statistically significant differences in any of the sleep macrostructure parameters studied (Table 3.2). For sleep spindle parameters, the spectral power was significantly different between groups, being higher in the intervention group (intervention:

8.89μV^2 [6.46–12.70], n = 83; control: 7.82μV^2 [4.75–11.40], n = 96; p = 0.04) (Table 3.2). In the subgroup analysis, spectral power remained significantly higher in the intervention group with greater differences between groups (intervention: 10.09μV^2 [6.89–13.67], n = 33; control: 7.86μV^2 [5.01–10.77], n = 57; p = 0.03). Similar to the main analysis, sleep macrostructure and other sleep spindle features were not statistically different.

Table 3.1 - Demographic data.

Demographics	Intervention			Control			p-value[b]
	n	*n* (%)[a]		*n*	*n* (%)[a]		
Registration and 2-week questionnaire							
Gestational age (weeks), median (IQR)	83	39.9	(39.0–40.9)	96	39.9	(38.9–41.0)	0.68
Birthweight (kg), median (IQR)	83	3.5	(3.2–3.8)	96	3.6	(3.3–3.8)	0.40
Sex, male	83	45	(54)	96	56	(58)	0.69[c]
Maternal age (years:months), median (IQR)	83	35:6	(32:4–37:8)	96	35:1	(32:9–37:7)	0.88
Maternal level of education	83			96			0.13[e]
Secondary school		2	(2)		7	(7)	
Third level certificate or diploma		19	(23)		17	(18)	
Degree or higher/graduate diploma		41	(49)		37	(39)	
Postgraduate		21	(25)		35	(36)	
Parents' annual net income after taxes	83			96			0.76[d]
€30 000 or less		5	(6)		8	(8)	
€30 001–€60 000		20	(24)		19	(20)	
€60 001 or above		37	(45)		40	(42)	
Prefer not to say/do not know		21	(25)		29	(30)	
4-month appointment and 4-month questionnaire							
Timing of recording, morning[f]	83	45	(54)	96	45	(47)	0.41[c]
Chronological age (weeks), median (IQR)	83	19.3	(18.3–20.4)	96	19.4	(18.6–20.4)	0.79
Weight (kg), median (IQR)	83	6.9	(6.5–7.6)	96	7.1	(6.4–7.8)	0.50
Type of feeding	80			96			0.51[d]
Breast fed		29	(36)		29	(30)	
Infant formula		38	(48)		54	(56)	
Both		13	(16)		13	(14)	
Fed on a regular schedule	80			96			0.60[d]
Yes, always		11	(14)		13	(14)	
Yes, try to		26	(33)		38	(40)	
No, fed on demand		43	(54)		45	(47)	
Duration of dermatosis with pruritus, if present	9			18			1.00[e]
Less than 6 weeks		5	(56)		9	(50)	
Between 6 weeks and less than 3 months		4	(44)		8	(44)	
Between 3 and 4 months		0	(0)		1	(6)	
Maternal EPDS, likelihood of depression	80			96			0.88[e]
Low (scored 0–9)		70	(88)		85	(89)	
Average (scored 10–12)		6	(8)		8	(8)	
High (scored >12)		4	(5)		3	(5)	
Maternal attachment score, median (IQR)	80	86.9	(83.0–90.5)	96	86.9	(82.7–90.5)	0.90

Descriptive statistics using median and interquartile range (IQR) or n (%). [a]Unless otherwise stated. [b]From Mann–Whitney U test unless otherwise stated. [c]From χ^2 test with continuity correction. [d]From Pearson's χ^2 test. [e]From Fisher's exact test. [f]Recording performed in morning or afternoon. Abbreviation: EPDS, Edinburgh Postnatal Depression Scale scores.

Table 3.2 - Sleep macrostructure and sleep spindle measures during the first sleep cycle, main and subgroup analysis.

Sleep parameters	Intervention			Control			p-value[a]
	n	Median (IQR)		*n*	Median (IQR)		
Main analysis							
Sleep macrostructure							
1st sleep cycle duration (minutes)	67	43.00	(38.50–50.50)	72	43.25	(37.00–51.88)	0.78
N1 (minutes)	67	8.00	(5.00–11.50)	72	7.75	(4.50–13.38)	0.66
N2 (minutes)	67	5.00	(3.00–8.00)	72	4.50	(2.50–6.50)	0.48
N3 (minutes)	67	21.00	(15.00–25.00)	72	21.50	(16.63–25.38)	0.47
NREM total (minutes)	67	33.50	(28.00–39.00)	72	35.50	(30.13–40.50)	0.29
REM (minutes)	67	9.00	(5.00–12.50)	72	6.50	(3.63–11.50)	0.24
REM (%)	67	21.18	(13.16–28.57)	72	17.44	(9.49–27.70)	0.15
Latency to sleep (minutes)	67	9.00	(5.00–15.00)	72	8.50	(4.00–14.00)	0.85
Latency to REM[b] (minutes)	62	34.75	(28.00–41.00)	70	36.00	(30.50–41.63)	0.36
Sleep spindles							
Frequency (Hz)	83	12.99	(12.80–13.24)	96	13.02	(12.79–13.27)	0.78
Duration (s)	83	3.17	(2.68–3.54)	96	2.92	(2.60–3.58)	0.27
Spectral power (μV^2)	83	8.89	(6.46–12.70)	96	7.82	(4.75–11.40)	0.04*
Brain symmetry index	83	0.20	(0.17–0.25)	96	0.20	(0.16–0.29)	0.70
Synchrony (%)	83	59.44	(53.80–63.82)	96	59.21	(53.19–63.80)	0.52
Number[c]	75	242.0	(185.0–282.0)	81	244.0	(194.0–286.5)	0.59
Density[c] (spindles/minute)	75	6.67	(5.53–8.05)	81	6.72	(5.79–8.02)	0.73
Subgroup analysis							
Sleep macrostructure							
1st sleep cycle duration (minutes)	29	44.00	(39.50–50.50)	41	45.00	(37.50–53.25)	0.80
N1 (minutes)	29	8.00	(5.25–12.25)	41	8.50	(5.50–15.50)	0.47
N2 (minutes)	29	5.50	(2.75–8.75)	41	5.00	(2.75–6.50)	0.38
N3 (minutes)	29	22.50	(14.00–26.25)	41	22.00	(16.50–25.00)	0.87
NREM total (minutes)	29	37.00	(30.50–42.75)	41	36.00	(30.50–45.75)	0.97
REM (minutes)	29	7.50	(4.75–12.25)	41	7.50	(4.50–12.25)	0.89
REM (%)	29	18.27	(12.52–26.07)	41	17.74	(10.71–26.96)	1.00
Latency to sleep (minutes)	29	9.50	(5.50–18.00)	41	9.00	(3.75–16.75)	0.59
Latency to REM[b] (minutes)	26	37.00	(31.13–44.50)	41	37.00	(31.50–44.00)	0.86
Sleep spindles							
Frequency (Hz)	33	13.00	(12.68–13.15)	57	13.02	(12.81–13.27)	0.21
Duration (s)	33	3.07	(2.67–3.50)	57	2.84	(2.55–3.56)	0.70
Spectral power (μV^2)	33	10.09	(6.89–13.67)	57	7.86	(5.01–10.77)	0.03*
Brain symmetry index	33	0.21	(0.18–0.28)	57	0.19	(0.16–0.26)	0.09
Synchrony (%)	33	59.14	(53.44–62.93)	57	59.27	(54.69–64.02)	0.59
Number[c]	32	249.5	(187.3–297.0)	47	253.0	(192.0–295.0)	0.83
Density[c] (spindles/minute)	32	6.54	(5.42–7.94)	47	6.78	(5.35–8.72)	0.57

[a]From Mann–Whitney U test; statistically significant differences ($p < 0.05$) marked with an asterisk. [b]Infants who met criteria for staging REM before N2 were excluded. [c]Infants who fell asleep before EEG were excluded. Abbreviations: EEG, electroencephalogram; IQR, interquartile range; REM, rapid eye movement.

qEEG features that were significantly different between the two groups in either the main or the subgroup analyses are presented in Table 3.3. The intervention group had higher magnitudes (i.e. spectral power and rEEG measures) across multiple frequency bands and sleep stages compared with the never-massaged group. In the subgroup analysis, most magnitude differences remained (Figure 3.2). N1 amplitude kurtosis in alpha, N3 delta 2 spectral flatness, REM theta, and N3 gamma relative powers were no longer statistically significant in the subgroup analysis. Within the subgroup analysis, some other statistical differences did emerge, namely higher standard deviation of rEEG in REM and N3 beta spectral differences in the intervention group and lower interhemispherical coherence (Figure 3.3) in regularly massaged infants. All results are shown in Supplementary Tables 3 and 4 – Appendix 7.

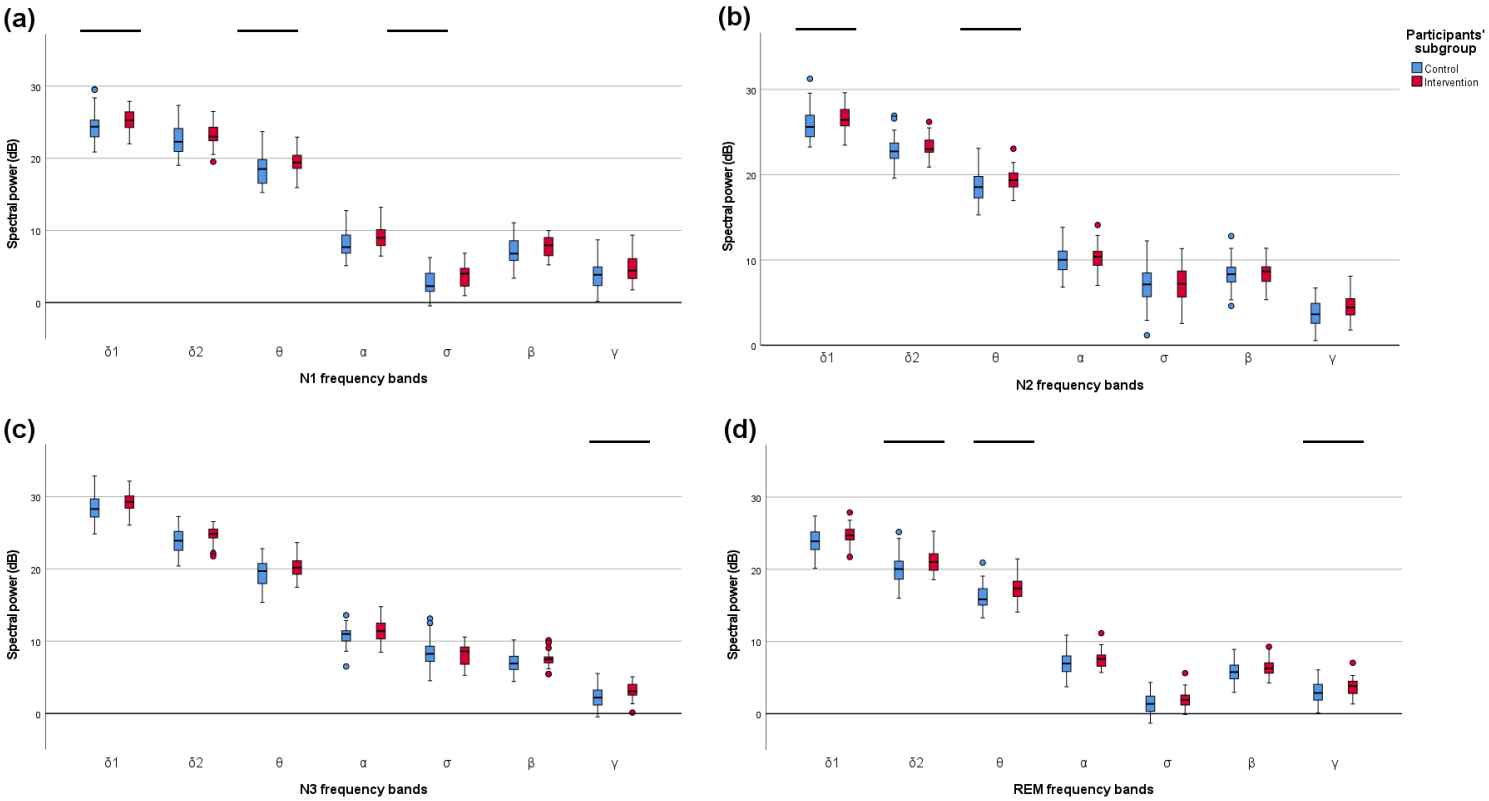


Figure 3.2 - Spectral power across frequency bands and sleep stages during the 1st cycle per group. Data from the subgroup analysis (i.e. the regularly massaged intervention group and never-massaged control infants). Horizontal lines indicate statistically significant differences between subgroups and circles indicate outliers. Units converted to decibels (dB; i.e. 10log10[spectral power]) to compare magnitudes in different frequency bands. (a–d) Spectral power during N1, N2, N3, and rapid eye movement (REM) respectively.

Table 3.3 - qEEG features with statistically significant differences between the two groups in either main or subgroup analyses during the first sleep cycle.

qEEG type	SS	FB	Intervention	Control	p-value[a]
			Median (IQR)		
Main analysis[b]					
Amplitude kurtosis	N1	α	3.49 (3.41–3.70)	3.43 (3.36–3.56)	0.04*
rEEG: lower margin (μV)	N1		63.33 (54.37–73.86)	57.22 (50.72–68.68)	0.02*
rEEG: median margin (μV)	N1		94.28 (81.97–110.07)	85.59 (75.11–100.30)	0.02*
rEEG: upper margin (μV)	N1		138.57 (119.52–164.36)	127.13 (112.28–147.95)	0.04*
Spectral power (μV^2)	N1	δ1	342.09 (269.90–458.32)	298.50 (215.89–408.80)	0.05
Spectral power (μV^2)	N1	δ2	216.52 (162.77–293.43)	178.46 (132.67–256.30)	0.04*
Spectral power (μV^2)	N1	θ	79.71 (66.27–127.88)	72.36 (49.37–96.39)	0.02*
Spectral power (μV^2)	N1	α	7.40 (5.95–10.67)	6.23 (5.00–8.31)	0.01*
Spectral power (μV^2)	N1	σ	2.30 (1.64–2.97)	1.80 (1.44–2.53)	0.02*
Coherence	N2	δ1	0.26 (0.19–0.29)	0.26 (0.21–0.31)	0.79
Coherence	N2	δ2	0.14 (0.11–0.17)	0.14 (0.12–0.17)	0.84
Coherence	N2	α	0.06 (0.04–0.09)	0.06 (0.04–0.08)	1.00
Coherence	N2	σ	0.09 (0.07–0.13)	0.09 (0.07–0.12)	0.82
Spectral power (μV^2)	N2	δ1	445.74 (370.56–616.77)	376.30 (286.23–532.56)	0.008*
Spectral power (μV^2)	N2	θ	81.35 (64.63–106.66)	73.53 (55.33–89.08)	0.03*
Spectral power (μV^2)	N2	β	7.60 (5.55–8.91)	6.11 (5.02–7.86)	0.04*
Spectral power (μV^2)	N2	γ	2.66 (2.12–3.41)	2.13 (1.84–2.88)	0.01*
Coherence	N3	δ1	0.23 (0.20–0.29)	0.26 (0.22–0.28)	0.30
Coherence	N3	δ2	0.14 (0.10–0.16)	0.13 (0.12–0.16)	0.78
Coherence	N3	σ	0.09 (0.08–0.12)	0.10 (0.08–0.12)	0.37
Coherence	N3	β	0.07 (0.05–0.10)	0.07 (0.06–0.10)	0.25
Coherence	N3	γ	0.06 (0.04–0.10)	0.07 (0.05–0.10)	0.26
rEEG median margin (μV)	N3		122.51 (108.06–131.99)	114.50 (99.35–128.60)	0.12
Relative spectral power (%)	N3	γ	0.16 (0.13–0.19)	0.13 (0.11–0.18)	0.02*
Spectral difference	N3	β	0.003 (0.002–0.005)	0.003 (0.002–0.005)	0.58
Spectral flatness	N3	δ2	0.89 (0.86–0.90)	0.88 (0.86–0.90)	0.04*
Spectral power (μV^2)	N3	β	5.47 (4.67–6.72)	4.70 (3.97–5.94)	0.004*
Spectral power (μV^2)	N3	γ	2.02 (1.70–2.52)	1.61 (1.29–1.98)	<0.001*
Amplitude skew	R	θ	0.015 (0.013–0.017)	0.017 (0.014–0.019)	<0.001*
Coherence	R	γ	0.04 (0.04–0.07)	0.05 (0.04–0.06)	0.26
rEEG lower margin (μV)	R		51.09 (45.86–58.42)	46.67 (42.34–54.56)	0.006*
rEEG median margin (μV)	R		76.09 (66.54–84.93)	70.44 (62.02–80.59)	0.03*
rEEG upper margin (μV)	R		109.69 (94.74–123.51)	101.63 (91.45–117.31)	0.06
rEEG SD (μV)	R		17.24 (14.70–19.60)	16.24 (14.16–18.64)	0.12
Spectral flatness	R	δ2	0.90 (0.88–0.92)	0.89 (0.87–0.91)	0.004*
Relative spectral power (%)	R	θ	9.87 (8.57–11.58)	9.04 (7.86–10.77)	0.03*
Spectral power (μV^2)	R	δ2	128.81 (94.43–164.94)	106.94 (82.57–151.24)	0.08
Spectral power (μV^2)	R	θ	48.19 (39.05–69.23)	39.89 (32.09–51.98)	0.003*
Spectral power (μV^2)	R	α	5.22 (4.39–6.49)	4.78 (3.69–5.88)	0.04*
Spectral power (μV^2)	R	σ	1.51 (1.27–1.80)	1.25 (1.02–1.70)	0.02*
Spectral power (μV^2)	R	β	4.11 (3.30–5.07)	3.64 (3.06–4.52)	0.02*
Spectral power (μV^2)	R	γ	2.28 (1.68–2.74)	1.89 (1.55–2.49)	0.02*
Subgroup analysis[c]					
Amplitude kurtosis	N1	α	3.47 (3.37–3.57)	3.43 (3.34–3.55)	0.20
rEEG lower margin (μV)	N1		63.60 (54.27–71.92)	56.22 (49.29–68.51)	0.07
rEEG median margin (μV)	N1		90.71 (83.17–106.61)	81.96 (71.57–104.79)	0.06
rEEG upper margin (μV)	N1		134.26 (123.25–158.84)	124.33 (105.98–149.89)	0.09
Spectral power (μV^2)	N1	δ1	336.24 (262.03–441.76)	274.61 (195.78–341.32)	0.04*
Spectral power (μV^2)	N1	δ2	200.34 (159.43–270.58)	169.37 (123.71–277.69)	0.10
Spectral power (μV^2)	N1	θ	87.73 (69.79–120.77)	71.11 (44.99–99.15)	0.02*

Spectral power (μV^2)	N1	α	7.94	(6.13–10.36)	5.88	(4.81–8.68)	0.05
Spectral power (μV^2)	N1	σ	2.53	(1.68–3.01)	1.70	(1.42–2.58)	0.03*
Coherence	N2	δ1	0.22	(0.16–0.29)	0.27	(0.21–0.30)	0.03*
Coherence	N2	δ2	0.13	(0.11–0.16)	0.15	(0.13–0.19)	0.03*
Coherence	N2	α	0.05	(0.03–0.06)	0.06	(0.04–0.08)	0.03*
Coherence	N2	σ	0.08	(0.06–0.10)	0.10	(0.07–0.13)	0.02*
Spectral power (μV^2)	N2	δ1	439.75	(368.76–596.72)	363.88	(275.66–502.29)	0.04*
Spectral power (μV^2)	N2	θ	86.18	(71.09–105.18)	71.48	(53.23–95.94)	0.04*
Spectral power (μV^2)	N2	β	7.33	(5.58–8.62)	6.81	(5.37–8.27)	0.48
Spectral power (μV^2)	N2	γ	2.78	(2.14–3.54)	2.30	(1.76–3.17)	0.07
Coherence	N3	δ1	0.20	(0.16–0.27)	0.26	(0.23–0.28)	0.003*
Coherence	N3	δ2	0.12	(0.10–0.15)	0.14	(0.12–0.16)	0.01*
Coherence	N3	σ	0.08	(0.06–0.11)	0.10	(0.08–0.12)	0.006*
Coherence	N3	β	0.06	(0.04–0.08)	0.07	(0.06–0.10)	0.04*
Coherence	N3	γ	0.05	(0.03–0.08)	0.06	(0.05–0.09)	0.03*
rEEG median margin (μV)	N3		125.36	(111.91–135.56)	112.31	(98.44–128.53)	0.05*
Relative spectral power (%)	N3	γ	0.16	(0.13–0.18)	0.14	(0.11–0.19)	0.47
Spectral difference	N3	β	0.004	(0.003–0.006)	0.003	(0.002–0.005)	0.04*
Spectral flatness	N3	δ2	0.90	(0.87–0.91)	0.88	(0.86–0.90)	0.10
Spectral power (μV^2)	N3	β	5.63	(4.96–6.65)	4.90	(4.04–6.25)	0.05
Spectral power (μV^2)	N3	γ	2.02	(1.78–2.62)	1.66	(1.30–2.11)	0.006*
Amplitude skew	R	θ	0.015	(0.013–0.017)	0.017	(0.015–0.019)	0.02*
Coherence	R	γ	0.04	(0.03–0.05)	0.05	(0.04–0.06)	0.03*
rEEG lower margin (μV)	R		52.97	(46.75–58.67)	45.72	(41.53–52.97)	0.007*
rEEG median margin (μV)	R		76.09	(67.05–87.24)	67.85	(61.64–78.15)	0.02*
rEEG upper margin (μV)	R		109.69	(97.32–125.32)	99.55	(88.16–118.56)	0.04*
rEEG SD (μV)	R		17.84	(14.84–19.71)	15.48	(13.62–19.29)	0.04*
Relative spectral power (%)	R	θ	10.29	(9.13–11.79)	9.38	(7.88–11.31)	0.17
Spectral flatness	R	δ2	0.91	(0.88–0.92)	0.88	(0.86–0.90)	0.01*
Spectral power (μV^2)	R	δ2	126.21	(95.81–165.02)	101.05	(72.07–145.70)	0.04*
Spectral power (μV^2)	R	θ	54.45	(41.47–69.48)	38.31	(31.71–54.07)	0.006*
Spectral power (μV^2)	R	α	5.76	(4.54–6.66)	4.95	(3.78–6.38)	0.10
Spectral power (μV^2)	R	σ	1.56	(1.31–1.82)	1.37	(1.07–1.77)	0.09
Spectral power (μV^2)	R	β	4.24	(3.62–5.14)	3.78	(3.01–4.84)	0.06
Spectral power (μV^2)	R	γ	2.41	(1.89–2.84)	1.94	(1.54–2.66)	0.02*

[a]From Mann–Whitney U test; statistically significant differences ($p < 0.05$) marked with an asterisk. [b]Intervention: n = 67; control: n = 72. [c]Regularly massaged intervention: n = 29; never-massaged control: n = 41. Abbreviations: EEG, electroencephalogram; FB, frequency bands: 0.5–2Hz (δ1), 2–4Hz (δ2), 4–8Hz (ϑ), 8–12Hz (α), 12–15Hz (σ), 15–30 (β), 30–45Hz (γ); IQR, interquartile range; qEEG, quantitative EEG; rEEG, range-EEG (frequency band 1–20Hz); SS, sleep stage.

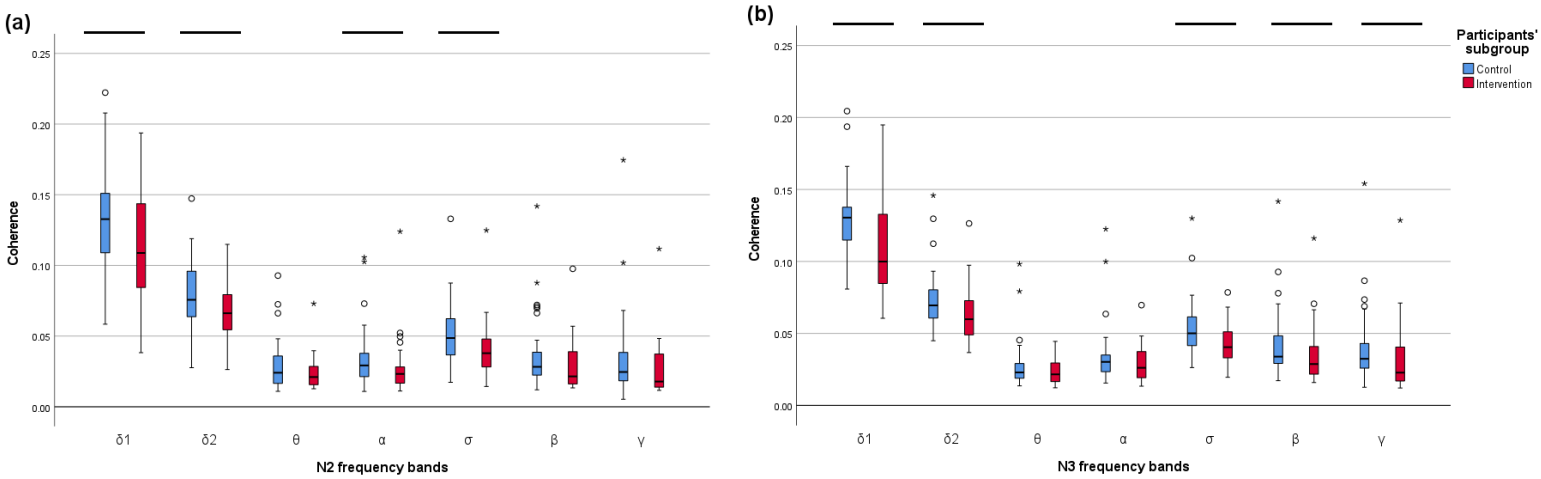


Figure 3.3 - Interhemispherical coherence across frequency bands during the 1st cycle per group. Data from the subgroup analysis (i.e., the regularly massaged intervention group and never-massaged control infants). Horizontal lines, indicate statistically significant differences between subgroups in the corresponding frequency ranges; circles and stars, outliers and extreme outliers respectively. (a, b) Coherence during N2 and N3 respectively.

3.3.3. Griffiths Scales of Child Development, Third Edition analysis

In the main analysis, intervention and control groups scored a median (IQR) of 117 (112–127) and 122 (112–127) at 18 months on Griffiths general development respectively ($p = 0.36$). There were no significant differences in any of the Griffiths scores at 4 and 18 months between groups in the main and subgroup analyses (Supplementary Table 5 – Appendix 7 and Table 3.4 respectively).

Table 3.4 - Neurodevelopmental outcomes at 18 months.

Griffiths III results at 18 months	Intervention			Control			p-value[a]
	n	Median (IQR)		*n*	Median (IQR)		
Main analysis							
General development[b]	81	117	(112–127)	92	122	(112–127)	0.36
Subscales							
A. Foundations of learning	81	114	(105–122)	92	114	(105–122)	0.67
B. Language and communication	81	109	(95–117)	92	109	(99–117)	0.53
C. Eye and hand coordination	81	114	(105–122)	92	118	(109–122)	0.23
D. Personal–social–emotional	81	118	(112–125)	92	122	(117–125)	0.28
E. Gross motor	81	130	(126–135)	92	135	(126–135)	0.15
Subgroup analysis							
General development[b]	33	122	(114.5–127)	56	122	(112–127)	0.33
Subscales							
A. Foundations of learning	33	114	(110–120)	56	114	(105–122)	0.59
B. Language and communication	33	109	(95–118.5)	56	104	(95–117)	0.44
C. Eye and hand coordination	33	118	(109–122)	56	116	(106–122)	0.66
D. Personal–social–emotional	33	122	(115–125)	56	118	(115–125)	0.26
E. Gross motor	33	130	(130–135)	56	135	(126–135)	0.90

[a]From Mann–Whitney U test. [b]A lower score indicates more severe neurodevelopmental delay. Abbreviations: Griffiths III; Griffiths Scales of Child Development, Third Edition; IQR, interquartile range.

3.4. Discussion

Previously, there has been no clear evidence of a beneficial effect of massage on cognitive development in term-born infants (95). This randomised control trial investigated the associations between routine parent-led massage during the first 4 months of age and neurodevelopment in a typically developing term-born cohort. This study has identified a higher background EEG magnitude across a range of frequencies and sleep stages, higher sleep spindle power, and lower interhemispherical coherence in regularly massaged infants at 4 months of age. The results may be generalizable to similar populations. Socioeconomics, maternal attachment, and likelihood of depression are factors that condition cognitive outcomes; no statistical differences were observed between groups (Table 3.1).

Massage typically coexists with other forms of social interaction (95), so both handling and social interactions are key elements. In animal models, neonatal handling promotes specific learning and memory formation, modulates stress responses, and conditions an array of behaviours (92). Maternal care is responsible for epigenetic modulation (463) and influences synaptogenesis (464). Children whose mothers show more positive

demonstrations of affection score better on performance and verbal IQ at later ages (465). Infant massage may decrease stress-related hormones and increase serotonin (454). This protective effect against stress may in itself influence neurodevelopment (54).

Even though massage may help induce sleep (454), evidence does not support its contribution to an overall daily increase of time spent asleep (95). Similarly, the present study showed that routine massage had no effect on total time asleep during the nap, on first sleep cycle length, or on the duration of individual sleep stages (Table 3.2).

We found higher spectral power values for sleep spindles in the intervention group than in the control group, and these differences increased in the subgroup analysis (Table 3.2). These group differences were not observed in the sigma band spectral power during N2 and N3, indicating that spindle power increase was not simply due to a general increase in background magnitude in this band. Existing literature shows an increasing sleep spindle magnitude in infancy(226); thus, these observations may be evidence of infant massage as an accelerator of brain maturation(58). Sleep spindle power is positively associated with axial diffusivity in the forceps minor, anterior corpus callosum, and tracts in the temporal lobe and thalamus (372). Axial diffusivity relates to the diffusion of water parallel to the axons. Although it is not entirely understood why increases in axial diffusivity can be present in early neurodevelopment, it is proposed to reflect decreased axonal tortuosity (466), or increased extra-axonal space(467, 468) due to pruning of superfluous connections (469). Functionally, sigma power has been positively associated with full-scale and fluid IQ (340) scores, while spindle-related frequency was negatively associated with perceptual reasoning, comprehension, and working memory (338), among others. Owing to the neuroplastic properties of spindles, they are thought to play a role in memory consolidation processes (470).

Results of the qEEG analysis indicated two major differences in the intervention group: the signal magnitude-related features, which showed a higher magnitude of signals, measured by rEEG margins and spectral power across nearly all frequency bands studied, largely replicated in the subgroup analysis; and lower levels of interhemispherical coherence (Table 3.3). Some of the qEEG statistically significant features were not preserved after subgroup analysis; this was probably because of the reduced number of participants and consequent loss of statistical power.

Previous studies of massage in infants born preterm have found differences in spectral power during active sleep. A parent-led massage study showed a dose association up to term equivalent age on global relative power, and increased localised alpha in the massage group (455) and in a study with a massage-expert, significant local and global absolute spectral power interactions between massage and non-massage groups were found before and after a massage routine performed by an expert (58). In line with previous observations, I also found effects of massage on spectral power in the term-born infants. Although changes in spectral power values of different frequency bands with maturation are regionally specific (413), overall, there is a net increase of the spectral power during the first half-year of life (471). In this context, the findings of increased EEG magnitude here observed further support the notion of more advanced brain maturation in the routinely massaged group, extending observations to cohorts of infants born at term (58, 455).

Subgroup analysis revealed lower interhemispherical coherence, a marker of connectivity, in the intervention group in nearly all frequency bands studied, particularly during NREM sleep (Table 3.3). Advanced functional axonal pruning of the corpus callosum connecting the two hemispheres, which occurs during early infancy, may explain this finding. Although connectivity changes vary across maturation, reflecting cycles of synaptic establishment and elimination (472) occurring at different time points in different brain regions; my thesis revealed a net decrease in interhemispherical connectivity in those infants who had regular parent-led massage during their first 4 months of age. Previous studies have noted decrements of interhemispherical connectivity in specific points of infancy and childhood (see, for example, Xiao *et al.* (473); Boersma *et al.* (474)). For example, from 5 to 7 years old, a general decrease of connectivity is accompanied by increased functional node clustering and path length and decreased heterogeneity of the synaptic weights; this indicates a possible transition from random to more organised network arrangement (474). In infants and toddlers, interhemispherical decreases of connectivity between homologous cortical areas may be expected as a result of neural segregation while integrating intrahemispherical areas of interest, accompanied by improvement of specific performances (473, 475). This chapter analyses demonstrated that changes were present in a resting state after providing continued environmental enrichment in the form of parental massage. The changes provided by continued massage sessions may have caused plastic changes that contributed to the differences in patterns seen ‘offline’ in the sleeping state.

The qEEG subgroup analysis also demonstrated differences in continuity (EEG skewness), and spectral distribution (spectral flatness and spectral differences) between groups. These results highlight that there are subtle changes in the EEG structure beyond the most evident sleep analysis that could only be observed through qEEG (195).

There were no statistically significant differences on Griffiths assessments at 4 months and 18 months between the two groups (Supplementary Table 5 – Appendix 7 and Table 3.4). I hypothesise that neurodevelopmental changes from routine massage are too subtle to be observable on Griffiths assessments at these early ages and that differences may only be evident at later time points, particularly school-age.

My thesis builds on knowledge about the effects of regular parent-led massage. So far, most studies of infant massage have focused on neurodevelopment in preterm cohorts. To my knowledge, this was the first study to report the effects of routine massage on the sleep EEG measures of typically developing term-born infants. With 179 infants included in this study up to 4 months, this is one of the largest and longest studies of infant massage. This study also included a diverse array of sleep measures that ranged from the duration of sleep stages, multiple sleep spindle features, and qEEG measurements. However, the approach in this exploratory study also implies that multiple analyses increase the probabilities of false-positive results. Because of this, I discuss general qEEG aspects instead of individual associations. The results were consistent, showing higher magnitude and lower coherence on intervention in several frequency ranges and sleep stages.

Before starting the massage routine, parents from the intervention group performed the massage on their children during the class with the International Association of Infant Massage-certified physiotherapist who corrected the technique if necessary. Parents were also invited to attend this class as a refresher if they had any further questions. However, the quality of massage on a day-to-day basis was not assessed, which can be considered a limitation. A further limitation is that laboratory adaptation sleep session was not performed owing to logistics. First ‘night’ effects can alter some macrostructure and spindle features. However, as all participant EEGs were recorded under the same conditions in this randomised controlled study, this should not influence the results of the comparative analysis. Owing to the high dropout rate, I compared demographic characteristics measured at registration between participants included in my thesis and those excluded (Supplementary tables 1 and 2 – Appendix 7). However, the sociodemographic data obtained at registration were limited.

Another limitation is the low compliance in the intervention group: only 33 out of 83 infants were regularly massaged, although all infants in the intervention group were massaged. One reason for non-compliance may have been a mismatch between expectations at recruitment and the actual reality of the considerable demands of being a new parent and accommodating additional regular massage into the daily routine. Although occasional phone calls of encouragement to parents may have helped retain some participants, future studies may achieve higher compliance if participants are enrolled after families are settled in their routines at home. Importantly, some group differences were still observed in the main analysis, indicating that less restrictive routine massage criteria may be acceptable to retain more participants and improve compliance. Further in-depth analysis should analyse the effects of massage by sex and relate infant massage to other neurodevelopmental assessments, including memory abilities, and examine the persistence of EEG changes observed in this study as participants age.

3.5. Conclusion

A parent-led massage routine during the first 4 months of age produced detectable changes in the sleep EEG. Those changes included higher spectral power in sleep spindles, higher magnitude of the EEG, and decreased interhemispherical coherence across most frequency bands and sleep stages. These findings indicate that environmental enrichment in the form of parental massage may be associated with a more mature neurodevelopmental profile observable in the infant sleep EEG at 4 months.

Chapter 4. Infant sleep electroencephalography features as markers of neurodevelopmental outcome at 18 months

4.1. Introduction

The first years of life, particularly infancy, represent exceptionally active periods of neurodevelopment. The brain doubles in volume, primarily due to the pronounced expansion of grey matter, reflecting both dendritic growth and the establishment of novel synapses (476, 477) with concurrent pruning of superfluous connections (478). Intense myelination (479) and functional changes take place (168, 480). There are critical periods during development in which there is an enhanced environment-dependent sensitivity for the establishment of synaptic connectivity that promotes long-lasting skill or trait acquisition (61). Notably, some essential critical periods associated with vision, auditory and language acquisition and the development of intra- and interpersonal skills occur during early years (61, 436). The psychologist Dr Ruth Griffiths exhaustively reviewed the behaviour of infants and young children whose work was first published in 1954 as *Baby Scales* (481). Successive observations resulted in the characterisation of stepwise milestones across locomotor, personal-social, hearing and speech, eye and hand coordination and performance areas. These studies provided the framework for the most recent Griffiths Developmental Scales: Griffiths III(438). Early identification of atypical trends in neurodevelopment may enable targeted intervention to help improve longer-term outcomes (482).

Sleep is ideal for studying existing EEG patterns with reduced influence from external inputs. Sleep is also integral to normal brain functioning (48, 138) and maturation (171). A meta-analysis on paediatric sleep duration (almost exclusively on studies during night-time sleep) show weak associations with several cognitive and behavioural domains (483). Meta-analyses of the relationship between sleep and cognitive features (memory and sustained attention) show different results in children and adults, possibly reflecting methodological and neurobiological differences (483). While sleep duration partially reflects some aspects of cognitive ability, other sleep features are important markers of outcome. Specific sleep patterns reflect and/or actively participate in brain maturation and cognitive ability (240, 245,

246, 308, 331, 423), supporting associations between learnt concepts (314) and, neurodevelopment (141, 335, 380, 414, 415, 420, 421, 484). For example, slow waves during NREM (non-rapid eye movement) sleep have been associated with homeostatic processes and synaptic down-selection (48, 138), with particular relevance during maturation (421); and properties of this slow rhythm such as anterior-posterior ratio and propagation are associated with myelination and anatomical brain connectivity (414, 485), and future skill learning (415). Beaugrand *et al.* however, described statistically non-significant results between anterior-posterior ratio at 6 months and development assessment scores; one possible explanation may be related to the relatively homogeneous cohort (416). The topographic distribution of sleep spindles (Figure 4.1) and other spindle characteristics are also related to previous learning activities (301-304, 306, 423), and appear to influence mental abilities (240, 307, 308, 331) and sleep maintenance (284, 285). Spindles in infancy are also believed to be involved in somatosensory mapping (141). Both sleep spindles and slow wave co-action appear to contribute to memory consolidation (135, 486). Despite all this evidence, comparisons between studies analysing sleep patterns and cognitive/neurodevelopmental trajectories are complicated by the use of different methodologies, different age groups, and generally low sample sizes (344, 380, 484, 487).

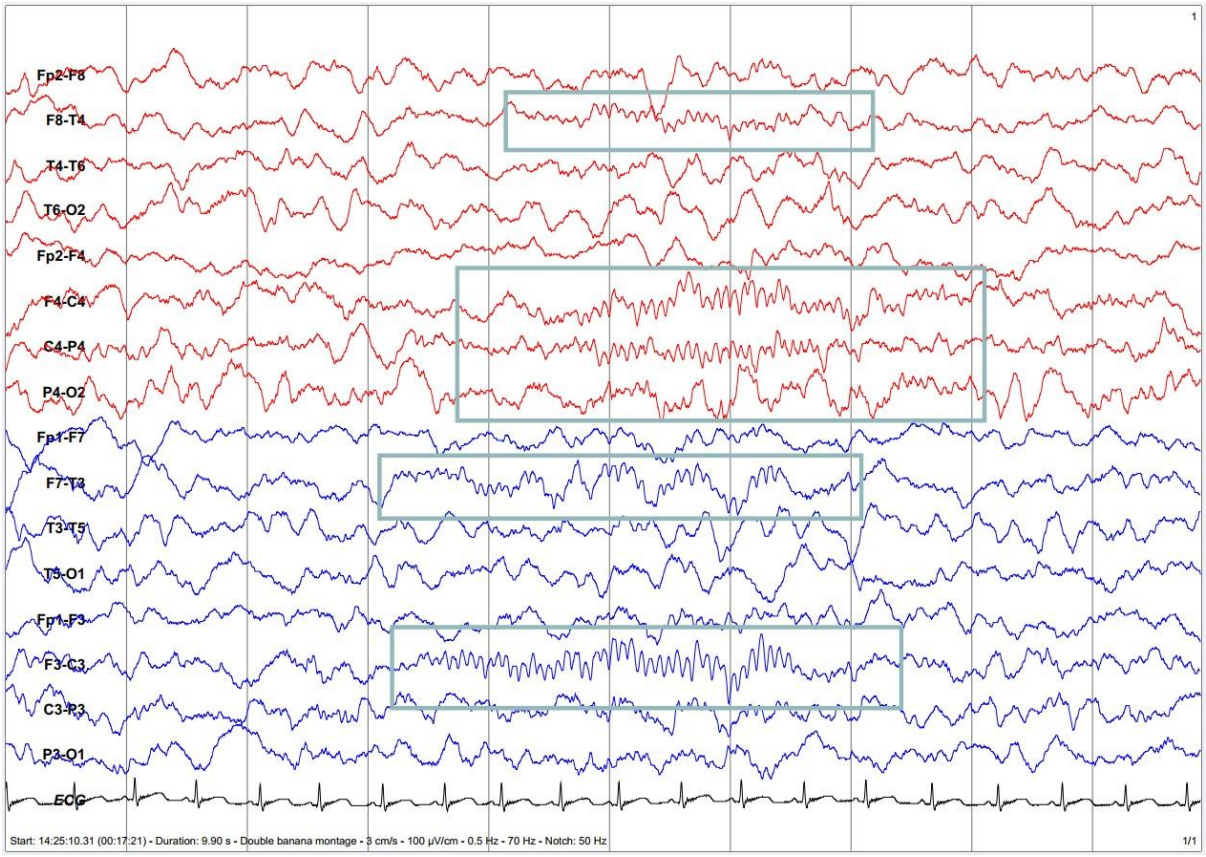


Figure 4.1 - Sleep spindles of a four-month old.
Sleep spindles (in grey boxes) are transients of sleep occurring as runs of 11-16Hz(121) activity during N2 and N3. Vertical lines represent 1-second intervals.

Acknowledging that neurodevelopment is a continuous process shaped by environment and biology, I aimed to explore EEG sleep features at 4 months as possible predictors of developmental outcome at 18 months.

4.2. Methods

4.2.1. Cohort

Infants born at or after 37 weeks of gestation were recruited at Cork University Maternity Hospital between 2017 and 2018 as part of the BabySMART (Study of Massage Therapy, Sleep And Neurodevelopment, registered identifier NCT03381027) study, a randomised controlled trial on sleep and massage intervention approved by the Research Ethics Committee of the Cork Teaching Hospitals. The infants were singletons, not requiring admission to the neonatal unit, and without suspected congenital or metabolic conditions.

Only data from infants in the non-intervention arm was analysed in this chapter and, to be included, infants had to have a sleep EEG recorded at approximately 4 months and Griffiths III assessments at 18-months. The participant's parents or guardians provided written informed consent and the study was approved by the Clinical Research Ethics Committee (CREC) of the Cork Teaching Hospitals.

4.2.2. EEG recording and analysis

EEG was recorded using the Lifelines iEEG (Lifelines Neuro, UK) system when infants were circa 4-months. The EEG electrodes included Fp1, Fp2, Fz, F3, F4, F7, F8, Cz, C3, C4, T3, T4, Pz, P3, P4, T5, T6, O1, O2, A1, A2, reference and ground, placed according to the 10-20 International System; impedances were maintained below 10 kΩ. The polysomnographic channels ECG, EOG, chin EMG and a movement sensor to record respiration were also applied. Appointments took place at the sleep lab of the INFANT Centre, University College Cork, Ireland during the daytime. Sleep was recorded during conditions of low noise and dimmed lights; the recording started once all electrodes were applied, and ended when the infant was fully awake. A clinical physiologist remained in the room to ensure optimum technical quality of the EEG throughout the recording.

Sleep staging was performed using the Nicolet (Natus, USA) sleep staging software according to the AASM 2.4 guidelines (121). Sleep macrostructure refers to the parameters derived from sleep staging; I analysed total sleep time, duration of sleep stages N1, N2, N3 and REM (rapid eye movement) sleep duration during the first cycle, and latencies to sleep (period from lights off to sleep onset) and REM (period from sleep onset to REM onset). Sleep spindles were visually identified and annotated from the left and right fronto-central EEG channels. Spindle number and duration were then directly extracted from the annotation list of the EEG review software (Stratus EEG, Iceland). Sleep spindle density was defined as the number of spindles per minute in NREM sleep during the first sleep cycle. Mean sleep spindle frequency, sleep spindle synchrony (calculated as the duration of overlap in both fronto-central channels, divided by the total spindle time for both channels, expressed as a percentage, Figure 4.2), brain symmetry index (BSI) (437) and spectral power were calculated for sleep spindles (121). qEEG features were calculated on the continuous EEG, namely

spectral power, and interhemispheric coherence. These features were calculated across all bipolar channels over the first sleep cycle for each sleep stage. Spectral power and interhemispheric coherence were calculated for 7 frequency bands: delta 1, 0.5-2Hz; delta 2, 2-4Hz; theta, 4-8Hz; alpha, 8-12Hz; sigma, 12-15Hz; beta, 15-30Hz, gamma, 30-45Hz. All qEEG features were calculated in MATLAB (The MathWorks Inc, Natick, Massachusetts, version 9.8.0 R2020a). Spectral power and coherence were generated using the NEURAL toolbox (195) for MATLAB. For further details on the sleep spindle and qEEG analyses, please refer to (Ventura *et al.* (488, 489)).

With the exception of total nap duration, sleep spindles, macrostructure and qEEG parameters were based exclusively on the first sleep cycle to avoid potential variability due to the reduction of sleep pressure across successive cycles. Other macrostructure and qEEG features analysis included only infants with a complete first sleep cycle recorded, i.e., infants who fell asleep after the recording started and had both NREM and REM recorded during the first sleep cycle. Infants who fell asleep before the recording started were also excluded from sleep spindle number and density analyses.

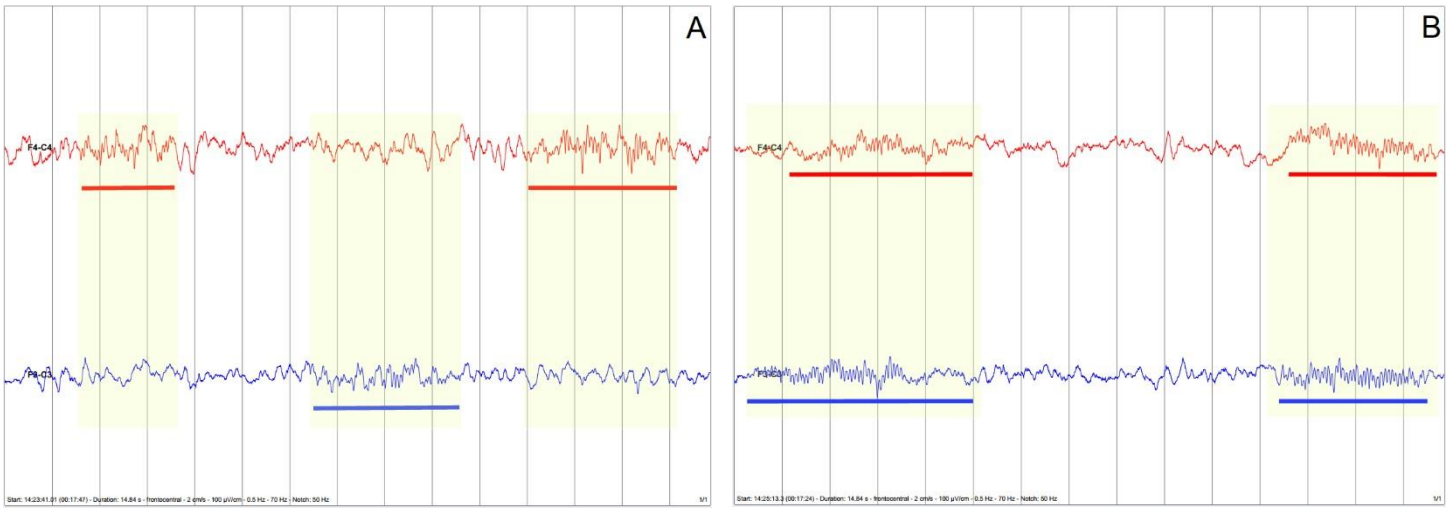


Figure 4.2 - Sleep spindle synchrony.
A) example of low synchrony; right (in red) and left (in blue) frontocentral spindles do not coincide in time. B) example of high synchrony; bilateral spindles overlap in time for the majority of their duration. Timebase of 2cm/s, vertical lines represent 1-second intervals.

4.2.3. Developmental outcome at 18 months and sociodemographic characterisation

Infants were assessed at the sleep lab using Griffiths III at 18 months, administered by a clinical neuropsychologist or paediatrician with training from the Association for Research

in Infant and Child Development (ARICD). The Griffiths III is a tool that assesses paediatric development progress and flags possible learning disorders at an age range from birth up to 6 years. It has been recently re-standardised for UK and Irish paediatric populations(438). Griffiths III assesses 5 domains of development: A – Foundations of Learning, B – Language and Communication; C – Eye and Hand Coordination; D – Personal-Social-Emotional and E – Gross Motor. Subscale A evaluates aspects of learning such as executive function, working memory and play. Subscale B evaluates expressive and receptive language; in preverbal children this subscale evaluates the children engagement to auditory stimuli and intention to communicate. Subscale C evaluates aspects that include fine motor and visuo-perceptual skills. Subscale D concerns the growing understanding of the concept of self, social interactions, and emotional responses. Subscale E assesses parameters like body coordination and strength. The GD score is derived from the average of all subscales scores. The developmental quotient of the infant of each subscale and GD scores were calculated for each infant. The developmental quotients are reported to have a mean (SD) of 100(15)(438). Lower quotients on the subscales and overall scale indicate developmental delay. Upper and lower thresholds of the Griffiths quotients are >150 and <50, respectively. No exclusions were performed based on the Griffiths III quotients as I aim to investigate the relationship between sleep features and developmental outcome for all infants.

For sociodemographic characterisation, I included information collected at study registration and in 2-week and 4-month questionnaires completed by parents. Maternal-infant postnatal attachment at 4 months was measured using the 19-item Maternal Postnatal Attachment Scale(458). Scores can range from 19 to 95, with higher scores representing greater maternal-infant attachment. Maternal postnatal depression at 4 months was measured using the 10-item Edinburgh Postnatal Depression Scale (EPDS) (459). Scores can range from 0 to 30 with higher scores representing a greater likelihood of depression.

4.2.4. Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics (version 26.0, IBM Corp., Armonk, NY, USA). Spearman's rank correlation coefficient was used to investigate and quantify relationships between the 4-months sleep features and Griffiths III developmental

quotients at 18-months. Partial Spearman's rank correlation coefficients were used to adjust for gestational age, ages at EEG and Griffiths III assessments, and sex. Socioeconomic factors, measured by maternal level of education and household income, were considered to have potential confounding effects on the relationships between sleep features and Griffiths III subscale quotients. To investigate this, associations between the sleep features, Griffiths III subscale quotients and socioeconomic factors were assessed. When both a sleep feature and a Griffiths III subscale quotient were associated with the same socioeconomic factor, a secondary analysis was performed calculating the Partial Spearman's Rank Correlation between the sleep feature and Griffiths III subscale quotient, adjusting for the previous confounders and the socioeconomic factor. Absolute correlations less than 0.1 represent a very small association, coefficients between 0.10 to 0.29 represent a small association, coefficients between 0.30 to 0.49 represent a medium association and coefficients of 0.5 and above represent a large association (490). Multiple hypothesis testing of correlation coefficients was not adjusted for in the analysis as the study was exploratory in nature and because reducing the probability of a type I error would increase the probability of a type II error (460, 461). Multivariable analysis was not performed due to the large number of independent variables investigated and the relatively small sample size.

4.3. Results

A total of 408 infants were enrolled in the BabySMART study of which, half (n=204) were randomised to the non-intervention group. Of those, 92 infants attended both the 4-month EEG appointment and had a non-pathologic sleep EEG recorded, and the 18-month Griffiths appointment (Figure 4.3). Table 4.1 presents the demographic characteristics of the 92 infants included in this study from which 40% (n=37) were females. The median (IQR) postnatal age was 4.4(4.3-4.7) months (range: 3.9-5.1) and 18.4(18.2-18.7) months (range: 17.8-19.8), at the 4- and 18-months appointments respectively. Table 4.2 presents the median (IQR) and observed range of Griffiths quotients at 18 months.

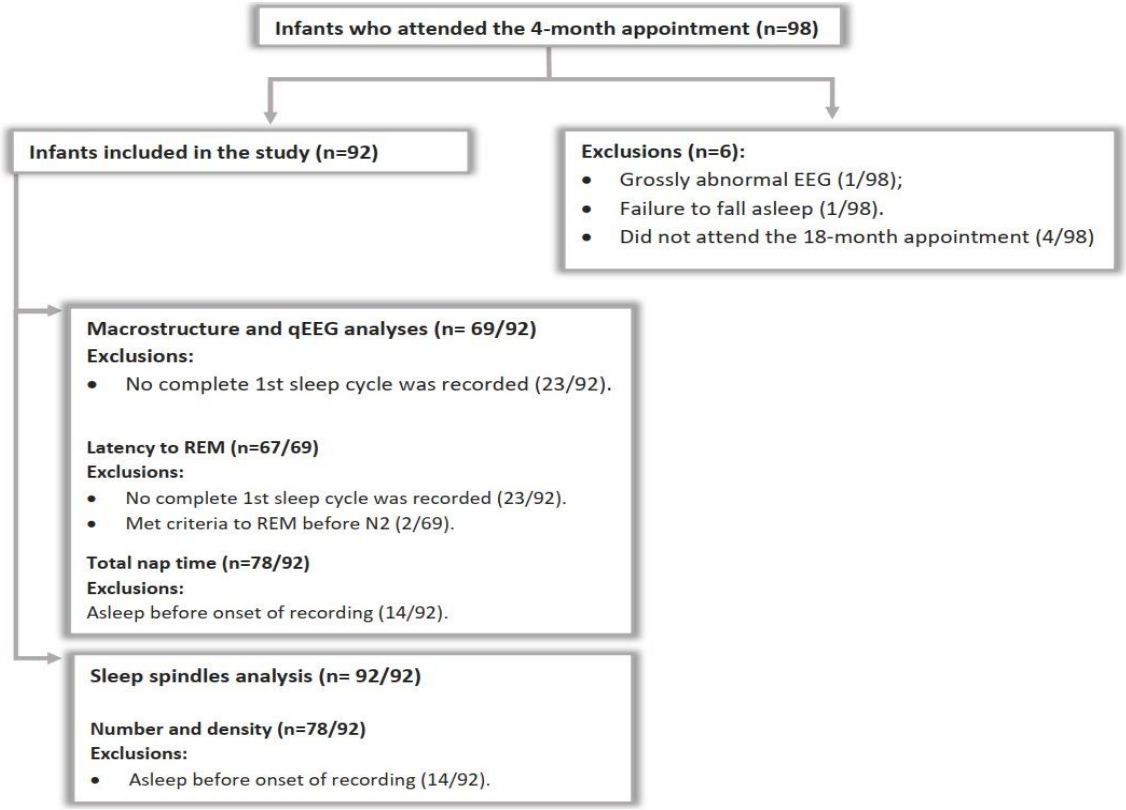


Figure 4.3 -Study flow chart.

Table 4.1 - Demographics, n=92.

Demographics	n(%)[a]	
Registration and 2-week questionnaire		
Sex – Female	37	(40.2)
Gestational age (weeks), mean (SD)	39.8	(1.2)
Weight at birth (Kg), mean (SD)	3.57	(0.46)
Maternal age (years), mean (SD)	35.1	(4.1)
Household net income/annum		
≤€30,000	7	(7.6)
€30,001-60,000	19	(20.7)
≥€60,001	39	(42.4)
Prefer not to say/ do not know	27	(29.3)
Highest level of education		
Secondary school	7	(7.6)
Third level certificate or diploma	17	(18.5)
Degree or higher diploma	36	(39.1)
Postgraduate qualification	32	(34.8)
4-month appointment and questionnaire		
Feeding at 4 months		
Breast Fed	26	(28.3)
Infant Formula	53	(57.6)
Both	13	(14.1)
Maternal attachment at 4 months[b], median (IQR)	86.8	(82.7-90.6)
Maternal EPDS at 4 months[c], median (IQR)	4	(1-8)

[a]Unless otherwise stated. [b]measured using the Maternal Postnatal Attachment Scale[49]. Scores can range from 19 to 95, with higher scores representing greater maternal-infant attachment. [c]measured using the Edinburgh Postnatal Depression Scale[50]. Scores can range from 0 to 30 with higher scores representing greater likelihood of depression.

Table 4.2 - Neurodevelopmental outcome at 18 months - Griffiths developmental quotients (DQ), n=92.

Griffiths III	Median	(IQR)	Observed range	
			Minimum	Maximum
Subscale A DQ, Foundations of Learning	114	(105-122)	68	130
Subscale B DQ, Language and Communication	109	(99-117)	67	145
Subscale C DQ, Eye and Hand Coordination	118	(109-122)	93	150
Subscale D DQ, Personal-Social-Emotional	122	(117-125)	88	132
Subscale E DQ, Gross Motor	135	(126-135)	50	150
GD DQ, General Development	122	(112-127)	84	141

Upper and lower thresholds of the Griffiths III quotients are >150 and <50, respectively. Low quotients indicate developmental delay.

Correlations between sleep macrostructure features at 4-months and Griffiths developmental quotients at 18-months are presented in Table 4.3. In the unadjusted analysis, a small positive association was observed between latency to sleep and the Foundations of Learning subscale. There was also a positive association between N3 duration and the Personal-Social-Emotional subscale. After adjustment, these associations remained significant and increased slightly in magnitude. A small negative association was observed between N2 duration and the Personal-Social-Emotional subscale in the unadjusted analysis, but this association was no longer statistically significant in the adjusted analysis.

Correlations between sleep spindle features at 4-months and Griffiths III developmental quotients at 18 months are presented in Table 4.4. Increased sleep spindle synchrony percentage was associated with lower quotients in the general development scale and three subscales; Eye and Hand Coordination, Personal-Social-Emotional and Gross Motor subscales. The strongest association was with the Gross Motor subscale. These associations increased slightly after adjustment for gestational age, ages at the assessments, and sex. Increased spindle duration was associated with decreased quotient value in both the Personal-Social-Emotional and Gross Motor subscales and these associations remained significant in the adjusted analysis.

Associations between Griffiths developmental quotients at 18-months and coherence, and spectral power at 4-months across the sleep stages are detailed in supplementary table 6 – Appendix 8 and summarised in Figure 4.4.

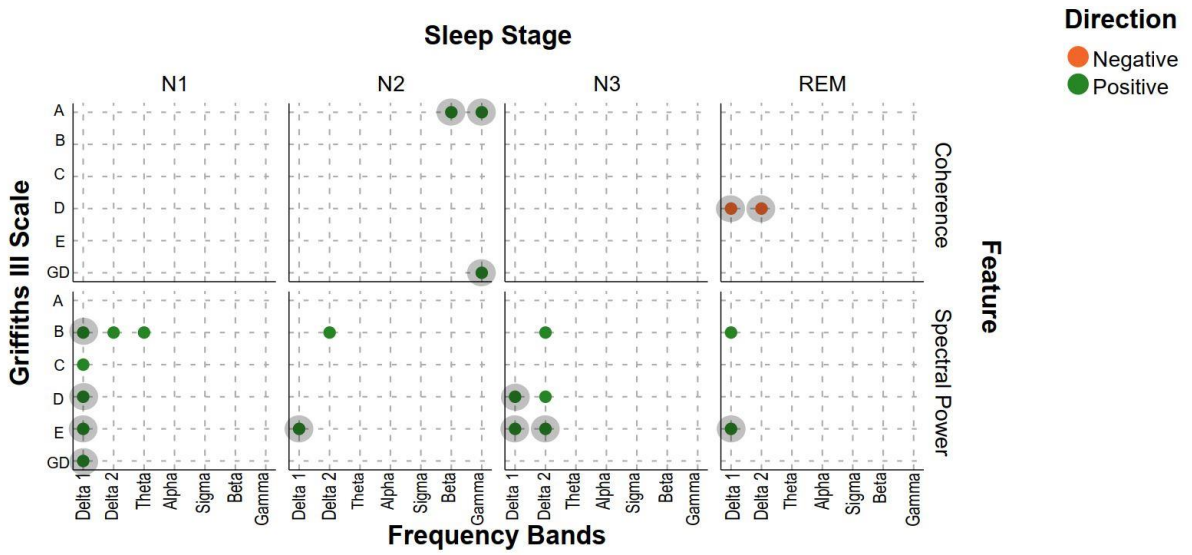


Figure 4.4 - Statistically significant associations between qEEG features and Griffiths developmental quotients.

Figure 4 presents statistically significant correlations between coherence and spectral power across the sleep stages, frequency bands and Griffiths developmental quotients (n=69). The majority (15/21) of statistically significant correlations observed were for spectral power in delta frequencies and indicated a small to medium positive association: N1 delta 1 and subscales B (r=0.318, p=0.008), C (r=0.252, p=0.037), D (r=0.362, p=0.002) and E (r=0.294, p=0.014) and general development (r=0.320, p=0.007); N1 delta 2 and subscale B (r=0.265, p=0.028); N2 delta 1 and E (r=0.243, p=0.044); N2 delta 2 and B (r=0.238, p=0.049); N3 Delta 1 and D (r=0.304, p=0.011) and E (r=0.304, p=0.011); N3 Delta 2 and B (r=0.267, p=0.027), D (r=0.244, p=0.043), E (r=0.278, p=0.021); REM Delta 1 B (r=0.238, p=0.049) and E (r=0.241, p=0.046). During N2, significant small positive correlations were seen in the coherence of higher frequencies spectrum and subscale A (Beta and Gamma) and general development quotients (Gamma). Negative small to medium associations were observed for the coherence of low frequencies during REM and subscale D. After adjustment, correlations remained statistically significant for coherence; spectral power was no longer statistically significant on B subscale (except on N1 delta 1), nor C subscale nor D subscale associations with N3 delta 2. Adjusted correlations were small to medium on both coherence and spectral power (supplementary table 6 – Appendix 8).

Table 4.3 - Association between macrostructure features and Griffiths developmental quotients at 18-months, n=69[a].

Griffiths scales/ macrostructure features (min)	Subscale A DQ, Foundations of Learning				Subscale B DQ, Language and Communication				Subscale C DQ, Eye and Hand Coordination				Subscale D DQ, Personal-Social-Emotional				Subscale E DQ, Gross Motor				GD DQ, General development			
	Spearman's Rank Correlation		Partial Spearman's Rank Correlation[b]		Spearman's Rank Correlation		Partial Spearman's Rank Correlation[b]		Spearman's Rank Correlation		Partial Spearman's Rank Correlation[b]		Spearman's Rank Correlation		Partial Spearman's Rank Correlation[b]		Spearman's Rank Correlation		Partial Spearman's Rank Correlation[b]		Spearman's Rank Correlation		Partial Spearman's Rank Correlation[b]	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Total nap duration[b]	-0.07	0.57	-0.11	0.37	-0.03	0.77	-0.08	0.51	-0.14	0.22	-0.19	0.11	-0.13	0.27	-0.18	0.13	-0.03	0.81	-0.06	0.61	-0.08	0.47	-0.15	0.20
Duration N1	-0.003	0.98	-0.002	0.99	-0.08	0.49	-0.13	0.32	0.05	0.67	0.05	0.72	-0.01	0.94	-0.04	0.76	0.04	0.72	0.04	0.76	0.08	0.54	0.08	0.55
Duration N2	-0.07	0.59	-0.04	0.73	-0.23	0.06	-0.18	0.16	-0.22	0.07	-0.17	0.17	-0.29*	0.02	-0.23	0.07	-0.06	0.60	-0.02	0.88	-0.14	0.26	-0.10	0.45
Duration N3	0.07	0.59	0.09	0.49	0.01	0.94	0.02	0.89	0.09	0.48	0.12	0.36	0.24*	0.05	0.27*	0.03	0.09	0.46	0.10	0.44	0.04	0.72	0.08	0.55
Duration REM	-0.08	0.53	-0.11	0.39	0.03	0.79	-0.01	0.94	-0.08	0.54	-0.14	0.27	-0.11	0.37	-0.17	0.17	0.03	0.79	0.01	0.91	-0.01	0.91	-0.08	0.55
Latency to sleep	0.27*	0.02	0.28*	0.03	-0.04	0.78	-0.05	0.68	0.06	0.66	0.04	0.73	0.10	0.40	0.10	0.45	0.04	0.75	0.04	0.75	0.06	0.64	0.04	0.74
Latency to REM[c]	-0.06	0.65	-0.03	0.81	-0.16	0.19	-0.18	0.15	0.10	0.41	0.12	0.33	0.10	0.44	0.10	0.42	0.06	0.65	0.07	0.59	0.04	0.77	0.05	0.69

*Partial Spearman's Rank Correlations are adjusted for postnatal age of both EEG and Griffiths at 18 months assessments, gestational age, and sex. *Coefficients whose p-value <0.05. [a]unless otherwise stated. [b]n=78, infants who fell asleep during EEG recording. [c]n=67, infants who had a full sleep cycle recorded and reached REM at the end of it.*

Table 4.4 - Association between sleep spindle features and Griffiths developmental quotients at 18-months, n=92[a].

Griffiths scales/ sleep spindle features	Subscale A DQ, Foundations of Learning				Subscale B DQ, Language and Communication				Subscale C DQ, Eye and Hand Coordination				Subscale D DQ, Personal-Social-Emotional				Subscale E DQ, Gross Motor				GD DQ, General development			
	Spearman's Rank Correlation		Partial Spearman's Rank Correlation[b]		Spearman's Rank Correlation		Partial Spearman's Rank Correlation[b]		Spearman's Rank Correlation		Partial Spearman's Rank Correlation[b]		Spearman's Rank Correlation		Partial Spearman's Rank Correlation[b]		Spearman's Rank Correlation		Partial Spearman's Rank Correlation[b]		Spearman's Rank Correlation		Partial Spearman's Rank Correlation[b]	
	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p	r	p
Synchrony (%)	-0.13	0.21	-0.15	0.16	-0.14	0.19	-0.16	0.15	-0.23*	0.03	-0.25*	0.02	-0.21*	0.05	-0.24*	0.03	-0.30*	0.004	-0.33*	0.001	-0.24*	0.02	-0.28*	0.01
Spectral power (μV^2)	-0.07	0.52	-0.10	0.37	0.13	0.23	0.09	0.42	-0.11	0.31	-0.17	0.12	-0.04	0.73	-0.10	0.37	-0.13	0.22	-0.13	0.25	-0.03	0.76	-0.09	0.38
Brain Symmetry Index	0.01	0.89	0.04	0.71	0.03	0.78	0.06	0.59	0.02	0.85	0.05	0.62	0.09	0.40	0.14	0.19	0.18	0.08	0.20	0.06	0.05	0.65	0.10	0.36
Duration (s)	-0.16	0.13	-0.15	0.17	0.02	0.86	0.03	0.76	-0.17	0.11	-0.16	0.14	-0.23*	0.03	-0.22*	0.04	-0.29*	0.01	-0.32*	0.003	-0.17	0.11	-0.15	0.18
Frequency (Hz)	0.03	0.79	0.01	0.92	-0.02	0.83	-0.06	0.61	0.09	0.38	0.06	0.55	-0.02	0.83	-0.06	0.61	0.09	0.38	0.09	0.40	0.09	0.38	0.06	0.56
Number[c]	-0.07	0.54	-0.03	0.78	-0.15	0.21	-0.10	0.41	-0.05	0.69	0.01	0.97	0.04	0.72	0.11	0.36	-0.08	0.48	-0.04	0.72	-0.13	0.25	-0.07	0.55
Density[c] (spindles/min)	-0.07	0.54	-0.07	0.57	-0.04	0.76	0.01	0.91	-0.11	0.35	-0.07	0.57	0.002	0.98	0.06	0.60	-0.11	0.34	-0.10	0.42	-0.15	0.19	-0.12	0.31

*Partial Spearman's Rank Correlations are adjusted for postnatal age of both EEG and Griffiths at 18 months assessments, gestational age, and sex. *coefficients whose p-value <0.05. [a]unless otherwise stated. [b]Partial Spearman's Rank Correlations are corrected for postnatal age of both EEG and Griffiths at 18-months assessments, gestational age, and sex.[c]n=78, infants who fell asleep during EEG recording.*

All participants provided information on maternal level of education and 65/92 participants provided information on household income. No associations were observed between Griffiths III and maternal level of education and hence maternal level of education was not a confounder in the relationships between sleep features and Griffiths subscale quotients. Griffiths was associated with household income on subscales B, C, D and General Development. No sleep spindles or macrostructure features were associated with household income. Associations between income and qEEG were only observed on N3 theta coherence. Partial Spearman's Rank Correlations between N3 theta coherence and the Griffiths subscales that were associated with household income were calculated (supplementary table 7 – Appendix 8); and the conclusions did not differ from the original adjusted analysis (supplementary table 6 – Appendix 8) where no statistically significant relationships were found.

4.4. Discussion

I previously described normative values for sleep macrostructure and sleep spindles in term-born infants at 4 months(488). In this chapter, I investigated associations between sleep EEG features at 4 months and Griffiths' quotients at 18 months in the same cohort of healthy term-born infants. EEG sleep macrostructure features, sleep spindles, and qEEG whole-brain measures: spectral power and interhemispheric coherence were analysed. Sleep EEG analysis, particularly sleep spindle synchrony and duration and delta power, showed correlations with Griffiths III. Intrinsic difficulties in designing and assessing psychometric tests for early infancy and the improvement with age have been discussed and described in the literature(491-494), and sleep EEG markers may be a good candidate to help track the trajectory of development in early infancy.

4.4.1. Sleep macrostructure

A meta-analysis in older children (≥5-year old) found sleep macrostructure presented very small but statistically significant correlations to cognitive and behavioural aspects. Namely, sleep duration had a positive correlation to cognitive performances and school

grades and, a negative correlation to behavioural problems(483). A systematic review exploring the association between diurnal napping in younger children (≤5 years), in parameters like presence and duration of the nap and difficulty in settling for sleep, and the cognitive performance and behaviour found conflicting results and warrants further research(495). Still, there is an indication that most studies on naps in children point out their protective role on new learning and on the generalisation of word meanings(496). Accumulation of learning experiences and consolidation of their abstract representations would, in turn, contribute to establishing and improving developmental skills. Active sleep and REM are associated with synaptic formation, protection and selective pruning in maturing animal models(49, 50) and activity during NREM may be correlated to synaptic renormalisation(421) and also systematic neuroplastic processes that lead to learning and memory(135, 486). REM and NREM sleep also show a complementary effect on brain maturation; both appearing to play a part on sensorimotor maturation(125, 140, 141) and visual learning(142). In this study and after adjustment for potential confounders, I found only two statistically significant correlations between the macrostructure properties of the first sleep cycle and future development. Those were positive associations between latency to sleep and Foundations of Learning subscale and, between duration of stage N3 and Personal-Social-Emotional subscale. Longer sleep latency may be a limited proxy of resistance to homeostatic pressure and to consequent increased availability in terms of time and predisposition to explore and learn; while deficits in N3 duration may mediate decreased positive mood, as suggested in non-depressed adults(497), and by consequence affect emotional regulation and receptivity for social interactions.

4.4.2. Sleep spindles

Sleep spindle properties change in parallel with brain development(357). Like slow wave activity, spindles are associated with learning and memory(486) and cognitive abilities(344), with in vitro studies showing that spindle-like patterns can cause synaptic potentiation or depression(296). Sleep spindles reflect both brain anatomical properties(372, 498) and expression of risk-genes associated with some neurodevelopmental disorders(381), such as CACNA1I which encodes a subunit of CAV3.3, a T-type calcium channel highly

expressed on the thalamic reticular nucleus (TRN) from which sleep spindle expression depends and is simultaneously a risk gene for schizophrenia(499); consequently, individuals with schizophrenia and their first-degree relatives show sleep spindle alterations(376, 377).

4.4.2.1. Spindle synchrony

Negative correlations were observed between spindle synchrony and General Development and subscales of Eye and Hand Coordination, Personal-Social-Emotional and Gross Motor skills (subscales C, D and E). Spindles are predominantly local phenomena(246, 255, 402). In adults, scalp EEGs have shown that hemispheric-specific spindle activity increases are related to previous procedural learning(301) and are correlated with overnight verbal memory encoding(306). Also, abstract reasoning abilities appear to be associated with sided predominance of spindle activity(240). As described by Louis *et al.* there is a trend of decreasing synchrony from 3 to 6 months (calculation of asymmetry in Louis *et al.* study was based on the interval between the middle of two contralateral spindles being more than 2 seconds)(226). As such, the observations on spindle synchrony may also reflect its maturational aspect.

4.4.2.2. Spindle duration

In infants after 3 months and toddlers, there is a downward trend in spindle duration linked to age(226, 335, 368, 369), which may indicate that the shortening of sleep spindles is associated with the normal course of brain maturation at this age. A multiplicity of factors induces spindle termination and thus determine spindle duration. Several of these factors may work together(456), some related to connectivity and synchronisation between cortical and thalamic structures. Corticothalamic inputs support thalamic propagation and influence the rhythmicity of thalamic spindle oscillations(422). In this manner, desynchronisation between thalamic and cortical networks during the waning phase of spindles progressively depolarises thalamic neurons, hindering the generation of a new spindle oscillation cycle(254). In addition, both strength and spread of the thalamocortical and corticothalamic networks are correlated with spindle duration(255). The thalamocortical functional connectivity to the sensorimotor area decreases during infancy(500) possibly associated with

increased functional network specialisation, potentially affecting spindle duration. In this chapter, I found a negative correlation between spindle duration and gross motor skills development, supporting the notion that decreases in sensorimotor neural connectivity in infancy may be associated with the process of progressive specialisation(28, 473, 500).

4.4.2.3. Sleep spindle associations with motor and personal-social-emotional skills

Altered sleep spindles have been reported in cerebral palsy, a condition characterised by motor deficits(375); also, interhemispherical spindle power asymmetry in perinatal unilateral brain injury in infancy has a high positive and negative predictive power for later development of unilateral cerebral palsy(501). Furthermore, on an early night study of 6 month-old infants Jaramillo *et al.* found a positive association between spindle density above 13.5Hz and mean spindle frequency, and gross motor skills reported by parents using Ages and Stages Questionnaire at 24 months after correcting for age and sex(333). Different age groups and methodologies may justify the different observations between the latter and the present studies. However, my thesis builds on evidence highlighting the role of sleep spindles in infancy and the maturation of motor skills(141).

In 5-year old children, nighttime number and frequency of spindles are positively correlated with stress-coping strategies, personal and social behaviour(502), number of spindles is also predictive of emotional and behavioural competencies one year later(503). In contrast, studies on spindles in younger children during naps or partial night sleep recordings found non-significant results on the association between the spindle features analysed and scales evaluating behavioural and social skills. Page *et al.*, reported no association between spindle frequency, amplitude, duration and density in children from 12 to 30 months and their current scores using Vineland Adaptive Behavior Scales–Second Edition(335). Jaramillo *et al.* found no association between spindles at 6 months, namely density above 13.5Hz and mean spindle frequency, during early NREM night sleep and parent reported personal-social skills at the time of recording and at 12 and 24 months(333). In the present analysis, I found that spindle duration and synchrony at 4 months were predictive of the Personal-Social-Emotional quotient at 18 months. Infancy and toddlerhood are marked by successive milestones in self-awareness, social and emotional development domains(504, 505). The default mode network has been correlated with self-referential processes(506, 507) and social competencies(508,

509). The infancy period coincides with the emergence of functional connectivity within default mode networks(510) and between the default mode networks and the thalamus(500) which may explain the associations of sleep spindles with later observed behaviour.

4.4.3. qEEG

4.4.3.1. Delta spectral power

Spectral power was the qEEG measure that showed the highest number of significant correlations to Griffiths developmental quotients at 18 months. When evaluating magnitude across the different EEG frequency bands through spectral power analysis, quotients of General Development and subscales B (Language and Communication), D (Personal-Social-Emotional) and E (Gross Motor) were positively associated with delta frequencies after adjustment. In a study by Kim *et al.* when 0.1-4Hz microelectrode-detected waves were divided into two classes based on the amplitudes of the up- and preceding down-states, they were found to have distinct and opposing roles(136), compatible with progressive down-selection of synapses(48, 136), or with synaptic strengthening(136, 511). According to the sleep homeostatic hypothesis, synaptic strength increases with wakefulness duration and learning activities. It has, however, an associated energy cost that leads to a need for renormalisation through net synaptic down-selection, ideally during brain "offline" periods(48). In an incompletely myelinated brain, with a high level of redundant synapses such as in the maturing brain, net synaptic down-selection may promote brain energy efficiency(171). Additionally, slow oscillations are also believed to facilitate temporal coupling with other oscillations such as sleep spindles and sharp wave ripples(313, 512), contributing to the transfer of particular memories traces from the hippocampus to the neocortex(486) (see Mason and Spencer(319) for discussion on the active system consolidation in infancy and childhood). In the paediatric population, slow wave activity is affected by prior duration of wakefulness, previous awaken stimuli exposure, and brain maturation(513) including myelination(414, 485). The daily slow wave activity gains, characteristic from childhood, are thought to assist the consolidation of abstract representations of memory traces and to allow generalisable consolidation of new learning in early development(513). The continued influence of incremental slow wave activity may facilitate a multitude of complex skill learning (415). Two subscales were not associated with delta power, those were Foundations of

Learning and Eye and Hand Coordination (subscales A and C). In line with my findings on subscale A, another developmental study found no association with the current cognitive skills of infants involved(334). In the light of the described function of slow waves, it may seem counterintuitive that Foundations of Learning is not associated with delta power. However, subscale A does not necessarily inform on long-term memory abilities, focusing instead on abilities to explore, and receive new information such as creative play and executive function. Subscale A maturation may be better reflected in parameters like coherence levels(514) as also demonstrated in this chapter.

In studies assessing 8-months-olds or young children, delta amplitude over frontal and occipital areas were positively associated with current fine motor abilities(334, 335). Despite these observations, the present study showed no association between delta power at 4 months and later Eye and Hand Coordination levels, possibly indicating that the previous findings of associations between fine motor skills and delta activity are specific to frontal and occipital regions, not extending to whole-brain analysis or to future development. I performed many correlation analyses in this study which increases the probability of false positives. I note however, all correlations were concentrated towards delta power, making the observations consistent and these associations are expected to be stronger amongst defined neurodiverse cohorts.

4.4.3.2. Interhemispheric coherence

After correcting for gestational and postnatal age at the time of the appointments and sex, two main trends amongst the significant results on interhemispheric coherence are noted. One was the interhemispheric coherence of fast rhythms which were positively correlated with subscale A at 18 months during N2. Subscale A assesses features related to learning aspects such as skills for learning, ways of thinking, working memory and play (438). Tasks requiring executive function (514) and associative learning (515) may produce functional changes in connectivity measured by EEG coherence. Neuronal coherence is hypothesised to reflect neuronal communication (516) with potential for changes in synaptic plasticity (517). Adding to these observations, this chapter shows that ‘offline’ aspects of global interhemispheric connectivity may partially indicate skills for learning at 18 months.

The second trend observed was the increased delta frequency interhemispheric disconnection during REM for infants who performed better on subscale E in early childhood.

This subscale focuses on personal-social-emotional aspects. Infants who score higher on this scale demonstrate higher independence, higher levels of empathy and tempered humour and seek more social interactions (518). Sleep has been associated with mood and emotional processing; its deprivation is related to decreased social and intrapersonal functioning (169, 519). Evidence suggests REM plays a critical role in sleep emotional processing, although there are discrepancies between studies regarding the direction of this modulation (144). Despite the associations described here on coherence and future development, it is important to highlight that global interhemispheric coherence accounted for only 2.98% of statistically significant correlation pairs of all potential correlation pairs analysed with uni- and multivariate analysis. Due to the high number of correlations analysed, results may be due to statistical error type I. Thus, the global interhemispheric coherence measured in this study should be regarded with caution.

4.4.4. Socioeconomic factors

Household income and highest level of maternal education were investigated as potential confounders in the relationships between the sleep features and Griffiths III quotients. Maternal education was not a confounder as it was not associated with Griffiths III quotients and hence I did not adjust for it in any analysis. Although household income was associated with Griffiths III quotients at 18 months, the association between sleep features and Griffiths III remained unaltered when adjusted for household income (Supplementary table 7 – Appendix 8). This analysis had less than three-quarters of the participants from the original qEEG analysis, as only 50/69 participants provided income information, which may have limited results. However, it should be noted that parental socioeconomics and alterations to this status affect childhood cognitive scores (520); consequently impacting adulthood risk for social exclusion and have important socioeconomic repercussions later in life (520). This way, early identification of risk for lower cognitive outcomes may allow psychosocial intervention with the potential for improving cognitive outcomes up to adulthood (521, 522), better psychosocial outcomes (522), education achievement (522) and job situation (523).

In summary, sleep spindle synchrony and duration, as well as, delta power present consistent small to medium associations with development at 18 months. Together, sleep spindles and delta power were associated with 4/5 Griffiths' subscales (the exception was Foundations of Learning) and general development quotients at 18 months. Macrostructure features and interhemispheric coherence, as analysed here, should be interpreted with caution due to the possibility of type I error.

4.4.5. Strengths/limitations

This paper analyses a diverse range of sleep EEG biomarkers in term-born infants and their association with future development. This is one of the largest cohorts relating future cognitive ability to EEG sleep in healthy infants.

I recorded sleep in the sleep lab, which allowed to control for environmental stimuli that could interfere with sleep and thus provided standardised conditions to the participants. However, due to practical logistic considerations, an adaptation nap was not performed. The first sleep in a lab correlates with lower sleep efficiency and higher proportions of light sleep in children compared to subsequent sleeps (196). In adolescents at least, sleep spectral power (524, 525) and sleep spindles parameters (238, 526) show good individual stability over consecutive nights, except for duration and density of slow spindles (453). Slow spindles are a type of spindle that emerges in populations older than my research participants at EEG recording (343), and for this reason, this factor will not affect the present observations. As noted by Beaugrand *et al*. (416) a desirable EEG marker of development should allow a feasible application to clinical settings. Therefore, studying sleep markers from the first sleep recording presents an advantage for adapting protocols to clinical settings.

This chapter objective was to examine a diverse range of EEG biomarkers and assess which features better predict development. However, multiple tests do increase the likelihood of type 1 error. Despite that, this chapter showed that statistically significant features gravitate towards sleep spindle synchrony and duration and delta spectral power, making these promising biomarkers for predicting development. These biomarkers may facilitate early identification of risk for adverse outcome in infants, which would enable early and targeted intervention.

There is a high prevalence of well-educated mothers and high-income families that consented for this study. Additionally, the median (IQR) values on maternal EPDS and attachment scale indicate a low likelihood of depression and high attachment scores in the maternal population study (Table 4.1), which also may influence positive aspects of developmental outcomes in this cohort(527-529). A possible limitation to this study is the underrepresentation of less favourable contexts.

I used Spearman's rank correlations in my analyses because some of the examined correlations did not meet the assumptions of normality and linearity required for Pearson's correlation. Spearman's rank correlation assesses monotonic relationships rather than linear ones. This is an initial study and future studies should include multivariable analysis of the sleep parameters identified in this study as being associated with neurodevelopment at 18 months.

Most of the statistically significant results showed small to medium associations, indicating that the predictive ability of the sleep parameters at 4 months to determine Griffiths III neurodevelopmental outcomes obtained approximately 14 months later is weak. Considering that neurodevelopment is dependent on ongoing environmental influences, but also that the diagnostic accuracy of psychometric tests improves with participants age, a follow-up study would be valuable to determine the strength of the correlations on the sleep patterns at 4 months in an older cohort.

For completeness, future studies should analyse sleep EEG features identified here on specific developmental conditions at early ages.

4.5. Conclusions

This chapter has shown that sleep, and in particular EEG sleep spindles and delta power, may be a useful biomarker of development in early childhood. Future studies aimed at identifying potential atypical development to facilitate early intervention, should consider the sleep biomarkers analysed here.

Chapter 5. EEG during sleep in moderate to late preterm infants and the association with neurodevelopment – a cohort study

5.1. Introduction

One in ten newborn infants are born preterm, of which more than 80% are moderate to late preterm (MLPs), defined as born between 32 and 36 completed weeks gestational age (GA)(530). Processes of rapid brain volume growth (531), cellular differentiation, dissolution of the subplate, the establishment of new synapses and ongoing gyrification (16) that typically occur prenatally must proceed in the extra-uterine environment in a premature neonate. Compared to gestational age-matched foetuses, premature birth triggers rapid brain surface area growth and gyrification, with the greatest differences observed at lower gestational ages (532). Early premature birth has also been associated with impaired glutamatergic neurogenesis (533). Several synaptic properties: synaptic density, ratio of axoshaft/axospine contacts, proportion of asymmetric synapses and synaptic length, differ between term and preterm neonates at equivalent corrected term age in the primate model, with some differences persisting at least up to the 3rd week at corrected age (41). Premature-born infants are vulnerable to different white and grey matter injuries (534, 535). Factors such as maternal and foetal morbidity and, demographic variables including socioeconomic status have a negative impact on developmental outcomes (37, 536). The preterm period is a time of particular vulnerability for infants when environmental factors can affect health and development (39, 66). The preterm population may be particularly exposed to many adverse factors. There is extensive literature on how pain and stress may affect the maturing brain (39); for example, persistent exposure to noise such as alarms can potentially limit tactile learning (537). Greater maternal presence and routine massage during hospitalisation may reduce the infant's stay (538, 539) and positively influence neurodevelopmental outcomes in early childhood (538, 540). Adequate sleep is critical for good brain development (52) and may be compromised during hospitalisation (51), and preterm-born children may show lower sleep quality (541). Preterm children may show lower deep-to-light sleep ratios, which are associated with behavioural and emotional difficulties (541) Furthermore, preterm children

are more sensitive to lower sleep efficiency than their term peers leading to poorer scores on IQ tests, arithmetic, selective attention and visuospatial memory tasks in the former group (542).

Sleep biomarkers provide insight into brain development. During the first hours after birth, extreme and very preterm infants show faster maturation of several qEEG features (215). The disappearance of tracé alternant and the emergence of the first sleep spindles occur approximately 12 and 8 days earlier, respectively, in very preterm-born infants (corrected age) compared with term-born infants (210). Other EEG differences between very preterm-born and term-born cohorts are still present in infancy (211) and adolescence (217) and correlate with developmental outcomes. For example, at term age, the spectral power ratio of medial to lateral brain regions during NREM sleep is lower in preterm cohorts and positively associated with motor performance (211). Adolescents who had a very premature birth are more likely to display a spectral profile with the highest delta and lowest relative theta, alpha and beta spectral powers. In turn, this profile is also associated with the lowest intelligence coefficient, academic performance, and motor functioning scores (217).

Even the late preterm subgroup of MLP neonates, a cohort that has been frequently overlooked due to lower risks compared to more immature preterm cohorts, shows higher mortality and morbidity indices compared to term-born neonates (543). During childhood, they are also at greater risk for poorer physical health and well-being, delays in language and communication development, grade repetition and attention deficit and hyperactivity disorder (536). Analysing infants from the same cohorts, Ryan *et al.* has recently shown that healthy MLP-born children scored lower than term-born children at 18 months in all Griffiths III subscales and general development scores after adjusting for sex (544). Given these results, the primary aim of this chapter's research was to investigate whether there are underlying sleep alterations namely, sleep spindles, sleep EEG macrostructure, and EEG background activity in the MLP cohort compared to the term-born cohort at 4 months corrected age. Similarly to the previous, this chapter characterises EEG background activity with spectral power which provides general information on the EEG rhythms magnitude and, coherence to measure functional connectivity. The secondary aim was to investigate possible correlations between sleep feature that differed between groups at 4 months and neurodevelopment at 18 months.

5.2. Methods

5.2.1. Cohorts

This study included healthy infants from two cohort studies, recruited from 2017 to 2018 at Cork University Maternity Hospital, which had both received ethical approval from the Cork Research Ethics Committee of the Cork Teaching Hospitals. The MLP cohort derived from an observational sleep study of healthy uncomplicated MLP infants admitted to the neonatal unit at birth (545). Parental informed consent was obtained in the neonatal unit. Inclusion criteria were gestational age at birth ≥32 weeks and up to 36^{+6} weeks, clinically stable, a normal physical and neurological clinical assessment and no confirmed or suspected genetic anomalies. Infants from singleton and multiple pregnancies were included in the original study, but only one infant per set of twins or triplets was included in the present study as sleep patterns are strongly influenced by family heredity(546). If all infants from the set met the inclusion criteria for the present study and had a complete first sleep cycle recorded, the infant to include was selected randomly. The term cohort derived from a randomised controlled trial of massage in infancy, BabySMART (ClinicalTrials.gov Identifier: NCT03381027); only the non-intervention arm was included in the present study. Parental consent was obtained post-delivery at the bedside. Inclusion criteria were gestational age at birth ≥37 weeks, not requiring admission to the neonatal unit, absence of confirmed or suspected metabolic or genetic anomalies and being a singleton(488).

5.2.2. EEG recording and data analysis

The term-born cohort sleep EEGs were recorded at approximately 4-months and the MLP cohort at 4 months corrected age. Sleep EEGs took place at the INFANT Research Centre Sleep lab using the Lifelines iEEG (Lifelines Neuro, UK) acquisition system. EEG electrodes were placed according to the 10-20 system at positions Fp1, Fp2, Fz, F3, F4, F7, F8, Cz, C3, C4, T3, T4, Pz, P3, P4, T5, T6, O1, O2, A1 and A2. Additional polysomnography measures for sleep staging included chin electromyography, electrooculogram, electrocardiogram, and a sensor

to monitor respiration. EEG quality was monitored using a modified double banana montage, timebase was set 3cm/s, high-pass and low-pass filters were respectively at 0.5 and 70Hz, and gain was set at 150µV/cm and adjusted according to needs. EEG was sampled at 500Hz. Synchronous video was recorded to further facilitate accurate sleep scoring. EEG appointments were individually booked, usually between 9.30 and 12.00 am for morning, and 1.30 and 4.00 pm for afternoon appointments, according to the infant's typical diurnal nap time to increase likelihood of sleep. Electrodes were typically placed during a feed to distract the child and induce drowsiness. Recording was continued for the duration of the nap.

Post-acquisition sleep staging was performed according to the AASM 2.4 guidelines using Nicolet (Natus, USA) sleep staging software, as previously described(488). Sleep macrostructure was calculated based on the sleep staging from first sleep cycle and included cycle and the different sleep stages durations, and latencies to sleep and REM. Sleep spindles were annotated in term and MLP cohorts using the EEG reviewing software (Stratus EEG, Iceland). Spindle density was calculated as the number of spindles per minute of NREM. The sleep spindle features of mean frequency, BSI (437), synchrony, and spectral power and the background qEEG measures of spectral power and electrode-pairs coherence, were calculated in MATLAB (version R2020a, The MathWorks Inc., USA). BSI, spectral power, and coherence were calculated using an open-source toolbox NEURAL version 0.4.3(195) for MATLAB. BSI represented differences in spectral power between the two hemispheres, with 75% overlap of a 30s sliding window. Spindle BSI, in particular, was calculated on selected spindle events using an additional 9 to 16Hz pass-band filter. Spindle synchrony was calculated as the percentage of time bilateral spindles coincided. Sleep spindle spectral power was calculated using the Welch power-spectral density method with a ½-second sliding window, based on their minimum length(121). In contrast to my previous papers(547, 548) (see Chapter 3 and Chapter 4) background spectral power was calculated using the Welch power-spectral density method with an 8-second instead of 2-second window offering a slightly higher smoothing effect of the data obtained. Some infants had more than one sleep cycle, but analysis was based on the first sleep cycle only, as decreasing sleep pressure with successive sleep cycles is known to affect certain sleep features (549, 550).

Background EEG spectral power and coherence are measures of EEG magnitude and functional connectivity respectively. These were separated into NREM and REM and into 7 frequency bands (Delta 1: 0.5-2Hz; Delta 2: 2-4Hz; Theta: 4-8Hz; Alpha: 8-12Hz; Sigma: 12-

15Hz; Beta: 15-30Hz; Gamma: 30-45Hz). Coherence was measured between electrode pairs as in Meijer *et al.*(551) and summarised by the maximum coherence value in the frequency band. Intrahemispherical coherence was analysed between pairs of bipolar channels representing regions of the cortex: A1-T5 and C3-P3, A1-T5 and T5-O1, A2-T6 and C4-P4, A2-T6 and T6-O2, C3-P3 and T5-O1, C4-P4 and T6-O2, Fp1-F3 and A1-T5, Fp1-F3 and C3-P3, Fp1-F3 and T5-O1, Fp2-F4 and A2-T6, Fp2-F4 and C4-P4, Fp2-F4 and T6-O2; and interhemispherical coherence was analysed between the following pairs of channels: A1-T5 and A2-T6, C3-P3 and C4-P4, F3-C3 and F4-C4, F7-A1 and F8-A2, F7-T3 and F8-T4, Fp1-F3 and Fp2-F4, Fp1-F7 and Fp2-F8, P3-O1 and P4-O2, T3-T5 and T4-T6, T5-O1 and T6-O2, A1-T5 and A2-T6, C3-P3 and C4-P4.

Infants who had a low number of spindles originating from frontocentral areas were excluded from the sleep spindle analysis. In addition, for sleep spindle density and number analysis, infants who were asleep before recording onset were excluded, and for spindle density infants with reduced montage were also excluded. Macrostructure and qEEG included the infants who had a full first sleep cycle recorded, i.e. falling asleep before recording started and having both NREM and REM. Additionally, for the latency to REM, infants were excluded if meeting criteria for REM before N2.

5.2.3. Neurodevelopmental assessments

Neurodevelopment was assessed using Griffiths III at approximately 18 months on the term cohort and 18 months adjusted age on the MLP cohort. Griffiths III was assessed by health professionals with certification from ARICD. Griffiths III encompasses 5 subscales of development and general development (GD), the latter calculated from these subscales. The subscales are A – foundations of learning, B – language and communication; C – eye and hand coordination; D – personal-social-emotional and E – gross motor(438). A developmental quotient was calculated for each infant on GD and Griffiths subscales. Griffiths III lower and upper quotient intervals are <50 and >150 respectively; lower values indicate poorer neurodevelopment.

5.2.4. Statistical analysis

IBM SPSS Statistics software (version 26.0, IBM Corp., Armonk, NY, USA) was used for the statistical analysis. Continuous variables were described using mean and standard deviation (SD) or median and interquartile range (IQR), and categorical variables using frequency and percentage. For comparisons between the term and preterm groups, the independent samples t-test or the Mann–Whitney U test were used for continuous variables, and the χ^2 test (with continuity correction for 2×2 tables) or, in the case of small expected counts, Fisher's exact test or Fisher-Freeman-Halton Exact Test (the later for tables larger than 2x2) for categorical variables. The secondary analysis investigated relationships between the 4-months sleep features that differed between the term and preterm groups and Griffiths III developmental quotients at 18-months. Infants who did not attend the Griffiths assessment at 18-months were excluded from this analysis. Spearman's rank correlation coefficients and Partial Spearman's rank correlation coefficients (adjusted for sex and multiple births) were used to investigate and quantify relationships. All tests were two-sided, and a p-value <0.05 was considered statistically significant.

5.3. Results

5.3.1. Demographics

After assessment for eligibility, 59 MLP and 96 term-born infants were included in the study. The number of infants included in the macrostructure, sleep spindles, background qEEG and secondary analyses are detailed in Figure 5.1. Due to COVID restrictions, 7/39 MLPs included on the secondary analysis had their Griffiths III assessment postponed to 20.37-21.78 months adjusted ages.

Demographic variables for both groups are presented in Table 5.1. The mean (SD) gestational age at birth for the MLP group was 34.5(1.3) weeks (n=59), while term controls were born at 39.8(1.2) weeks (n=96) (Table 5.1). As expected, MLP infants were lighter at birth (p<0.001), but no weight differences were found at 4 months corrected age compared to the term cohort (p=0.15). Type of feeding at 4 months was significantly different between

the two groups (p=0.006), with a higher percentage of MLPs exclusively fed on infant formula (81.4% (48/59) vs 56.3% (54/96) and a lower percentage of MLPs exclusively breastfed [11.9% (7/59) vs 30.2% (29/96)] compared to term infants. Also, unlike the term-born group, the majority of MLPs had been introduced to solid food [61.0% (36/59) vs 27.1% (26/96), p<0.001].

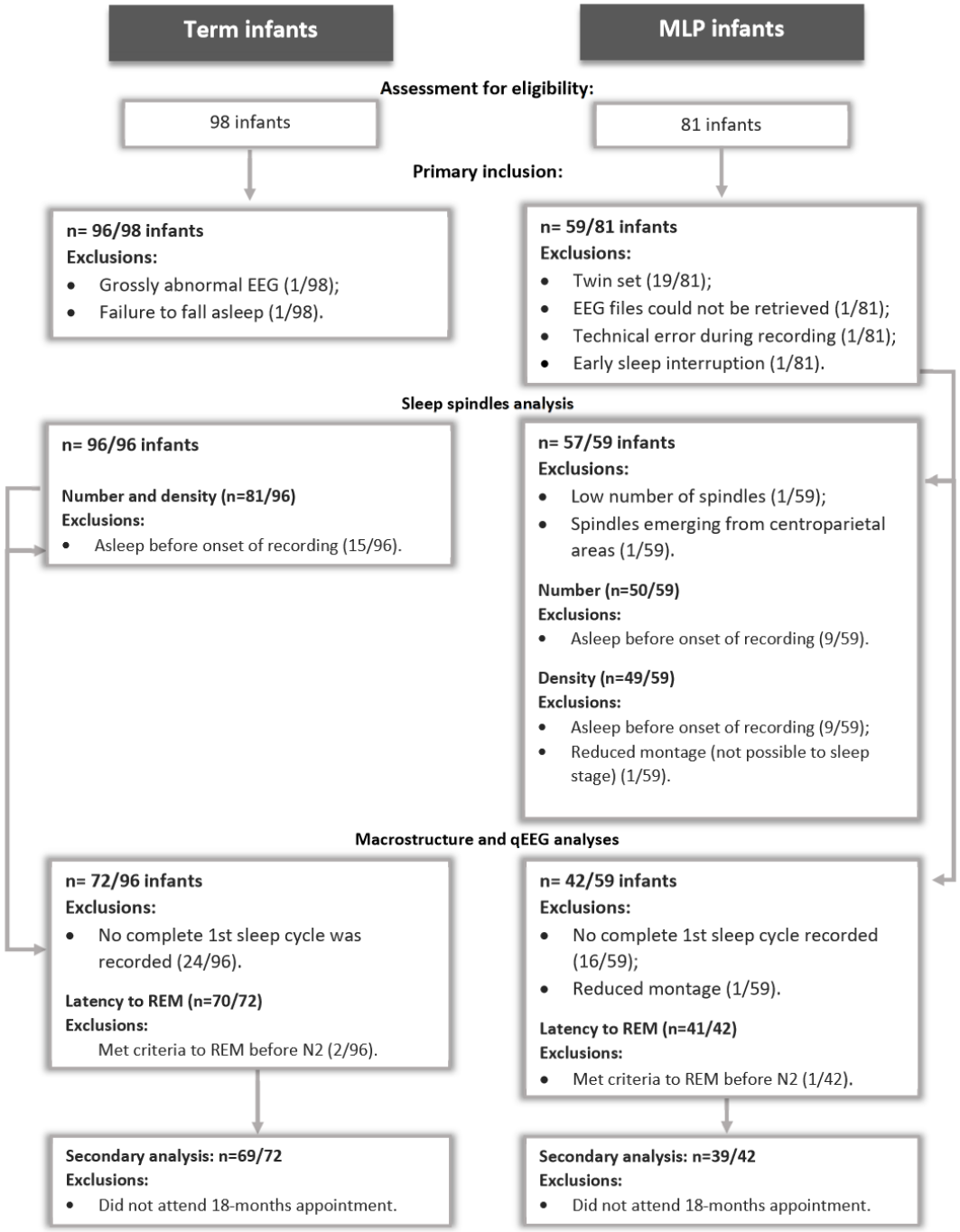


Figure 5.1 - Flow diagram detailing number of infants included/excluded in each analysis.

Table 5.1 - Demographic data.

	Term infants			MLP infants			p-value[b]
	n	mean	(SD)[a]	n	mean	(SD)[a]	
Maternal characteristics							
Maternal age (years)	96	34.4	(4.2)	53	35.5	(4.8)	0.13
Nationality - Irish, n (%)	96	85	(88.5)	59	55	(93.2)	0.50[c]
Smoker, n (%)	96	3	(3.1)	58	1	(1.7)	1.00[d]
Professional category	94			43			0.48[e]
Professional		60	(63.8)		25	(58.1)	
Lower professional		2	(2.1)		2	(4.7)	
Managerial/ Technical/ Admin		14	(14.9)		6	(14.0)	
Non-Manual		11	(11.7)		5	(11.6)	
Manual		5	(5.3)		1	(2.3)	
Student		1	(1.1)		3	(7.0)	
Unemployed		1	(1.1)		1	(2.3)	
Infant data - at birth							
Sex - Female n (%)	96	40	(41.7)	59	30	(50.8)	0.34[c]
Weight (kg)	96	3.57	(0.46)	59	2.17	(0.45)	<0.001*
Infant characteristics - at the 4 months appointment							
Chronological age (weeks)	96	19.6	(1.3)	59	24.5	(1.9)	<0.001*
Postmenstrual age (weeks)	96	59.4	(1.8)	59	59.0	(1.4)	0.14
Weight (Kg)	96	7.09	(0.90)	59	6.87	(0.84)	0.15
Type of feeding, n (%)	96			59			0.006[f]*
Breast feeding		29	(30.2)		7	(11.9)	
Infant Formula		54	(56.3)		48	(81.4)	
Mix of both		13	(13.5)		4	(6.8)	
Introduction to solid foods – Yes, n (%)	96	26	(27.1)	59	36	(61.0)	<0.001[c]*
Infant characteristics - at the 18 months appointment							
Chronological age (months), median (IQR)	92	18.4	(18.2 to 18.7)	54	19.7	(19.4 to 20.4)	<0.001[g]*
Adjusted age (months), median (IQR)	92	18.4	(18.2 to 18.7)	54	18.4	(18.1 to 19.3)	0.52[g]*

Descriptive statistics using mean and standard deviation (SD) or median and interquartile range (IQR) or n (%). Statistically significant differences ($p < 0.05$) marked with an asterisk. [a]Unless otherwise stated. [b]From independent samples t-test unless otherwise stated. [c]From $\chi 2$ test with continuity correction. [d]From Fisher's exact test. [e]From Fisher-Freeman-Halton Exact Test. [f]From Pearson's $\chi 2$ test. [g]From Mann-Whitney U test.

5.3.2. Macrostructure

Both macrostructure and qEEG analysis included only infants with a complete first sleep cycle recorded, corresponding to 72/96 in the term cohort and 43/59 in the MLP cohort. A further MLP infant was excluded due to a reduced montage EEG, incompatible with sleep staging according to the AASM rules (Figure 5.1). Hence, 72 term infants and 42 MLP infants are included in the comparison of macrostructure features between the two groups. For latency to REM, infants who met the criteria for REM before N2 were excluded (n=2 term infants and n=1 MLP infant).

N2 duration was significantly shorter ($p<0.001$) in the preterm cohort [median (IQR): 2.75 (1.88 to 4.63) minutes, n=42] compared to the term cohort [median (IQR): 4.50 (2.50 to 6.50) mins, n=72]. There were no statistically significant differences in any of the other macrostructure features studied (Table 5.2).

5.3.3. Sleep spindles

Sleep spindles were manually annotated for 96 infants in the term cohort and 59 infants in the preterm cohort. Two MLP infants were excluded from spindle analysis because of a short 1st sleep cycle that only allowed for the recording of two spindles (n=1) and because of a low number of frontocentral spindles (n=1). Hence, 96 term infants and 57 MLP infants are included in the comparison of sleep spindle features between the two groups (Figure 5.1). There were no statistically significant differences in any of the sleep spindle features studied (Table 5.2).

Table 5.2 - Macrostructure and sleep spindles measures.

Sleep features	Term infants		MLP infants		p-value[a]
	n	median (IQR)	n	median (IQR)	
Macrostructure					
1st cycle duration (min)	72	43.25 (37.00 to 51.88)	42	42.00 (35.88 to 47.63)	0.28
NREM duration (minutes)	72	35.50 (30.13 to 40.50)	42	34.25 (28.88 to 38.75)	0.23
N1 duration (minutes)	72	7.75 (4.50 to 13.38)	42	8.25 (5.00 to 11.13)	0.92
N2 duration (minutes)	72	4.50 (2.50 to 6.50)	42	2.75 (1.88 to 4.63)	<0.001*
N3 duration (minutes)	72	21.50 (16.63 to 25.38)	42	22.00 (17.13 to 26.63)	0.55
REM duration (minutes)	72	6.50 (3.63 to 11.50)	42	7.25 (3.75 to 11.75)	0.81
Latency to sleep (minutes)	72	8.50 (4.00 to 14.00)	42	8.25 (3.88 to 16.13)	0.86
Latency to REM (minutes)[d]	70	36.00 (30.50 to 41.63)	41	36.00 (29.00 to 41.00)	0.46
Spindles					
Density (spindles/minutes)[b]	81	6.72 (5.79 to 8.03)	49	6.83 (5.84 to 7.91)	0.95
Number[c]	81	244.0 (194.0 to 286.5)	50	227.5 (186.3 to 279.0)	0.27
BSI	96	0.20 (0.16 to 0.29)	57	0.23 (0.19 to 0.32)	0.12
Synchrony (%)	96	59.21 (53.19 to 63.81)	57	56.52 (51.50 to 61.90)	0.17
Spectral Power (µV2)	96	7.79 (4.72 to 11.47)	57	6.51 (4.90 to 9.48)	0.42
Frequency (Hz)	96	13.02 (12.79 to 13.27)	57	12.93 (12.64 to 13.18)	0.11
Duration (s)	96	2.92 (2.60 to 3.58)	57	3.17 (2.64 to 3.59)	0.47

Statistically significant differences ($p < 0.05$) marked with an asterisk. [a]From Mann-Whitney U. [b]Infants who fell asleep before onset of recording and had a reduced montage (not possible to sleep stage) were excluded. [c]Infants who fell asleep before onset of recording were excluded. [d]Infants who had met the criteria for REM before N2 were excluded.

5.3.4. qEEG features

5.3.4.1. Spectral power

Spectral power analysis revealed group differences exclusively during NREM sleep. These were higher (absolute) spectral power in delta 1 and 2 in the MLP group (Table 5.3).

Table 5.3 - Whole brain absolute spectral power.

Frequency Bands	Term infants (n=72) median (IQR)		MLP infants (n=42) median (IQR)		p-value[a]
NREM					
Delta 1 (μV^2)	593.34	(449.21 to 749.36)	702.49	(557.46 to 840.67)	0.011*
Delta 2 (μV^2)	215.32	(164.78 to 283.39)	245.31	(194.17 to 327.77)	0.028*
Theta (μV^2)	80.91	(61.60 to 95.84)	81.75	(67.72 to 111.15)	0.13
Alpha (μV^2)	10.19	(7.93 to 12.72)	10.60	(9.10 to 14.78)	0.053
Sigma (μV^2)	4.75	(3.72 to 6.42)	5.23	(3.99 to 6.78)	0.15
Beta (μV^2)	4.81	(3.97 to 6.07)	5.36	(4.24 to 6.51)	0.082
Gamma (μV^2)	1.67	(1.37 to 2.06)	1.70	(1.40 to 2.19)	0.54
REM					
Delta 1 (μV^2)	285.83	(209.19 to 365.81)	330.34	(256.53 to 386.98)	0.058
Delta 2 (μV^2)	107.80	(77.38 to 138.32)	111.36	(93.81 to 152.35)	0.40
Theta (μV^2)	39.29	(31.78 to 49.64)	42.32	(37.07 to 58.21)	0.11
Alpha (μV^2)	4.84	(4.00 to 6.38)	5.07	(4.45 to 6.00)	0.27
Sigma (μV^2)	1.31	(1.11 to 1.75)	1.43	(1.27 to 1.73)	0.16
Beta (μV^2)	3.75	(3.20 to 4.79)	3.89	(3.22 to 5.11)	0.49
Gamma (μV^2)	1.86	(1.47 to 2.56)	1.94	(1.42 to 2.41)	0.98

Statistically significant differences ($p < 0.05$) marked with an asterisk. [a]From Mann-Whitney U Test.

5.3.4.2. Coherence

All coherence results are presented in Supplementary table 1 – Appendix 9. Table 5.4 is restricted to the statistically significant differences in coherence between groups, coherence values were multiplied by 100 to improve readability. A schematic representation of the statistically significant associations is presented in Supplementary figure 1 – Appendix 10. MLP infants showed altered functional connectivity, which were either enhanced or reduced compared to term infants. Two patterns stand out: frontal lobes connections, i.e. within and, between other brain regions and frontal lobes, were particularly associated with enhanced coherence in the preterms and, most of the lower coherences in the MLP group were within interhemispherical parietooccipital connectivities.

Table 5.4 - Statistically significant results from coherence analysis.

Frequency Bands	Channels	Term infants (n=72) Median (IQR) x10²		MLP infants (n=42) Median (IQR) x10²		p-value[a]
NREM Interhemispherical Coherence						
Delta 1	P3-O1 & P4-O2	90	(89 to 92)	89	(86 to 90)	0.002
Delta 2	P3-O1 & P4-O2	88	(87 to 90)	87	(85 to 88)	0.001
Theta	P3-O1 & P4-O2	89	(88 to 90)	88	(87 to 89)	0.013
Alpha	P3-O1 & P4-O2	89	(87 to 90)	88	(87 to 89)	0.009
Sigma	P3-O1 & P4-O2	88	(86 to 89)	86	(85 to 88)	0.002
Beta	P3-O1 & P4-O2	94	(94 to 95)	94	(93 to 94)	0.035
Beta	A1-T5 & A2-T6	94	(94 to 95)	95	(94 to 96)	0.002
Gamma	P3-O1 & P4-O2	94	(94 to 95)	94	(93 to 94)	0.011
Gamma	A1-T5 & A2-T6	94	(93 to 94)	94	(94 to 95)	0.047
NREM Intrahemispherical Coherence						
Delta 1	Fp2-F4 & T6-O2	79	(78 to 80)	80	(79 to 82)	0.039
Delta 2	Fp2-F4 & T6-O2	81	(80 to 81)	82	(81 to 83)	0.002
Alpha	Fp2-F4 & T6-O2	86	(86 to 87)	86	(86 to 88)	0.027
Sigma	Fp1-F3 & A1-T5	87	(86 to 89)	86	(85 to 87)	0.009
Beta	C3-P3 & T5-O1	93	(92 to 93)	93	(93 to 94)	0.039
Beta	Fp2-F4 & A2-T6	93	(93 to 93)	93	(93 to 94)	0.019
Gamma	Fp1-F3 & A1-T5	93	(93 to 93)	93	(93 to 94)	0.028
REM Interhemispherical Coherence						
Delta 1	P3-O1 & P4-O2	91	(87 to 93)	89	(85 to 91)	0.029
Delta 1	T3-T5 & T4-T6	87	(85 to 88)	88	(85 to 90)	0.033
Delta 2	Fp1-F7 & Fp2-F8	87	(85 to 90)	89	(87 to 90)	0.050
Sigma	Fp1-F3 & Fp2-F4	87	(86 to 89)	89	(87 to 90)	0.003
Sigma	A1-T5 & A2-T6	87	(86 to 90)	90	(87 to 92)	0.003
Beta	Fp1-F3 & Fp2-F4	94	(93 to 95)	95	(94 to 95)	0.029
Beta	A1-T5 & A2-T6	95	(94 to 96)	96	(94 to 97)	0.010
REM Intrahemispherical Coherence						
Delta 1	A2-T6 & T6-O2	84	(82 to 87)	83	(81 to 85)	0.028
Delta 1	A1-T5 & C3-P3	88	(86 to 89)	89	(87 to 92)	0.034
Delta 2	A2-T6 & C4-P4	91	(89 to 92)	92	(89 to 94)	0.036
Delta 2	A1-T5 & C3-P3	91	(89 to 92)	93	(91 to 94)	0.002
Theta	A2-T6 & T6-O2	90	(88 to 92)	88	(87 to 90)	0.008
Theta	Fp2-F4 & A2-T6	89	(87 to 90)	88	(86 to 89)	0.023
Sigma	Fp2-F4 & A2-T6	85	(83 to 87)	86	(84 to 89)	0.009
Beta	Fp1-F3 & T5-O1	93	(92 to 94)	94	(93 to 94)	0.037
Gamma	A2-T6 & T6-O2	95	(95 to 96)	95	(94 to 96)	0.005
Gamma	Fp2-F4 & T6-O2	93	(92 to 94)	94	(93 to 94)	0.023

[a]From Mann-Whitney U Test

5.3.5. Sleep features and Griffiths III

Unadjusted and adjusted results for Griffiths III scores at 18 months and 4-month sleep features that differed between groups are presented in supplementary tables 2a and 2b – Appendix 9, respectively. Multiple births were considered for the adjusted analysis as co-sleeping with a young infant may induce an external sleep deprivation effect. The macrostructure feature N2 duration was not associated with the GD or with any subscale.

Delta 1 and 2 spectral power were positively correlated with Griffiths III subscale D (personal-social-emotional subscale) in the unadjusted and adjusted analysis. Delta 1 and 2 were positively associated with subscale E (gross motor) after adjusting for sex and multiple births. Delta 2 was also positively associated with subscale B (language and communication) in the unadjusted analysis but the association did not remain after adjusting for age and multiple births.

Coherence also showed associations with Griffiths III. During NREM sleep, coherence between P3-O1 and P4-O2 areas was positively associated with subscale E (gross motor) and GD for gamma in both the unadjusted and adjusted analyses. Coherence between Fp1-F3 and A1-T5 during NREM was negatively associated with subscale A (foundations of learning) in the gamma band before and after adjustment for sex and multiple births. In REM sleep, delta 1 coherence between P3-O1 and P4-O2 areas was positively associated with subscale E (gross motor) in both the adjusted and unadjusted analysis. Coherence for delta 1 between T3-T5 and T4-T6 was negatively correlated with subscales C (eye and hand coordination), D (personal-social-emotional subscale) and GD before and after adjustment. Also, in REM sleep, A1-T5 and C3-P3 in delta 2 and Fp2-F4 and T6-02 in gamma were negatively associated with subscales D (personal-social-emotional subscale) and E (gross motor) and GD.

5.4. Discussion

Despite MLP infants being at lower risk for adverse outcome than earlier preterm counterparts, they still present higher morbidity and mortality than term-born cohorts. A previous study by Ryan *et al.* demonstrated MLPs from this cohort have suboptimal neurodevelopment measured by Griffiths III compared to the term-born cohort (544). The morbidity observed may be a result of altered brain function. My thesis adds to the now-growing body of literature on the MLP group by examining EEG activity in infancy beyond the neonatal period. My thesis demonstrates that at 4 months corrected age, quantitative differences in several sleep EEG biomarkers are seen relative to term-born counterparts, namely, shorter N2 duration, higher delta power in NREM and altered connectivity. A secondary analysis showed that, from these, delta spectral power and aspects of connectivity were associated with Griffiths III at 18 months. The results may be generalizable to similar populations.

5.4.1. Macrostructure

No group differences were noted for total NREM and REM duration and cycle length (Table 5.2). These results are consistent with those observed between very preterm and term-born infants during daytime naps at 3 months (corrected age for preterm infants)(211). Similarly, an overnight study comparing very preterm- and term-born infants at postmenstrual ages up to 55 weeks showed no differences in percentages or duration of active and quiet sleep. However, in the latter study, other variables, i.e. episodes of quiet sleep lasting at least 10 minutes and duration of intermediate sleep, were greater in preterm cohorts(552). Statistical differences between groups relating NREM and REM durations may become more evident as infants age, with preterm groups presenting higher NREM and lower REM percentages at 6 months chronological age (214).

The only group differences within the 1st sleep cycle macrostructure were in the N2 duration. In both groups, NREM sleep was marked by predominant delta activity, including slow wave activity (defined as 0.5-2Hz activity of >75 μV measured from peak-to-peak over frontal to contralateral auricular/mastoid electrodes channels (121)). This factor contributed

to the fast transition from N2 to N3 observed and the consequent low N2 median duration in both cohorts. In the MLP cohort, median N2 duration was almost 2 minutes shorter, which *per se* may not be clinically significant, but is consistent with the findings of higher delta spectral power in MLP infants (Table 5.3). No association was observed between N2 and Griffiths III quotients (Supplementary tables 2a and 2b – Appendix 9).

The observed macrostructural differences may alter as infants age and reach school-age years. A systematic review on preterm sleep at school-age found that distributions of sleep stages do not differ between groups within most studies(553), although some exceptions were noted. Perkinson-Gloor *et al.* reported longer overnight N2 and shorter N3 in 6-10-year-olds born earlier than 32 weeks gestational age(541). Five to 12-year-old children who were born extremely and early prematurely with appropriate birthweight for gestational age had shorter N2 and shorter NREM than term-born peers, while children born preterm with foetal growth restriction showed higher duration and percentage of N2 to term infants with appropriate birthweight for gestational age. Differences possibly point to complex neuropathology driving sleep alterations underlying prematurity (554). This study also found shorter and less efficient sleep in preterm-born with appropriate birthweight for gestational age compared to term peers(554). In teenage years, very preterm and term-born teenagers do not show differences in overnight sleep efficiency and architecture(498).

5.4.2. Sleep Spindles

Extreme and very preterm infants at term age show reduced thalamic volume(555) and thalamocortical connectivity to widespread cortical areas(556). In addition, lower gestational age at birth is also positively associated with decreased neurite density in local (non-core) connections including links from the thalamus to the frontal cortex(557). The thalamocortical connectivity impairment in preterm infants is negatively associated with cognitive scores at 2 months and with degree of prematurity at birth(558). Despite these reported alterations, no difference in the number or morphology of sleep spindles in the MLP group comparing to the term group was found at 4 months.

Sleep spindles are associated to protection against sleep fragmentation(285, 287) and to neuroplastic properties either of depression or potentiation(296-298). The association

between the ability to learn different tasks and sleep spindle properties has been extensively demonstrated. The learning tasks associated include motor sequences(301, 302), visuospatial memory(303), spatial orientation(304, 305), and verbal, including learning word-pairs(175, 245, 306-308). In particular, the number of spindles may be associated with the refinement of the sensorimotor mapping in infancy(141). The previous chapter demonstrated that sleep spindles properties at 4 months, namely synchrony and duration are associated with eye and hand coordination, personal-social-emotional and gross motor subscales and GD quotients at 18 months(548). Ryan *et al.*, 2023 noted that these MLPs had significantly lower scores compared to the term-born infants in the referred subscales and GD at 18 months(544). This chapter demonstrates that both observations are likely unrelated as no statistical difference in sleep spindle properties was observed between these two groups at 4 months. It is crucial, however, to understand if the lack of spindle properties differences between groups holds true during nocturnal sleep.

5.4.3. qEEG features

5.4.3.1. Spectral power

The MLP infant group had higher delta power. Delta waves are associated with neurodevelopment(413-415, 485, 548, 559). Two different theories relate slow waves to synaptic renormalisation(48, 406) and contribution to memory consolidation(135, 486). The increased NREM delta activity in the MLP groups may be explained by two alternative hypotheses.

The first hypothesis is related to delta activity as a surrogate sleep pressure marker. As discussed above, some nocturnal polysomnographic studies in school-age children born preterm point to less nocturnal time spent in deep sleep(541), more awakenings(541, 542) or the time spent awake after sleep onset(554). Additionally, children born preterm with appropriate birthweight for gestational age may show lower sleep efficiency and total time asleep than their term peers(554). These observations may be at least in part related to higher rates of sleep disordered breathing(560). It is possible to speculate that overnight lower sleep quality reported in preterm children may drive increased sleep pressure evidenced by the

present findings of increased delta activity during the first cycle of the day nap, similar to that observed in nocturnal recordings on pre-school children with sleep disordered breathing(561). However, results deriving from the association between delta power and Griffiths III point in a different direction to the first hypothesis. Delta 1 and delta 2 power were positively associated with subscales D (personal-social-emotional) and E (gross motor) quotients after adjusting for sex and multiple births. Delta 2's positive association with subscale B (language and communication) was only significant in the unadjusted analysis. Increased delta may point to compensatory mechanisms in place or may be due to chronological age. Local slow wave activity correlates to age and previous experience-dependent activities(559). Slow wave amplitude and particularly slope reflect synaptic strength and are highest at the beginning of sleep and expected to decrease over the night(394, 420); this is thought to reflect a process of synapse downscaling as part of synaptic homeostatic regulation, occurring during sleep(48, 394, 407). However, mean slow wave amplitude and slope over the entire night increase across infancy(420) and may only reverse approaching puberty(412, 421). These daily gains in slow wave magnitude and slope indicate a bias towards establishing rather than eliminating synapses in early life(420). Despite sleep recordings of the MLP infants being at the same corrected age as terms at 4 months EEG assessments, MLP infants had greater chronological age and, therefore, had more opportunities for extrauterine experience-dependent neuroplastic changes, which may explain higher delta in the preterm cohort. Facilitating opportunities for naps and promoting sleep hygiene routines may attenuate adverse neurocognitive outcomes associated with prematurity.

5.4.3.2. Coherence

Brain connectivity, particularly functional brain connectivity, undergoes considerable reorganisation because of developmental processes with increasingly more efficient networks with high global integration and local clustering(562). Changes in connectivity are heterogeneous across time(472) and regions(475). Similar to previous findings between term and preterm infants in the neonatal period(23), there were group differences in connectivity at 4 months which changed between sleep states, highlighting the dynamic variations of functional network connectivity between the two states(563). Numerous differences were found between the preterm and term groups. Most of the differences highlighted in Table 5.4

were related to higher frontal connectivity in the preterm compared to the term cohort, particularly for lower and higher frequency ranges. These results are also comparable to observations in previous sleep EEG studies conducted in the neonatal period(23, 564). The magnitude of overall EEG interhemispherical connectivity decreases with gestational age in the preterm population(551) and across postmenstrual age in frontopolar regions (within and between other regions) in preterm infants close to term-equivalent age, in both quiet and active sleep(565). My thesis expands the observations from previous studies, demonstrating that alterations in frontal connectivity are not exclusive to extremely and very preterm-born infants, with a cohort exclusively composed by MLP also significantly differing from term-born infants and, are still present at 4 months corrected age. This characteristic of frontal hyperconnectivity in preterm cohorts may extend into school-age years, at least during awake resting-state(566). An MRI-based connectome study revealed preterms exhibit relatively fewer general connection weights, interpreted asfewer white matter resources for network construction(567). This white matter resources paucity is hypothesised of being compensated by preferentially allocating them to networks with higher rich-club indices, abundant in frontal areas, to protect global connectivity (566, 567) . The connectivity differences between preterm and term-born involving the frontal regions in resting-states may be associated with adverse neonatal experiences(566) and may underpin behavioural(566), motor(23) and cognitive deficits(23, 566). Strategies such as routine parent-led massage(547) and Family Nurture Intervention which consists of series of interventions such as skin-to-skin contact or neonate-directed maternal vocalisations during NICU stay, have a strong parental bonding and physical stimuli components, which may help overcome the alterations in brain connectivity(565) and support preterm infants' brain development(568).

We observed relatively lower connectivity between P3-O1 and P4-O2 channels in the preterm group. Although difficult to pinpoint due to the low spatial resolution of the EEG, a possible explanation may reside in the connectivity-strengthening delays within the posterior default mode network that show important developmental changes in populations from 4-9 months(569). The default mode network represents several interconnected areas, shown to be highly activated during the brain's resting state. Its structures are involved in self-referential and emotional regulation processes and recollection, and this network is thought to play a role in the brain's functional integration(570). Its integrity is associated with cognitive and language scores at 12-months(571) and behavioural and emotional regulation

in adulthood(572). Also, early hypoconnectivity of the default mode network to other structures during sleep is observed in pathologies such as autism in general and with a subtype displaying the greatest difficulties in social-visual engagement(573). Lower connectivity within the default mode network was shown to be increased in early-life chronic stress(574). Although all efforts are made to reduce discomfort during the hospital stay, infants admitted to the neonatal unit are exposed to bright lights, and a higher level of noise with sleep cycles being often disrupted(51). MLPs in the neonatal unit may experience a higher number of medical procedures and handling and longer periods of parental separation than their term-born counterparts. In this way, moderate-to-late preterm birth may constitute a risk factor for early stress. Adequate parenting strategies such as those involving calm and secure attachment may mitigate deficits within the default mode network connectivity(574, 575) and reduce the risk of developing a psychopathology(575). The secondary analysis, however, shows that at 18 months, correlations between P3-O1 and P4-O2 connectivity and Griffiths III occur only for NREM gamma (gross motor and GD) and REM delta 1 (gross motor) before and after adjusting for sex and multiple births. Associations of NREM connectivity between P3-O1 and P4-O2 in delta 2 and gross motor and, in gamma and foundations of learning were only significant in the unadjusted analysis.

Altered connectivity in the MLP group compared to the term groups is associated in general with worst outcomes in Griffiths III at 18 months involving all subscales but B, and GD, mostly in the delta range. These coherence results should be interpreted with caution due to multiple comparisons.

5.4.4. Study strengths and limitations

I demonstrate that MLP infants show several altered sleep biomarkers that may reflect abnormal brain function and which may impact on neurodevelopment. My thesis extends the knowledge base about sleep EEG macrostructure, microstructure, and background qEEG in term and MLP infants (see for example(210-212, 215)). During the neonatal period, differences observed between preterm and term infants may arise both from the effect immaturity or altered neurodevelopment. While it is important to understand early signs of divergent brain maturation trends, observations from the neonatal period may raise the question of possible permeation of confounding factors associated with birth stress and

physiological adjustment to extrauterine life. This is particularly important when comparing term infants with premature infants at term-age, when birth stress and extrauterine adaptation is more recent in one group than the other. Nevertheless, it is also important to compare infant brain development at equivalent adjusted age. Studying infants at 4 months allows the comparison of early brain maturation trends at equivalent ages for terms and MLP infants while overcoming the potential inequalities of a very recent birth in one of the groups.

This study does have some limitations. Due to the nature of the studies from which both cohorts derived, the EEG reviewer could not be blinded to group assignment. Demographic analysis (Table 5.1), revealed that the two groups were predominantly fed on infant formula but a higher proportion of MLP infants were on formula and were introduced to solid foods earlier compared to term controls. Food composition impacts infant sleep quantity and quality (576, 577) and is also associated with IQ scores (578). However, macrostructure features such as shorter latency to sleep or longer REM reported in the literature to be associated with this factor were not significantly different between MLP and term infants. All efforts were made to book appointments at 18 months adjusted age but 7/39 MLP assessments had to be postponed due to COVID restrictions; this may not constitute a significant limitation considering that Griffiths III quotients are adjusted for age.

5.5. Conclusion

Through analyses of multiple sleep EEG features, this chapter identified altered brain maturational trends in the sleep biomarkers of MLP infants, which reflect early changes in brain organisation and function associated with neurodevelopment.

Chapter 6. Summary and Conclusions

6.1. Motivation and aims

Sleep properties in infancy evolve very dynamically, reflecting the progress of brain maturation. During infancy, sleep stages become more defined as prominent patterns such as sleep spindles emerge, and sleep onset shifts to NREM. Also, features like the ratio of NREM to REM, sleep spindles, and slow wave properties change with maturation. This stage of life also coincides with a period of intensive learning as infants become more independent and in control of their own movements and start developing their personalities.

Sleep properties not only reflect brain maturational changes, but they are also shown to be active agents of fundamental processes such as learning and memory consolidation, while maintaining synaptic homeostasis through down-selection of the superabundant synapses that characterise the young brain; Furthermore, these also show alterations in specific neurologic conditions. Consequently, sleep features are good candidates for assessing neurodevelopment in an early period of life when behavioural-based neurodevelopment assessments are constrained in healthy infants by their limited range of abilities and cooperativity. A measure of early trends of atypical brain maturation creates the opportunity to implement early strategies in a timeframe characterised by high neuroplasticity, allowing infants to reach their full potential. This PhD thesis represents a step towards this goal.

I have performed original, exploratory research with the aim of characterising normal infant sleep, physiological differences affecting sleep arising from environmental enrichment, specifically routine parent-led massage, the predictive value of 4-months sleep markers for neurodevelopment at 18 months and, the sleep features of MLP preterm-born infants and whether these are distinct from term-born infants at equivalent postmenstrual age. With this purpose, 96 full-term non-intervention infants (care-as-normal), 83 full-term intervention infants (routine massage), and 59 MLP healthy infants were recruited shortly after birth. They had video EEG in sleep at 4-5 months corrected age, recorded during their habitual diurnal nap time. Initial EEG analysis consisted of manual identification of the sleep spindles and sleep staging. Sleep spindles were marked over bilateral fronto-central channels coinciding with the maximal amplitude channels. Sleep staging was according to sleep staging rules for children from the American AASM Manual for Scoring Sleep and Associated Events version 2.4,

applicable to children of 2 months corrected age and older. qEEG analysis of the sleep spindles features, apart from frequency and synchrony, and background EEG was also performed using a bespoke MATLAB Toolbox *NEURAL*.

With this project, I aimed to explore some important issues:

1) What are the characteristics of electrical sleep patterns in neurotypical infants? With this study, I aimed to describe sleep macrostructure and sleep spindles during naps in the healthy term-born infant, establishing a benchmark for comparison with potentially abnormal developmental trajectories and thus, facilitating early intervention.

2) Does parent-led routine massage lead to neurophysiologic changes indicative of a more mature profile in the healthy full-term infant? Literature suggests that massage may produce these changes in the preterm population. I aimed to extend this knowledge to the healthy full-term cohort. Massage is an accessible method of environmental enrichment, and by consequence has the potential to reach a wide populational sector, independent of socioeconomic status. Infancy, in turn, provides a valuable window of opportunity for therapeutic intervention as a period of rapid neurodevelopmental advancement.

3) How do infant sleep features relate to future neurodevelopment? I correlated infant sleep features with future neurodevelopment through the Griffiths III assessment scores at 18 months. Knowing how sleep features relate prognostically to aspects of future neurodevelopment may facilitate early targeted intervention in at-risk infants.

4) How do quantitative sleep EEG features differ between MLP and full-term infants? Although most sleep studies after the neonatal period focus on earlier preterm populations due to their relatively higher risk, MLPs still have an increased risk of lower cognitive, behavioural and academic outcomes compared to full-term infants. As sleep is a promising tool for assessing neurodevelopmental prognosis, I aimed to explore whether sleep EEG differs in MLP groups relative to their term-born peers.

6.2. Main results

The introductory chapter consists of a literature review of the current knowledge on brain development, sensitive periods, and environmental enrichment. This chapter also aimed to specifically explore existing knowledge as to how and why sleep features such as sleep spindles and slow waves relate to brain maturation and cognitive function. Despite a considerable body of previous research, much concerning the sleep characteristics in early infancy remains unknown.

As a result, chapter 2 aimed to characterise sleep in neurotypical infants. I conducted a detailed analysis on a large cohort of infants with Griffiths III general development quotients >85 at 18 months to describe daytime sleep macrostructure and sleep spindles in healthy-term infants, establishing a benchmark for comparison.

Chapter 2 demonstrated that, although sleep onset starts shifting from active to quiet sleep after 44 weeks of age(168), at 4 months of age 2/68 infants still reached REM before N2, suggesting that this is a feature in which maturation may still be in progress at 4 months of age. Chapter 2 confirms infant's shorter sleep cycle duration compared to adults (approximately 90 minutes), similar to observations by Stern *et al.*(579). The sleep cycle had a median duration of 42.8 minutes and about 1/3 of the infants progressed to a second sleep cycle. Up to this date, many of the sleep spindle characteristics in infancy analysed in this work had been carried out on cohorts of very limited size. This chapter describes, for example, a median density of 6.6 spindles per minute during NREM sleep. Sleep spindles are of approximately 2.9 seconds in length and may decrease with postmenstrual age. It also provided evidence that, unlike in older children and adults, at 4 months, there are not two distinguishable sleep spindle peak frequencies, which are generally associated with slow and fast spindles, detected over frontal and central electrodes. The appearance of two distinct spindle peaks in older children may be considered another marker of development reflecting either thalamic or corticothalamic structures and their interactions.

My research was the first to report sex differences in infant sleep spindles. Studying sleep spindle spectral power instead of its proxy sigma power, I confirm the trend-level observations from Jenni *et al.*(451), that in contrast to what is expected in adult populations(360), spindle spectral power decreases from the first to the second cycle in

infancy; and by employing a larger cohort than the former study, I demonstrate statistical significance and extend the findings to 4 months infants. Local increments of spindle spectral power are observed after learning tasks. A possible explanation is that the great number of novel learning experiences present at this age drives major neuroplastic changes in which spindles may play a fundamental role, and this may be particularly evident during early sleep.

Chapter 3 describes a randomised controlled trial exploring whether a standardised environmental enrichment, in the form of parent-led massage during the first 4 months of age, leads to functional brain changes on the EEG. Parents from the intervention group learned the massage according to the International Association of Infant Massage through a certified staff member. These parents were asked to perform the massage 3 times daily for at least 15 minutes and keep a record of the massages. The control arm had standard care. Groups' demographics, including sex, were not statistically different. A secondary analysis compared the regularly-massaged intervention subgroup with the never-massaged non-intervention subgroup. An infant was regularly massaged if (s)he received 2 or more massages lasting 5 minutes or longer for at least 4 days per week in 10 out of 13 weeks (week 4 to week 16); never-massaged infants were identified from the questionnaire. The sleep variables of interest were again sleep macrostructure, sleep spindles, and exploratory qEEG background analyses that included EEG magnitude, spectral distribution, signal continuity, and interhemispherical connectivity.

Routine massage was not associated with changes in sleep macrostructure. Sleep spindles' power, by contrast, was significantly higher in the intervention group; sleep spindles' power is a feature that increases with maturation and may be a marker of maturation related to pruning of superfluous synapses.

The exploratory analysis of the qEEG background features revealed two main trends: 1) An increased EEG magnitude (spectral power) across all sleep states and frequency ranges, accompanying the maturational trajectory of this age group, and in this manner, extending observations from previous studies on preterm massage; 2) Interhemispherical functional connectivity (coherence) was reduced in the intervention subgroup analysis, which is hypothesised to be associated with advanced axonal pruning of the corpus callosum connections towards increased organisation of the network arrangement.

Lastly, no differences were found between the Griffiths III assessments of the two groups. The challenge of adequately assessing neurodevelopment through behavioural repertoire in healthy young cohorts may have precluded these observations.

Chapter 4 explores the potential of infant sleep features to predict neurodevelopment at 18 months. The variables of interest were sleep macrostructure, sleep spindles, and the qEEG features of the background EEG, magnitude and interhemispheric connectivity. I assessed neurodevelopment using Griffiths III general development and its subscales.

After adjusting for sex, gestational age at birth and exact age at the assessments, I found, a positive association between latency to sleep and the foundations of learning subscale within sleep macrostructure, which, although surprising, may speculatively be related to resistance to sleep due to extended availability to explore and learn. I also found a positive association between the duration of N3 and the personal-social-emotional subscale. N3 duration is a modulator of positive mood (497), and shorter duration of N3 may disturb emotional regulation and reduce receptivity for socialisation; in the long-term, consistent N3 deficits in early development may directly and indirectly (the latter through missed opportunities for socialisation) modulate experience-dependent neuroplastic changes in these domains.

Sleep spindle duration was also negatively associated with personal-social-emotional and gross motor subscales, while spindle synchrony was also negatively associated with these subscales and, in addition, eye-and-hand coordination and general development scores. These may be age group-specific markers as both sleep spindles features have been previously reported to decrease (at a trend level for interhemispherical synchrony) within this age group. Duration, in particular, is determined by the spread and strength of thalamocortical and corticothalamic networks; its decrease during this phase is probably associated with functional network specialisation.

Lastly, I found interesting results in the exploratory qEEG analysis of the background. EEG magnitude analysis revealed delta power associations spanning most Griffiths III neurodevelopmental subscales. This observation can be explained in the light of the two popular sleep theories: sleep homeostasis hypothesis and active systems consolidation (see section 1.4 and 1.5.7.4 Learning and long-term memory formation). I also observed two sleep stage-dependent patterns, 1) an interhemispheric connectivity of N2 fast activity associated

with foundations of learning and general development and, 2) a pattern of interhemispheric dysconnectivity on REM delta activity associated with personal-social-emotional subscales. These connectivity analyses suggest complex functional interactions that should however be interpreted with caution, due to the probability of statistical error type I as a consequence of multiple comparisons.

Finally, Chapter 5 explored MLP sleep by comparing the sleep features between MLP and term-born infant cohorts. The features of interest were the same as previous. This time however, coherence studies were analysed between inter- and intra-hemispherical pairs of channels to study connectivity patterns across the different brain regions and the background magnitude was analysed with a Welch power-spectral density method with an 8-second instead of 2-second window used in previous analyses, to promote an increased smoothing effect of the data obtained.

The macrostructure analysis suggested a statistically shorter N2 duration in the MLP group; this difference may not be clinically significant but reflects a higher presence of slow waves, a factor that leads to a faster transition from N2 to N3 sleep and that is in line with the delta power magnitude analysis, further discussed.

Sleep spindles were morphologically and numerically similar between groups. Although sleep spindles are associated with neurodevelopment, these appear to be unrelated to neurodevelopmental differences between the MLP and the term cohorts at 4 months observed in a previous study from the INFANT centre. Other variables should be taken into consideration for neurodevelopment prediction in MLPs, as discussed below.

Delta magnitude was increased in the MLP group. The most probable explanation is related to the fact that delta magnitude increases during infancy, which may be related to extrauterine experience-dependent neuroplastic changes and may represent a positive balance between stablishing and strenghtening synapses rather than pruning and weakening. Although both cohorts are at similar postmenstrual ages at the time of the assessments, MLPs are chronologically older. This is translated into a positive association between delta magnitude and personal-social-emotional and gross motor Griffiths III subscales.

Lastly, the MLP showed a diverse landscape of altered functional connectivity when compared to term-born infants. Curiously, lower connectivity in the MLP cohort is mostly found between the bilateral parieto-occipital channels and may be associated with

connectivity within posterior default network structures. In earlier preterm neonatal studies, hyperconnectivity has been observed, particularly involving frontal regions, both within frontal lobes and between frontal and other regions; this study extends these findings to the present MLP infant group. In extremely low gestational age groups, this frontal hyperconnectivity is still patent in school-age children at least during awake-resting, and is associated with previous adverse neonatal experiences and childhood cognitive and behavioural problems(566). Importantly, frontal hyperconnectivity in preterms has been demonstrated to be mitigated through environmental enrichment interventions(565), which have also been shown to improve neurodevelopmental and behavioural scores(568). In general, the altered connectivity observed in the MLP in comparison to term peers, which was correlated with Griffiths, was associated to worse MLP neurodevelopmental outcomes.

6.2.1. On multiple comparisons

EEG is comprised of highly complex time-varying and multi-dimensional biosignal recordings. The exploratory analyses included multiple measures I considered relevant for the description of the background EEG across the different frequency bands and sleep stages. I believe the strength of my thesis' exploratory studies is in the fine detail of the analyses to capture the complexity of the EEG signal. I believe this fine detail will be very useful for future research in this area.

Authors like Rothman and Althouse have discussed the analysis of multiple comparisons and why this should not be performed as a standard procedure in all studies(460, 461). Considering their arguments, I therefore decided not to adjust for multiple comparisons because 1) of the exploratory nature of the research queries; and 2) this analysis penalises true observations found in large studies. By reducing the probability of type I error, I would increase the probability of type II error, where a true observation might be disregarded. Given these reasons, it was decided to allow readers to exercise their own judgement, making explicit the potential for type I errors while still presenting the exploratory analyses in fine detail, and commenting on general trends, like for example increased spectral power and decreased interhemispherical coherence on chapter 3.

6.3. Strengths and limitations

There are several limitations to my thesis. Chapter 3 reports a randomised controlled trial but blinding was only partially achieved: the personnel performing sleep recordings, sleep analyses and Griffiths assessments were blinded to allocation and, also participants were obviously too young to realise their group allocation; however, due to the nature of the intervention, caregivers, which doubled-up as massage providers in the intervention arm, could not be blinded to group allocation. Chapter 5 derives from the comparison between two cohorts, the BabySMART control arm and preterm cohorts, whose EEG recordings and analyses were performed separately. Due to this, it was not possible to conceal groups from the EEG recording personnel and reviewer, nor could the caregivers be blinded. In both cases, this could lead to bias. In chapter 3, it is possible that the parents from the control group, being part of this study became more aware of the importance of environmental enrichment and sought to compensate for not being in the massage group in other ways as for example increased parental attention. In chapter 5, although the EEG analysis is protected by the tendentially objective set of rules that characterise its interpretation and the staff were consciously vigilant of possible bias, I cannot totally exclude subconscious bias.

No sleep adaptation session was performed before the analysed sleep recording. It is known that sleep macrostructure features differ between the adaptative sleep and the following sleep recordings, and some sleep spindles aspects (duration and density of slow spindles) require more than one sleep session for good individual stability, at least in the adolescent population. When aiming to establish benchmark sleep values for comparison and explore biomarkers predictive of neurodevelopment these should be of feasible application in clinical settings; thus, basing the data on the first sleep recording is desirable for this purpose. This factor is also unlikely to affect group comparisons (chapters 3 and 5) as group sleep was recorded under the same conditions.

Although unintentional, infants arising from medium and high socioeconomic contexts predominated in my thesis research when considering parameters of maternal education and

family income. Infants from less favourable socioeconomic backgrounds are thus relatively underrepresented.

Since recruitment, there was a high proportion of dropouts, particularly from the massage arm. To assess the risk of bias, I compared data at registration between included and excluded infants, I observed mothers from excluded infants were significantly younger (2.6 years on average). Socioeconomic status information was obtained at a later stage (2 weeks appointment), which limited data from the excluded infants who dropped out by this stage (n= 93 of excluded infants); statistical analysis revealed no group differences for maternal education, family income and number of children in the household (data not showed).

This thesis also has a number of strengths. In a field dominated by lower sample sizes, see for example Ujma, 2018(344), one of the greatest strengths throughout this work is the large cohorts included. To my knowledge, this thesis presented the largest cohort study on sleep spindles at 4 months, and employed the largest cohort in a trial relating a massage protocol in infancy and sleep EEG biomarkers. In the same way, and to my knowledge, this is the largest cohort of healthy infants in a study exploring sleep EEG as a potential neurodevelopmental biomarker.

I employed an accessible, non-invasive technique, the EEG. I used objective quantitative measures that reflect brain maturity during a period of reduced interference from incoming environmental stimuli and minimal/absent contamination from muscle and movement artefact, i.e. sleep. Recordings in the sleep lab enabled controlled conditions of temperature, low light and noise and a standard environment for all the sleep recordings. These factors will allow reproducibility of the method and future application to clinical settings.

Another advantage is the fine detail of my analyses by using multichannel EEG, which offered a good spatial coverage and combined several aspects of sleep EEG that range from sleep macrostructure, different parameters of the manually-annotated sleep spindles and different background qEEG features across different sleep stages, providing a holistic picture of infant sleep.

During this phase of development, sleep features are rapidly evolving. Another strength of this study is the tight age range; consequently age-driven variation could be reduced to a minimum.

Specialised staff were in charge of the assessments and massage trainings. Sleep recordings were performed by either experienced neurophysiologists or trained paediatric health personnel and EEG analysis was performed by an experienced neurophysiologist. In the same way, Griffiths III analysis was performed by a paediatric neuropsychologist or paediatricians, all ARICD-trained practitioners to perform the Griffiths assessments. The massage learning sessions were taught by a paediatric physiotherapist certified by the International Association of Infant Massage.

Finally, this work expanded knowledge on the impact of massage on sleep biomarkers by analysing term-born typically developing infants. I analysed the correlation between several new sleep biomarkers and neurodevelopment, and I demonstrated that sleep EEG alterations accompany moderate-to-late premature infants after the neonatal period.

6.4. Future directions and clinical implications

This work revealed promising observations. The next step in this work should be confirmatory studies, and particularly multicentre studies, to ascertain the generalisability of my conclusions.

Early targeted intervention of infants at risk for abnormal neurodevelopmental trajectory offers the most promising results due to the neuroplasticity observed in early life. However, the identification of this risk early in otherwise healthy infants is still a challenge due to the limited behavioural repertoire that characterises early infancy. In these cases, objective, quantitative and sensitive measures are needed. This thesis joins other recent studies demonstrating the potential for the identification of abnormal brain development. These early studies may pave the way for future clinical application. However, further studies are needed. For example, future studies should investigate how the identified sleep

biomarkers relate to specific neurodevelopmental conditions. Another approach would be to use machine learning algorithms to explore how sleep biomarkers collectively relate to neurodevelopment.

Parental-led massage is a form of environmental enrichment that may promote brain development from at least two directions, namely somatosensory stimulation and social bonding. This technique is economically accessible, allowing broad access to different populations. I showed that massage was associated with neurophysiological changes suggestive of advanced brain maturation in a typically developing term-born population. However, some questions remain to be answered in future studies. The first is whether the Griffiths neurodevelopmental assessments can capture group differences in later stages of development when more complex cognitive skills can be assessed. The second is the persistence of the sleep biomarkers differences at later stages.

MLP children are at higher risk of behavioural and cognitive problems than their term peers(38). I provided evidence that further attention should be given to the MLP group. I showed that several sleep characteristics with a divergent trajectory in this group remain after the neonatal period, and these may either reflect dysfunction of networks and structures or, due to the role sleep has on development, be an intermediate process for later disruption. Family Nurture Intervention consists of a set of measures that ranges from family education to promotion of skin-to-skin contact. It has shown to reduce sleep connectivity alterations in more premature cohorts (565). Using a MLP-exclusive cohort, I showed that altered sleep connectivity is not exclusive to more premature-born children and these alterations extend beyond the neonatal period. The alterations were mostly related to worse neurodevelopmental outcomes. Future studies should evaluate if Family Nurture Intervention could benefit MLP-exclusive cohorts.

6.5. Final remarks

I have provided a detailed profile of the electrographic features of sleep structure in early infancy and highlighted the potential for EEG, a non-invasive assessment tool whose

quantitative data may be objectively measured, to act as a marker of neurodevelopment. This knowledge offers the opportunity to detect deviation from the normal developmental trajectory, potentially before other phenotypical expressions become obvious. Such early detection allows targeted intervention in a period of great neuroplasticity, both in preterm-born populations that are known to be more at risk of lower neurodevelopment outcomes, but also in term-born populations. One such intervention is routine massage, which, for the first time, was demonstrated to be associated with increased maturation of neurophysiologic sleep features in the healthy term-born population.

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Appendices

Appendix 1 – Electroencephalographic sleep macrostructure and sleep spindles in early infancy

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ORIGINAL ARTICLE

Electroencephalographic sleep macrostructure and sleep spindles in early infancy

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Abstract

Study Objectives: Sleep features in infancy are potential biomarkers for brain maturation but poorly characterized. We describe normative values for sleep macrostructure and sleep spindles at 4–5 months of age.

Methods: Healthy term infants were recruited at birth and had daytime sleep electroencephalograms (EEGs) at 4–5 months. Sleep staging was performed and five features were analyzed. Sleep spindles were annotated and seven quantitative features were extracted. Features were analyzed across sex, recording time (am/pm), infant age, and from first to second sleep cycles.

Results: We analyzed sleep recordings from 91 infants, 41% females. Median (interquartile range [IQR]) macrostructure results: sleep duration 49.0 (37.8–72.0) min (n = 77); first sleep cycle duration 42.8 (37.0–51.4) min; rapid eye movement (REM) percentage 17.4 (9.5–27.7)% (n = 68); latency to REM 36.0 (30.5–41.1) min (n = 66). First cycle median (IQR) values for spindle features: number 241.0 (193.0–286.5), density 6.6 (5.7–8.0) spindles/min (n = 77); mean frequency 13.0 (12.8–13.3) Hz, mean duration 2.9 (2.6–3.6) s, spectral power 7.8 (4.7–11.4) μV^2, brain symmetry index 0.20 (0.16–0.29), synchrony 59.5 (53.2–63.8)% (n = 91). In males, spindle spectral power (μV^2) was 24.5% lower (p = .032) and brain symmetry index 24.2% higher than females (p = .011) when controlling for gestational and postnatal age and timing of the nap. We found no other significant associations between studied sleep features and sex, recording time (am/pm), or age. Spectral power decreased (p < .001) on the second cycle.

Conclusion: This normative data may be useful for comparison with future studies of sleep dysfunction and atypical neurodevelopment in infancy.

Clinical Trial Registration: BABY SMART (Study of Massage Therapy, Sleep And neurodevelopMenT) (BabySMART)

URL: https://clinicaltrials.gov/ct2/show/results/NCT03381027?view=results.

ClinicalTrials.gov Identifier: NCT03381027

Statement of Significance

Sleep is fundamental at any stage of life but has particular importance during the first year. Sleep quality influences the architecture of neural connections and circuits, which are essential for memory consolidation and learning. During infancy, there is an unparalleled dynamic evolution of sleep that accompanies the progress of brain maturation. This study describes normative data for sleep macrostructure and sleep spindle features at 4 to 5 months and compares those parameters across sex, time of the day, first and second sleep cycle, and possible trends across gestational, postmenstrual, and postnatal ages, providing a benchmark for comparison with future studies of atypical neurodevelopment in infancy.

Key words: neurodevelopment; sleep; spindles; macrostructure; infant; EEG; REM; NREM; nap

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Introduction

Some of the most important sleep waveforms recorded on the electroencephalogram (EEG) are sleep spindles. These transients, defined by the American Academy of Sleep Medicine (AASM) as runs of 11–16 Hz oscillatory activity occurring over central locations during N2 and N3 stages of sleep [1], arise from the thalamus [2] during a state of relative hyperpolarization [3]. Spindle properties are dependent on intra-thalamic interactions, particularly between the reticular nucleus and thalamocortical neurons, and amongst thalamic and cortical structures [4–7]. In humans, the first spindles can be detected on surface EEG as early as 6 to 9 weeks postnatal age (PNA) [8].

Since early analysis by Gibbs and Gibbs [9], it has been extensively demonstrated that there are two spindle frequencies: slow spindles, prevalent over the frontal areas, and fast sleep spindles, prevalent over the central/centroparietal areas, in children [10–12] and adults [13–17]. The two types of spindles correspond to different underlying dynamics [13, 17, 18], cortical and subcortical activations [19], and may reflect specific processes of learning and cognitive ability [13, 16, 18, 20–22]. Different studies have used different cut-off frequency points for the two types of sleep spindles, and this inconsistency may be related to interindividual differences [23].

Sleep spindle features are partially dependent on genetic factors [24], including sex [23, 25]. However, several environmental factors can influence sleep spindle parameters, including circadian rhythm and sleep pressure [23, 25–29]; activities where learning might be involved [30–35]; cognitive abilities; and pathological conditions such as Alzheimer's [36], Parkinson's [37], attention deficit hyperactivity disorder [38], autism spectrum disorder [39], schizophrenia [40, 41], and some cases of cerebral palsy [42]. A possible explanation linking some manifestations of neurological diseases to alterations in spindle parameters may reside in the fact that several "at-risk" genes for these pathologies are highly expressed in the thalamic reticular nucleus [43], which is integral to spindle generation.

With aging, there is an early shift of the maximal spindle-related power toward anterior locations [44], and evolution of several individual sleep spindle parameters such as density, frequency, duration, and amplitude [12, 25, 45]. The most marked changes occur during infancy but continue to a lesser extent until adulthood, accompanying major brain maturation processes, further to this, sleep spindles might present properties that actively contribute to some synaptic development [46] (for review see Ref. [47]).

In the pediatric population, slower and fast sleep spindles appear to play different roles in the varying factors that compose intelligence [10, 48–50]. However, no association was observed in a cohort from 12 to 30 months in a series of tests that included socialization, hand-and-eye coordination, and other motor functions [51]. Similar to the adult population [52], there has been no evidence that sleep spindles in children are related to verbal cognitive ability with the exception of narrative memory [48, 49]. Also, like adult studies, there seem to be sex differences regarding these associations in populations as young as 4 to 8 years old [10]. It has also been demonstrated that there are reciprocal interactions between sleep spindle characteristics such as number [30, 31, 34], density [32, 35, 53], spectral power [30, 33], amplitude [53], duration [53] and relationship with slow waves [18], and the ability to learning different tasks.

At term, the proportion of time spent in active sleep is at its highest [54] and sleep tends to initiate with this sleep stage. The shift to quiet sleep onset occurs after the 44th week of postmenstrual age (PMA) [55, 56]. During infancy, latency to rapid eye movement stage (REM) increases and becomes more dependent on duration of the previous wakefulness period [57], while the proportion of active to quiet sleep and, subsequently, REM:non-rapid eye movement (NREM) sleep continues to decrease [54, 58]. In infancy, REM:NREM proportions from daytime naps maturate earlier relative to nocturnal proportions [59]. From 4 months of age, NREM sleep can be further classified into stage 1 (N1), stage 2, (N2), and stage 3 (N3), as the stages become increasingly differentiated [60].

Sleep is theorized to endorse homeostatic balance at the cellular level [61] and to prevent neuroinflammation [62–64]. Sleep reshapes the maturing brain [65, 66]; in a period of life with less efficient neural networks, sleep in thought to contribute to a general energy saving by reducing the number of redundant synapses, and enhancing strategic synapses, thus promoting learning and memory consolidation [63]. It allows somatosensory and motor development [67, 68], prolongs stimuli recognition in infants [69–71], and contributes to mood regulation [72]. Poor quality of sleep during development is associated with higher risk for the development of psychopathological symptoms [73] and lower cognitive outcomes later in life [62].

The process of neurodevelopment during the first months of life is dynamic with changes in sleep macrostructure and sleep spindles reflecting some of these alterations. These features may provide early biomarkers for development. Sleep spindles, in particular, show a unique individual *fingerprint* [15] and are affected by different neurologic pathologies. The normative values for the 4 to 5-month age group may offer a benchmark with which to assess a potentially abnormal developmental trajectory. In this study, we analyze the macrostructure of sleep, as well as sleep spindles of ex-term infants at 4 to 5 months of age, possible influences of gender, period of the day the nap occurs, and differences from first to second sleep cycle. The sleep parameter trends are also analyzed across gestational age (GA), PMA, and PNA.

Methods

This study was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals. The guardians of the participants were approached for consent in the postnatal ward at Cork University Maternity Hospital between 2017 and 2018. The criteria for inclusion were being healthy, singleton, and born after 37 weeks of GA.

Infants were enrolled into the BabySMART study, a randomized controlled study investigating the impact of an intervention (infant massage) on sleep and neurodevelopment in early infancy. EEG analysis was performed blinded to group allocation. Only the data from the nonintervention group was used in this analysis. Infants participated in a sleep EEG recording at approximately 4–5 months of age. Appointments occurred during the day, either during the morning or the afternoon, generally between 9.30 and 12.00 am, and 1.30 and 4.00 pm respectively. The soundproof room was at a comfortable temperature according to parents' judgment and lights dimmed; guardians could opt to let the infant sleep on their lap, the buggy, or in a cot. During EEG electrode placement, feeding

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was encouraged to distract the infants from the procedure and soothe them.

EEG recording

Video-EEGs were recorded using a Lifelines (Lifelines Neuro, UK) EEG acquisition system. Electrode placement followed the international 10–20 system and included FP2, FP1, F8, F7, F4, F3, Fz, A2, A1, T4, T3, C4, C3, Cz, T8, T7, P4, P3, Pz, O2, O1, reference, and ground. Disposable cup EEG electrodes were used and impedances were maintained below 10 kΩ. Additional polygraphy, including electrocardiogram, electrooculogram, submental electromyogram, and respiration, was recorded. The EEG was sampled at 500 Hz. The EEG was recorded for the sleep duration and finished after the baby fully woke and was no longer able to return to sleep. A clinical physiologist remained in the room during the recording to promptly address any technical issues that might arise and to flag any incidental EEG findings.

EEG analysis

The post-acquisition EEG analysis of sleep macrostructure and sleep spindles was performed by an experienced clinical physiologist (S.V.). EEG was reviewed using standard high- and low-pass filters of 0.5 and 70 Hz respectively.

Sleep staging was performed according to the AASM guidelines version 2.4, in 30-second epochs and annotated using sleep staging software (Nicolet, Natus). Sleep spindles present over F4-C4 and F3-C3 EEG channels were visually identified post-acquisition on Stratus EEG (Kvikna, Iceland) software and were marked on the EEG with duration annotations, populating an events list that could be exported as a text file for further analysis. The spindle parameters analyzed were the number of spindles, density (number of sleep spindles per minute of NREM), duration (s), median sleep spindle spectral power (μV^2), mean spindle frequency (Hz), brain symmetry index [74] (a measure of divergence of inter-hemispheric power), and synchrony. This data was segregated by sleep cycles. Spectral power and mean frequency were calculated from power spectral density (PSD) estimates of each spindle. These measures used a periodogram PSD. Brain symmetry index was calculated over a 30 s epoch with 75% overlap; non-spindle activity in this epoch was ignored. This measure used a Welch PSD with a 0.5 s Hamming window with 75% overlap. All PSD estimates were limited to the range 9 to 16 Hz and spindles of <0.5 s in duration were ignored. The median values of number of spindles, duration, median spindle spectral power, and mean spindle frequency were calculated as a summary measure per infant. Synchrony, a measure of coincidental contralateral sleep spindles, was calculated as the percentage overlap between spindles in the left and right hemispheres. Duration of spindles was obtained from the sleep spindles individual annotations and density was calculated based on the number of sleep spindles and the duration of NREM sleep during each sleep cycle. Mean frequency and synchrony were calculated using Matlab (The Mathworks Inc, Natick, MA, version 9.8.0 R2020a); brain symmetry index and spectral power of the spindles were calculated using NEURAL software (a neonatal EEG feature set in Matlab, version 0.4.3) [75].

Griffiths III is a neurodevelopmental assessment tool, designed to be administered to children from birth to 5 years and 11 months [76]. Infants with an overall Griffiths developmental quotient (GDQ) of 85 or lower at 18 months were considered to have developmental delay and were excluded from this study. Infants were also excluded if their GDQ score was not available at 18 months, a sleep recording was not obtained or EEG abnormalities were found. Further exclusions were applied depending on the amount of sleep data recorded. As there were some infants that fell asleep before the recording initiated, those infants were excluded for calculations of spindle number and density due to the potential for missing data. Only infants with a complete first sleep cycle were considered for the macrostructure analyses. Here, complete sleep cycle meant that infants only fell asleep after the onset of the polysomnography and had both NREM and REM sleep, with a requirement for N2 and/or N3 during NREM state being present. We noted exceptional cases where REM sleep occurred before meeting criteria for N2; to avoid joining the norm with the exception on the measures of latency to REM, these infants were excluded from these specific variable analyses. For comparison between first and second cycle, the previous rules still applied on the micro- and macrostructure; additionally, for microstructure analysis, we included infants who had NREM recorded for both sleep cycles, and for macrostructure, infants who had at least two complete sleep cycles.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (version 26.0, IBM Corp., Armonk, NY). Continuous variables were described using mean and standard deviation (SD) or median and interquartile range (IQR) and categorical variables were described using frequency and percentage. Relationships between continuous variables were initially investigated using Pearson's correlation coefficient.

Univariable and multivariable linear regression was used to investigate factors associated with sleep features. The factors investigated were age (GA and PNA), sex, and period of the day. Some sleep features were log-transformed prior to the regression analyses: spindle spectral power and brain symmetry index and macrostructural metrics of time in REM, first cycle duration, latency to sleep, that is, time from the period of lights off at the start of EEG to onset of sleep, and to REM variables. The regression coefficients (B) and their corresponding 95% CIs were back-transformed and presented in the original units.

Differences in sleep parameters between the first and second sleep cycle were investigated using the Wilcoxon signed-rank test. All tests were two-sided and a p-value < .05 was considered statistically significant. Sleep spindles annotations analyzed in this study were compared with annotations of a second-rater using the Kappa statistic.

Results

Ninety-eight infants had an EEG recording at 4 to 5 months of age. Of those, two were excluded, as one did not fall asleep and the other had an abnormal EEG. We further excluded four infants who did not have a Griffith's-III neurodevelopmental assessment at 18 months and one infant whose GDQ score was equal or lower than 85 (Figure 1). Table 1 describes the demographic information of the 91 infants included in this study.

Analysis of diurnal sleep macrostructure

A median (IQR) total recording time of 65.0 (49.5–87.5) min was obtained for 91 infants in the study; 42 recordings (46.2%)

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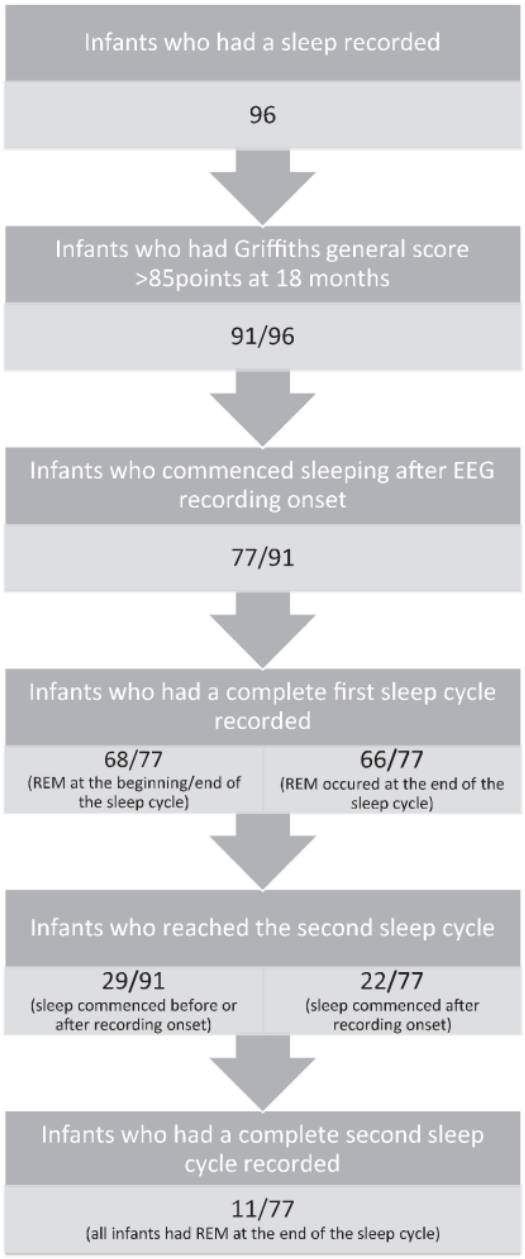


Figure 1. Flow diagram detailing numbers of participants in analysis.

occurred during the morning time. In total, 14 infants fell asleep during electrode application. The total nap duration for those who fell asleep after the recording started had a median (IQR) of 49.0 (37.8–72.0) min (n = 77). Of those 77 infants, 68 had a complete first sleep cycle recorded. Twenty-nine infants out of the total 91 reached the second sleep cycle. When including infants that fell asleep after the recording started (n = 77), only 22/77 (28.6%) reached at least the second sleep cycle; 5/77 (7.8%) achieved and 3/77 (3.9%) completed the third cycle. 1/77 infant reached the 4th sleep cycle (Figure 1). Two infants out of 77 (2.6%) reached REM stage before meeting the criteria for N2.

Table 1. Demographics

Characteristics	Mean (SD)* (n = 91)
Sex—female, n (%)	37 (40.7)
Weight at birth (kg)	3.57 (0.46)
Gestational age (weeks)	39.8 (1.2)
PNA at the appointment (weeks)	19.5 (1.3)
PMA at the appointment (weeks)	59.3 (1.8)
Timing of the nap—afternoon, n (%)	49 (53.8)
Mother's ethnicity, n (%)	
White Irish	82 (90.1)
Non-Irish White	6 (6.6)
Asian	2 (2.2)
Latin-American	1 (1.1)
Father's ethnicity, n (%)	
White Irish	82 (90.1)
Non-Irish White	6 (6.6)
Asian	2 (2.2)
Arabic	1 (1.1)

Gestational age is referred as the time from mothers' last menstrual cycle to delivery, PNA as age at the occasion of the EEG recording counted from birth and postmenstrual age as the sum of both.[34]
*Unless otherwise stated.
PNA, postnatal age; PMA, postmenstrual age.

Table 2 represents the duration of sleep stages during the first complete cycle and latencies to sleep and REM (n = 68). Figures 2–5 illustrate the typical sleep stages from N1 to REM of a participant.

Of the 96 infants with a clinically normal EEG and with sleep recorded, 5 were excluded from the study due to lacking or having a low score on Griffiths and, 15 were excluded from macrostructure analysis due to falling asleep before the recording onset. As an indicator of sample size calculation for future studies, ignoring the mentioned exclusions, 72/96 infants (75.0%) reached a complete sleep cycle (N1 may not have been recorded). Two further infants had REM before reaching N2 and N3 out of the 81 whose sleep onset was recorded (2.5%).

Analysis of diurnal sleep spindles

To calculate interobserver agreement for detection of sleep spindles, the first sleep cycles of 65/96 infants were annotated by two raters and compared. Kappa scores for spindle identification were 0.82 and 0.80 for right and left fronto-central spindles respectively, which confirm that sleep spindles are discrete and readily identifiable waveforms. 50.3% of the sleep spindles were right-sided. From the 91 participants, we recorded a total of 29 947 sleep spindles. For the first cycle, 21 867 spindles were recorded. Table 3 provides the characterization of the sleep spindles during the first sleep cycle across infants. The distribution of sleep spindle frequencies (Hz) is shown in Figure 6.

Sleep parameter regression analysis

Results from the univariable and multivariable linear regression analyses between sleep spindle parameters at 4 to 5 months during the first sleep cycle and GA, PNA, sex, and timing of the nap are presented in Table 4. In the univariable analysis, GA was negatively correlated with synchrony [B(95% CI): −1.307(−2.583 to −0.031), p = .045] but this relationship

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Table 2. Duration of sleep stages on the first cycle

	Median (IQR) (n = 68)	
Sleep stages	Time (minutes)	Time (% from cycle duration)
Latency to sleep	8.5 (4.0–14.0)	—
Cycle duration	42.8 (37.0–51.4)	—
NREM	35.5 (30.1–40.5)	82.6 (72.3–90.5)
N1	8.0 (4.5–13.4)	18.8 (13.3–28.0)
N2	4.5 (2.5–6.5)	10.9 (6.0–16.5)
N3	20.3 (16.5–25.4)	46.0 (38.3–59.7)
REM	6.5 (3.6–11.5)	17.4 (9.5–27.7)
Latency to REM*	36.0 (30.5–41.1)	—

*n = 66, infants who meet criteria for staging REM before N2 were excluded.

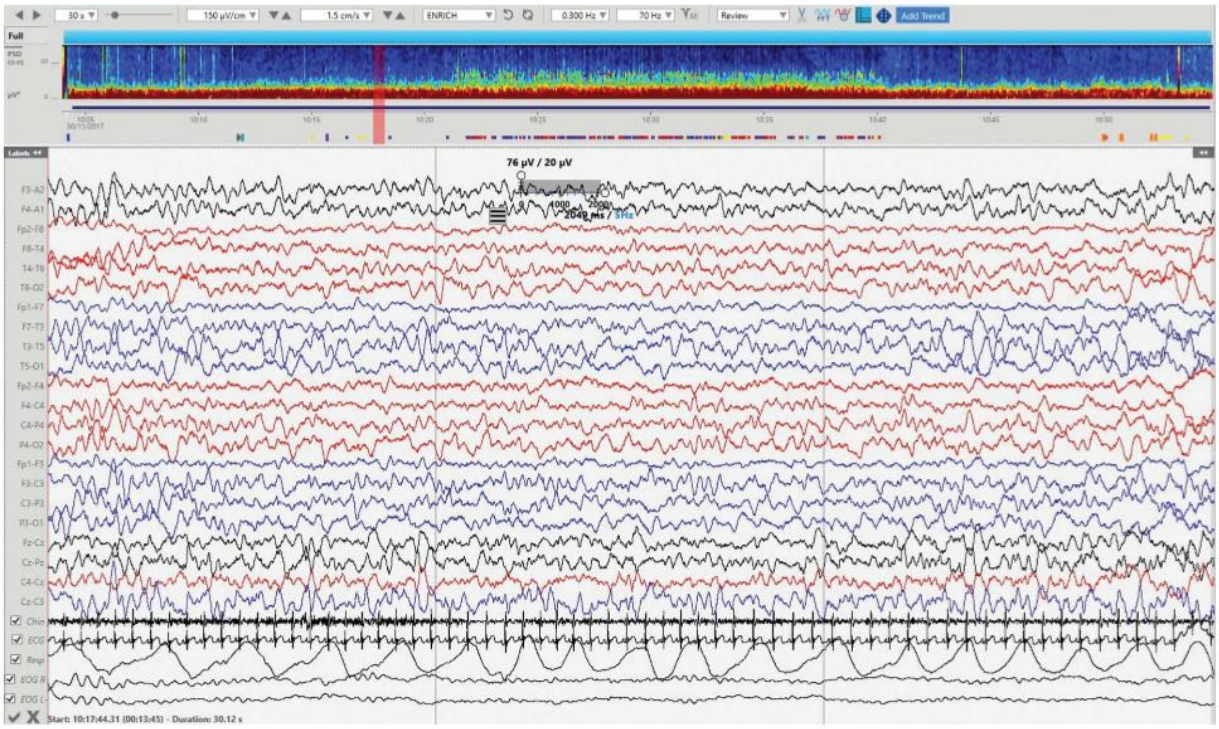


Figure 2. N1 sleep stage with its characteristic relative low amplitude and mixed frequencies and slow eye movement (intrusion of frontal activity is present on the EOG channels). Incipient vertex sharp waves are present in the central regions. Gridlines represent 10 s-intervals. Power spectrum density (PSD) window on top of the EEG represent spectral power of frequencies from 0.5 to 30 Hz (y axis) across the approximately 1 h of recording (x axis). Red colors represent the highest spectral power for a particular frequency and time on the spectrum; colors close to dark blue represent the lower spectral power.

was no longer statistically significant in multivariable analysis [B(95% CI): −1.216(−2.547 to 0.115), p = .073]. Likewise, PMA and spindle duration were negatively correlated [B(95% CI): −0.086 (−0.158 to −0.015), p = .018] in the univariable analysis, losing statistical significance when controling for sex and timing of the nap (results not included). Sleep spindle spectral power and brain symmetry index differed by sex in the univariable analyses with significantly higher power and lower brain symmetry index in females (p = .022 and p = .010, respectively). These differences remained statistically significant in the multivariable analyses (p = .032 and p = .011, respectively). Comparisons of sleep parameters by timing of the nap (morning or afternoon) demonstrated lower spindle duration during afternoons in the unadjusted analysis (p = .034) but the difference was not significant in the adjusted analysis (p = .115). No significant relationships between the factors and the macrostructure outcomes were found in the univariable or multivariable analyses (Table 5).

To study sleep macrostructure across the first two cycles, further exclusion was made to only include infants who had two complete sleep cycles (n = 11). No statistically significant differences were observed in sleep macrostructure parameters between cycles (Table 6). Changes in sleep spindle parameters along with the reduction of sleep pressure from the first to second cycle were investigated using the data of 29 infants who reached a second sleep cycle. Sleep spindle spectral power decreased significantly on the second cycle with (p < .001, Table 6).

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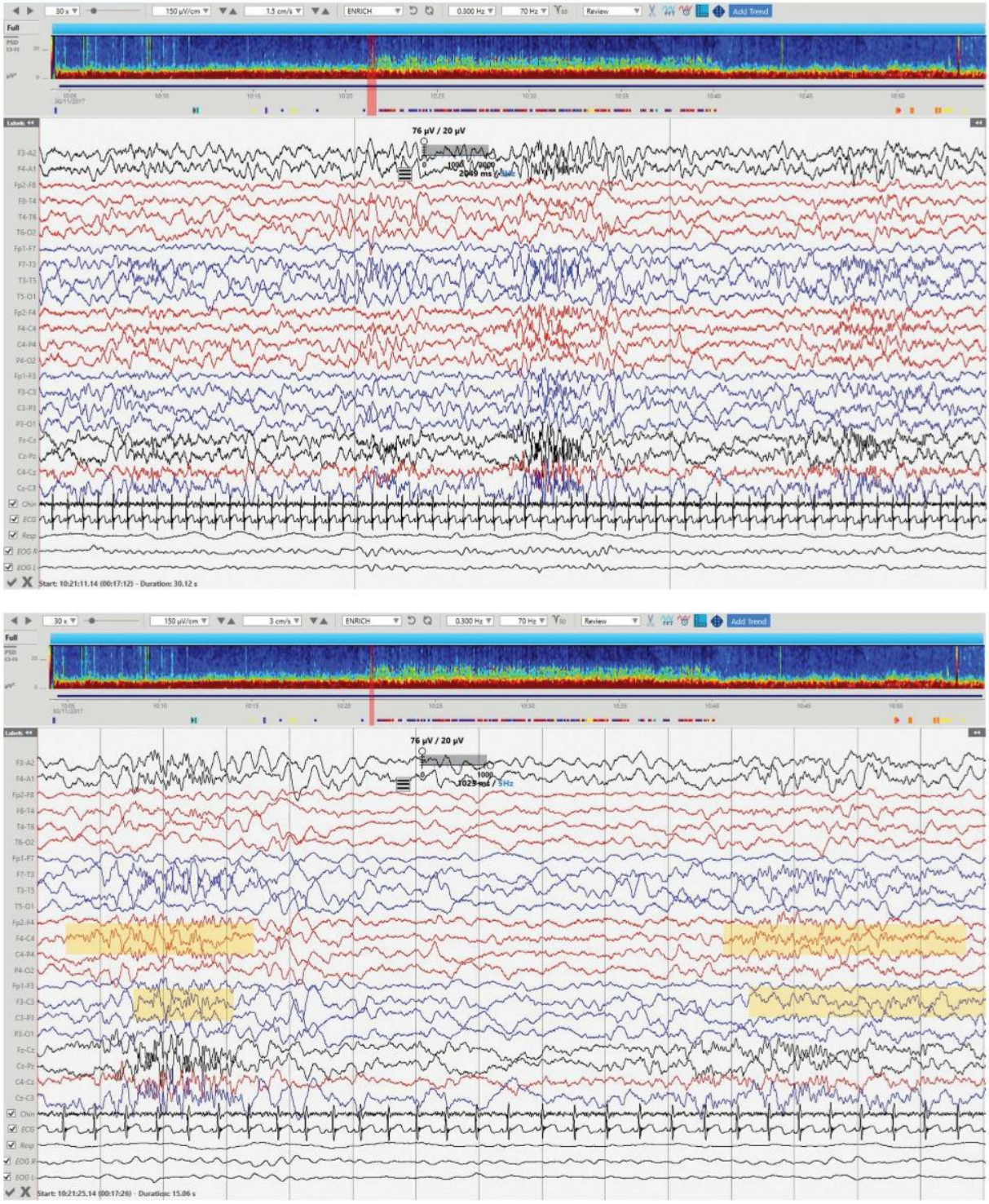


Figure 3. N2 sleep stage. Sleep spindles marked the onset of this epoch. Top EEG: timebase = 1.5 cm/s, gridlines correspond to 10 s intervals. Bottom EEG: detail of the previous page to evidence spindles, timebase = 3cm/s, gridlines correspond to 1 s intervals. Right and left fronto-central sleep spindles in yellow.

Discussion

We have described sleep macrostructure and sleep spindle parameters in the largest cohort to date of infants from 4 to 5 months of age. Sleep spindle spectral power decreased from the first to the second sleep cycle. From multivariable analysis, we noted that two-spindle features differed by gender:

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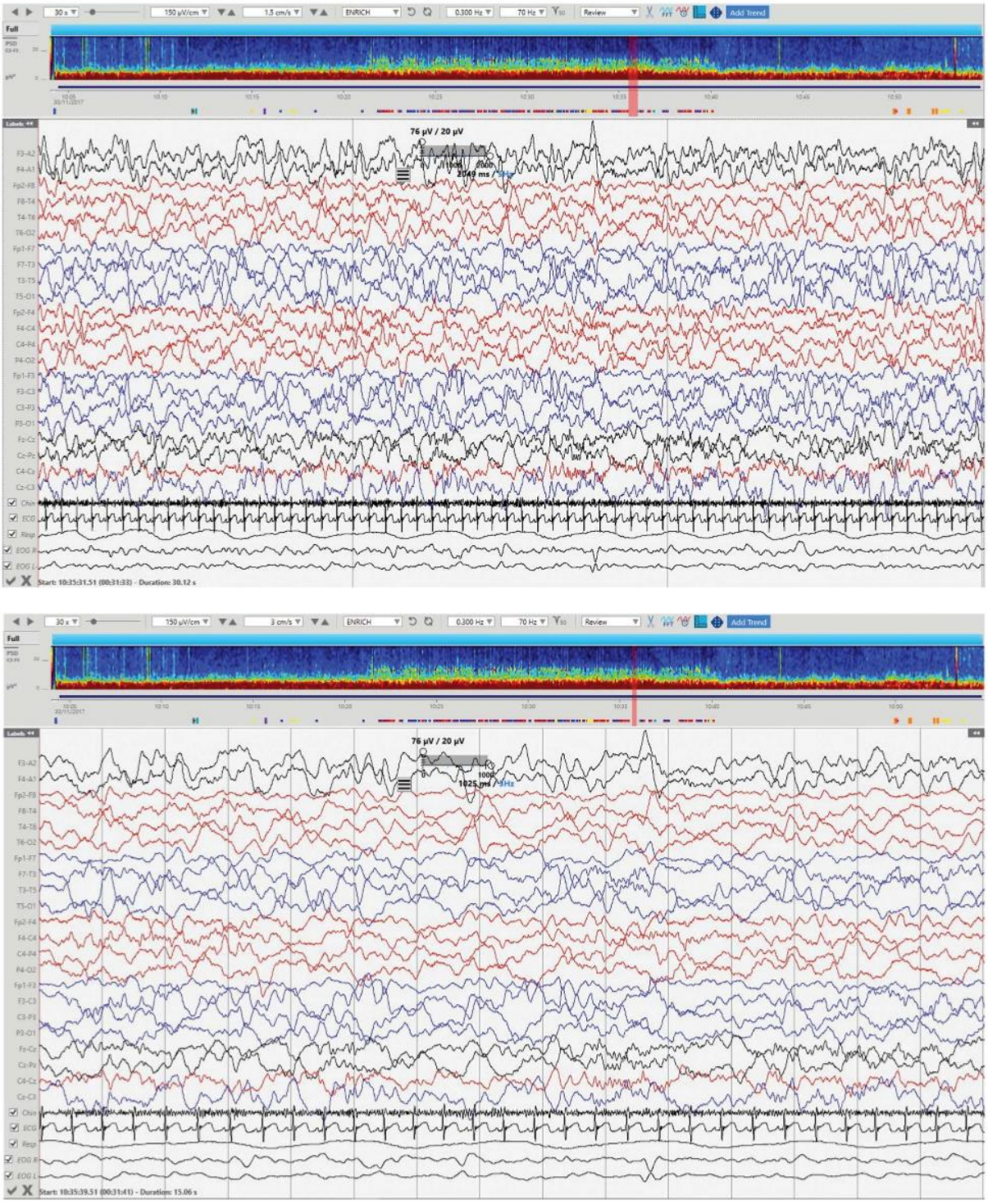


Figure 4. N3 sleep stage. High amplitude slow waves are a hallmark of this stage (intrusions of frontal high amplitude activity present on the EOG channels). Top EEG: timebase =1.5cm/s, gridlines denote 10 s intervals. Bottom EEG: detail of the previous page, timebase = 3cm/s, gridlines denote 1 s intervals.

spindle spectral power and symmetry. There were no significant correlations between spindle features and time of recording (am/pm), GA and chronological age. No association were observed between sleep macrostructure analysis and sex, time of recording (am/pm), GA, and chronological age. This data serves as baseline normative values, which can be used in future studies to compare with infants at risk of abnormal neurodevelopment.

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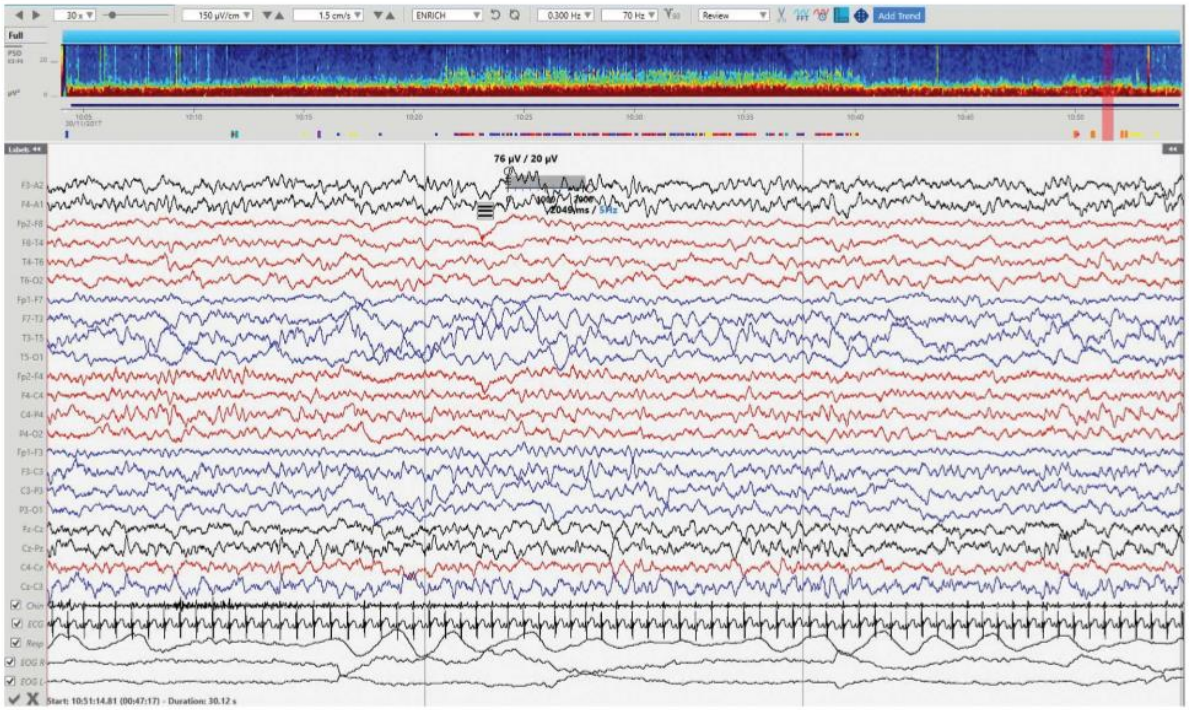


Figure 5. REM sleep stage with relatively low amplitude activities, accompanying conjugate rapid eye movements, irregular respiration rate and lower chin EMG.

Table 3. Analysis of sleep spindle features during the first sleep cycle

Sleep spindle parameters	Median (IQR) (n = 91)
Frequency (Hz)	13.0 (12.8–13.3)
Duration (s)	2.9 (2.6–3.6)
Spectral Power (μV^2)	7.8 (4.7–11.4)
Brain symmetry index	0.20 (0.16–0.29)
Synchrony (%)	59.5 (53.2–63.8)
Number*	241.0 (193.0–286.5)
Density* (spindles/min)	6.6 (5.7–8.0)

*Infants who fell asleep before EEG started were excluded (n = 77).

Normative values for sleep macrostructure and sleep spindles at 4 to 5 months and their maturational evolution

Around the 44th week of PMA, sleep onset starts shifting from REM to NREM sleep [55]. In this study, only two infants reached REM sleep before reaching N2. As reviewed by de Weerd *et al.* [77], this is in keeping with other studies that did note a complete REM onset of sleep in some infants of this age group. Schulz *et al.* hypothesized that increased latency to REM in infancy is dependent on the establishment of the circadian rhythms through means of body temperature circadian amplitude variation and reduction of sleep fragmentation [57].

In the present study, the median (IQR) sleep latency was 8.5 (4.0–14.0) min [mean (SD) of 12.7 (15.8) min]. This latency is shorter in comparison with studies based on parent perception [78, 79]. Our results for sleep latency are similar to other studies when compared using the mean instead of the median (we opted to use the median as sleep latency data has a skewed distribution). Considering the high SD of the latency to sleep in the discussed papers and of the present study, it is clear that there is a high variance in sleep latencies for this age group which may further explain the different results. Additionally, differences in sleep latency may be explained by our use of polysomnographic recordings which provide a more accurate and objective measure of sleep onset, with all our recordings carried out in a temperature-controlled low noise sleep lab. At the same time, feeding during electrode placement in our study may have had a soothing effect that increased the infants' predisposition to fall asleep, even before turning off lights and suspension of other stimuli. In fact, 14 infants fell asleep during the lights-on period while electrodes were being placed.

To date, the majority of studies seeking to characterize the evolution of sleep spindle parameters during the first year of life have included only small cohorts of infants. Nevertheless, these pioneer studies allow insight into the trends and values of different sleep spindle parameters during this neurodevelopmentally rich phase of life. During the first 6 months, the increasingly dominant sleep spindle frequencies range from 12.0 to 14.0Hz [8]. This finding is in accordance with our study, in which the median sleep spindle frequency was 13.0Hz with a narrow IQR of 12.8 to 13.3Hz, suggesting it is well conserved across infants. In contrast, spindles of slower rhythms (<12 Hz) become significantly less common across the same period [8]. Particularly after the first year, and at least, until reaching the 4-year mark, slow spindles reverse this tendency and increase in density [12]. It is widely accepted that two individual types of sleep spindles develop during childhood; fast spindles over more centroparietal areas and slow spindles over more anterior areas, that have different dynamics [19, 80] and subserve different functions (see for example Refs. [33, 53]). In the present study, the fronto-central channels were chosen to manually annotate the sleep spindles due to their

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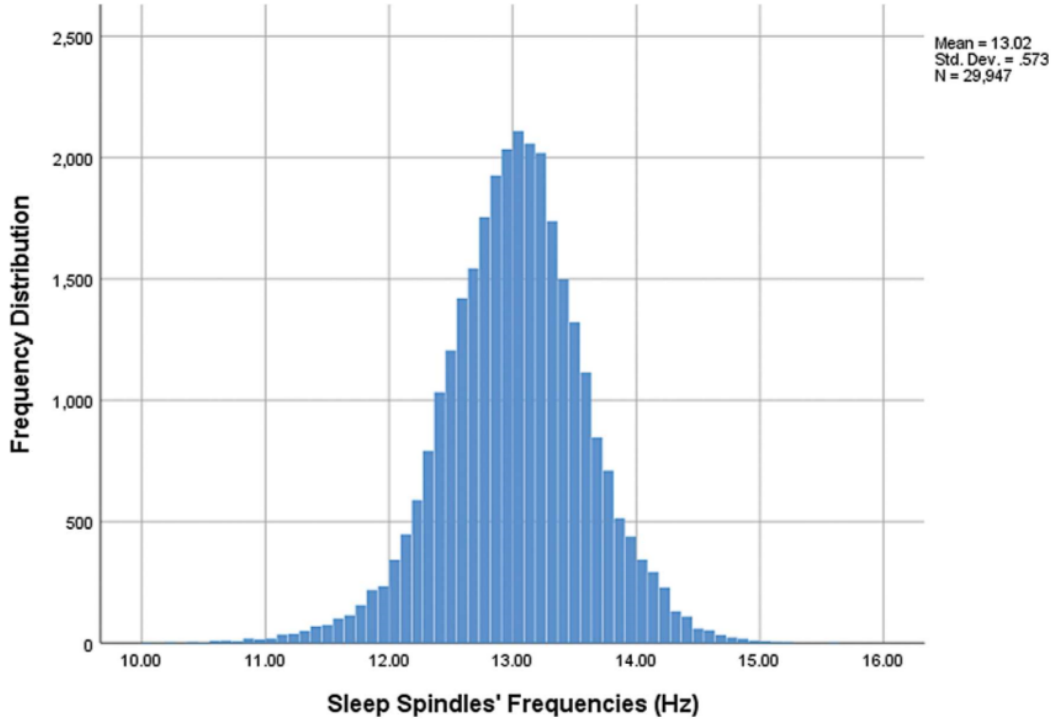


Figure 6. Distribution of sleep spindle frequencies (Hz) from all sleep cycles recorded.

predominance in this area on EEG visual inspection. However, if two sleep spindle types with distinct frequencies were present in these EEGs, we would expect some level of intrusion of fast sleep spindles through central electrodes and to be able to detect these two distinct frequency peaks on these channels. However, the histogram of the distribution of mean frequencies of each spindle (Figure 6), does not show obvious evidence of a second peak. The infants' young age may explain the absence of a clear double peak and thus, its presence may constitute a marker of neurodevelopment. This finding is consistent with previous observations which suggest that the two types of sleep spindles do not coexist before the first year of life [12, 51]. Nevertheless, it remains a possibility that the two potential peaks were concealed by intra-individual differences, driven by plotting N2 and N3 spindles simultaneously, and inter-individual differences in frequency [15, 80] and further investigation on this matter may be required.

In the first 4 years, sleep spindle density, is highest between 4 and 12 months [12]. Sleep spindle synchrony and asynchrony values fluctuate during the first year with the highest sleep spindle asynchrony in earlier infancy [8, 81]. Symmetry is expected to be lowest between 4 and 5 months of age [8]. We identified two previous studies addressing spindle synchrony and one on spindle symmetry in infants [8, 81]. However, in our study, we used different methods to calculate symmetry and synchrony to these studies and our results may not be directly comparable. In the former studies, spindles were considered asymmetrical if one of the spindles were twice the amplitude of the contralateral side and were considered asynchronous if two contralateral spindle mid-points were 2s apart. We used the brain symmetry index which has been used before in adults [82] and we consider it to be a more accurate metric of spindle symmetry than the binary system used previously in infants. We also considered the percentage of coincidental contralateral spindles less arbitrary than the 2-second rule for the synchrony calculation. Along with asymmetry, from infancy to approximately 2 years of age, spindle duration was reported to reach its maximum between 3 and 5 months of age, decreasing thereafter [8, 45, 81]. In keeping with these findings, spindle duration in our group showed a weak but statistically significant association to PMA with a decrease in the short period of time from 55 + 1 to 63 weeks PMA (Table 4), for each week of PMA, there is a decrease of 0.09 s in the spindle duration (p = .018). However, after controlling for sex and timing of recording, (results not included) correlations lost statistical significance.

We also found that interhemispheric synchrony of sleep spindles was weakly correlated with GA (Table 4) with synchrony decreasing 1.31% for each increment of a week on the GA at birth (Table 4). However, this correlation was lost after correcting for PNA, sex, and time of recording.

Relationship between sex and sleep parameters

From simple linear regression analysis, spectral power was 25.4% lower (p = .022) and BSI 23.9% (p = .010) higher in males. These values changed to 24.5% (p = .032) lower and 24.2% (p = .011) higher in males respectively after controlling for GA, PNA, sex, and timing of the nap (Table 4). Gender differences in some sleep spindle parameters have previously been directly [28] and indirectly observed, both before [10] and after puberty [83] in older cohorts. Fast spindle density is higher in female cohorts [28] and may be partly related to menstrual cycle [84]. Also, the evolution of sleep spindle frequency and density across aging is different for both sexes [25]. To our

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Table 4. Univariable and multivariable linear regression analysis of the sleep spindle features on GA, PMA, PNA, sex, and timing of the nap

Dependent Variable	Independent Variable		Univariable analysis (n = 91)				Multivariable analysis (n = 91)		
			r/median (IQR)	B	95% CI	P†	B	95% CI	P‡
Frequency (Hz)	GA		0.024	0.007	(–0.053 to 0.066)	.825	–0.004	(–0.066 to 0.058)	.906
	PMA		0.066	0.013	(–0.028 to 0.054)	.534			
	PNA		0.068	0.018	(–0.038 to 0.074)	.520	0.010	(–0.048 to 0.068)	.723
	Sex	Female (ref)	13.1 (12.8 to 13.3)						
		Male	13.0 (12.8 to 13.2)	–0.051	(–0.197 to 0.096)	.494	–0.041	(–0.191 to 0.110)	.593
	Timing of the nap	Morning (ref)	13.0 (12.7 to 13.2)						
		Afternoon	13.0 (12.9 to 13.3)	0.101	(–0.042 to 0.244)	.165	0.098	(–0.053 to 0.249)	.202
Duration (min)	GA		–0.169	–0.086	(–0.192 to 0.020)	.109	–0.064	(–0.171 to 0.043)	.240
	PMA		–0.246*	–0.086	(–0.158 to –0.015)	.018*			
	PNA		–0.179	–0.086	(–0.185 to 0.014)	.090	–0.068	(–0.168 to 0.033)	.185
	Sex	Female (ref)	2.7 (2.6 to 3.5)						
		Male	3.1 (2.6 to 3.6)	0.158	(–0.104 to 0.421)	.233	0.109	(–0.152 to 0.370)	.409
	Timing of the nap	Morning (ref)	3.2 (2.7 to 3.7)						
		Afternoon	2.8 (2.6 to 3.4)	–0.276	(–0.530 to –0.022)	.034*	–0.210	(–0.473 to 0.052)	.115
Spectral Power (μV^2)§	GA		0.168	1.088	(0.980 to 1.206)	.112	1.079	(0.970 to 1.198)	.158
	PMA		0.167	1.059	(0.986 to 1.137)	.113			
	PNA		0.072	1.034	(0.937 to 1.141)	.500	1.010	(0.915 to 1.115)	.839
	Sex	Female (ref)	8.9 (5.9–12.2)						
		Male	6.4 (4.3–10.3)	0.746	(0.581 to 0.958)	.022*	0.755	(0.584 to 0.976)	.032*
	Timing of the nap	Morning (ref)	7.2 (4.2–10.3)						
		Afternoon	8.2 (5.0–11.6)	1.119	(0.868 to 1.442)	.380	1.052	(0.813 to 1.361)	.697
BSI§	GA		0.090	1.029	(0.962 to 1.102)	.396	1.021	(0.954 to 1.093)	.545
	PMA		0.017	1.004	(0.958 to 1.051)	.875			
	PNA		–0.062	0.981	(0.920 to 1.046)	.561	0.993	(0.931 to 1.058)	.822
	Sex	Female (ref)	0.18 (0.16–0.24)						
		Male	0.24 (0.17–0.32)	1.239	(1.054 to 1.456)	.010*	1.242	(1.052 to 1.465)	.011*
	Timing of the nap	Morning (ref)	0.19 (0.16–0.27)						
		Afternoon	0.22 (0.17–0.30)	1.108	(0.941 to 1.305)	.216	1.108	(0.939 to 1.309)	.222
Synchrony	GA		–0.211*	–1.307*	(–2.583 to –0.031)	.045*	–1.216	(–2.547 to 0.115)	.073
	PMA		–0.144	–0.613	(–1.500 to 0.273)	.173			
	PNA		0.001	0.004	(–1.223 to 1.231)	.994	–0.067	(–1.313 to 1.178)	.915
	Sex	Female (ref)	60.4 (53.4 to 64.6)						
		Male	59.1 (53.0 to 63.6)	–1.386	(–4.585 to 1.813)	.392	–1.547	(–4.785 to 1.690)	.345
	Timing of the nap	Morning (ref)	61.4 (54.6 to 65.0)						
		Afternoon	58.2 (52.2 to 63.7)	–1.724	(–4.868 to 1.420)	.279	–1.047	(–4.301 to 2.206)	.524
Number‖	GA		0.095	5.787	(–8.203 to 19.777)	.413	3.410	(–11.072 to 17.892)	.640
	PMA		–0.050	–2.180	(–12.103 to 7.743)	.663			
	PNA		–0.157	–9.078	(–22.204 to 4.048)	.172	–9.648	(–23.303 to 4.007)	.163
	Sex	Female (ref)	237.5 (179.0 to 305.8)						
		Male	245.0 (208.0 to 285.0)	–0.200	(–34.134 to 33.734)	.991	–4.750	(–39.596 to 30.095)	.787
	Timing of the nap	Morning (ref)	230.5 (192.8 to 287.3)						
		Afternoon	253.0 (200.0 to 287.0)	18.152	(–14.911 to 51.214)	.278	17.448	(–16.737 to 51.632)	.312
Density (spindles/min)‖	GA		0.015	0.021	(–0.307 to 0.348)	.900	0.006	(–0.340 to 0.351)	.974
	PMA		–0.009	–0.009	(–0.240 to 0.223)	.940			
	PNA		–0.025	–0.034	(–0.344 to 0.276)	.826	–0.048	(–0.373 to 0.278)	.771
	Sex	Female (ref)	6.9 (5.3 to 8.8)						
		Male	6.6 (5.8 to 7.9)	–0.117	(–0.908 to 0.674)	.769	–0.142	(–0.972 to 0.689)	.735
	Timing of the nap	Morning (ref)	6.7 (5.3 to 8.0)						
		Afternoon	6.6 (5.8 to 8.1)	0.081	(–0.696 to 0.857)	.837	0.082	(–0.733 to 0.897)	.842

Pearson correlation coefficient (r); reference variable (ref); females: n = 37; males: n = 54; morning: n = 42; afternoon: n = 49, unless stated otherwise.
*P < .05.
†From simple regression analysis.
‡From multiple regression analysis..
§Log-transformed prior to analysis and then back-transformed.
‖Infants who fell asleep before EEG were excluded (n total= 77; females: n = 30; males: n = 47; morning: n = 34; afternoon: n = 43).

knowledge, this is the first time that sex differences have been reported in sleep spindle power and spindle symmetry in infancy. These differences at this young age may be driven by different hormonal [85], and brain connectivity profiles [86]. In light of these findings, future studies should consider sex differences when studying sleep spindle power and symmetry properties in infants. No gender differences were found in the sleep macrostructure parameters.

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Table 5. Univariable and multivariable linear regression analysis of the sleep macrostructure features on GA, PMA, PNA, sex, and timing of the nap

			Univariable analysis (n = 68)				Multivariable analysis (n = 68)		
			r/median (IQR)	B	95% CI	P†	B	95% CI	P‡
Time in NREM (min)§	GA		0.029	1.006	(0.955 to 1.060)	.814	1.003	(0.951 to 1.059)	.905
	PMA		−0.095	0.986	(0.951 to 1.022)	.443			
	PNA		−0.166	0.965	(0.917 to 1.016)	.177	0.966	(0.915 to 1.019)	.197
	Sex	Female (ref)	36.5 (26.0–40.5)						
		Male	34.5 (30.5–42.8)	1.041	(0.916 to 1.184)	.533	1.025	(0.899 to 1.170)	.707
	Timing of the nap	Morning (ref)	34.0 (29.5–39.5)						
		Afternoon	36.0 (30.2–43.0)	1.051	(0.928 to 1.192)	.429	1.055	(0.926 to 1.201)	.414
Time in REM (min)	GA		−0.030	−0.157	(−1.436 to 1.122)	.807	−0.181	(−1.522 to 1.161)	.789
	PMA		0.055	0.199	(−0.695 to 1.094)	.658			
	PNA		0.109	0.571	(−0.710 to 1.852)	.377	0.531	(−0.804 to 1.866)	.430
	Sex	Female (ref)	7.5 (4.4 to 13.6)						
		Male	6.2 (2.9 to 11.5)	−0.870	(−4.039 to 2.299)	.585	−0.674	(−3.976 to 2.628)	.685
	Timing of the nap	Morning (ref)	7.5 (4.5 to 11.5)						
		Afternoon	6.0 (3.5 to 11.5)	−0.218	(−3.317 to 2.881)	.889	−0.198	(−3.436 to 3.041)	.903
%REM	GA		−0.014	−0.118	(−2.196 to 1.960)	.910	−0.053	(−2.193 to 2.087)	.961
	PMA		0.104	0.617	(−0.830 to 2.064)	.397			
	PNA		0.164	1.397	(−0.667 to 3.461)	.181	1.284	(−0.847 to 3.414)	.233
	Sex	Female (ref)	17.7 (10.7 to 29.1)						
		Male	17.2 (7.5 to 25.8)	−2.899	(−8.008 to 2.209)	.261	−2.315	(−7.583 to 2.954)	.383
	Timing of the nap	Morning (ref)	18.0 (10.7 to 28.0)						
		Afternoon	17.1 (7.9 to 26.9)	−1.970	(−6.979 to 3.039)	.435	−2.120	(−7.288 to 3.047)	.415
Cycle duration (min)§	GA		0.021	1.004	(0.953 to 1.058)	.866	1.002	(0.949 to 1.059)	.937
	PMA		−0.042	0.994	(0.958 to 1.031)	.735			
	PNA		−0.081	0.983	(0.932 to 1.036)	.511	0.982	(0.930 to 1.037)	.506
	Sex	Female (ref)	44.0 (35.1–52.4)						
		Male	41.8 (37.4–51.1)	1.007	(0.884 to 1.146)	.920	0.999	(0.872 to 1.143)	.983
	Timing of the nap	Morning (ref)	43.5 (36.5–51.0)						
		Afternoon	42.5 (37.5–51.8)	1.028	(0.906 to 1.166)	.663	1.030	(0.902 to 1.176)	.660
Latency to Sleep (min)§	GA		0.071	1.062	(0.863 to 1.306)	.566	1.016	(0.821 to 1.258)	.880
	PMA		0.094	1.057	(0.915 to 1.221)	.448			
	PNA		0.063	1.055	(0.857 to 1.300)	.608	1.028	(0.831 to 1.272)	.795
	Sex	Female (ref)	8.8 (3.5–23.9)						
		Male	7.5 (4.7–13.6)	0.800	(0.479 to 1.334)	.387	0.810	(0.479 to 1.372)	.428
	Timing of the nap	Morning (ref)	7.0 (4.0–12.0)						
		Afternoon	9.0 (3.5–23.2)	1.470	(0.897 to 2.406)	.124	1.453	(0.867 to 2.434)	.153
Latency to REM (min)§,ǂ	GA		0.133	1.032	(0.973 to 1.095)	.289	1.026	(0.965 to 1.092)	.404
	PMA		−0.010	0.998	(0.958 to 1.041)	.936			
	PNA		−0.147	0.966	(0.910 to 1.024)	.239	0.963	(0.907 to 1.024)	.222
	Sex	Female (ref)	36.8 (28.9–42.0)						
		Male	35.0 (30.6–40.8)	1.010	(0.871 to 1.170)	.898	1.000	(0.860 to 1.161)	.996
	Timing of the nap	Morning (ref)	35.0 (29.0–40.0)						
		Afternoon	36.5 (30.5–43.5)	1.069	(0.927 to 1.234)	.353	1.060	(0.913 to 1.231)	.436

Pearson correlation coefficient (r); reference variable (ref); females: n = 26; males: n = 42; morning: n = 31; afternoon: n = 37.
†From simple regression analysis.
‡From multiple regression analysis.
§Log-transformed prior to analysis and then back-transformed.
ǂInfants who did not have a complete sleep cycle recorded or meet criteria for staging REM before N2 were excluded (n total = 66; females: n = 26; males: n = 40, morning: n = 31; afternoon: n = 35).

Analysis of the relationship between sleep pressure and sleep time, and sleep features

We compared the sleep macrostructure and sleep spindle parameters between the morning and the afternoon recordings to determine if the time of recordings and circadian rhythm influence sleep patterns during daytime. Results from univariable analysis indicated that spindle duration was 0.28 min shorter in the afternoon (p = .034) but, after correcting for sex, GA and PNA, the correlation was no longer significant (Table 4). Also, no differences were found in sleep macrostructure (Table 5). As expected, the proportions of REM/NREM sleep in our diurnal study were smaller than those reported during night sleep, although the duration of a typical sleep cycle is similar [54]. This nocturnal/diurnal contrast in REM and NREM sleep proportions contrast has been described previously not only in adults but also in infants by Louis *et al.* [59] and might be at least partially related to the oscillation of body temperature across the day [87] (for a review [88]).

Similarly, although some macrostructural sleep elements are expected to change across sleep [88], no changes from the first to second sleep cycle were observed in this study which might be due to the narrow sleep time-frame of our analysis

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Table 6. Comparison of macrostructure and sleep spindle parameters' values from first to second sleep cycles

	Median (IQR)		
	First cycle	Second cycle	P†
Macrostructure analysis (n = 11)‡			
Time in NREM (min)	28 (20.0–36.0)	30 (27.5–37.0)	.285
Time in REM (min)	10.5 (4.5–14.5)	7.5 (3.5–12.5)	.533
%REM	29.7 (13.8–33.0)	16.7 (11.6–29.4)	.213
Cycle duration (min)	37 (29.0–43.0)	40.5 (34.5–45.0)	.929
Latency to REM (min)	28.5 (21.5–36)	31 (27.5–37.5)	.213
Sleep spindle analysis (n = 29)			
Frequency (Hz)	13 (12.8–13.2)	13 (12.8–13.3)	.689
Duration (s)	2.9 (2.6–3.6)	2.9 (2.7–3.4)	.496
Spectral power (μV^2)	8.2 (4.9–14.5)	6.4 (4.4–12.2)	<.001*
Brain Symmetry Index	0.18 (0.16–0.26)	0.19 (0.16–0.26)	.627
Synchrony (%)	58.2 (52.6–61.6)	57.2 (52.6–61.6)	.358
Number§	227 (174.2–246.5)	237.5 (170.0–302.5)	.200
Density§ (spindles/min)	6.4 (5.4–7.6)	7.6 (6.1–9.2)	.082

†From Wilcoxon test for paired samples.
‡Infants who did not have two complete sleep cycles recorded were excluded.
§n = 22 due to additional exclusion of infants who fell asleep during electrode placement.
P < .05 marked with an asterisk (*).

(Table 6). Adult studies have shown sleep spindles to be sensitive to reductions in sleep pressure through consecutive sleep cycles [23, 25, 28] and increases following sleep deprivation [24, 27, 89]. In our infants (Table 6), one sleep spindle parameter, spectral power, decreased in response to a decrease in sleep pressure from the first to the second NREM period. This contrasts with adult studies that found an increase in the spindle-related spectral power concurrent with a decrease in delta activity across sleep cycles [27, 90]. No studies to date have investigated spindle spectral power across consecutive sleep cycles in infants and Louis *et al.* showed no significant changes in amplitude across cycles in 12 infants studied [8]; hence this is an area that warrants further research.

Strengths

We used polysomnography in this study, which is considered the gold-standard for sleep studies; additionally, good spatial coverage was assured by the use of 21 active electrodes. Recording in the sleep laboratory allowed for a standardized environment and control for factors such as light, noise, and temperature. A clinical physiologist was present at all times during the recording to address any technical problems.

All infants had a Griffiths-III assessment performed by certified clinical neuropsychologist/pediatricians at 18-months, ensuring that the infants included in this study had no neurodevelopmental impairment.

Spindles were identified as discrete features and selected for analysis manually by an experienced clinical physiologist which would be considered the gold standard for spindle detection and allowed correct detection of the spindle segments, as opposed to spectral power analysis of sigma frequencies or more rudimentary spindle-detection algorithms. High agreement scores were obtained when comparing a subset of these EEGs with the markings from an EEG-trained research nurse.

The present study included a large cohort of infants and described the sleep macrostructure at 4 to 5 months of age and is the biggest infant cohort reported to date describing sleep spindles and this will allow comparison with futures studies on maturation and neurodevelopment.

Our study resulted in the detailed description of the sleep spindles using parameters that generally are not studied together: frequency, duration, spectral power, brain symmetry index, synchrony, number and density, permitting a broader picture of the several elements that constitute the sleep spindles. To our knowledge, this was also the first study to report sex differences in sleep spindle parameters in infancy and future studies relating sleep spindles to neurodevelopmental pathologies will have to take account of those differences.

Spindles result from complex interactions between the thalamus and the cortex; they depend on the numerical extent of cortical neurons involved as well as the cortical layers involved and pathways [91]. Surface EEG, in turn, is only able to detect post-synaptic potentials from the most superficial region of the cortex. Detection of potentials is also dependent on the position of the neurons, as biological dipoles, on the cortical gyri, and the number of synchronized firing neurons [92]. As a result, only a fraction of the spindle oscillations generated are indirectly detectable by this technique. Nevertheless, the study of spindles with EEG is an accessible, real-time, noninvasive technique with promising results reflecting cognitive abilities [10, 16, 20, 21, 83] and neurologic conditions [36–41]

Limitations

There are a number of limitations to our study. First, we could not control for the fact that some of the infants fell asleep during the trip to the sleep lab; effects of a short nap during the car journey may have affected sleep pressure. As seen in Table 6, sleep spindle power changes from the first to the second cycle. For this reason, a possible reduction of sleep pressure resulting from a nap during the trip to our lab may have affected sleep spindle power.

Although sleep spindle identification kappa scores were high between two raters, sleep staging was only performed by one

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neurophysiology trained rater and this may constitute a limitation to our study.

Despite being the biggest cohort of infants reported to date which provides a detailed analysis of sleep spindles, the number of infants may still be a limitation for group comparisons by gender and time of the day and particularly for comparisons between the first and second sleep cycle. Given the sample size, only medium/large effect sizes could be detected.

It was only possible to record one polysomnography per infant. Due to this, there was no previous infant adaptation to the laboratory. Results from first recording sessions may differ from results from other consecutive sessions [60]. Regarding sleep spindles, it is accepted that, based on the EEG spectra, they are fairly constant and represent an individual signature [15]. In a study of twelve adolescents, not all parameters presented good intra-individual reliability with one single recording; those parameters were slow spindle density and slow spindle duration [93]. There is no study so far on the intra-individual reliability of sleep spindles in infants. However, considering that only slow sleep spindles showed lower intra-individual reliability in adolescents [93] and that these types of spindles tend to appear toward toddlerhood [12], we do not believe this had a major impact on our sleep spindle analysis.

To conclude, in this study we characterize normative values for daytime sleep macrostructure and sleep spindles from 4 to 5 months of age. Spindle spectral power decreased from the first to the second sleep cycle. Sex differences in the sleep spindle spectral power and symmetry were the only independent variables studied from the first sleep cycle that remained statistically significant after controlling for GA and PNA at the recording, and time of nap. For this reason, sex differences should be taken into consideration in future studies in this age group. These values may provide a benchmark for future studies focusing on early recognition of risk of atypical neurodevelopmental trajectory.

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Disclosure Statement

None declared.

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Appendix 2 – Parent-led massage and sleep EEG for term-born infants: A randomised controlled parallel-group study

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ORIGINAL ARTICLE

Parent-led massage and sleep EEG for term-born infants: A randomized controlled parallel-group study

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Abstract

Aim: To examine the impact of parent-led massage on the sleep electroencephalogram (EEG) features of typically developing term-born infants at 4 months.

Method: Infants recruited at birth were randomized to intervention (routine parent-led massage) and control groups. Infants had a daytime sleep EEG at 4 months and were assessed using the Griffiths Scales of Child Development, Third Edition at 4 and 18 months. Comparative analysis between groups and subgroup analysis between regularly massaged and never-massaged infants were performed. Groups were compared for sleep stage, sleep spindles, quantitative EEG (primary analysis), and Griffiths using the Mann–Whitney *U* test.

Results: In total, 179 out of 182 infants (intervention: 83 out of 84; control: 96 out of 98) had a normal sleep EEG. Median (interquartile range) sleep duration was 49.8 minutes (39.1–71.4) (n = 156). A complete first sleep cycle was seen in 67 out of 83 (81%) and 72 out of 96 (75%) in the intervention and control groups respectively. Groups did not differ in sleep stage durations, latencies to sleep and to rapid eye movement sleep. Sleep spindle spectral power was greater in the intervention group in main and subgroup analyses. The intervention group showed greater EEG magnitudes, and lower interhemispherical coherence on subgroup analyses. Griffiths assessments at 4 months (n = 179) and 18 months (n = 173) showed no group differences in the main and subgroup analyses.

Interpretation: Routine massage is associated with distinct functional brain changes at 4 months.

Early infancy is a period of intense learning and development including the processes of neural proliferation, differentiation, migration, myelination, and circuit formation.[1] During this crucial time, we may expect to see the most marked effects of environment-dependent enrichment. Environmental enrichment occurs when stimuli are introduced into the environment, within the appropriate timeframe, positively affecting development through epigenetics and neural plasticity.[2] Infant massage can be considered an environmental enrichment as it involves tactile stimulation and social bonding.[3] Studies suggest that infant massage may counterbalance some negative outcomes resulting from adverse events in early life,[4] and accelerate preterm neurodevelopment,[3,5,6] although some changes fade over the second half of infancy.[3] However, much remains unknown about the possible benefits of infant massage on the neurodevelopment of term-born, low-risk infants.

A considerable part of infant life is spent in sleep, with term-born infants sleeping more than two-thirds of the day. Important sleep transitions related to maturation occur

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Abbreviations: NREM, non-rapid eye movement; REM, rapid eye movement.

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during infancy. For most infants, by 4 months of age, sleep onset no longer starts in rapid eye movement (REM) but in non-rapid eye movement (NREM) and sleep stages become more mature, with three different NREM stages being readily identifiable. Sleep spindles are well established by 4 months of age as a result of thalamic and cortical interactions and their features reflect processes of ageing, memory consolidation, and cognitive skills.[7] In addition, other sleep electroencephalogram (EEG) features such as EEG power spectrum characteristics also evolve with ageing, particularly in early life, and these changes are partly associated with neurodevelopment.[8]

The primary aim of this study was to explore the impact of massage on infant neurodevelopment using EEG assessment of sleep parameters such as sleep macrostructure, sleep spindles, and quantitative EEG (qEEG).

What this paper adds

- Routine massage of infants is associated with differences in sleep electroencephalogram biomarkers at 4 months.
- Massaged infants had higher sleep spindle spectral power, greater sleep EEG magnitudes, and lower interhemispherical coherence.
- No differences between groups were observed in total nap duration or first cycle macrostructure.

METHOD

Participants

Neonates were recruited post-delivery at Cork University Maternity Hospital, Cork, Ireland, during 2017 to 2018 to the BabySMART study (Study of Massage Therapy, Sleep And neurodevelopMenT, registered identifier NCT03381027), a randomized controlled parallel-group trial to evaluate the impact of massage intervention on infant neurodevelopment. The study was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals, Cork, Ireland. Inclusion criteria were gestational age greater than or equal to 37 weeks and singleton birth; exclusion criteria included admission to a neonatal intensive care unit, and suspected congenital or metabolic anomalies. Written informed consent was obtained from all parents or guardians. Infants were randomly assigned (1:1) to the intervention (daily massage) group or the control (routine care) group. Block randomization (with varying block sizes of two, four, and six) was used to allocate infants to each group. The randomization list was generated by a biostatistician using the 'ralloc' procedure in Stata. Sequentially numbered, opaque, sealed envelopes ensured concealment. Owing to the nature of the intervention, the recruiting research personnel, clinical team, and families of the infants were aware of group allocation. EEG recordings, neurodevelopmental assessments, and statistical analysis were performed blinded to group allocation.

Massage

A massage training session of about 45 minutes at the 2-week appointment and optional review classes during the study were provided to parents in the intervention group by a physiotherapist, certified by the International Association of Infant Massage (massage instructions booklet in Appendix S1). From 2 weeks of age until the 4-month appointment, parents were asked to perform a massage routine three times daily for at least 15 minutes on at least two body areas and to keep a massage diary. The parents were given 'Johnson's® Head-to-Toe® extra moisturizing baby cream' and parents were encouraged to use it during the infant massage but could use other products if they preferred. The control group were requested to use standard care throughout the study.

Assessments

The EEG recording technique has been described in detail in Ventura et al.[9] Briefly, at 4 months, EEGs were recorded using the Lifelines EEG (Lifelines Neuro, UK) acquisition system. An EEG was recorded using disposable electrodes placed according to the 10–20 system at positions Fp1, Fp2, Fz, F3, F4, F7, F8, Cz, C3, C4, T3, T4, Pz, P3, P4, T5, T6, O1, O2, A1 and A2, plus reference, and ground with impedances less than 10kΩ. Extra channels included chin electromyography, electrooculogram, electrocardiogram, and a movement sensor to monitor respiration. The recording started as soon as all the electrodes were applied and ended when the infant was fully awake. During acquisition, quality of the recording was monitored using 24 bipolar EEG channels in a modified double-banana montage and 5 extra polygraphy channels. The appointments coincided with their normal diurnal nap time to increase the likelihood of sleep and were performed in the INFANT Research Centre sleep laboratory to reduce the influence of external stimuli.

Post-acquisition, sleep staging was performed according to American Academy of Sleep Medicine 2.4 definitions and rules[10] using a sleep staging application (Nicolet, Natus, USA); sleep spindles were annotated over left and right fronto-central regions with StratusEEG (Kvikna, Iceland) software. Both sleep stages and sleep spindles were identified manually by SV. All analyses were restricted to the first sleep cycle to avoid potential effects of decreasing sleep pressure on sleep features in subsequent sleep cycles. Parameters in the analysis included sleep spindles, sleep macrostructure derived from the sleep staging data such as sleep stage duration and latencies, and qEEG features, a set of objective biomarkers that capture key characteristics of the background EEG pattern.

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Infants who fell asleep during electrode placement were excluded from sleep spindle number and density calculations because of a potential for missing spindles. Analysis of macrostructure features, except total sleep time, and qEEG was only performed for infants who had a complete first sleep cycle recorded. Within infants with a complete sleep cycle, those who had REM sleep before reaching criteria for staging N2 were excluded from the latency to REM analysis.

Spindle number and duration were calculated for each infant. Spindle density was defined as the number of sleep spindles per minute of NREM sleep. Mean frequency, brain symmetry index,[11] synchrony, and spectral power of sleep spindles were calculated using MATLAB (version R2020a, The MathWorks Inc., Natick, MA, USA). Spindle brain symmetry index was determined per epoch of 30 seconds using a sliding window with 75% overlap and an additional 9 Hz to 16 Hz passband filter to reduce the background activity present during a sleep spindle event. Synchrony calculation was based on the percentage of coincidental contralateral sleep spindles.[9]

Owing to the complexity of the EEG it was not possible to fully represent all EEGs by a finite set of features.[12] Instead, we characterized salient attributes of this complex, time-varying, multi-dimensional signal by generating multiple features that captured different overarching characteristics of the EEG. These features, generated using an open-source MATLAB toolbox (NEURAL version 0.4.3),[13] were divided into four groups: EEG magnitude, spectral distribution, continuity, and connectivity. Each group contained multiple features, described as follows.

The EEG magnitude group included features of spectral power and range-EEG (rEEG). Spectral power quantified EEG power in different frequency bands and rEEG quantified peak-to-peak amplitude. Four features were extracted from the rEEG: standard deviation (SD), median, and the 5th and 95th centiles of the rEEG; these last two are known as the lower (5th) and upper (95th) margins. The spectral distribution group captures frequency characteristics of the EEG which are independent of amplitude and power. This group included fractal dimension, an estimate of the slope of the power spectrum; spectral flatness, a measure of disorder (entropy) of the spectra; and spectral difference, a measure of change of the spectra over time. The continuity group captured deviations from a continuous EEG activity pattern. Features in this group included the skewness and kurtosis of the EEG and the rEEG asymmetry measure. The last group included measures of interhemispherical connectivity: brain symmetry index, a measure to quantify differences in spectral power across the hemispheres; and interhemispherical coherence, a measure of coupling calculated across bilateral electrode pairs.

Where applicable, features were further subdivided into a standard set of frequency bands: delta 1, 0.5 Hz to 2 Hz; delta 2, 2 Hz to 4 Hz; theta, 4 Hz to 8 Hz; alpha, 8 Hz to 12 Hz; sigma, 12 Hz to 15 Hz; beta, 15 Hz to 30 Hz; and gamma, 30 Hz to 45 Hz. Fractal dimension was calculated across the full bandwidth (0.5–45 Hz) and rEEG was pre-filtered to the 1 Hz to 20 Hz bandwidth, as detailed elsewhere.[13] All features were calculated for the different sleep stages. qEEG and spindle features estimated on short-time segments were summarized by a median value over time.

Infant neurodevelopmental outcome was assessed by a trained psychologist at 4 months and 18 months of age respectively using the Griffiths Scales of Child Development, Third Edition. The Griffiths Scales of Child Development, Third Edition measures overall general development (GD) and five neurodevelopmental areas: foundations of learning (A), language and communication (B), eye and hand coordination (C), personal–social–emotional (D), and gross motor abilities (E), with lower scores indicating more severe developmental delay. Sociodemographic questionnaires were given to the parents to complete at 2-week, 4-month, and 18-month appointments (data of the 18-month questionnaire not shown). At 4 months mothers also completed the Maternal Postnatal Attachment Scale, a 19-item questionnaire with possible scores ranging from 19 to 95, the latter score indicating the greatest maternal attachment[14] and Edinburgh Postnatal Depression Scale, a 10-item questionnaire with possible scores ranging from 0 to 30, the latter score indicating greatest likelihood of depression.[15]

Statistical analysis

For statistical analysis we used IBM SPSS Statistics (version 26.0, IBM Corp., Armonk, NY, USA). Continuous variables were described using median and interquartile range (IQR), and categorical variables using frequency and percentage. For comparisons between groups, a Mann–Whitney *U* test was used for continuous variables and a χ^2 test (with continuity correction for 2×2 tables) or Fisher's exact test (in the case of small expected counts) for categorical variables. All tests were two-sided and $p < 0.05$ was considered statistically significant.

We included a subgroup analysis where regularly massaged infants in the intervention group were compared to never-massaged infants in the control group. Participants in the intervention group were considered to massage regularly if the infant received at least two massage sessions, that lasted at least 5 minutes, over at least 4 days within each week for at least 10 weeks from week 4 to the end of week 16. Never-massaged control infants were identified on the basis of questionnaire responses from the parents.

We did not adjust for multiple comparisons in the analysis owing to the exploratory nature of this study and because reducing the probability of type I error would increase the probability of type II error.[16,17]

An a priori sample size calculation indicated that a sample of 150 infants (75 per group) was necessary for an independent-samples *t*-test to detect a difference of 6% in mean active sleep between the intervention and control groups, assuming a mean of 52% in the control group, a standard deviation of 13%,[18] a power of 80%, a level of significance of 0.05, and a two-tailed test.

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RESULTS

Demographics

A total of 408 newborn infants were consented to the study and randomized to intervention and control groups ($n = 204$ per group). At 4 months of age, 84 infants remained in the intervention group and 98 in the control group. Three infants were excluded from the analysis: two females, one from each group, who did not sleep during the EEG, and a male infant in the control group who had a grossly abnormal EEG and was referred for further clinical assessment. Hence, 179 infants were included in the main analysis (83 from the intervention and 96 from the control group) (Figure S1). Demographic variables were similar between groups (Table 1). To assess the risk of bias, we compared demographic characteristics provided at registration between (1) infants included ($n = 179$) and those excluded in our analysis ($n = 229$) and (2) within those excluded, infants allocated to intervention ($n = 121$) and control ($n = 108$) groups (Tables S1 and S2). Maternal age was significantly lower in the excluded group than in the included group (mean 32 years 4 months [SD 5 years 3 months] vs 34 years 11 months [4 years 0 months], $p < 0.001$). No other statistically significant differences were observed. For the subgroup analyses, 33 regularly massaged infants in the intervention group and 57 never-massaged control infants were included (Figure S2). By 18 months, six infants were lost to follow-up. Hence, for the comparison of neurodevelopmental outcome at 18 months, 173 infants were included in the main analysis (81 from the intervention and 92 from the control group) and 89 infants were included in the subgroup analysis (33 from the intervention and 56 from the control group).

Sleep analyses

For those who had sleep onset recorded ($n = 156$), median (IQR) total sleep time was 49.8 minutes (39.1–71.4) with no significant differences between groups; the intervention group slept 50.5 minutes (40.0–68.0), $n = 75$; the control group 49.0 minutes (37.8–72.5), $n = 81$ ($p = 0.76$). Similarly, in the subgroup analysis, there was no statistically significant difference ($p = 0.68$) between total sleep time of regularly massaged intervention (50.0 minutes [42.6–62.4], $n = 32$) and never-massaged control infants (50.0 minutes [37.0–72.0], $n = 47$).

There were no statistically significant differences in any of the sleep macrostructure parameters studied (Table 2). For sleep spindle parameters, the spectral power was significantly different between groups, being higher in the intervention group (intervention: 8.89μV^2 [6.46–12.70], $n = 83$; control: 7.82μV^2 [4.75–11.40], $n = 96$; $p = 0.04$) (Table 2). In the subgroup analysis, spectral power remained significantly higher in the intervention group with greater differences between groups (intervention: 10.09μV^2 [6.89–13.67], $n = 33$; control: 7.86μV^2 [5.01–10.77], $n = 57$; $p = 0.03$). Similar to the main analysis, sleep macrostructure and other sleep spindle features were not statistically different.

qEEG features that were significantly different between the two groups in either the main or the subgroup analyses are presented in Table 3. We found higher magnitudes (i.e. spectral power and rEEG measures) across multiple frequency bands and sleep stages in the intervention group compared with the never-massaged group. In the subgroup analysis, most magnitude differences remained (Figure 1). N1 amplitude kurtosis in alpha, N3 delta 2 spectral flatness, REM theta, and N3 gamma relative powers were no longer statistically significant in the subgroup analysis. Within the subgroup analysis, some other statistical differences did emerge, namely higher standard deviation of rEEG in REM and N3 beta spectral differences in the intervention group and lower interhemispherical coherence (Figure 2) in regularly massaged infants. All results are shown in Tables S3 and S4.

Griffiths Scales of Child Development, Third Edition analysis

In the main analysis, intervention and control groups scored a median (IQR) of 117 (112–127) and 122 (112–127) at 18 months on Griffiths general development respectively ($p = 0.36$). There were no significant differences in any of the Griffiths scores at 4 and 18 months between groups in the main and subgroup analyses (Table S5 and Table 4 respectively).

DISCUSSION

To date, there is still no clear evidence of a beneficial effect of massage on cognitive development in term-born infants.[19] This randomized controlled study investigated the associations between routine parent-led massage during the first 4 months of age and neurodevelopment in a typically developing term-born cohort. This study has identified a higher background EEG magnitude across a range of frequencies and sleep stages, higher sleep spindle power, and lower interhemispherical coherence in regularly massaged infants at 4 months of age. The results may be generalizable to similar populations. Socioeconomics, maternal attachment, and likelihood of depression are factors that condition cognitive outcomes; no statistical differences were observed between groups (Table 1).

Massage typically coexists with other forms of social interaction,[19] so both handling and social interactions are key elements. In animal models, neonatal handling promotes specific learning and memory formation, modulates stress responses, and conditions an array of behaviours.[20] Maternal care is responsible for epigenetic modulation[21] and influences synaptogenesis.[22] Children whose mothers show more positive demonstrations of affection score better on performance and verbal IQ at later ages.[23] Infant massage

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TABLE 1 Demographic data.

Demographics	Intervention n	Intervention n (%)[b]		Control n	Control n (%)[b]		p[a]
Registration and 2-week questionnaire							
Gestational age (weeks), median (IQR)	83	39.9	(39.0–40.9)	96	39.9	(38.9–41.0)	0.68
Birthweight (kg), median (IQR)	83	3.5	(3.2–3.8)	96	3.6	(3.3–3.8)	0.40
Sex, male	83	45	(54)	96	56	(58)	0.69[c]
Maternal age (years:months), median (IQR)	83	35:6	(32:4–37:8)	96	35:1	(32:9–37:7)	0.88
Maternal level of education	83			96			0.13[d]
Secondary school		2	(2)		7	(7)	
Third level certificate or diploma		19	(23)		17	(18)	
Degree or higher/graduate diploma		41	(49)		37	(39)	
Postgraduate		21	(25)		35	(35)	
Parents' annual net income after taxes	83			96			0.76[e]
€30 000 or less		5	(6)		8	(8)	
€30 001–€60 000		20	(24)		19	(20)	
€60 001 or above		37	(45)		40	(42)	
Prefer not to say/do not know		21	(25)		29	(29)	
4-month appointment and 4-month questionnaire							
Timing of recording, morning[f]	83	45	(54)	96	45	(47)	0.41[c]
Chronological age (weeks), median (IQR)	83	19.3	(18.3–20.4)	96	19.4	(18.6–20.4)	0.79
Weight (kg), median (IQR)	83	6.9	(6.5–7.6)	96	7.1	(6.4–7.8)	0.50
Type of feeding	80			96			0.51[e]
Breast fed		29	(35)		29	(29)	
Infant formula		38	(48)		54	(56)	
Both		13	(16)		13	(14)	
Fed on a regular schedule	80			96			0.60[e]
Yes, always		11	(14)		13	(14)	
Yes, try to		26	(33)		38	(40)	
No, fed on demand		43	(54)		45	(47)	
Duration of dermatosis with pruritus, if present	9			18			1.00[d]
Less than 6 weeks		5	(56)		9	(50)	
Between 6 weeks and less than 3 months		4	(44)		8	(44)	
Between 3 and 4 months		0	(0)		1	(6)	
Maternal EPDS, likelihood of depression	80			96			0.88[d]
Low (scored 0–9)		70	(88)		85	(89)	
Average (scored 10–12)		6	(8)		8	(8)	
High (scored >12)		4	(5)		3	(5)	
Maternal attachment score, median (IQR)	80	86.9	(83.0–90.5)	96	86.9	(82.7–90.5)	0.90

Descriptive statistics using median and interquartile range (IQR) or *n* (%).

Abbreviation: EPDS, Edinburgh Postnatal Depression Scale scores.

[a]From Mann–Whitney *U* test unless otherwise stated.

[b]Unless otherwise stated.

[c]From χ^2 test with continuity correction.

[d]From Fisher's exact test.

[e]From Pearson's χ^2 test.

[f]Recording performed in morning or afternoon.

TABLE 2 Sleep macrostructure and sleep spindle measures during the first sleep cycle, main and subgroup analysis.

	Intervention			Control			
Sleep parameters	***n***	**Median (IQR)**		***n***	**Median (IQR)**		***p*** [a]
Main analysis							
Sleep macrostructure							
1st sleep cycle duration (minutes)	67	43.00	(38.50–50.50)	72	43.25	(37.00–51.88)	0.78
N1 (minutes)	67	8.00	(5.00–11.50)	72	7.75	(4.50–13.38)	0.66
N2 (minutes)	67	5.00	(3.00–8.00)	72	4.50	(2.50–6.50)	0.48
N3 (minutes)	67	21.00	(15.00–25.00)	72	21.50	(16.63–25.38)	0.47
NREM total (minutes)	67	33.50	(28.00–39.00)	72	35.50	(30.13–40.50)	0.29
REM (minutes)	67	9.00	(5.00–12.50)	72	6.50	(3.63–11.50)	0.24
REM (%)	67	21.18	(13.16–28.57)	72	17.44	(9.49–27.70)	0.15
Latency to sleep (minutes)	67	9.00	(5.00–15.00)	72	8.50	(4.00–14.00)	0.85
Latency to REM[b] (minutes)	62	34.75	(28.00–41.00)	70	36.00	(30.50–41.63)	0.36
Sleep spindles							
Frequency (Hz)	83	12.99	(12.80–13.24)	96	13.02	(12.79–13.27)	0.78
Duration (s)	83	3.17	(2.68–3.54)	96	2.92	(2.60–3.58)	0.27
Spectral power (μV^2)	83	8.89	(6.46–12.70)	96	7.82	(4.75–11.40)	0.04*
Brain symmetry index	83	0.20	(0.17–0.25)	96	0.20	(0.16–0.29)	0.70
Synchrony (%)	83	59.44	(53.80–63.82)	96	59.21	(53.19–63.80)	0.52
Number[c]	75	242.0	(185.0–282.0)	81	244.0	(194.0–286.5)	0.59
Density[c] (spindles/minute)	75	6.67	(5.53–8.05)	81	6.72	(5.79–8.03)	0.73
Subgroup analysis							
Sleep macrostructure							
1st sleep cycle duration (minutes)	29	44.00	(39.50–50.50)	41	45.00	(37.50–53.25)	0.80
N1 (minutes)	29	8.00	(5.25–12.25)	41	8.50	(5.50–15.50)	0.47
N2 (minutes)	29	5.50	(2.75–8.75)	41	5.00	(2.75–6.50)	0.38
N3 (minutes)	29	22.50	(14.00–26.25)	41	22.00	(16.50–25.00)	0.87
NREM total (minutes)	29	37.00	(30.50–42.75)	41	36.00	(30.50–45.75)	0.97
REM (minutes)	29	7.50	(4.75–12.25)	41	7.50	(4.50–12.25)	0.89
REM (%)	29	18.27	(12.52–26.07)	41	17.74	(10.71–26.96)	1.00
Latency to sleep (minutes)	29	9.50	(5.50–18.00)	41	9.00	(3.75–16.75)	0.59
Latency to REM[b] (minutes)	26	37.00	(31.13–44.50)	41	37.00	(31.50–44.00)	0.86
Sleep spindles							
Frequency (Hz)	33	13.00	(12.68–13.15)	57	13.02	(12.81–13.27)	0.21
Duration (s)	33	3.07	(2.67–3.50)	57	2.84	(2.55–3.56)	0.70
Spectral power (μV^2)	33	10.09	(6.89–13.67)	57	7.86	(5.01–10.77)	0.03*
Brain symmetry index	33	0.21	(0.18–0.28)	57	0.19	(0.16–0.26)	0.09
Synchrony (%)	33	59.14	(53.44–62.93)	57	59.27	(54.69–64.02)	0.59
Number[c]	32	249.5	(187.3–297.0)	47	253.0	(192.0–295.0)	0.83
Density[c] (spindles/minute)	32	6.54	(5.42–7.94)	47	6.78	(5.35–8.72)	0.57

Abbreviations: EEG, electroencephalogram; IQR, interquartile range; NREM, non-rapid eye movement; REM, rapid eye movement.

[a]From Mann–Whitney *U* test; statistically significant differences ($p < 0.05$) marked with an asterisk.

[b]Infants who met criteria for staging REM before N2 were excluded.

[c]Infants who fell asleep before EEG were excluded.

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TABLE 3 qEEG features with statistically significant differences between the two groups in either main or subgroup analyses during the first sleep cycle.

			Intervention		Control		
qEEG type	SS	FB	Median (IQR)		Median (IQR)		p[a]
Main analysis[b]							
Amplitude kurtosis	N1	α	3.49	(3.41–3.70)	3.43	(3.36–3.56)	0.04*
rEEG: lower margin (μV)	N1		63.33	(54.37–73.86)	57.22	(50.72–68.68)	0.02*
rEEG: median margin (μV)	N1		94.28	(81.97–110.07)	85.59	(75.11–100.30)	0.02*
rEEG: upper margin (μV)	N1		138.57	(119.52–164.36)	127.13	(112.28–147.95)	0.04*
Spectral power (μV^2)	N1	δ1	342.09	(269.90–458.32)	298.50	(215.89–408.80)	0.05
Spectral power (μV^2)	N1	δ2	216.52	(162.77–293.43)	178.46	(132.67–256.30)	0.04*
Spectral power (μV^2)	N1	θ	79.71	(66.27–127.88)	72.36	(49.37–96.39)	0.02*
Spectral power (μV^2)	N1	α	7.40	(5.95–10.67)	6.23	(5.00–8.31)	0.01*
Spectral power (μV^2)	N1	σ	2.30	(1.64–2.97)	1.80	(1.44–2.53)	0.02*
Coherence	N2	δ1	0.26	(0.19–0.29)	0.26	(0.21–0.31)	0.79
Coherence	N2	δ2	0.14	(0.11–0.17)	0.14	(0.12–0.17)	0.84
Coherence	N2	α	0.06	(0.04–0.09)	0.06	(0.04–0.08)	1.00
Coherence	N2	σ	0.09	(0.07–0.13)	0.09	(0.07–0.12)	0.82
Spectral power (μV^2)	N2	δ1	445.74	(370.56–616.77)	376.30	(286.23–532.56)	0.008*
Spectral power (μV^2)	N2	θ	81.35	(64.63–106.66)	73.53	(55.33–89.08)	0.03*
Spectral power (μV^2)	N2	β	7.60	(5.55–8.91)	6.11	(5.02–7.86)	0.04*
Spectral power (μV^2)	N2	γ	2.66	(2.12–3.41)	2.13	(1.84–2.88)	0.01*
Coherence	N3	δ1	0.23	(0.20–0.29)	0.26	(0.22–0.28)	0.30
Coherence	N3	δ2	0.14	(0.10–0.16)	0.13	(0.12–0.16)	0.78
Coherence	N3	σ	0.09	(0.08–0.12)	0.10	(0.08–0.12)	0.37
Coherence	N3	β	0.07	(0.05–0.10)	0.07	(0.06–0.10)	0.25
Coherence	N3	γ	0.06	(0.04–0.10)	0.07	(0.05–0.10)	0.26
rEEG median margin (μV)	N3		122.51	(108.06–131.99)	114.50	(99.35–128.60)	0.12
Relative spectral power (%)	N3	γ	0.16	(0.13–0.19)	0.13	(0.11–0.18)	0.02*
Spectral difference	N3	β	0.003	(0.002–0.005)	0.003	(0.002–0.005)	0.58
Spectral flatness	N3	δ2	0.89	(0.86–0.90)	0.88	(0.86–0.90)	0.04*
Spectral power (μV^2)	N3	β	5.47	(4.67–6.72)	4.70	(3.97–5.94)	0.004*
Spectral power (μV^2)	N3	γ	2.02	(1.70–2.52)	1.61	(1.29–1.98)	<0.001*
Amplitude skew	R	θ	0.015	(0.013–0.017)	0.017	(0.014–0.019)	<0.001*
Coherence	R	γ	0.04	(0.04–0.07)	0.05	(0.04–0.06)	0.26
rEEG lower margin (μV)	R		51.09	(45.86–58.42)	46.67	(42.34–54.56)	0.006*
rEEG median margin (μV)	R		76.09	(66.54–84.93)	70.44	(62.02–80.59)	0.03*
rEEG upper margin (μV)	R		109.69	(94.74–123.51)	101.63	(91.45–117.31)	0.06
rEEG SD (μV)	R		17.24	(14.70–19.60)	16.24	(14.16–18.64)	0.12
Spectral flatness	R	δ2	0.90	(0.88–0.92)	0.89	(0.87–0.91)	0.004*
Relative spectral power (%)	R	θ	9.87	(8.57–11.58)	9.04	(7.86–10.77)	0.03*
Spectral power (μV^2)	R	δ2	128.81	(94.43–164.94)	106.94	(82.57–151.24)	0.08
Spectral power (μV^2)	R	θ	48.19	(39.05–69.23)	39.89	(32.09–51.98)	0.003*
Spectral power (μV^2)	R	α	5.22	(4.39–6.49)	4.78	(3.69–5.88)	0.04*
Spectral power (μV^2)	R	σ	1.51	(1.27–1.80)	1.25	(1.02–1.70)	0.02*
Spectral power (μV^2)	R	β	4.11	(3.30–5.07)	3.64	(3.06–4.52)	0.02*
Spectral power (μV^2)	R	γ	2.28	(1.68–2.74)	1.89	(1.55–2.49)	0.02*

(Continues)

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TABLE 3 (Continued)

qEEG type	SS	FB	Intervention Median (IQR)		Control Median (IQR)		p[a]
Subgroup analysis[c]							
Amplitude kurtosis	N1	α	3.47	(3.37–3.57)	3.43	(3.34–3.55)	0.20
rEEG lower margin (μV)	N1		63.60	(54.27–71.92)	56.22	(49.29–68.51)	0.07
rEEG median margin (μV)	N1		90.71	(83.17–106.61)	81.96	(71.57–104.79)	0.06
rEEG upper margin (μV)	N1		134.26	(123.25–158.84)	124.33	(105.98–149.89)	0.09
Spectral power (μV^2)	N1	δ1	336.24	(262.03–441.76)	274.61	(195.78–341.32)	0.04*
Spectral power (μV^2)	N1	δ2	200.34	(159.43–270.58)	169.37	(123.71–277.69)	0.10
Spectral power (μV^2)	N1	θ	87.73	(69.79–120.77)	71.11	(44.99–99.15)	0.02*
Spectral power (μV^2)	N1	α	7.94	(6.13–10.36)	5.88	(4.81–8.68)	0.05
Spectral power (μV^2)	N1	σ	2.53	(1.68–3.01)	1.70	(1.42–2.58)	0.03*
Coherence	N2	δ1	0.22	(0.16–0.29)	0.27	(0.21–0.30)	0.03*
Coherence	N2	δ2	0.13	(0.11–0.16)	0.15	(0.13–0.19)	0.03*
Coherence	N2	α	0.05	(0.03–0.06)	0.06	(0.04–0.08)	0.03*
Coherence	N2	σ	0.08	(0.06–0.10)	0.10	(0.07–0.13)	0.02*
Spectral power (μV^2)	N2	δ1	439.75	(368.76–596.72)	363.88	(275.66–502.29)	0.04*
Spectral power (μV^2)	N2	θ	86.18	(71.09–105.18)	71.48	(53.23–95.94)	0.04*
Spectral power (μV^2)	N2	β	7.33	(5.58–8.62)	6.81	(5.37–8.27)	0.48
Spectral power (μV^2)	N2	γ	2.78	(2.14–3.54)	2.30	(1.76–3.17)	0.07
Coherence	N3	δ1	0.20	(0.16–0.27)	0.26	(0.23–0.28)	0.003*
Coherence	N3	δ2	0.12	(0.10–0.15)	0.14	(0.12–0.16)	0.01*
Coherence	N3	σ	0.08	(0.06–0.11)	0.10	(0.08–0.12)	0.006*
Coherence	N3	β	0.06	(0.04–0.08)	0.07	(0.06–0.10)	0.04*
Coherence	N3	γ	0.05	(0.03–0.08)	0.06	(0.05–0.09)	0.03*
rEEG median margin (μV)	N3		125.36	(111.91–135.56)	112.31	(98.44–128.53)	0.05*
Relative spectral power (%)	N3	γ	0.16	(0.13–0.18)	0.14	(0.11–0.19)	0.47
Spectral difference	N3	β	0.004	(0.003–0.006)	0.003	(0.002–0.005)	0.04*
Spectral flatness	N3	δ2	0.90	(0.87–0.91)	0.88	(0.86–0.90)	0.10
Spectral power (μV^2)	N3	β	5.63	(4.96–6.65)	4.90	(4.04–6.25)	0.05
Spectral power (μV^2)	N3	γ	2.02	(1.78–2.62)	1.66	(1.30–2.11)	0.006*
Amplitude skew	R	θ	0.015	(0.013–0.017)	0.017	(0.015–0.019)	0.02*
Coherence	R	γ	0.04	(0.03–0.05)	0.05	(0.04–0.06)	0.03*
rEEG lower margin (μV)	R		52.97	(46.75–58.67)	45.72	(41.53–52.97)	0.007*
rEEG median margin (μV)	R		76.09	(67.05–87.24)	67.85	(61.64–78.15)	0.02*
rEEG upper margin (μV)	R		109.69	(97.32–125.32)	99.55	(88.16–118.56)	0.04*
rEEG SD (μV)	R		17.84	(14.84–19.71)	15.48	(13.62–19.29)	0.04*
Relative spectral power (%)	R	θ	10.29	(9.13–11.79)	9.38	(7.88–11.31)	0.17
Spectral flatness	R	δ2	0.91	(0.88–0.92)	0.88	(0.86–0.90)	0.01*
Spectral power (μV^2)	R	δ2	126.21	(95.81–165.02)	101.05	(72.07–145.70)	0.04*
Spectral power (μV^2)	R	θ	54.45	(41.47–69.48)	38.31	(31.71–54.07)	0.006*
Spectral power (μV^2)	R	α	5.76	(4.54–6.66)	4.95	(3.78–6.38)	0.10
Spectral power (μV^2)	R	σ	1.56	(1.31–1.82)	1.37	(1.07–1.77)	0.09
Spectral power (μV^2)	R	β	4.24	(3.62–5.14)	3.78	(3.01–4.84)	0.06
Spectral power (μV^2)	R	γ	2.41	(1.89–2.84)	1.94	(1.54–2.66)	0.02*

Abbreviations: EEG, electroencephalogram; FB, frequency bands: 0.5–2 Hz (δ1), 2–4 Hz (δ2), 4–8 Hz (θ), 8–12 Hz (α), 12–15 Hz (σ), 15–30 (β), 30–45 Hz (γ); IQR, interquartile range; qEEG, quantitative EEG; rEEG, range-EEG (frequency band 1–20 Hz); SS, sleep stage.

[a]From Mann–Whitney U test; statistically significant differences ($p < 0.05$) marked with an asterisk.

[b]Intervention: $n = 67$; control: $n = 72$.

[c]Regularly massaged intervention: $n = 29$; never-massaged control: $n = 41$.

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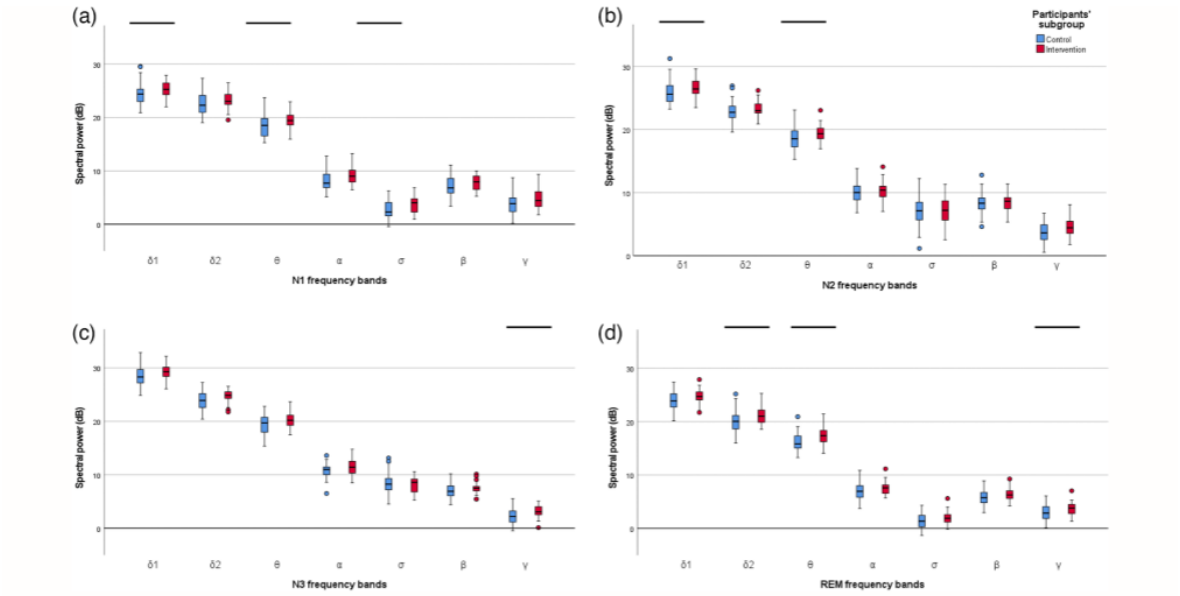


FIGURE 1 Spectral power across frequency bands and sleep stages during the 1st cycle on the basis of the subgroup analysis (i.e. the regularly massaged intervention group and never-massaged control infants). Horizontal lines indicate statistically significant differences between subgroups and circles indicate outliers. Units converted to decibels (dB; i.e. $10\log_{10}$[spectral power]) to compare magnitudes in different frequency bands. (a–d) Spectral power during N1, N2, N3, and rapid eye movement (REM) respectively.

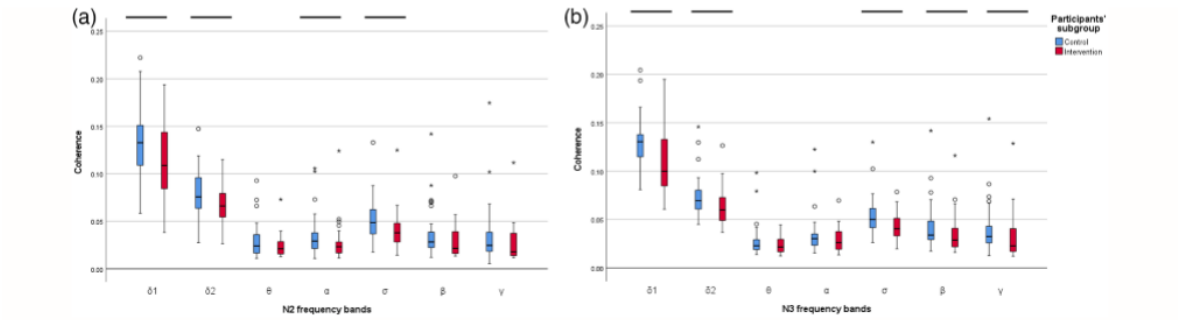


FIGURE 2 Interhemispherical coherence across frequency bands during the first cycle on the basis of the subgroup analysis (i.e. the regularly massaged intervention group and never-massaged control infants). Horizontal lines, indicate statistically significant differences between subgroups in the corresponding frequency ranges; circles and stars, outliers and extreme outliers respectively. (a, b) Coherence during N2 and N3 respectively.

may decrease stress-related hormones and increase serotonin.[4] This protective effect against stress may in itself influence neurodevelopment.[24]

Even though massage may help induce sleep,[4] evidence does not support its contribution to an overall daily increase of time spent asleep.[19] Similarly, in our study, routine massage had no effect on total time asleep during the nap, on first sleep cycle length, or duration of individual sleep stages (Table 2).

We found higher spectral power values for sleep spindles in the intervention group than in the control group, and these differences increased in the subgroup analysis (Table 2). These group differences were not observed in the sigma band spectral power during N2 and N3, indicating that spindle power increase was not simply due to a general increase in background magnitude in this band. Existing literature shows an increasing sleep spindle magnitude in infancy;[25] thus, these observations may be evidence of infant massage as an accelerator of brain maturation.[5] Sleep spindle power is positively associated with axial diffusivity in the forceps minor, anterior corpus callosum, and tracts in the temporal lobe and thalamus.[26] Axial diffusivity relates to the diffusion of water parallel to the axons. Although it is not entirely understood why increases in axial diffusivity can be present in early neurodevelopment, it is proposed to reflect decreased axonal tortuosity,[27] or increased extra-axonal

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TABLE 4 Neurodevelopmental outcomes at 18 months.

Griffiths III results at 18 months	Intervention			Control			p^a
	n	Median (IQR)		*n*	Median (IQR)		
Main analysis							
General development[b]	81	117	(112–127)	92	122	(112–127)	0.36
Subscales							
A. Foundations of learning	81	114	(105–122)	92	114	(105–122)	0.67
B. Language and communication	81	109	(95–117)	92	109	(99–117)	0.53
C. Eye and hand coordination	81	114	(105–122)	92	118	(109–122)	0.23
D. Personal–social–emotional	81	118	(112–125)	92	122	(117–125)	0.28
E. Gross motor	81	130	(126–135)	92	135	(126–135)	0.15
Subgroup analysis							
General development[b]	33	122	(114.5–127)	56	122	(112–127)	0.33
Subscales							
A. Foundations of learning	33	114	(110–120)	56	114	(105–122)	0.59
B. Language and communication	33	109	(95–118.5)	56	104	(95–117)	0.44
C. Eye and hand coordination	33	118	(109–122)	56	116	(106–122)	0.66
D. Personal–social–emotional	33	122	(115–125)	56	118	(115–125)	0.26
E. Gross motor	33	130	(130–135)	56	135	(126–135)	0.90

Abbreviations: Griffiths III, Griffiths Scales of Child Development, Third Edition; IQR, interquartile range.

[a]From Mann–Whitney *U* test.

[b]A lower score indicates more severe neurodevelopmental delay.

space[28,29] due to pruning of superfluous connections.[30] Functionally, sigma power has been positively associated with full-scale and fluid IQ[31] scores, while spindle-related frequency was negatively associated with perceptual reasoning, comprehension, and working memory,[32] among others. Owing to the neuroplastic properties of spindles, they are thought to play a role in memory consolidation processes.[33]

Results of qEEG indicated two major differences in the intervention group: the signal magnitude-related features, which showed a higher magnitude of signals, measured by rEEG margins and spectral power across nearly all frequency bands studied, largely replicated in the subgroup analysis; and lower levels of interhemispherical coherence (Table 3). Some of the qEEG statistically significant features were not preserved after subgroup analysis; this was probably because of the reduced number of participants and consequent loss of statistical power.

Previous studies of massage in infants born preterm have found differences in spectral power during active sleep. A parent-led massage study showed a dose association up to term equivalent age on global relative power, and increased localized alpha in the massage group[6] and in a study with a massage-expert, significant local and global absolute spectral power interactions between massage and non-massage groups were found before and after a massage routine performed by an expert.[5] In line with previous observations, we also found effects of massage on spectral power in our group of term-born infants. Although changes in spectral power values of different frequency bands with maturation are regionally specific,[8] overall, there is a net increase of the spectral power during the first half-year of life.[34] In this context, our findings of increased EEG magnitude further support the notion of more advanced brain maturation in the routinely massaged group, extending observations to cohorts of infants born at term.[5,6]

Subgroup analysis revealed lower interhemispherical coherence, a marker of functional connectivity, in the intervention group in nearly all frequency bands studied, particularly during NREM sleep (Table 3). Advanced functional axonal pruning of the corpus callosum connecting the two hemispheres, which occurs during early infancy, may explain this finding. Although connectivity changes vary across maturation, reflecting cycles of synaptic establishment and elimination[35] occurring at different time points in different brain regions; our study revealed a net decrease in interhemispherical connectivity in those infants who had regular parent-led massage during their first 4 months of life. Previous studies have noted decrements of interhemispherical connectivity in specific points of infancy and childhood (see, for example, Xiao et al.[36]; Boersma et al.[37]). For example, from 5 to 7 years old, a general decrease of connectivity is accompanied by increased functional node clustering and path length and decreased heterogeneity of the synaptic weights; this indicates a possible transition from random to more organized network arrangement.[37] In infants and toddlers, interhemispherical decreases of connectivity between homologous

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cortical areas may be expected as a result of neural segregation while integrating intrahemispherical areas of interest, accompanied by improvement of specific performances.[36,38] In our cohort, the changes were seen in a resting state after providing continued environmental enrichment in the form of parental massage. The changes provided by continued massage sessions may have caused plastic changes that contributed to the differences in patterns seen 'offline' in the sleeping state.

Our qEEG subgroup analysis also demonstrated differences in continuity (EEG skewness), and spectral distribution (spectral flatness and spectral differences) between groups. These results highlight that there are subtle changes in the EEG structure beyond the most evident sleep analysis that could only be observed through qEEG.[13]

There were no statistically significant differences on Griffiths assessments at 4 months and 18 months between the two groups (Table S5 and Table 4). We hypothesize that neurodevelopmental changes from routine massage are too subtle to be observable on Griffiths assessments at these early ages and that differences may only be evident at later time points, particularly school-age.

This study builds on knowledge about the effects of regular parent-led massage. So far, most studies of infant massage have focused on neurodevelopment in preterm cohorts. To our knowledge, this is the first study to report the effects of routine massage on the sleep EEG measures of typically developing term-born infants. With 179 infants included in this study up to 4 months, this is one of the largest and longest studies of infant massage. This study also included a diverse array of sleep measures that ranged from the duration of sleep stages, multiple sleep spindle features, and qEEG measurements. However, the approach in this exploratory study also implies that multiple analyses increase the probabilities of false-positive results. Because of this, we discuss general qEEG aspects instead of individual associations. The results were consistent, showing higher magnitude and lower coherence on intervention in several frequency ranges and sleep stages.

Before starting the massage routine, parents from the intervention group performed the massage on their children during the class with the International Association of Infant Massage-certified physiotherapist who corrected the technique if necessary. Parents were also invited to attend this class as a refresher if they had any further questions. However, we did not assess the quality of massage on a day-to-day basis, which can be considered a limitation of our study. A further limitation is that we did not perform a laboratory adaptation sleep session owing to logistics. First 'night' effects can alter some macrostructure and spindle features. However, as all participant EEGs were recorded under the same conditions in this randomized controlled study, this should not influence the results of the comparative analysis. Owing to the high dropout rate, we compared demographic characteristics measured at registration between participants included in our study and those excluded (Tables S1 and S2). However, the sociodemographic data obtained at registration were limited. Another limitation is the low compliance in the intervention group: only 33 out of 83 infants were regularly massaged, although all infants in the intervention group were massaged. One reason for non-compliance may have been a mismatch between expectations at recruitment and the actual reality of the considerable demands of being a new parent and accommodating additional regular massage into the daily routine. Although occasional phone calls of encouragement to parents may have helped retain some participants, future studies may achieve higher compliance if participants are enrolled after families are settled in their routines at home. Importantly, some group differences were still observed in the main analysis, indicating that less restrictive routine massage criteria may be acceptable to retain more participants and improve compliance. Further in-depth analysis should analyse the effects of massage by sex and relate infant massage to other neurodevelopmental assessments, including memory abilities, and examine the persistence of EEG changes observed in this study as participants age.

CONCLUSION

A parent-led massage routine during the first 4 months of age produced detectable changes in the sleep EEG. Those changes included higher spectral power in sleep spindles, higher magnitude of the EEG, and decreased interhemispherical coherence across most frequency bands and sleep stages. These findings indicate that environmental enrichment in the form of parental massage may be associated with a more mature neurodevelopmental profile observable in the infant sleep EEG at 4 months.

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DATA AVAILABILITY STATEMENT

It is not possible to share the datasets for the current study. The clinical data is collected under a written proxy consent from the participants' guardians/parents, which did not include permission for sharing or open data. To be allowed to share this data under Irish Health Research Regulations we are required to re-consent families or to obtain approval by the Health Regulation Consent Declaration Committee.

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SUPPORTING INFORMATION

The following additional material may be found online:
Appendix S1: Massage booklet.
Table S1: Sociodemographic comparisons between excluded and included participants according to data provided at registration.
Table S2: Sociodemographic comparisons between excluded participants from intervention and control according to data provided at registration.
Table S3: qEEG features during the first sleep cycle.
Table S4: qEEG features during the first sleep cycle – subgroup analysis.
Table S5: Neurodevelopmental outcomes at 4 months.
Figure S1: Main analysis flow diagram.
Figure S2: Subgroup analysis flow diagram.

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Appendix 3 – Clinical Research Ethics Committee approval

UCC
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Coláiste na hOllscoile Corcaigh, Éire
University College Cork, Ireland

COISTE EITICE UM THAIGHDE CLINICIÚIL
Clinical Research Ethics Committee

Lancaster Hall,
6 Little Hanover Street,
Cork,
Ireland.

Our Ref: ECM 3 (ggg) 04/04/17 & ECM 3 (hhh) 06/06/17

31st May 2017

Professor Geraldine Boylan
Director of INFANT
INFANT Centre
5th Floor
Cork University Maternity Hospital
Wilton
Cork

Re: BABY SMART (Study of massage therapy, sleep and neurodevelopment).

The Chairman approved the following:

- Cover Letter dated 17th May 2017
- Study Protocol Version 1 dated 9th March 2017.

Full approval is now granted to begin this study.

Yours sincerely

Professor Michael G Molloy
Chairman
Clinical Research Ethics Committee
of the Cork Teaching Hospitals

The Clinical Research Ethics Committee of the Cork Teaching Hospitals, UCC, is a recognised Ethics Committee under Regulation 7 of the European Communities (Clinical Trials on Medicinal Products for Human Use) Regulations 2004, and is authorised by the Department of Health and Children to carry out the ethical review of clinical trials of investigational medicinal products. The Committee is fully compliant with the Regulations as they relate to Ethics Committees and the conditions and principles of Good Clinical Practice

Ollscoil na hÉireann, Corcaigh - National University of Ireland, Cork.

Appendix 4 – Parents' Consent Form

Participant's Informed Consent Form

BABY SMART (Study of Massage therapy, sleep And neurodevelopMenT)

If you wish to take part in this research project having read the information leaflet please read and sign this form.

Participant Name:_____ **Participant DOB:**_____ **Study number:**_____	**Project Title**: BabySMART **Principal Investigator**: Prof. Geraldine Boylan, **Contact Details**: 0879607875/0214205014

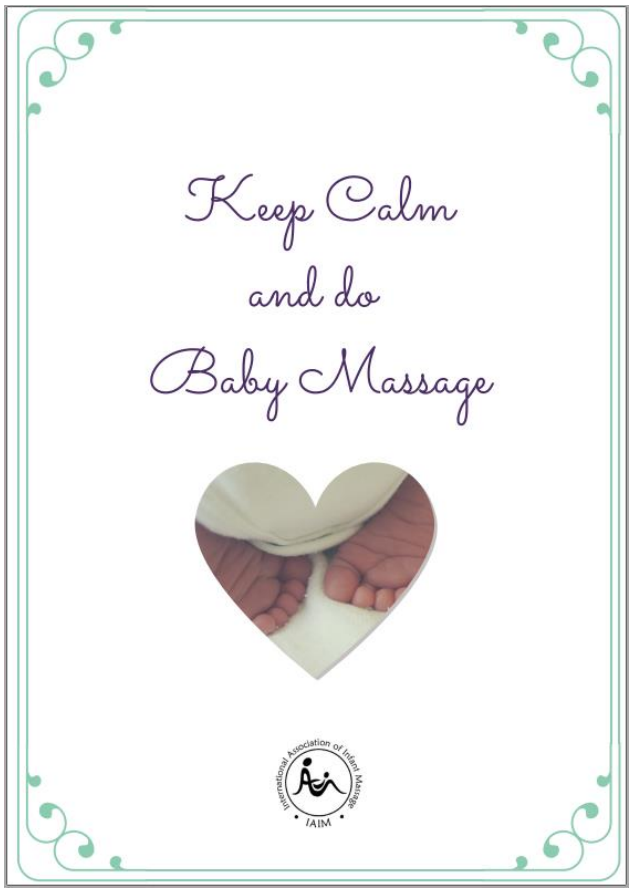
	Please tick the appropriate box	**Yes**	**No**
1.	I have read the information leaflet about this research and have been given a copy to keep. The information has been fully explained to me and I have been able to ask questions. I understand why the research is being done and any risks involved.	☐	☐
2.	I am aware that participation is voluntary and I may withdraw my consent at any time. I am aware that my decision not to continue participation at any stage will not restrict my access to health care services normally available to me.	☐	☐
3.	I agree to allow my baby to take part in the follow up visits required at approximately 2 weeks, 4 months and 18 months of age to measure their growth, health and development. These visits will involve parental questionnaires, development assessments and measurements of growth, and health.	☐	☐
4.	I agree to allow the ENRICH research team to carry out a video EEG recording of my baby's sleep, at the 4 month visit.	☐	☐
5.	I give permission to the ENRICH research team to ask me to complete a questionnaire about my own postnatal health. Based on my responses, I understand that the research team may contact me or need to refer me to my GP.	☐	☐
6.	I consent to have my baby assigned to an enrichment programme, which may involve a structured pre-sleep routine.	☐	☐
7.	I agree that the ENRICH research team may contact my GP with any relevant health issues that may arise for me and/or my baby.	☐	☐
8.	I give permission for my baby's anonymised video EEG and all information collected about my baby to be stored for possible future research, unrelated to this study *without my further consent being required* but subject to approval by a research ethics committee.	☐	☐
9.	I have been assured that all information collected pertaining to me and my baby will be kept confidential.	☐	☐
10.	I understand that the Investigators have adequate insurances as required by law.	☐	☐
11.	I give permission for information collected (not including video EEG) about my baby to be shared anonymously with the industry partner (Johnson & Johnson) who has co-funded this study	☐	☐

Signed:_____ Date:_____
Name of research participants parent

Signed:_____ Date:_____
Name of person taking consent

V4 20 Jul 2017

Appendix 5 – Baby massage instruction leaflet



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Relaxation Exercise for Parents

To help yourself relax, take a slow, deep breath.
Continue breathing slowly and deeply as you let go of tension throughout your body. Since many of us hold tension in our shoulders and neck, it may help to slowly rotate your neck from side to side. Gently lift and roll your shoulders. Breathe deeply throughout the massage to increase your enjoyment and help your baby learn how to relax.

What you will need for your massage session:

1. Towel or blanket
2. Spare nappies
3. Change of clothes for your baby

Baby mats and Lotion will be provided

ENRICH Infant Sleep Study | Ospideal Maithreachais na hOllscoile Corcaigh Cork University Maternity Hospital | Infant

Create a special time for you and your baby each day. Be flexible: some days you may have more time than others. Perhaps you have a quiet half hour when your baby awakens from their nap, maybe you have a few minutes while changing their nappy or just before their bath.

Be sensitive to your baby's moods and try to choose a time when your baby is not too tired or hungry. Your baby will not be able to relax and respond to you if their needs are not being met.

Observe your baby and watch for a time when baby is alert, quiet, and receptive. This is the best time to start the massage interaction. The goal is to try and incorporate three 15 minute massages every day.

Create a space that is comfortable, warm, and free from distractions. Warmth is especially important for newborns because they tend to lose body heat rapidly. Find a sunny spot on your bed, use a space heater to warm the room, or you may do the massage with baby partially clothed.

You may want to have a soft blanket to place over your baby during the massage. A soft pillow to place baby on provides a feeling of security and warmth for young infants. Be relaxed and make sure you are comfortable.

Sit in a way that is restful; try sitting cross-legged on a couch or bed with baby in front of you or lean back against the wall with baby lying on your extended legs. Be aware of your needs and create a time for massage when you feel relaxed enough to put your concerns and distractions aside for a while. Many mothers and fathers find themselves relaxing and letting go of tension along with their babies during a massage.

ENRICH Infant Sleep Study | Ospideal Maithreachais na hOllscoile Corcaigh Cork University Maternity Hospital | Infant

Touch Relaxation

Touch Relaxation may be used throughout infancy and childhood, either with the massage interaction or on its own, separate from the massage. It is a valuable skill to help reduce stress and increase your child's feeling of well-being.

Place your hands on the body part that you wish your baby to let go and relax. Gently and softly pat the area, giving baby a touch cue with a verbal cue to "let go" or "relax. As soon as you feel the baby soften and relax, give your baby acknowledgment verbally and with your facial expression, "That's wonderful".

The purpose of Touch Relaxation is to teach your baby how they can relax and release tension. It is a conditional response which is learned through the repetition of consistent messages communicated to your baby with your voice and touch.

Infant Cues

Your baby uses cues to tell you what he needs. At times, your baby may be "willing' to engage with you; at other times he may be unwilling. There may be a mixture of both willingness and unwillingness cues. Watch your baby and respond to the cues that appear most prominent. As you interact with your baby, your baby may change the cues they are giving you. For example, they may express some unwillingness cues, such as turning away, fussing and avoiding eye contact. You respond by reducing the amount of stimulation, perhaps pausing and just holding the area being massaged.

Your baby reacts by turning back toward you, engaging in eye and face contact, and letting you know that they are ready to resume the massage interaction. At other times, their cues will signal the need for a cuddle break or the need to end the massage.

Willingness Clues:
- Eye contact
- Looking at your face
- Stilling (stopping moving)
- Smooth movement of arms and legs
- Turning eyes or head toward you
- Smiling
- Babbling, talking, cooing sounds
- Raising head or reaching out to you
- Eyes wide and bright
- Hand to mouth

Unwillingness Cues
- Fussiness/Crying
- Turning head away
- Coughing/choking sounds
- Frowning or grimacing face
- Strong back arching
- Eyes turning away or dull looking
- Spitting up/vomiting
- Pulling away/squirming/kicking
- Hiccoughs

Remember it is not necessary to do the whole massage regime each time, better to pick the areas that you know you baby prefers and do these for a minimum of **15 minutes, three times a day** until your 4-month visit.

Listening To Your Baby

Remember, in listening to your baby . . . Take long, deep breaths and relax. Clear your thoughts and place all your attention on your baby. It may be appropriate to stop the massage and just focus your baby; maintaining your touch by placing your hands gently and securely on their body. Connect with your baby, and if possible, establish eye-to-eye contact. They may tell their story through various types of cries or by making sounds. Usually, this is accompanied by a willingness to make eye contact at the same time, as though they are reaching out to be seen and heard.

As your baby needs, offer the comfort and security of rocking, caressing, or walking. If your baby has shared and released feelings through strong crying, make certain you give the support of loving reassurance and nurturing touch. Afterwards, you may choose to resume the massage interaction, depending on the needs of both you and your baby.

LEGS & FEET

Indian Milking



Begin by massaging your baby's legs. Babies of any age usually enjoy having their legs massaged. When you massage your baby's legs, do all the strokes on one leg, then repeat the same strokes on the other leg. By doing this, you gradually encourage relaxation in each of your baby's legs. It may help to hold your hand in a "C" shape while stroking your baby's leg. Continue repeating the milking stroke alternating your hands so that you first stroke the outside and then the inside of the leg.

Hug & Glide



Using the "C" shape of your hands, starting at your baby's hip and holding baby's leg with both hands close together, alternate a gentle squeezing and twisting motion from your baby's hip to ankle.

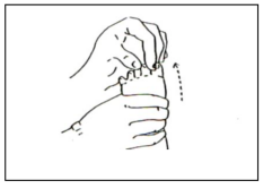
Thumb over Thumb



Begin by stroking the bottom of baby's foot from the heel to the toes with your thumbs, one after another.

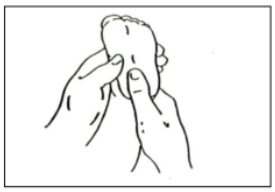
"Swish swash, swish swash"

Toe Roll



Gently roll each toe. This is a great time to recite:
"This little piggy went to market,
This little piggy stayed home,
This little piggy had a massage
And this little piggy did, too.
This little piggy said, wee, all the way home"

Walking



Press gently all over the bottom of your baby's foot with your thumbs.

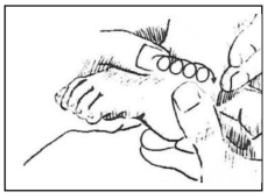
"Cho cho choo choo" / hungry caterpillar

Stroking Top of Foot



Stroke the top of the foot toward the ankle.

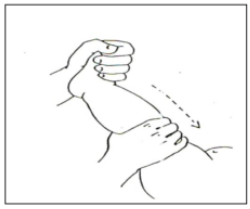
Ankle Circles



Gently circle you baby's ankles using your thumbs

Swedish Milking

Support your baby's foot with your hand. Using a "C" shaped hand, stroke your baby's leg from the ankle to the hip with a smooth downward stroke. Alternate hands to massage both sides of your baby's leg.



Rolling

Roll baby's lower leg between your hands and then the upper leg between your hands.

"Wibbly wobbly wibbly wobbly jelly on a plate".



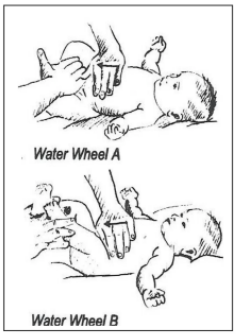
STROKES FOR THE STOMACH

These strokes help tone the intestinal system and relieve gas and constipation. It is very important that these strokes be done in a circular clockwise direction on your baby's stomach. If your baby is lying on their back, imagine that your baby's head is at 12 o'clock (on the face of a clock) and that your baby's feet are at 6 o'clock. Your hands move in the same direction as the hands of a clock.

Water Wheel

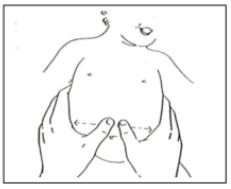
Using the sides of your hands, make paddling strokes on your baby's stomach, one hand following the other, as if you were scooping sand toward yourself. Start below the ribs and move into the lower abdomen. Stroke hand over hand with a gentle but firm pressure.

"Row row row your boat"



Thumb to Sides

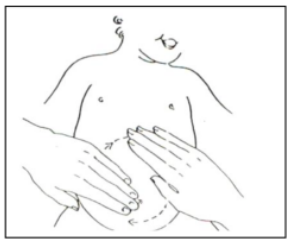
With your thumbs at the level of baby's belly button, stroke out to the sides. Be gentle and do not poke.



Sun & Moon

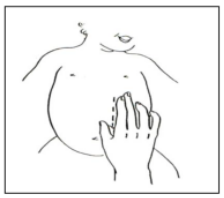
Your left hand begins by making a full circle on baby's stomach, moving clockwise. Your right and makes a half circle or crescent moon in a clockwise direction starting at about 10 o'clock on your baby's tummy and stroke down to 4 o'clock.

Then lift your right hand up and over your left hand, making the continuous circular "Sun strokes. Massage around and around with your hands like the hands of a clock.

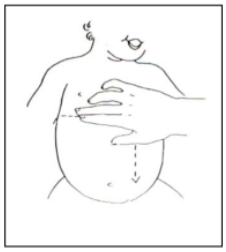


I Love You

This is a three-part stroke which spells out the message. "I love you" to your baby. Babies love a high-pitched voice saying "I love you" as you share this stroke.



"I" Make a vertical I-shaped stroke on the left side of your baby's stomach.



"Love" Make a backward upside down L-shaped stroke moving from your left to right as if you are reading words on a page.



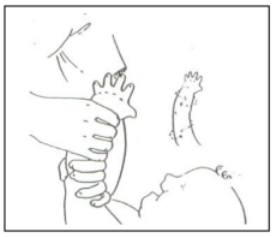
"You" Make an upside down U-shaped stroke from your left to right.

STROKES FOR THE ARMS AND HANDS

These strokes are similar to those for the legs and feet.
Begin Touch Relaxation to let your baby know you would like to massage their arms and hands.
Never forcibly pull your baby's arms away from their body to do a stroke, but gently stroke the arm allowing them to relax and gradually extend their arm to you. Continue to use Touch relaxation throughout the massage to encourage your baby to let go and relax their arm.

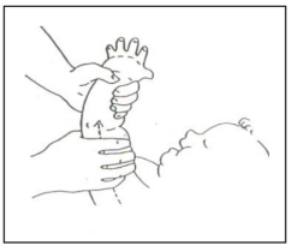
Indian Milking

Hold baby's wrist with in your hand.
With your other hand in the "C" shape, start at the shoulder and stroke smoothly down to the hand.
Alternate hands and repeat the stroke.
Continue this stroke, alternating hands, making contact with the inner and other areas of the arm. Imagine stress and tension leaving baby's body through their fingertips.



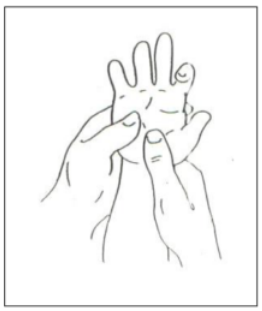
Squeeze and Twist

Begin at your baby's upper arm, use the "C" shape of your hands and slowly squeeze and twist both hands moving down baby's arm to their wrist using gentle pressure.



Open Hand

Stroke baby's palm gently with your thumbs. If your baby is making a tight fist in a reflex response, gently stroke the tops of the fingers to encourage them to relax and open the hand to you.



Stroke Each Finger

Gently squeeze and stroke each tiny finger between your thumb and fingers.

Recite "*This Little Piggy Went to Market....*" or other rhymes.



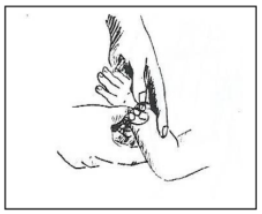
Top of Hand

Gently stroke the top of baby's hand from the wrist to the fingers.



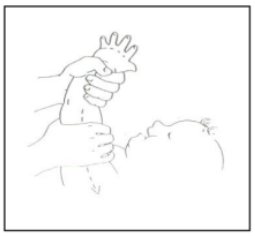
Small Circles around Wrist

Massage your baby's wrist by making small circles all around it, front and back



Swedish Milking

Support baby's wrist, and stroke up from the wrist to the shoulder. Continue this stroke, alternating your hands, using firm, smooth strokes



STROKES FOR THE BACK

The back massage strokes are very relaxing, and are favourites of both babies and toddlers. Lay your baby on their stomach, either on the floor on a soft quilt or on your lap with your legs out-stretched. Talk to your baby while they are on their tummies. Your voice contact is important since they can't see you.

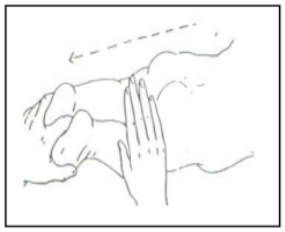
Back and Forth

Begin with both hands together at the top of baby's back. Glide your relaxed hands back and forth, in opposite directions, going down their back to the buttocks, then up to their shoulders, and back down again.



Swooping to Bottom & Ankles

Cup baby's buttocks with one hand. Beginning at baby's neck, your other hand glides down smoothly to the buttocks. Repeat this swooping stroke several times. Repeat cupping your baby's feet with one hand, your other hand glides down smoothly to the feet several times.

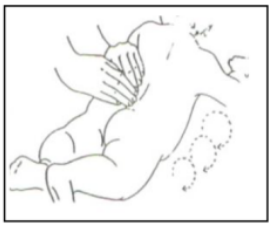


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Small Circles All Over Back

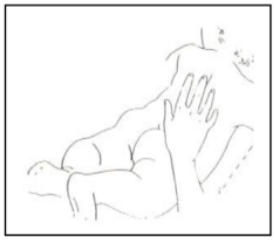
Use your fingertips to massage small circles on both sides of baby's spine moving all over their back.
Be sure to massage small circles on baby's hips and buttocks, too.

Avoid doing these circles directly on the spine.



Combing

With your hand open and fingers spread apart, gently "comb" your baby's back starting at their neck and moving to their buttocks. Your strokes become gradually lighter each time. Repeat several times.
End with a very light "feather" touch. You may also begin with baby's head and stroke down the back.



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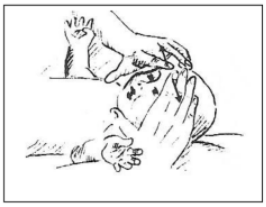
STROKES FOR THE FACE

A baby's face can have a great deal of tension from sucking, teething, and crying. The strokes for the face can help relieve these tensions.

Ask Permission. Begin by making small circles on the sides of baby's head as a cue that you would like to massage the face. Because the face is a sensitive area, for some babies, it takes time to become comfortable receiving a massage on the face. Proceed slowly and observe baby's cues.

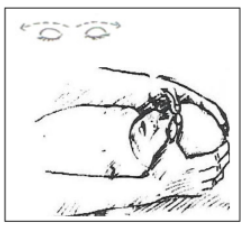
Open Book

Using your fingertips, start at the centre of baby's forehead and gently stroke out to the side, as if smoothing the pages of a book.



Relax your eyes

Place your thumbs lightly on the inner corners of the eyebrows and stroke outwards



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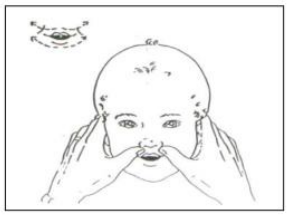
Stroke down Sides of Nose

With your thumbs or forefingers at the centre of the brows, stroke down on either side of the nose and then stop and press gently. This can help clear baby's sinuses.



Smile on Upper and Lower Lips

With your thumbs, stroke across the upper lip moving into the cheek, then stroke across the lower lip and into the cheek. This is like drawing a smile on your baby's face, and helps soothe the muscles used for sucking.



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Small Circles around Jaw

Make small circles around baby's jaw with your fingertips. Use a very gentle touch. These strokes can soothe the muscles used for sucking and may help relieve some of the discomfort of teething.



Over Ears and Under Chin

Finish the facial massage by stroking with your fingertips in front and around the backs of baby's ears, then continue down under the chin. This stroke helps relax the jaw and massages the lymph nodes in this area.



Remember it is not necessary to do the whole massage regime each time, better to pick the areas that you know you baby prefers and do these for a minimum of 15 minutes three times a day, until your 4 month visit.

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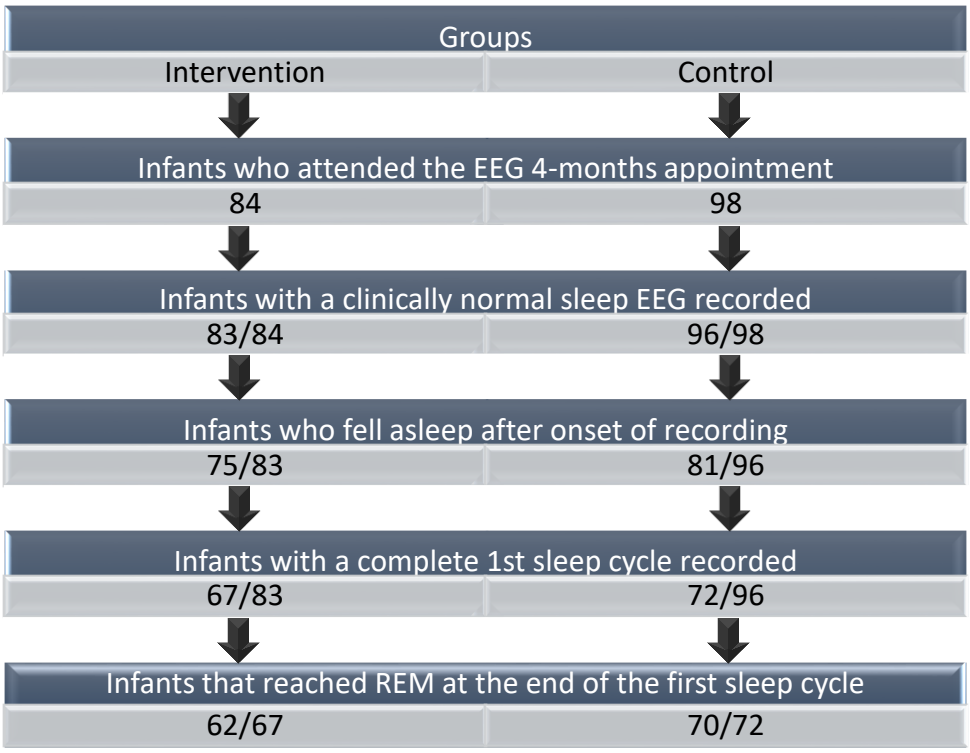
babysmart@ucc.ie

www.infantcentre.ie

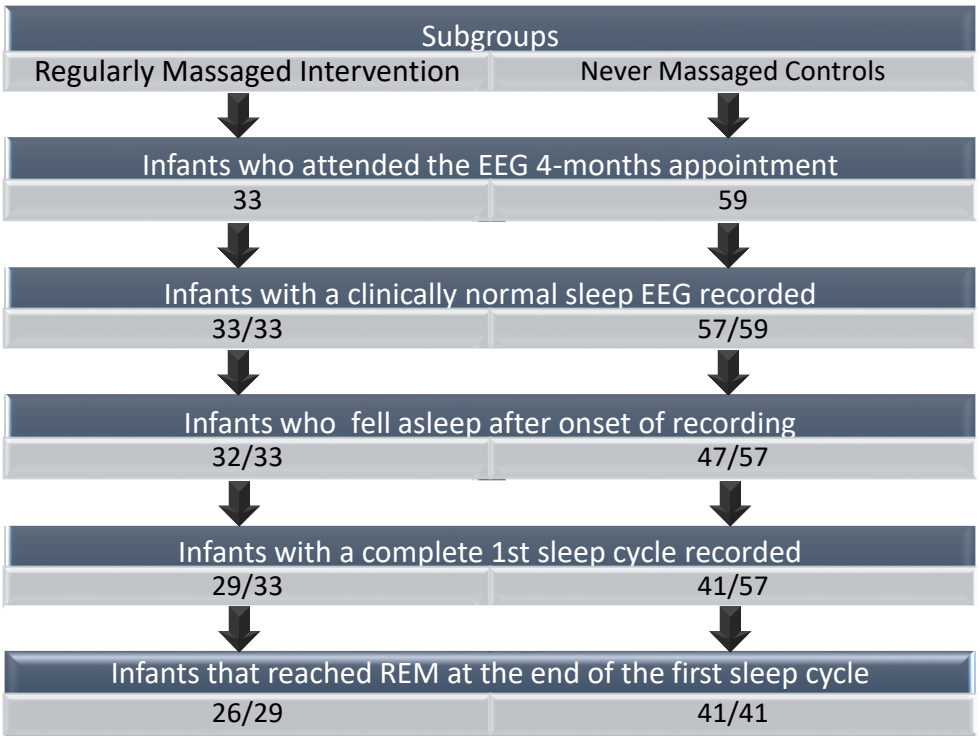
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Appendix 6 – Supplementary figures from chapter 3

Supplementary Figure 1 - Main analysis flow diagram.



Supplementary Figure 2 - Subgroup analysis flow diagram.



Appendix 7 – Supplementary tables from chapter 3

Supplementary Table 1 - Sociodemographic comparison between excluded and included participants according to data provided at registration.

	Excluded (n=229)		Included (n=179)		
	Mean	**(SD)**	**Mean**	**(SD)**	***p*[a]**
Maternal age (years:months)	32:4	(5:3)	34:11	(3:12)	<0.001
Gestational age (weeks)	39.7	(1.2)	39.8	(1.2)	0.31
Birthweight (Kg)	3.5	(0.5)	3.5	(0.5)	0.28
	n	**(%)**	**n**	**(%)**	***p*[b]**
Sex-male	122	(53.3)	101	(56.4)	0.53
Mode of delivery					0.75
Spontaneous Vaginal Delivery	85	(37.1)	67	(37.4)	
Assisted Vaginal Delivery	47	(20.5)	39	(21.8)	
Scheduled Caesarean Section	52	(22.7)	45	(25.1)	
Emergency Caesarean Section	45	(19.7)	28	(15.6)	

[a]From independent samples t-test; [b]From Pearson Chi-Square.

Supplementary Table 2 - Sociodemographic comparison between excluded participants from intervention and control according to data provided at registration.

	Intervention (n=121)		Control (n=108)		
	Mean	**(SD)**	**Mean**	**(SD)**	***p*[a]**
Maternal age (years:months)	32:4	(5:5)	32:5	(5:1)	0.91
Gestational age (weeks)	39.7	(1.1)	39.7	(1.2)	0.94
Birthweight (Kg)	3.5	(0.5)	3.5	(0.6)	0.77
	n	**(%)**	**n**	**(%)**	***p*value[b]**
Sex-male	64	(52.9)	58	(53.7)	0.90
Mode of delivery					0.19
Spontaneous Vaginal Delivery	50	(41.3)	35	(32.4)	
Assisted Vaginal Delivery	27	(22.3)	20	(18.5)	
Scheduled Caesarean Section	26	(21.5)	26	(24.1)	
Emergency Caesarean Section	18	(14.9)	27	(25.0)	

[a]From independent samples t-test; [b]From Pearson Chi-Square.

Supplementary Table 3 - qEEG features during the first sleep cycle.

qEEG type	SS	FB	Median (IQR) Intervention (n=67)	Median (IQR) Controls (n=72)	*p*[a]
Amplitude kurtosis	N1	δ1	3.24 (3.19-3.28)	3.23 (3.15-3.29)	0.45
Amplitude kurtosis	N1	δ2	3.35 (3.28-3.44)	3.35 (3.28-3.42)	0.94
Amplitude kurtosis	N1	θ	3.30 (3.24-3.37)	3.29 (3.23-3.34)	0.44
Amplitude kurtosis	N1	α	3.49 (3.41-3.70)	3.43 (3.36-3.56)	0.04*
Amplitude kurtosis	N1	σ	3.48 (3.35-3.70)	3.41 (3.31-3.59)	0.22
Amplitude kurtosis	N1	β	3.30 (3.22-3.45)	3.27 (3.22-3.41)	0.81
Amplitude kurtosis	N1	γ	3.29 (3.23-3.40)	3.29 (3.24-3.38)	0.92
Amplitude skew	N1	δ1	0.14 (0.14-0.16)	0.15 (0.14-0.16)	0.68
Amplitude skew	N1	δ2	0.020 (0.017-0.022)	0.020 (0.017-0.023)	0.99
Amplitude skew	N1	θ	0.017 (0.015-0.020)	0.017 (0.015-0.019)	0.31
Amplitude skew	N1	α	0.004 (0.004-0.005)	0.004 (0.004-0.005)	0.81
Amplitude skew	N1	σ	0.001 (0.001-0.002)	0.001 (0.001-0.002)	0.62
Amplitude skew	N1	β	0.006 (0.005-0.007)	0.006 (0.005-0.008)	0.67
Amplitude skew	N1	γ	0.026 (0.024-0.031)	0.027 (0.024-0.034)	0.44
Brain Symmetry Index	N1	δ1	0.18 (0.12-0.21)	0.16 (0.12-0.24)	0.89
Brain Symmetry Index	N1	δ2	0.13 (0.10-0.17)	0.12 (0.10-0.17)	0.78
Brain Symmetry Index	N1	θ	0.11 (0.10-0.15)	0.11 (0.10-0.17)	0.74
Brain Symmetry Index	N1	α	0.13 (0.10-0.17)	0.13 (0.09-0.20)	0.95
Brain Symmetry Index	N1	σ	0.14 (0.11-0.20)	0.14 (0.10-0.20)	0.84
Brain Symmetry Index	N1	β	0.15 (0.11-0.20)	0.15 (0.12-0.21)	0.94
Brain Symmetry Index	N1	γ	0.16 (0.11-0.19)	0.16 (0.12-0.23)	0.57
Coherence	N1	δ1	0.20 (0.18-0.26)	0.22 (0.18-0.27)	0.19
Coherence	N1	δ2	0.14 (0.12-0.18)	0.15 (0.12-0.19)	0.40
Coherence	N1	θ	0.06 (0.04-0.08)	0.06 (0.05-0.09)	0.20
Coherence	N1	α	0.05 (0.04-0.07)	0.06 (0.04-0.08)	0.53
Coherence	N1	σ	0.05 (0.04-0.07)	0.05 (0.04-0.08)	0.64
Coherence	N1	β	0.04 (0.03-0.07)	0.05 (0.03-0.07)	0.47
Coherence	N1	γ	0.036 (0.030-0.052)	0.039 (0.028-0.057)	0.59
Fractal dimension	N1		1.25 (1.22-1.29)	1.25 (1.22-1.31)	0.65
rEEG: asymmetry	N1		0.21 (0.18-0.23)	0.21 (0.19-0.23)	0.68
rEEG: lower margin (μV)	N1		63.33 (54.37-73.86)	57.22 (50.72-68.68)	0.02*
rEEG: median margin (μV)	N1		94.28 (81.97-110.07)	85.59 (75.11-100.30)	0.02*
rEEG: upper margin (μV)	N1		138.57 (119.52-164.36)	127.13 (112.28-147.95)	0.04*
rEEG: SD (μV)	N1		23.64 (19.20-26.59)	20.83 (17.48-25.34)	0.05
Relative spectral power (%)	N1	δ1	51.70 (46.73-58.20)	53.00 (48.45-57.37)	0.45
Relative spectral power (%)	N1	δ2	32.06 (28.16-35.19)	31.43 (27.26-34.61)	0.53
Relative spectral power (%)	N1	θ	11.85 (9.36-14.53)	11.60 (9.79-13.37)	0.55
Relative spectral power (%)	N1	α	1.07 (0.92-1.24)	1.07 (0.92-1.16)	0.65
Relative spectral power (%)	N1	σ	0.31 (0.27-0.36)	0.31 (0.26-0.37)	0.99
Relative spectral power (%)	N1	β	0.81 (0.63-0.98)	0.75 (0.59-1.11)	1.00
Relative spectral power (%)	N1	γ	0.39 (0.28-0.50)	0.36 (0.29-0.55)	0.97
Spectral difference	N1	δ1	0.004 (0.002-0.006)	0.004 (0.002-0.006)	0.96

Spectral difference	N1	δ2	0.010	(0.009-0.011)	0.010	(0.009-0.011)	0.58
Spectral difference	N1	θ	0.004	(0.004-0.005)	0.004	(0.004-0.005)	0.46
Spectral difference	N1	α	0.008	(0.008-0.009)	0.009	(0.008-0.010)	0.08
Spectral difference	N1	σ	0.013	(0.012-0.014)	0.014	(0.012-0.015)	0.24
Spectral difference	N1	β	0.010	(0.008-0.011)	0.010	(0.009-0.011)	0.24
Spectral difference	N1	γ	0.010	(0.009-0.011)	0.010	(0.009-0.011)	0.87
Spectral edge frequency	N1		4.75	(4.50-5.00)	4.75	(4.00-5.00)	0.99
Spectral flatness	N1	δ1	0.77	(0.65-0.86)	0.80	(0.55-0.89)	0.77
Spectral flatness	N1	δ2	0.95	(0.93-0.96)	0.95	(0.92-0.96)	0.18
Spectral flatness	N1	θ	0.61	(0.57-0.65)	0.61	(0.59-0.65)	0.83
Spectral flatness	N1	α	0.89	(0.88-0.91)	0.90	(0.89-0.91)	0.30
Spectral flatness	N1	σ	0.97	(0.97-0.97)	0.97	(0.97-0.97)	0.90
Spectral flatness	N1	β	0.95	(0.94-0.96)	0.95	(0.94-0.96)	0.49
Spectral flatness	N1	γ	0.94	(0.93-0.95)	0.94	(0.93-0.95)	0.45
Spectral power (μV^2)	N1	δ1	342.09	(269.90-458.32)	298.50	(215.89-408.80)	0.05
Spectral power (μV^2)	N1	δ2	216.52	(162.77-293.43)	178.46	(132.67-256.30)	0.04*
Spectral power (μV^2)	N1	θ	79.71	(66.27-127.88)	72.36	(49.37-96.39)	0.02*
Spectral power (μV^2)	N1	α	7.40	(5.95-10.67)	6.23	(5.00-8.31)	0.01*
Spectral power (μV^2)	N1	σ	2.30	(1.64-2.97)	1.80	(1.44-2.53)	0.02*
Spectral power (μV^2)	N1	β	6.07	(4.21-7.68)	4.84	(3.78-6.91)	0.06
Spectral power (μV^2)	N1	γ	2.69	(2.09-3.57)	2.42	(1.76-3.16)	0.08
Amplitude kurtosis	N2	δ1	3.25	(3.17-3.33)	3.23	(3.16-3.33)	0.48
Amplitude kurtosis	N2	δ2	3.32	(3.26-3.45)	3.32	(3.23-3.42)	0.38
Amplitude kurtosis	N2	θ	3.40	(3.32-3.52)	3.39	(3.32-3.48)	0.32
Amplitude kurtosis	N2	α	3.68	(3.59-3.84)	3.67	(3.55-3.93)	0.79
Amplitude kurtosis	N2	σ	4.59	(4.31-5.03)	4.61	(4.17-5.02)	0.65
Amplitude kurtosis	N2	β	3.74	(3.47-4.08)	3.76	(3.50-4.18)	0.72
Amplitude kurtosis	N2	γ	3.32	(3.26-3.42)	3.31	(3.24-3.41)	0.36
Amplitude skew	N2	δ1	0.15	(0.14-0.17)	0.14	(0.13-0.15)	0.08
Amplitude skew	N2	δ2	0.021	(0.019-0.022)	0.021	(0.018-0.024)	0.96
Amplitude skew	N2	θ	0.017	(0.015-0.019)	0.017	(0.016-0.021)	0.38
Amplitude skew	N2	α	0.004	(0.004-0.005)	0.004	(0.004-0.006)	0.62
Amplitude skew	N2	σ	0.001	(0.001-0.001)	0.001	(0.001-0.001)	0.71
Amplitude skew	N2	β	0.019	(0.014-0.031)	0.022	(0.014-0.037)	0.43
Amplitude skew	N2	γ	0.028	(0.025-0.035)	0.027	(0.024-0.032)	0.22
Brain Symmetry Index	N2	δ1	0.13	(0.10-0.19)	0.14	(0.10-0.21)	0.33
Brain Symmetry Index	N2	δ2	0.10	(0.09-0.16)	0.10	(0.08-0.16)	0.98
Brain Symmetry Index	N2	θ	0.10	(0.08-0.13)	0.11	(0.08-0.16)	0.67
Brain Symmetry Index	N2	α	0.12	(0.09-0.17)	0.12	(0.08-0.19)	0.43
Brain Symmetry Index	N2	σ	0.16	(0.13-0.20)	0.16	(0.14-0.22)	0.53
Brain Symmetry Index	N2	β	0.12	(0.10-0.19)	0.15	(0.10-0.21)	0.22
Brain Symmetry Index	N2	γ	0.13	(0.10-0.19)	0.16	(0.10-0.21)	0.60
Coherence	N2	δ1	0.26	(0.19-0.29)	0.26	(0.21-0.31)	0.79
Coherence	N2	δ2	0.14	(0.11-0.17)	0.14	(0.12-0.17)	0.84
Coherence	N2	θ	0.06	(0.04-0.07)	0.05	(0.04-0.06)	0.54
Coherence	N2	α	0.06	(0.04-0.09)	0.06	(0.04-0.08)	1.00

Coherence	N2	σ	0.09	(0.07-0.13)	0.09	(0.07-0.12)	0.82
Coherence	N2	β	0.06	(0.04-0.09)	0.06	(0.05-0.09)	0.62
Coherence	N2	γ	0.05	(0.03-0.09)	0.05	(0.04-0.09)	0.55
Fractal dimension	N2		1.28	(1.26-1.32)	1.28	(1.25-1.31)	0.58
rEEG: asymmetry	N2		0.22	(0.20-0.25)	0.22	(0.20-0.24)	0.43
rEEG: lower margin (μV)	N2		67.30	(58.68-77.31)	63.70	(58.07-68.97)	0.09
rEEG: median margin (μV)	N2		99.81	(90.28-113.65)	94.45	(85.03-106.25)	0.09
rEEG: upper margin (μV)	N2		153.06	(136.04-168.66)	144.02	(125.58-166.05)	0.13
rEEG: SD (μV)	N2		24.95	(22.63-27.90)	23.35	(21.09-27.38)	0.17
Relative spectral power (%)	N2	δ1	60.48	(54.59-63.74)	59.03	(54.03-63.47)	0.36
Relative spectral power (%)	N2	δ2	26.70	(24.14-29.9)	27.10	(25.38-30.46)	0.27
Relative spectral power (%)	N2	θ	9.42	(7.71-11.13)	9.25	(7.88-11.18)	0.61
Relative spectral power (%)	N2	α	1.18	(1.01-1.35)	1.17	(0.91-1.40)	0.85
Relative spectral power (%)	N2	σ	0.58	(0.42-0.86)	0.67	(0.46-0.92)	0.33
Relative spectral power (%)	N2	β	0.84	(0.67-1.01)	0.80	(0.65-1.01)	0.69
Relative spectral power (%)	N2	γ	0.31	(0.26-0.39)	0.30	(0.25-0.39)	0.36
Spectral difference	N2	δ1	0.004	(0.002-0.006)	0.004	(0.002-0.007)	0.47
Spectral difference	N2	δ2	0.009	(0.008-0.010)	0.009	(0.008-0.010)	0.89
Spectral difference	N2	θ	0.005	(0.004-0.005)	0.005	(0.004-0.006)	0.11
Spectral difference	N2	α	0.008	(0.007-0.009)	0.008	(0.007-0.009)	0.38
Spectral difference	N2	σ	0.002	(0.001-0.006)	0.002	(0.001-0.007)	0.74
Spectral difference	N2	β	0.004	(0.002-0.007)	0.005	(0.002-0.007)	0.82
Spectral difference	N2	γ	0.01	(0.009-0.011)	0.01	(0.009-0.011)	0.46
Spectral edge frequency	N2		4.50	(4.00-5.38)	4.50	(4.00-5.44)	0.65
Spectral flatness	N2	δ1	0.74	(0.58-0.88)	0.78	(0.64-0.91)	0.31
Spectral flatness	N2	δ2	0.92	(0.90-0.93)	0.91	(0.89-0.93)	0.10
Spectral flatness	N2	θ	0.70	(0.68-0.73)	0.70	(0.68-0.73)	0.70
Spectral flatness	N2	α	0.91	(0.90-0.92)	0.91	(0.90-0.92)	0.83
Spectral flatness	N2	σ	0.93	(0.87-0.95)	0.92	(0.83-0.95)	0.35
Spectral flatness	N2	β	0.92	(0.89-0.94)	0.91	(0.89-0.93)	0.53
Spectral flatness	N2	γ	0.93	(0.92-0.94)	0.93	(0.92-0.94)	0.44
Spectral power (μV^2)	N2	δ1	445.74	(370.56-616.77)	376.30	(286.23-532.56)	0.008*
Spectral power (μV^2)	N2	δ2	200.05	(169.28-279.62)	189.00	(157.70-248.84)	0.10
Spectral power (μV^2)	N2	θ	81.35	(64.63-106.66)	73.53	(55.33-89.08)	0.03*
Spectral power (μV^2)	N2	α	10.57	(8.00-14.28)	9.37	(7.63-11.95)	0.13
Spectral power (μV^2)	N2	σ	5.47	(3.57-7.81)	5.05	(3.38-8.48)	0.86
Spectral power (μV^2)	N2	β	7.60	(5.55-8.91)	6.11	(5.02-7.86)	0.04*
Spectral power (μV^2)	N2	γ	2.66	(2.12-3.41)	2.13	(1.84-2.88)	0.01*
Amplitude kurtosis	N3	δ1	3.16	(3.12-3.22)	3.16	(3.12-3.20)	0.36
Amplitude kurtosis	N3	δ2	3.20	(3.15-3.24)	3.18	(3.14-3.23)	0.28
Amplitude kurtosis	N3	θ	3.31	(3.26-3.41)	3.31	(3.27-3.37)	0.44
Amplitude kurtosis	N3	α	3.58	(3.53-3.70)	3.60	(3.51-3.70)	0.86
Amplitude kurtosis	N3	σ	4.74	(4.38-5.12)	4.71	(4.45-5.18)	0.73
Amplitude kurtosis	N3	β	3.80	(3.60-4.08)	3.88	(3.63-4.15)	0.24
Amplitude kurtosis	N3	γ	3.33	(3.29-3.40)	3.32	(3.26-3.43)	0.58
Amplitude skew	N3	δ1	0.13	(0.13-0.14)	0.13	(0.12-0.14)	0.24

Amplitude skew	N3	δ2	0.021	(0.019-0.022)	0.021	(0.020-0.023)	0.10
Amplitude skew	N3	θ	0.017	(0.016-0.018)	0.017	(0.015-0.018)	0.97
Amplitude skew	N3	α	0.004	(0.003-0.004)	0.004	(0.004-0.004)	0.95
Amplitude skew	N3	σ	0.001	(0.001-0.001)	0.001	(0.001-0.001)	0.82
Amplitude skew	N3	β	0.035	(0.021-0.044)	0.033	(0.024-0.047)	0.81
Amplitude skew	N3	γ	0.027	(0.025-0.030)	0.027	(0.025-0.030)	0.85
Brain Symmetry Index	N3	δ1	0.12	(0.09-0.16)	0.12	(0.10-0.16)	0.38
Brain Symmetry Index	N3	δ2	0.09	(0.08-0.12)	0.09	(0.08-0.12)	0.46
Brain Symmetry Index	N3	θ	0.09	(0.08-0.14)	0.10	(0.08-0.13)	0.40
Brain Symmetry Index	N3	α	0.10	(0.08-0.14)	0.11	(0.09-0.15)	0.15
Brain Symmetry Index	N3	σ	0.15	(0.13-0.18)	0.15	(0.13-0.19)	0.98
Brain Symmetry Index	N3	β	0.12	(0.09-0.16)	0.14	(0.10-0.18)	0.38
Brain Symmetry Index	N3	γ	0.12	(0.09-0.17)	0.14	(0.10-0.21)	0.31
Coherence	N3	δ1	0.23	(0.20-0.29)	0.26	(0.22-0.28)	0.30
Coherence	N3	δ2	0.14	(0.10-0.16)	0.13	(0.12-0.16)	0.78
Coherence	N3	θ	0.05	(0.04-0.07)	0.05	(0.04-0.06)	0.67
Coherence	N3	α	0.06	(0.05-0.09)	0.06	(0.05-0.08)	0.63
Coherence	N3	σ	0.09	(0.08-0.12)	0.10	(0.08-0.12)	0.37
Coherence	N3	β	0.07	(0.05-0.10)	0.07	(0.06-0.10)	0.25
Coherence	N3	γ	0.06	(0.04-0.10)	0.07	(0.05-0.10)	0.26
Fractal dimension	N3		1.22	(1.20-1.24)	1.21	(1.19-1.23)	0.06
rEEG: asymmetry	N3		0.21	(0.20-0.23)	0.20	(0.19-0.22)	0.09
rEEG: lower margin (μV)	N3		82.62	(73.91-90.11)	78.92	(69.29-87.63)	0.21
rEEG: median margin (μV)	N3		122.51	(108.06-131.99)	114.50	(99.35-128.60)	0.12
rEEG: upper margin (μV)	N3		179.12	(158.14-193.59)	166.32	(148.24-188.94)	0.12
rEEG: SD (μV)	N3		28.21	(24.90-30.77)	25.83	(23.39-30.38)	0.13
Relative spectral power (%)	N3	δ1	67.63	(63.95-71.19)	67.59	(64.87-70.04)	0.89
Relative spectral power (%)	N3	δ2	22.98	(20.86-24.85)	23.52	(21.22-24.89)	0.54
Relative spectral power (%)	N3	θ	7.38	(6.11-8.84)	7.25	(5.98-8.55)	0.71
Relative spectral power (%)	N3	α	0.97	(0.81-1.15)	0.94	(0.75-1.15)	0.06
Relative spectral power (%)	N3	σ	0.46	(0.38-0.65)	0.44	(0.38-0.62)	0.63
Relative spectral power (%)	N3	β	0.42	(0.37-0.53)	0.40	(0.32-0.48)	0.12
Relative spectral power (%)	N3	γ	0.16	(0.13-0.19)	0.13	(0.11-0.18)	0.02*
Spectral difference	N3	δ1	0.005	(0.003-0.008)	0.006	(0.004-0.008)	0.18
Spectral difference	N3	δ2	0.009	(0.008-0.009)	0.009	(0.008-0.009)	0.56
Spectral difference	N3	θ	0.005	(0.005-0.006)	0.005	(0.005-0.006)	0.44
Spectral difference	N3	α	0.008	(0.007-0.008)	0.008	(0.007-0.008)	0.76
Spectral difference	N3	σ	0.001	(0.001-0.002)	0.001	(0.001-0.003)	0.92
Spectral difference	N3	β	0.003	(0.002-0.005)	0.003	(0.002-0.005)	0.58
Spectral difference	N3	γ	0.010	(0.009-0.011)	0.010	(0.009-0.011)	0.29
Spectral edge frequency	N3		4.25	(3.50-4.50)	4.00	(3.50-4.50)	0.87
Spectral flatness	N3	δ1	0.81	(0.69-0.87)	0.85	(0.74-0.89)	0.10
Spectral flatness	N3	δ2	0.89	(0.86-0.90)	0.88	(0.86-0.90)	0.04*
Spectral flatness	N3	θ	0.72	(0.71-0.73)	0.72	(0.71-0.73)	0.65
Spectral flatness	N3	α	0.90	(0.89-0.91)	0.89	(0.88-0.90)	0.16
Spectral flatness	N3	σ	0.89	(0.85-0.94)	0.91	(0.83-0.93)	0.70

Spectral flatness	N3	β	0.89	(0.86-0.91)	0.88	(0.86-0.90)	0.21
Spectral flatness	N3	γ	0.93	(0.93-0.95)	0.94	(0.92-0.94)	0.96
Spectral power (μV^2)	N3	δ1	790.94	(659.41-1010.90)	708.47	(529.62-979.90)	0.12
Spectral power (μV^2)	N3	δ2	296.95	(223.94-333.35)	254.89	(190.75-330.95)	0.18
Spectral power (μV^2)	N3	θ	101.31	(78.02-125.91)	89.99	(67.67-118.69)	0.19
Spectral power (μV^2)	N3	α	13.83	(9.93-17.01)	11.85	(9.46-14.13)	0.11
Spectral power (μV^2)	N3	σ	6.45	(5.01-8.41)	5.99	(4.32-8.12)	0.18
Spectral power (μV^2)	N3	β	5.47	(4.67-6.72)	4.70	(3.97-5.94)	0.004*
Spectral power (μV^2)	N3	γ	2.02	(1.70-2.52)	1.61	(1.29-1.98)	<0.001*
Amplitude kurtosis	R	δ1	3.21	(3.18-3.32)	3.20	(3.13-3.30)	0.21
Amplitude kurtosis	R	δ2	3.28	(3.20-3.34)	3.24	(3.18-3.29)	0.06
Amplitude kurtosis	R	θ	3.33	(3.28-3.38)	3.33	(3.29-3.43)	0.38
Amplitude kurtosis	R	α	3.35	(3.30-3.45)	3.37	(3.31-3.46)	0.58
Amplitude kurtosis	R	σ	3.29	(3.23-3.39)	3.30	(3.24-3.44)	0.32
Amplitude kurtosis	R	β	3.29	(3.21-3.38)	3.30	(3.21-3.37)	0.47
Amplitude kurtosis	R	γ	3.30	(3.23-3.38)	3.30	(3.26-3.46)	0.29
Amplitude skew	R	δ1	0.15	(0.14-0.16)	0.15	(0.14-0.17)	0.51
Amplitude skew	R	δ2	0.023	(0.022-0.026)	0.024	(0.022-0.027)	0.29
Amplitude skew	R	θ	0.015	(0.013-0.017)	0.017	(0.014-0.019)	<0.001*
Amplitude skew	R	α	0.004	(0.003-0.005)	0.004	(0.003-0.005)	0.56
Amplitude skew	R	σ	0.001	(0.001-0.002)	0.001	(0.001-0.002)	0.06
Amplitude skew	R	β	0.006	(0.005-0.007)	0.006	(0.005-0.008)	0.51
Amplitude skew	R	γ	0.028	(0.025-0.031)	0.028	(0.025-0.033)	0.45
Brain Symmetry Index	R	δ1	0.14	(0.12-0.19)	0.14	(0.10-0.20)	0.37
Brain Symmetry Index	R	δ2	0.13	(0.10-0.18)	0.11	(0.09-0.18)	0.22
Brain Symmetry Index	R	θ	0.11	(0.09-0.15)	0.12	(0.09-0.17)	0.79
Brain Symmetry Index	R	α	0.11	(0.09-0.17)	0.11	(0.10-0.16)	0.95
Brain Symmetry Index	R	σ	0.12	(0.10-0.17)	0.12	(0.09-0.17)	0.94
Brain Symmetry Index	R	β	0.12	(0.10-0.16)	0.12	(0.09-0.16)	0.62
Brain Symmetry Index	R	γ	0.12	(0.10-0.17)	0.12	(0.10-0.18)	0.78
Coherence	R	δ1	0.27	(0.22-0.34)	0.28	(0.21-0.32)	0.77
Coherence	R	δ2	0.22	(0.16-0.27)	0.22	(0.17-0.27)	0.97
Coherence	R	θ	0.12	(0.09-0.14)	0.12	(0.09-0.15)	0.70
Coherence	R	α	0.09	(0.07-0.12)	0.09	(0.08-0.11)	0.65
Coherence	R	σ	0.07	(0.06-0.11)	0.08	(0.06-0.11)	0.47
Coherence	R	β	0.06	(0.04-0.09)	0.06	(0.05-0.08)	0.62
Coherence	R	γ	0.04	(0.04-0.07)	0.05	(0.04-0.06)	0.26
Fractal dimension	R		1.30	(1.27-1.34)	1.30	(1.27-1.34)	0.84
rEEG: asymmetry	R		0.19	(0.17-0.21)	0.19	(0.16-0.21)	0.39
rEEG: lower margin (μV)	R		51.09	(45.86-58.42)	46.67	(42.34-54.56)	0.006*
rEEG: median margin (μV)	R		76.09	(66.54-84.93)	70.44	(62.02-80.59)	0.03*
rEEG: upper margin (μV)	R		109.69	(94.74-123.51)	101.63	(91.45-117.31)	0.06
rEEG: SD (μV)	R		17.24	(14.70-19.60)	16.24	(14.16-18.64)	0.12
Relative spectral power (%)	R	δ1	61.75	(56.65-65.88)	61.56	(58.40-64.72)	0.93
Relative spectral power (%)	R	δ2	25.43	(22.04-29.05)	25.33	(22.88-28.62)	0.66
Relative spectral power (%)	R	θ	9.87	(8.57-11.58)	9.04	(7.86-10.77)	0.03*

Relative spectral power (%)	R	α	1.15	(1.01-1.33)	1.12	(1.00-1.34)	0.82
Relative spectral power (%)	R	σ	0.32	(0.28-0.38)	0.33	(0.29-0.40)	0.97
Relative spectral power (%)	R	β	0.85	(0.72-1.08)	0.86	(0.70-1.14)	0.99
Relative spectral power (%)	R	γ	0.44	(0.36-0.56)	0.45	(0.35-0.57)	0.80
Spectral difference	R	δ1	0.003	(0.001-0.005)	0.003	(0.002-0.005)	0.98
Spectral difference	R	δ2	0.009	(0.008-0.01)	0.009	(0.008-0.01)	0.95
Spectral difference	R	θ	0.005	(0.005-0.006)	0.006	(0.005-0.006)	0.86
Spectral difference	R	α	0.009	(0.008-0.009)	0.009	(0.008-0.01)	0.75
Spectral difference	R	σ	0.014	(0.013-0.015)	0.014	(0.012-0.014)	0.94
Spectral difference	R	β	0.01	(0.01-0.011)	0.01	(0.01-0.011)	0.82
Spectral difference	R	γ	0.009	(0.008-0.01)	0.01	(0.008-0.011)	0.37
Spectral edge frequency	R		4.50	(4.00-5.00)	4.50	(4.00-5.00)	0.75
Spectral flatness	R	δ1	0.76	(0.55-0.87)	0.73	(0.61-0.88)	0.83
Spectral flatness	R	δ2	0.90	(0.88-0.92)	0.89	(0.87-0.91)	0.004*
Spectral flatness	R	θ	0.70	(0.68-0.74)	0.72	(0.68-0.75)	0.31
Spectral flatness	R	α	0.89	(0.88-0.91)	0.89	(0.88-0.90)	0.42
Spectral flatness	R	σ	0.97	(0.97-0.97)	0.97	(0.96-0.97)	0.59
Spectral flatness	R	β	0.96	(0.96-0.97)	0.96	(0.95-0.97)	0.42
Spectral flatness	R	γ	0.93	(0.92-0.94)	0.93	(0.92-0.94)	0.17
Spectral power (μV^2)	R	δ1	275.09	(230.66-358.18)	252.67	(194.83-330.12)	0.05
Spectral power (μV^2)	R	δ2	128.81	(94.43-164.94)	106.94	(82.57-151.24)	0.08
Spectral power (μV^2)	R	θ	48.19	(39.05-69.23)	39.89	(32.09-51.98)	0.003*
Spectral power (μV^2)	R	α	5.22	(4.39-6.49)	4.78	(3.69-5.88)	0.04*
Spectral power (μV^2)	R	σ	1.51	(1.27-1.80)	1.25	(1.02-1.70)	0.02*
Spectral power (μV^2)	R	β	4.11	(3.30-5.07)	3.64	(3.06-4.52)	0.02*
Spectral power (μV^2)	R	γ	2.28	(1.68-2.74)	1.89	(1.55-2.49)	0.02*

qEEG = Quantitative EEG; Sp. power = Spectral power; Sp. rel. power = Spectral relative power. FB = Frequency bands: 0.5-2Hz (δ1). 2-4Hz (δ2). 4-8Hz (θ). 8-12Hz (α). 12-15Hz (σ). 15-30 (β). 30-45Hz (γ); SS: sleep stage; rEEG = range-EEG. rEEG frequency band: 1-20Hz. [a]from Mann-Whitney U test, statistically significant differences (p<0.05) marked with an asterisk (*).

Supplementary Table 4 - qEEG features during the first sleep cycle – subgroup analysis.

qEEG type	SS	FB	Median (IQR) Intervention (n=29)		Median (IQR) Controls (n=41)		*p*[a]
Amplitude kurtosis	N1	δ1	3.22	(3.19-3.29)	3.24	(3.15-3.28)	0.82
Amplitude kurtosis	N1	δ2	3.35	(3.28-3.40)	3.35	(3.28-3.43)	0.68
Amplitude kurtosis	N1	θ	3.31	(3.24-3.37)	3.29	(3.23-3.35)	0.43
Amplitude kurtosis	N1	α	3.47	(3.37-3.57)	3.43	(3.34-3.55)	0.20
Amplitude kurtosis	N1	σ	3.48	(3.33-3.56)	3.38	(3.30-3.58)	0.32
Amplitude kurtosis	N1	β	3.29	(3.22-3.40)	3.26	(3.21-3.41)	0.95
Amplitude kurtosis	N1	γ	3.29	(3.23-3.43)	3.30	(3.22-3.38)	0.97
Amplitude skew	N1	δ1	0.15	(0.14-0.16)	0.15	(0.14-0.16)	0.83
Amplitude skew	N1	δ2	0.021	(0.019-0.023)	0.020	(0.018-0.022)	0.34
Amplitude skew	N1	θ	0.017	(0.015-0.019)	0.016	(0.014-0.018)	0.29
Amplitude skew	N1	α	0.004	(0.004-0.005)	0.004	(0.004-0.005)	0.30

Amplitude skew	N1	σ	0.001	(0.001-0.002)	0.001	(0.001-0.002)	0.74
Amplitude skew	N1	β	0.006	(0.005-0.007)	0.006	(0.005-0.007)	0.58
Amplitude skew	N1	γ	0.026	(0.023-0.031)	0.026	(0.023-0.033)	0.75
Brain Symmetry Index	N1	δ1	0.18	(0.14-0.21)	0.16	(0.11-0.26)	0.60
Brain Symmetry Index	N1	δ2	0.14	(0.10-0.17)	0.11	(0.09-0.14)	0.14
Brain Symmetry Index	N1	θ	0.12	(0.10-0.14)	0.11	(0.09-0.15)	0.48
Brain Symmetry Index	N1	α	0.14	(0.09-0.18)	0.11	(0.09-0.21)	0.55
Brain Symmetry Index	N1	σ	0.16	(0.12-0.21)	0.13	(0.10-0.23)	0.52
Brain Symmetry Index	N1	β	0.15	(0.12-0.19)	0.14	(0.12-0.22)	0.76
Brain Symmetry Index	N1	γ	0.16	(0.11-0.18)	0.16	(0.12-0.25)	0.41
Coherence	N1	δ1	0.20	(0.17-0.24)	0.22	(0.20-0.27)	0.09
Coherence	N1	δ2	0.14	(0.12-0.18)	0.15	(0.13-0.20)	0.26
Coherence	N1	θ	0.06	(0.04-0.07)	0.05	(0.04-0.08)	0.58
Coherence	N1	α	0.05	(0.04-0.07)	0.06	(0.04-0.08)	0.57
Coherence	N1	σ	0.04	(0.04-0.07)	0.05	(0.03-0.07)	0.94
Coherence	N1	β	0.04	(0.03-0.06)	0.04	(0.03-0.06)	0.56
Coherence	N1	γ	0.04	(0.02-0.06)	0.04	(0.03-0.05)	0.48
Fractal dimension	N1		1.28	(1.22-1.30)	1.25	(1.22-1.32)	0.83
rEEG: asymmetry	N1		0.20	(0.18-0.21)	0.20	(0.19-0.23)	0.49
rEEG: lower margin (μV)	N1		63.60	(54.27-71.92)	56.22	(49.29-68.51)	0.07
rEEG: median margin (μV)	N1		90.71	(83.17-106.61)	81.96	(71.57-104.79)	0.06
rEEG: upper margin (μV)	N1		134.26	(123.25-158.84)	124.33	(105.98-149.89)	0.09
rEEG: SD (μV)	N1		23.64	(19.07-25.97)	20.17	(16.82-25.37)	0.07
Relative spectral power (%)	N1	δ1	50.77	(46.69-59.48)	52.34	(48.25-56.48)	0.73
Relative spectral power (%)	N1	δ2	32.09	(28.26-35.63)	30.45	(28.59-34.12)	0.58
Relative spectral power (%)	N1	θ	12.09	(9.76-15.07)	11.93	(10.05-14.05)	0.59
Relative spectral power (%)	N1	α	1.12	(1.01-1.30)	1.10	(0.99-1.27)	0.70
Relative spectral power (%)	N1	σ	0.34	(0.29-0.36)	0.33	(0.27-0.38)	0.85
Relative spectral power (%)	N1	β	0.83	(0.65-1.07)	0.78	(0.64-1.15)	0.99
Relative spectral power (%)	N1	γ	0.39	(0.32-0.55)	0.37	(0.32-0.55)	0.90
Spectral difference	N1	δ1	0.004	(0.003-0.005)	0.004	(0.002-0.006)	0.99
Spectral difference	N1	δ2	0.01	(0.009-0.011)	0.01	(0.009-0.010)	0.39
Spectral difference	N1	θ	0.004	(0.004-0.005)	0.005	(0.004-0.005)	0.60
Spectral difference	N1	α	0.008	(0.008-0.009)	0.008	(0.008-0.009)	1.00
Spectral difference	N1	σ	0.013	(0.012-0.014)	0.013	(0.012-0.014)	0.70
Spectral difference	N1	β	0.01	(0.009-0.011)	0.01	(0.009-0.011)	0.99
Spectral difference	N1	γ	0.01	(0.009-0.011)	0.01	(0.009-0.011)	0.70
Spectral edge frequency	N1		5.00	(4.50-5.25)	5.00	(4.50-5.50)	0.70
Spectral flatness	N1	δ1	0.78	(0.66-0.84)	0.83	(0.57-0.88)	0.49
Spectral flatness	N1	δ2	0.95	(0.93-0.97)	0.94	(0.92-0.96)	0.24
Spectral flatness	N1	θ	0.64	(0.58-0.66)	0.63	(0.60-0.66)	0.76
Spectral flatness	N1	α	0.90	(0.88-0.91)	0.90	(0.89-0.91)	0.82
Spectral flatness	N1	σ	0.97	(0.97-0.97)	0.97	(0.97-0.97)	0.58
Spectral flatness	N1	β	0.95	(0.94-0.96)	0.95	(0.94-0.96)	0.94
Spectral flatness	N1	γ	0.93	(0.93-0.95)	0.94	(0.93-0.95)	0.52
Spectral power (μV2)	N1	δ1	336.24	(262.03-441.76)	274.61	(195.78-341.32)	0.04*

Spectral power (μV2)	N1	δ2	200.34	(159.43-270.58)	169.37	(123.71-277.69)	0.10
Spectral power (μV2)	N1	θ	87.73	(69.79-120.77)	71.11	(44.99-99.15)	0.02*
Spectral power (μV2)	N1	α	7.94	(6.13-10.36)	5.88	(4.81-8.68)	0.05
Spectral power (μV2)	N1	σ	2.53	(1.68-3.01)	1.70	(1.42-2.58)	0.03*
Spectral power (μV2)	N1	β	6.21	(4.35-7.98)	4.78	(3.81-7.26)	0.11
Spectral power (μV2)	N1	γ	2.78	(2.13-4.09)	2.44	(1.72-3.27)	0.08
Amplitude kurtosis	N2	δ1	3.25	(3.18-3.30)	3.23	(3.16-3.29)	0.49
Amplitude kurtosis	N2	δ2	3.31	(3.30-3.45)	3.31	(3.23-3.39)	0.16
Amplitude kurtosis	N2	θ	3.41	(3.31-3.52)	3.37	(3.32-3.47)	0.34
Amplitude kurtosis	N2	α	3.65	(3.51-3.84)	3.66	(3.54-3.85)	0.93
Amplitude kurtosis	N2	σ	4.50	(4.27-4.97)	4.66	(4.18-5.01)	0.49
Amplitude kurtosis	N2	β	3.63	(3.44-3.89)	3.79	(3.49-4.17)	0.27
Amplitude kurtosis	N2	γ	3.32	(3.25-3.41)	3.31	(3.24-3.42)	0.92
Amplitude skew	N2	δ1	0.15	(0.13-0.16)	0.14	(0.13-0.15)	0.18
Amplitude skew	N2	δ2	0.020	(0.018-0.023)	0.021	(0.018-0.023)	0.52
Amplitude skew	N2	θ	0.017	(0.014-0.019)	0.017	(0.016-0.020)	0.36
Amplitude skew	N2	α	0.004	(0.004-0.005)	0.004	(0.003-0.005)	0.43
Amplitude skew	N2	σ	0.001	(0.001-0.001)	0.001	(0.001-0.001)	0.95
Amplitude skew	N2	β	0.019	(0.012-0.027)	0.021	(0.012-0.029)	0.60
Amplitude skew	N2	γ	0.027	(0.024-0.034)	0.027	(0.024-0.032)	0.67
Brain Symmetry Index	N2	δ1	0.13	(0.10-0.17)	0.14	(0.11-0.21)	0.59
Brain Symmetry Index	N2	δ2	0.10	(0.09-0.15)	0.10	(0.08-0.14)	0.43
Brain Symmetry Index	N2	θ	0.10	(0.08-0.13)	0.11	(0.08-0.14)	0.92
Brain Symmetry Index	N2	α	0.12	(0.09-0.16)	0.12	(0.08-0.17)	0.98
Brain Symmetry Index	N2	σ	0.16	(0.13-0.20)	0.16	(0.13-0.23)	0.98
Brain Symmetry Index	N2	β	0.13	(0.10-0.18)	0.15	(0.11-0.22)	0.52
Brain Symmetry Index	N2	γ	0.14	(0.10-0.19)	0.15	(0.10-0.19)	0.83
Coherence	N2	δ1	0.22	(0.16-0.29)	0.27	(0.21-0.30)	0.03*
Coherence	N2	δ2	0.13	(0.11-0.16)	0.15	(0.13-0.19)	0.03*
Coherence	N2	θ	0.04	(0.03-0.06)	0.05	(0.03-0.07)	0.47
Coherence	N2	α	0.05	(0.03-0.06)	0.06	(0.04-0.08)	0.03*
Coherence	N2	σ	0.08	(0.06-0.10)	0.10	(0.07-0.13)	0.02*
Coherence	N2	β	0.04	(0.03-0.08)	0.06	(0.04-0.08)	0.13
Coherence	N2	γ	0.04	(0.03-0.08)	0.05	(0.04-0.08)	0.08
Fractal dimension	N2		1.28	(1.26-1.32)	1.28	(1.25-1.32)	0.86
rEEG: asymmetry	N2		0.23	(0.20-0.25)	0.22	(0.20-0.25)	0.63
rEEG: lower margin (μV)	N2		67.17	(61.26-74.00)	63.76	(58.43-68.91)	0.11
rEEG: median margin (μV)	N2		100.22	(90.87-108.56)	93.41	(85.34-107.39)	0.13
rEEG: upper margin (μV)	N2		156.89	(137.28-166.47)	142.90	(126.22-163.07)	0.11
rEEG: SD (μV)	N2		25.86	(22.78-27.82)	23.14	(20.84-27.00)	0.09
Relative spectral power (%)	N2	δ1	58.88	(53.29-63.74)	57.78	(53.79-62.29)	0.71
Relative spectral power (%)	N2	δ2	27.18	(23.90-30.38)	27.54	(25.29-30.60)	0.60
Relative spectral power (%)	N2	θ	10.20	(8.21-11.93)	9.56	(8.05-11.21)	0.40
Relative spectral power (%)	N2	α	1.28	(1.02-1.55)	1.22	(1.04-1.42)	0.68
Relative spectral power (%)	N2	σ	0.67	(0.41-0.88)	0.67	(0.46-0.90)	0.69
Relative spectral power (%)	N2	β	0.88	(0.68-1.13)	0.83	(0.67-1.05)	0.69

Relative spectral power (%)	N2	γ	0.36	(0.26-0.41)	0.31	(0.25-0.40)	0.55
Spectral difference	N2	δ1	0.004	(0.003-0.007)	0.004	(0.002-0.007)	0.73
Spectral difference	N2	δ2	0.009	(0.008-0.010)	0.009	(0.008-0.010)	0.87
Spectral difference	N2	θ	0.005	(0.004-0.005)	0.005	(0.005-0.006)	0.08
Spectral difference	N2	α	0.008	(0.007-0.008)	0.008	(0.007-0.009)	0.79
Spectral difference	N2	σ	0.002	(0.001-0.005)	0.002	(0.001-0.005)	0.99
Spectral difference	N2	β	0.005	(0.002-0.007)	0.005	(0.002-0.006)	0.46
Spectral difference	N2	γ	0.009	(0.008-0.01)	0.009	(0.009-0.011)	0.86
Spectral edge frequency	N2		4.75	(4.38-5.50)	4.75	(4.19-5.50)	0.84
Spectral flatness	N2	δ1	0.78	(0.61-0.90)	0.79	(0.67-0.91)	0.52
Spectral flatness	N2	δ2	0.92	(0.90-0.94)	0.91	(0.89-0.93)	0.09
Spectral flatness	N2	θ	0.70	(0.68-0.73)	0.71	(0.69-0.73)	0.30
Spectral flatness	N2	α	0.92	(0.90-0.93)	0.91	(0.90-0.92)	0.83
Spectral flatness	N2	σ	0.93	(0.88-0.94)	0.93	(0.86-0.95)	0.77
Spectral flatness	N2	β	0.92	(0.89-0.94)	0.91	(0.89-0.92)	0.22
Spectral flatness	N2	γ	0.92	(0.91-0.93)	0.93	(0.92-0.94)	0.21
Spectral power (μV2)	N2	δ1	439.75	(368.76-596.72)	363.88	(275.66-502.29)	0.04*
Spectral power (μV2)	N2	δ2	200.05	(179.98-256.07)	188.03	(154.44-236.65)	0.10
Spectral power (μV2)	N2	θ	86.18	(71.09-105.18)	71.48	(53.23-95.94)	0.04*
Spectral power (μV2)	N2	α	10.95	(8.55-12.65)	10.05	(7.65-12.85)	0.25
Spectral power (μV2)	N2	σ	5.25	(3.65-7.72)	5.17	(3.69-7.33)	0.77
Spectral power (μV2)	N2	β	7.33	(5.58-8.62)	6.81	(5.37-8.27)	0.48
Spectral power (μV2)	N2	γ	2.78	(2.14-3.54)	2.30	(1.76-3.17)	0.07
Amplitude kurtosis	N3	δ1	3.16	(3.12-3.21)	3.16	(3.13-3.20)	0.73
Amplitude kurtosis	N3	δ2	3.20	(3.14-3.24)	3.20	(3.14-3.23)	0.80
Amplitude kurtosis	N3	θ	3.34	(3.26-3.44)	3.31	(3.26-3.38)	0.29
Amplitude kurtosis	N3	α	3.64	(3.51-3.75)	3.59	(3.52-3.69)	0.43
Amplitude kurtosis	N3	σ	4.62	(4.36-5.28)	4.80	(4.49-5.16)	0.64
Amplitude kurtosis	N3	β	3.71	(3.50-4.04)	3.90	(3.6-4.12)	0.13
Amplitude kurtosis	N3	γ	3.33	(3.28-3.40)	3.32	(3.26-3.41)	1.00
Amplitude skew	N3	δ1	0.13	(0.13-0.14)	0.13	(0.12-0.14)	0.18
Amplitude skew	N3	δ2	0.021	(0.020-0.022)	0.021	(0.020-0.023)	0.43
Amplitude skew	N3	θ	0.016	(0.016-0.019)	0.016	(0.015-0.018)	0.52
Amplitude skew	N3	α	0.004	(0.003-0.004)	0.004	(0.004-0.004)	0.92
Amplitude skew	N3	σ	0.001	(0.001-0.001)	0.001	(0.001-0.001)	0.50
Amplitude skew	N3	β	0.03	(0.02-0.04)	0.04	(0.02-0.05)	0.49
Amplitude skew	N3	γ	0.027	(0.025-0.031)	0.027	(0.025-0.030)	0.73
Brain Symmetry Index	N3	δ1	0.12	(0.08-0.16)	0.12	(0.10-0.15)	0.71
Brain Symmetry Index	N3	δ2	0.09	(0.07-0.13)	0.09	(0.08-0.12)	0.93
Brain Symmetry Index	N3	θ	0.09	(0.08-0.14)	0.10	(0.08-0.13)	0.77
Brain Symmetry Index	N3	α	0.10	(0.08-0.15)	0.11	(0.09-0.14)	0.53
Brain Symmetry Index	N3	σ	0.15	(0.13-0.19)	0.15	(0.13-0.18)	0.24
Brain Symmetry Index	N3	β	0.12	(0.09-0.17)	0.14	(0.11-0.18)	0.49
Brain Symmetry Index	N3	γ	0.14	(0.09-0.18)	0.15	(0.11-0.20)	0.46
Coherence	N3	δ1	0.20	(0.16-0.27)	0.26	(0.23-0.28)	0.003*
Coherence	N3	δ2	0.12	(0.10-0.15)	0.14	(0.12-0.16)	0.01*

Coherence	N3	θ	0.04	(0.03-0.06)	0.05	(0.04-0.06)	0.41
Coherence	N3	α	0.05	(0.04-0.08)	0.06	(0.05-0.07)	0.14
Coherence	N3	σ	0.08	(0.06-0.11)	0.10	(0.08-0.12)	0.006*
Coherence	N3	β	0.06	(0.04-0.08)	0.07	(0.06-0.10)	0.04*
Coherence	N3	γ	0.05	(0.03-0.08)	0.06	(0.05-0.09)	0.03*
Fractal dimension	N3		1.22	(1.19-1.24)	1.21	(1.19-1.23)	0.48
rEEG: asymmetry	N3		0.21	(0.20-0.22)	0.20	(0.19-0.22)	0.33
rEEG: lower margin (μV)	N3		85.31	(77.03-93.32)	77.30	(68.26-87.70)	0.07
rEEG: median margin (μV)	N3		125.36	(111.91-135.56)	112.31	(98.44-128.53)	0.05*
rEEG: upper margin (μV)	N3		183.56	(163.67-198.13)	165.81	(145.03-192.53)	0.07
rEEG: SD (μV)	N3		28.88	(25.98-31.33)	26.68	(23.03-30.61)	0.07
Relative spectral power (%)	N3	δ1	66.17	(62.97-72.31)	66.20	(64.18-69.68)	1.00
Relative spectral power (%)	N3	δ2	24.10	(20.65-25.68)	23.52	(21.16-25.29)	0.91
Relative spectral power (%)	N3	θ	8.19	(6.01-9.23)	7.77	(6.44-8.99)	0.56
Relative spectral power (%)	N3	α	1.10	(0.81-1.26)	1.01	(0.82-1.22)	0.64
Relative spectral power (%)	N3	σ	0.50	(0.36-0.67)	0.53	(0.39-0.72)	0.65
Relative spectral power (%)	N3	β	0.43	(0.36-0.56)	0.43	(0.32-0.52)	0.55
Relative spectral power (%)	N3	γ	0.16	(0.13-0.18)	0.14	(0.11-0.19)	0.47
Spectral difference	N3	δ1	0.007	(0.003-0.008)	0.006	(0.004-0.007)	0.49
Spectral difference	N3	δ2	0.009	(0.008-0.010)	0.008	(0.008-0.009)	0.30
Spectral difference	N3	θ	0.005	(0.005-0.006)	0.005	(0.005-0.006)	0.87
Spectral difference	N3	α	0.008	(0.007-0.009)	0.008	(0.007-0.008)	0.94
Spectral difference	N3	σ	0.001	(0.001-0.003)	0.001	(0.001-0.003)	0.61
Spectral difference	N3	β	0.004	(0.003-0.006)	0.003	(0.002-0.005)	0.04*
Spectral difference	N3	γ	0.010	(0.009-0.011)	0.010	(0.009-0.011)	0.31
Spectral edge frequency	N3		4.50	(4.00-5.00)	4.25	(4.00-4.75)	0.56
Spectral flatness	N3	δ1	0.86	(0.68-0.89)	0.84	(0.74-0.90)	0.68
Spectral flatness	N3	δ2	0.90	(0.87-0.91)	0.88	(0.86-0.90)	0.10
Spectral flatness	N3	θ	0.72	(0.71-0.74)	0.73	(0.71-0.73)	0.90
Spectral flatness	N3	α	0.90	(0.89-0.91)	0.90	(0.88-0.92)	0.95
Spectral flatness	N3	σ	0.89	(0.86-0.93)	0.90	(0.83-0.93)	0.96
Spectral flatness	N3	β	0.90	(0.87-0.91)	0.88	(0.85-0.90)	0.22
Spectral flatness	N3	γ	0.93	(0.92-0.94)	0.93	(0.92-0.94)	0.76
Spectral power (μV2)	N3	δ1	845.28	(684.52-1023.81)	672.84	(521.95-943.03)	0.07
Spectral power (μV2)	N3	δ2	305.59	(266.26-357.43)	245.35	(180.44-330.48)	0.07
Spectral power (μV2)	N3	θ	104.40	(83.89-130.01)	93.33	(62.12-119.95)	0.11
Spectral power (μV2)	N3	α	13.87	(10.63-17.83)	12.56	(10.06-14.36)	0.10
Spectral power (μV2)	N3	σ	7.22	(4.79-8.54)	6.68	(4.95-8.53)	0.75
Spectral power (μV2)	N3	β	5.63	(4.96-6.65)	4.90	(4.04-6.25)	0.05
Spectral power (μV2)	N3	γ	2.02	(1.78-2.62)	1.66	(1.30-2.11)	0.006*
Amplitude kurtosis	R	δ1	3.19	(3.18-3.29)	3.20	(3.13-3.31)	0.73
Amplitude kurtosis	R	δ2	3.29	(3.20-3.37)	3.24	(3.17-3.29)	0.11
Amplitude kurtosis	R	θ	3.33	(3.29-3.39)	3.33	(3.28-3.43)	0.74
Amplitude kurtosis	R	α	3.38	(3.30-3.44)	3.37	(3.31-3.47)	0.71
Amplitude kurtosis	R	σ	3.33	(3.24-3.40)	3.33	(3.24-3.46)	0.49
Amplitude kurtosis	R	β	3.30	(3.22-3.37)	3.28	(3.20-3.37)	0.85

Amplitude kurtosis	R	γ	3.31	(3.26-3.37)	3.32	(3.26-3.46)	0.63
Amplitude skew	R	δ1	0.15	(0.14-0.16)	0.15	(0.14-0.16)	0.31
Amplitude skew	R	δ2	0.023	(0.021-0.027)	0.024	(0.022-0.027)	0.36
Amplitude skew	R	θ	0.015	(0.013-0.017)	0.017	(0.015-0.019)	0.02*
Amplitude skew	R	α	0.004	(0.003-0.005)	0.004	(0.003-0.005)	0.72
Amplitude skew	R	σ	0.001	(0.001-0.002)	0.001	(0.001-0.002)	0.20
Amplitude skew	R	β	0.006	(0.005-0.007)	0.006	(0.005-0.008)	0.89
Amplitude skew	R	γ	0.028	(0.025-0.032)	0.028	(0.025-0.032)	0.99
Brain Symmetry Index	R	δ1	0.14	(0.12-0.19)	0.15	(0.10-0.20)	0.41
Brain Symmetry Index	R	δ2	0.13	(0.10-0.16)	0.11	(0.08-0.16)	0.10
Brain Symmetry Index	R	θ	0.10	(0.09-0.14)	0.12	(0.09-0.13)	0.73
Brain Symmetry Index	R	α	0.11	(0.08-0.17)	0.12	(0.09-0.15)	0.63
Brain Symmetry Index	R	σ	0.12	(0.09-0.16)	0.12	(0.09-0.17)	0.91
Brain Symmetry Index	R	β	0.12	(0.09-0.14)	0.12	(0.09-0.15)	0.69
Brain Symmetry Index	R	γ	0.12	(0.10-0.17)	0.12	(0.10-0.16)	0.92
Coherence	R	δ1	0.25	(0.21-0.33)	0.29	(0.24-0.32)	0.18
Coherence	R	δ2	0.22	(0.17-0.25)	0.24	(0.20-0.27)	0.21
Coherence	R	θ	0.11	(0.08-0.14)	0.12	(0.10-0.15)	0.30
Coherence	R	α	0.08	(0.06-0.10)	0.09	(0.08-0.12)	0.10
Coherence	R	σ	0.06	(0.05-0.09)	0.08	(0.07-0.11)	0.07
Coherence	R	β	0.05	(0.04-0.07)	0.06	(0.05-0.08)	0.21
Coherence	R	γ	0.04	(0.03-0.05)	0.05	(0.04-0.06)	0.03*
Fractal dimension	R		1.30	(1.28-1.33)	1.31	(1.27-1.36)	0.58
rEEG: asymmetry	R		0.20	(0.17-0.22)	0.19	(0.16-0.21)	0.61
rEEG: lower margin (μV)	R		52.97	(46.75-58.67)	45.72	(41.53-52.97)	0.007*
rEEG: median margin (μV)	R		76.09	(67.05-87.24)	67.85	(61.64-78.15)	0.02*
rEEG: upper margin (μV)	R		109.69	(97.32-125.32)	99.55	(88.16-118.56)	0.04*
rEEG: SD (μV)	R		17.84	(14.84-19.71)	15.48	(13.62-19.29)	0.04*
Relative spectral power (%)	R	δ1	60.47	(56.4-65.83)	61.48	(58.09-63.88)	0.94
Relative spectral power (%)	R	δ2	25.73	(22.24-29.06)	26.01	(23.00-28.26)	0.82
Relative spectral power (%)	R	θ	10.29	(9.13-11.79)	9.38	(7.88-11.31)	0.17
Relative spectral power (%)	R	α	1.19	(1.03-1.39)	1.23	(1.10-1.47)	0.52
Relative spectral power (%)	R	σ	0.34	(0.29-0.39)	0.34	(0.30-0.42)	0.68
Relative spectral power (%)	R	β	0.89	(0.76-1.07)	0.94	(0.70-1.29)	0.76
Relative spectral power (%)	R	γ	0.44	(0.37-0.55)	0.48	(0.37-0.61)	0.64
Spectral difference	R	δ1	0.003	(0.002-0.005)	0.003	(0.002-0.005)	0.80
Spectral difference	R	δ2	0.009	(0.008-0.010)	0.009	(0.008-0.010)	0.32
Spectral difference	R	θ	0.005	(0.005-0.006)	0.006	(0.005-0.006)	0.22
Spectral difference	R	α	0.008	(0.008-0.009)	0.009	(0.008-0.010)	0.8
Spectral difference	R	σ	0.014	(0.013-0.014)	0.013	(0.012-0.014)	0.57
Spectral difference	R	β	0.010	(0.009-0.011)	0.010	(0.009-0.011)	0.83
Spectral difference	R	γ	0.009	(0.008-0.010)	0.009	(0.008-0.010)	0.99
Spectral edge frequency	R		4.50	(4.00-5.50)	4.75	(4.00-5.38)	0.83
Spectral flatness	R	δ1	0.77	(0.55-0.85)	0.74	(0.64-0.86)	0.90
Spectral flatness	R	δ2	0.91	(0.88-0.92)	0.88	(0.86-0.90)	0.01*
Spectral flatness	R	θ	0.71	(0.68-0.74)	0.73	(0.69-0.76)	0.13

Spectral flatness	R	α	0.89	(0.88-0.91)	0.89	(0.88-0.91)	0.98
Spectral flatness	R	σ	0.97	(0.96-0.97)	0.97	(0.96-0.97)	0.85
Spectral flatness	R	β	0.96	(0.96-0.97)	0.96	(0.95-0.97)	0.97
Spectral flatness	R	γ	0.93	(0.92-0.94)	0.93	(0.92-0.94)	0.32
Spectral power (μV2)	R	δ1	294.06	(246.12-363.25)	243.60	(186.81-332.77)	0.07
Spectral power (μV2)	R	δ2	126.21	(95.81-165.02)	101.05	(72.07-145.70)	0.04*
Spectral power (μV2)	R	θ	54.45	(41.47-69.48)	38.31	(31.71-54.07)	0.006*
Spectral power (μV2)	R	α	5.76	(4.54-6.66)	4.95	(3.78-6.38)	0.10
Spectral power (μV2)	R	σ	1.56	(1.31-1.82)	1.37	(1.07-1.77)	0.09
Spectral power (μV2)	R	β	4.24	(3.62-5.14)	3.78	(3.01-4.84)	0.06
Spectral power (μV2)	R	γ	2.41	(1.89-2.84)	1.94	(1.54-2.66)	0.02*

qEEG = Quantitative EEG; Sp. power = Spectral power; Sp. rel. power = Spectral relative power. FB = Frequency bands: 0.5-2Hz (δ1). 2-4Hz (δ2). 4-8Hz (θ). 8-12Hz (α). 12-15Hz (σ). 15-30 (β). 30-45Hz (γ); SS: sleep stage; rEEG = range-EEG. rEEG frequency band: 1-20Hz. [1]from Mann-Whitney U test, statistically significant differences (p<0.05) marked with an asterisk (*).

Supplementary Table 5 - Neurodevelopmental outcomes at 4 months.

Griffiths III results at 4 months	**Intervention**			**Control**			***p*[a]**
	n	**Median (IQR)**		**n**	**Median (IQR)**		
Main Analysis							
General Development[b]	83	128	(120-132)	96	128	(120-135)	0.43
Subscales							
A. Foundations of Learning	83	123	(109-135)	96	119	(109-129)	0.63
B. Language and Communication	83	131	(120-136)	96	131	(125-142)	0.09
C. Eye and Hand Coordination	83	120	(114-126)	96	120	(111-126)	0.50
D. Personal-Social-Emotional	83	128	(120-133)	96	128	(120-133)	0.79
E. Gross Motor	83	121	(113-131)	96	124	(117-131)	0.49
Subgroup Analysis							
General Development[b]	33	124	(120-133.5)	57	128	(120-135)	0.57
Subscales							
A. Foundations of Learning	33	123	(109-135)	57	119	(109-129)	0.37
B. Language and Communication	33	125	(118-136)	57	131	(124.5-142)	0.05
C. Eye and Hand Coordination	33	117	(110-123)	57	120	(110-123)	0.78
D. Personal-Social-Emotional	33	124	(121.5-131)	57	128	(120-133)	0.57
E. Gross Motor	33	121	(113-131)	57	124	(115-131)	0.80

[a]From Mann-Whitney U test. [b]A lower score indicates more severe neurodevelopmental delay.

Appendix 8 – Supplementary tables from chapter 4

Supplementary Table 6 - Association between qEEG parameters and Griffiths quotients at 18 months, n=69.

Sleep stage	qEEG feature	Frequency band	Unadjusted analysis (n=69) Spearman's Rank Correlation		Adjusted analysis (n=69) Partial Spearman's Rank Correlation[1]	
			r	p-value	r	p-value
		Foundations of Learning				
N1	Coherence	Delta 1	0.091	0.457	0.087	0.492
N1	Coherence	Delta 2	-0.065	0.596	-0.040	0.751
N1	Coherence	Theta	-0.025	0.838	-0.011	0.932
N1	Coherence	Alpha	0.100	0.416	0.102	0.420
N1	Coherence	Sigma	0.068	0.579	0.061	0.627
N1	Coherence	Beta	0.038	0.755	0.016	0.901
N1	Coherence	Gamma	0.020	0.869	-0.006	0.963
N1	Spectral Power	Delta 1	0.043	0.724	0.008	0.950
N1	Spectral Power	Delta 2	-0.076	0.536	-0.114	0.367
N1	Spectral Power	Theta	-0.100	0.413	-0.132	0.295
N1	Spectral Power	Alpha	-0.128	0.295	-0.153	0.223
N1	Spectral Power	Sigma	-0.076	0.533	-0.091	0.469
N1	Spectral Power	Beta	-0.003	0.978	0.008	0.949
N1	Spectral Power	Gamma	0.070	0.568	0.087	0.489
N2	Coherence	Delta 1	0.067	0.585	0.050	0.690
N2	Coherence	Delta 2	-0.086	0.484	-0.056	0.655
N2	Coherence	Theta	-0.034	0.781	-0.043	0.732
N2	Coherence	Alpha	0.096	0.434	0.081	0.523
N2	Coherence	Sigma	0.071	0.561	0.056	0.659
N2	Coherence	Beta	0.286*	0.017	0.277*	0.026
N2	Coherence	Gamma	0.298*	0.013	0.296*	0.017
N2	Spectral Power	Delta 1	0.109	0.373	0.100	0.430
N2	Spectral Power	Delta 2	0.017	0.890	0.011	0.931
N2	Spectral Power	Theta	-0.044	0.719	-0.048	0.704
N2	Spectral Power	Alpha	0.088	0.470	0.097	0.441
N2	Spectral Power	Sigma	0.098	0.424	0.088	0.484
N2	Spectral Power	Beta	0.089	0.467	0.075	0.550
N2	Spectral Power	Gamma	0.039	0.750	0.021	0.867
N3	Coherence	Delta 1	-0.078	0.525	-0.078	0.535
N3	Coherence	Delta 2	-0.026	0.832	0.007	0.956
N3	Coherence	Theta	-0.059	0.631	-0.045	0.724
N3	Coherence	Alpha	0.019	0.874	0.021	0.868

N3	Coherence	Sigma	0.009	0.939	0.033	0.795
N3	Coherence	Beta	0.151	0.215	0.161	0.199
N3	Coherence	Gamma	0.180	0.138	0.186	0.138
N3	Spectral Power	Delta 1	0.051	0.678	0.032	0.799
N3	Spectral Power	Delta 2	0.036	0.770	0.013	0.919
N3	Spectral Power	Theta	0.007	0.956	-0.005	0.970
N3	Spectral Power	Alpha	-0.008	0.947	0.001	0.995
N3	Spectral Power	Sigma	-0.127	0.299	-0.123	0.329
N3	Spectral Power	Beta	-0.043	0.726	-0.057	0.654
N3	Spectral Power	Gamma	-0.024	0.846	-0.047	0.711
REM	Coherence	Delta 1	-0.028	0.818	-0.052	0.681
REM	Coherence	Delta 2	-0.132	0.279	-0.145	0.248
REM	Coherence	Theta	0.006	0.962	0.001	0.991
REM	Coherence	Alpha	-0.076	0.534	-0.085	0.501
REM	Coherence	Sigma	0.068	0.577	0.039	0.756
REM	Coherence	Beta	0.062	0.610	0.049	0.701
REM	Coherence	Gamma	0.007	0.952	-0.023	0.858
REM	Spectral Power	Delta 1	-0.018	0.883	-0.019	0.881
REM	Spectral Power	Delta 2	-0.039	0.748	-0.045	0.722
REM	Spectral Power	Theta	-0.113	0.357	-0.109	0.388
REM	Spectral Power	Alpha	-0.045	0.714	-0.050	0.693
REM	Spectral Power	Sigma	-0.018	0.884	-0.023	0.856
REM	Spectral Power	Beta	-0.012	0.920	-0.030	0.813
REM	Spectral Power	Gamma	0.080	0.512	0.074	0.559
		Language and Communication				
N1	Coherence	Delta 1	-0.102	0.406	-0.076	0.550
N1	Coherence	Delta 2	-0.126	0.300	-0.086	0.497
N1	Coherence	Theta	-0.082	0.502	-0.079	0.533
N1	Coherence	Alpha	-0.043	0.724	-0.037	0.772
N1	Coherence	Sigma	-0.069	0.575	-0.065	0.605
N1	Coherence	Beta	-0.030	0.804	-0.027	0.828
N1	Coherence	Gamma	-0.007	0.954	-0.010	0.935
N1	Spectral Power	Delta 1	0.318*	0.008	0.279*	0.024
N1	Spectral Power	Delta 2	0.265*	0.028	0.216	0.085
N1	Spectral Power	Theta	0.253*	0.036	0.238	0.057
N1	Spectral Power	Alpha	0.236*	0.050	0.220	0.079
N1	Spectral Power	Sigma	0.171	0.160	0.151	0.229
N1	Spectral Power	Beta	0.082	0.502	0.073	0.556
N1	Spectral Power	Gamma	0.114	0.353	0.104	0.411
N2	Coherence	Delta 1	-0.089	0.466	-0.101	0.424
N2	Coherence	Delta 2	-0.143	0.242	-0.126	0.319
N2	Coherence	Theta	-0.106	0.388	-0.121	0.338
N2	Coherence	Alpha	-0.041	0.740	-0.040	0.754

N2	Coherence	Sigma	-0.023	0.849	-0.047	0.707
N2	Coherence	Beta	0.120	0.326	0.121	0.337
N2	Coherence	Gamma	0.074	0.548	0.078	0.535
N2	Spectral Power	Delta 1	0.092	0.454	0.071	0.574
N2	Spectral Power	Delta 2	0.238*	0.049	0.207	0.083
N2	Spectral Power	Theta	0.216	0.074	0.200	0.110
N2	Spectral Power	Alpha	0.163	0.180	0.174	0.165
N2	Spectral Power	Sigma	0.095	0.436	0.093	0.463
N2	Spectral Power	Beta	0.074	0.545	0.022	0.864
N2	Spectral Power	Gamma	0.127	0.298	0.079	0.534
N3	Coherence	Delta 1	-0.170	0.161	-0.127	0.314
N3	Coherence	Delta 2	-0.168	0.168	-0.100	0.428
N3	Coherence	Theta	-0.204	0.093	-0.173	0.168
N3	Coherence	Alpha	-0.010	0.932	0.040	0.754
N3	Coherence	Sigma	-0.147	0.228	-0.074	0.557
N3	Coherence	Beta	0.016	0.897	0.062	0.626
N3	Coherence	Gamma	0.028	0.821	0.054	0.667
N3	Spectral Power	Delta 1	0.198	0.104	0.144	0.254
N3	Spectral Power	Delta 2	0.267*	0.027	0.220	0.078
N3	Spectral Power	Theta	0.227	0.061	0.198	0.113
N3	Spectral Power	Alpha	0.218	0.073	0.215	0.085
N3	Spectral Power	Sigma	0.111	0.363	0.139	0.270
N3	Spectral Power	Beta	0.202	0.095	0.176	0.162
N3	Spectral Power	Gamma	0.093	0.447	0.045	0.722
REM	Coherence	Delta 1	-0.071	0.561	-0.103	0.416
REM	Coherence	Delta 2	-0.115	0.346	-0.145	0.250
REM	Coherence	Theta	-0.093	0.447	-0.101	0.424
REM	Coherence	Alpha	-0.062	0.611	-0.079	0.530
REM	Coherence	Sigma	-0.034	0.781	-0.068	0.592
REM	Coherence	Beta	0.042	0.734	0.042	0.739
REM	Coherence	Gamma	0.048	0.698	0.015	0.907
REM	Spectral Power	Delta 1	0.238*	0.049	0.222	0.075
REM	Spectral Power	Delta 2	0.216	0.075	0.191	0.127
REM	Spectral Power	Theta	0.215	0.076	0.213	0.088
REM	Spectral Power	Alpha	0.176	0.149	0.167	0.184
REM	Spectral Power	Sigma	0.141	0.247	0.126	0.317
REM	Spectral Power	Beta	0.070	0.568	0.024	0.849
REM	Spectral Power	Gamma	0.105	0.389	0.062	0.623
		Eye and Hand Coordination				
N1	Coherence	Delta 1	0.078	0.526	0.099	0.435
N1	Coherence	Delta 2	-0.112	0.359	-0.077	0.543
N1	Coherence	Theta	-0.024	0.844	-0.001	0.995
N1	Coherence	Alpha	-0.015	0.902	-0.001	0.992

N1	Coherence	Sigma	0.012	0.924	0.020	0.872
N1	Coherence	Beta	0.052	0.669	0.054	0.667
N1	Coherence	Gamma	0.037	0.766	0.029	0.818
N1	Spectral Power	Delta 1	0.252*	0.037	0.202	0.106
N1	Spectral Power	Delta 2	0.109	0.374	0.036	0.777
N1	Spectral Power	Theta	0.091	0.455	0.040	0.752
N1	Spectral Power	Alpha	0.050	0.685	-0.001	0.993
N1	Spectral Power	Sigma	0.035	0.778	-0.012	0.926
N1	Spectral Power	Beta	-0.032	0.793	-0.063	0.616
N1	Spectral Power	Gamma	0.033	0.789	0.017	0.893
N2	Coherence	Delta 1	0.072	0.555	0.053	0.678
N2	Coherence	Delta 2	-0.137	0.262	-0.118	0.348
N2	Coherence	Theta	-0.021	0.866	-0.035	0.782
N2	Coherence	Alpha	0.093	0.448	0.094	0.455
N2	Coherence	Sigma	0.065	0.595	0.043	0.736
N2	Coherence	Beta	0.221	0.069	0.223	0.075
N2	Coherence	Gamma	0.234	0.053	0.242	0.052
N2	Spectral Power	Delta 1	0.110	0.369	0.090	0.476
N2	Spectral Power	Delta 2	0.046	0.706	0.012	0.925
N2	Spectral Power	Theta	0.072	0.556	0.041	0.744
N2	Spectral Power	Alpha	0.079	0.518	0.073	0.564
N2	Spectral Power	Sigma	-0.014	0.910	-0.026	0.834
N2	Spectral Power	Beta	0.013	0.919	-0.045	0.721
N2	Spectral Power	Gamma	0.078	0.524	0.019	0.882
N3	Coherence	Delta 1	-0.061	0.621	-0.029	0.819
N3	Coherence	Delta 2	-0.174	0.153	-0.122	0.332
N3	Coherence	Theta	-0.135	0.270	-0.103	0.412
N3	Coherence	Alpha	-0.042	0.730	-0.002	0.986
N3	Coherence	Sigma	-0.078	0.524	-0.008	0.946
N3	Coherence	Beta	0.069	0.573	0.111	0.379
N3	Coherence	Gamma	0.088	0.473	0.115	0.363
N3	Spectral Power	Delta 1	0.126	0.301	0.074	0.559
N3	Spectral Power	Delta 2	0.114	0.350	0.049	0.697
N3	Spectral Power	Theta	0.069	0.574	0.016	0.897
N3	Spectral Power	Alpha	0.013	0.916	-0.015	0.905
N3	Spectral Power	Sigma	-0.171	0.159	-0.170	0.176
N3	Spectral Power	Beta	0.030	0.807	-0.016	0.901
N3	Spectral Power	Gamma	0.064	0.603	0.005	0.968
REM	Coherence	Delta 1	-0.036	0.768	-0.075	0.554
REM	Coherence	Delta 2	-0.095	0.437	-0.131	0.300
REM	Coherence	Theta	-0.015	0.901	-0.025	0.841
REM	Coherence	Alpha	0.011	0.927	<0.001	1.000
REM	Coherence	Sigma	0.039	0.750	-0.001	0.993

REM	Coherence	Beta	0.105	0.390	0.105	0.405
REM	Coherence	Gamma	0.111	0.364	0.076	0.548
REM	Spectral Power	Delta 1	0.097	0.429	0.085	0.503
REM	Spectral Power	Delta 2	0.014	0.910	-0.021	0.870
REM	Spectral Power	Theta	0.102	0.404	0.096	0.449
REM	Spectral Power	Alpha	0.080	0.516	0.049	0.701
REM	Spectral Power	Sigma	0.064	0.600	0.029	0.822
REM	Spectral Power	Beta	0.008	0.946	-0.061	0.627
REM	Spectral Power	Gamma	0.068	0.577	0.018	0.889
		Personal-Social-Emotional				
N1	Coherence	Delta 1	-0.038	0.755	-0.007	0.959
N1	Coherence	Delta 2	-0.144	0.237	-0.097	0.442
N1	Coherence	Theta	0.018	0.886	0.046	0.715
N1	Coherence	Alpha	0.027	0.826	0.045	0.724
N1	Coherence	Sigma	0.023	0.854	0.042	0.738
N1	Coherence	Beta	-0.006	0.958	0.001	0.995
N1	Coherence	Gamma	0.019	0.880	0.019	0.878
N1	Spectral Power	Delta 1	0.362*	0.002	0.327*	0.008
N1	Spectral Power	Delta 2	0.227	0.060	0.168	0.182
N1	Spectral Power	Theta	0.173	0.154	0.145	0.251
N1	Spectral Power	Alpha	0.148	0.226	0.116	0.359
N1	Spectral Power	Sigma	0.125	0.307	0.096	0.447
N1	Spectral Power	Beta	0.116	0.341	0.115	0.361
N1	Spectral Power	Gamma	0.154	0.207	0.152	0.225
N2	Coherence	Delta 1	0.074	0.548	0.066	0.602
N2	Coherence	Delta 2	-0.080	0.512	-0.051	0.684
N2	Coherence	Theta	0.058	0.636	0.056	0.659
N2	Coherence	Alpha	0.079	0.517	0.093	0.459
N2	Coherence	Sigma	0.065	0.598	0.046	0.713
N2	Coherence	Beta	0.098	0.425	0.100	0.427
N2	Coherence	Gamma	0.185	0.127	0.209	0.095
N2	Spectral Power	Delta 1	0.163	0.182	0.152	0.227
N2	Spectral Power	Delta 2	0.162	0.183	0.137	0.278
N2	Spectral Power	Theta	0.153	0.209	0.135	0.284
N2	Spectral Power	Alpha	0.011	0.926	0.011	0.933
N2	Spectral Power	Sigma	-0.126	0.303	-0.141	0.262
N2	Spectral Power	Beta	0.065	0.595	0.008	0.948
N2	Spectral Power	Gamma	0.182	0.134	0.133	0.291
N3	Coherence	Delta 1	-0.217	0.073	-0.184	0.142
N3	Coherence	Delta 2	-0.225	0.063	-0.158	0.208
N3	Coherence	Theta	-0.106	0.387	-0.057	0.652
N3	Coherence	Alpha	0.081	0.509	0.147	0.241
N3	Coherence	Sigma	-0.078	0.524	0.009	0.940

N3	Coherence	Beta	0.044	0.718	0.099	0.433
N3	Coherence	Gamma	0.094	0.442	0.134	0.288
N3	Spectral Power	Delta 1	0.304*	0.011	0.253*	0.042
N3	Spectral Power	Delta 2	0.244*	0.043	0.188	0.135
N3	Spectral Power	Theta	0.140	0.253	0.099	0.432
N3	Spectral Power	Alpha	0.051	0.676	0.030	0.810
N3	Spectral Power	Sigma	-0.166	0.172	-0.159	0.207
N3	Spectral Power	Beta	0.139	0.256	0.102	0.419
N3	Spectral Power	Gamma	0.206	0.089	0.158	0.207
REM	Coherence	Delta 1	-0.244*	0.044	-0.296*	0.017
REM	Coherence	Delta 2	-0.332*	0.005	-0.386*	0.001
REM	Coherence	Theta	-0.134	0.273	-0.147	0.244
REM	Coherence	Alpha	-0.062	0.611	-0.082	0.515
REM	Coherence	Sigma	0.013	0.917	-0.025	0.846
REM	Coherence	Beta	0.090	0.460	0.100	0.430
REM	Coherence	Gamma	0.121	0.322	0.091	0.471
REM	Spectral Power	Delta 1	0.202	0.096	0.192	0.126
REM	Spectral Power	Delta 2	0.055	0.655	0.019	0.878
REM	Spectral Power	Theta	0.131	0.283	0.126	0.317
REM	Spectral Power	Alpha	0.154	0.207	0.133	0.290
REM	Spectral Power	Sigma	0.136	0.265	0.114	0.366
REM	Spectral Power	Beta	0.104	0.396	0.050	0.690
REM	Spectral Power	Gamma	0.219	0.070	0.177	0.159
		Gross Motor				
N1	Coherence	Delta 1	0.033	0.787	0.044	0.730
N1	Coherence	Delta 2	-0.049	0.687	-0.018	0.886
N1	Coherence	Theta	0.089	0.466	0.113	0.369
N1	Coherence	Alpha	0.107	0.381	0.113	0.369
N1	Coherence	Sigma	0.178	0.144	0.192	0.125
N1	Coherence	Beta	0.183	0.131	0.189	0.132
N1	Coherence	Gamma	0.221	0.068	0.225	0.071
N1	Spectral Power	Delta 1	0.294*	0.014	0.287*	0.020
N1	Spectral Power	Delta 2	0.231	0.056	0.227	0.069
N1	Spectral Power	Theta	0.200	0.100	0.221	0.077
N1	Spectral Power	Alpha	0.123	0.316	0.131	0.297
N1	Spectral Power	Sigma	0.092	0.451	0.100	0.430
N1	Spectral Power	Beta	0.022	0.857	0.038	0.766
N1	Spectral Power	Gamma	0.085	0.488	0.100	0.427
N2	Coherence	Delta 1	0.018	0.884	0.011	0.928
N2	Coherence	Delta 2	-0.028	0.816	-0.002	0.990
N2	Coherence	Theta	0.165	0.176	0.171	0.174
N2	Coherence	Alpha	0.136	0.264	0.141	0.263
N2	Coherence	Sigma	-0.037	0.763	-0.046	0.717

N2	Coherence	Beta	0.141	0.247	0.141	0.262
N2	Coherence	Gamma	0.128	0.295	0.137	0.275
N2	Spectral Power	Delta 1	0.243*	0.044	0.249*	0.046
N2	Spectral Power	Delta 2	0.123	0.314	0.123	0.331
N2	Spectral Power	Theta	0.110	0.369	0.124	0.326
N2	Spectral Power	Alpha	0.031	0.801	0.047	0.710
N2	Spectral Power	Sigma	-0.188	0.122	-0.196	0.117
N2	Spectral Power	Beta	0.005	0.967	-0.010	0.937
N2	Spectral Power	Gamma	0.134	0.272	0.125	0.319
N3	Coherence	Delta 1	-0.079	0.521	-0.072	0.570
N3	Coherence	Delta 2	0.063	0.609	0.109	0.389
N3	Coherence	Theta	0.180	0.140	0.215	0.085
N3	Coherence	Alpha	0.123	0.314	0.142	0.258
N3	Coherence	Sigma	-0.032	0.796	-0.004	0.977
N3	Coherence	Beta	0.113	0.354	0.132	0.295
N3	Coherence	Gamma	0.118	0.334	0.132	0.293
N3	Spectral Power	Delta 1	0.283*	0.018	0.269*	0.031
N3	Spectral Power	Delta 2	0.278*	0.021	0.271*	0.029
N3	Spectral Power	Theta	0.174	0.153	0.186	0.138
N3	Spectral Power	Alpha	0.074	0.546	0.092	0.465
N3	Spectral Power	Sigma	-0.134	0.273	-0.129	0.306
N3	Spectral Power	Beta	0.094	0.443	0.088	0.484
N3	Spectral Power	Gamma	0.179	0.141	0.168	0.181
REM	Coherence	Delta 1	-0.067	0.582	-0.086	0.495
REM	Coherence	Delta 2	-0.051	0.679	-0.061	0.628
REM	Coherence	Theta	0.038	0.759	0.039	0.759
REM	Coherence	Alpha	-0.002	0.990	-0.010	0.939
REM	Coherence	Sigma	0.075	0.538	0.059	0.642
REM	Coherence	Beta	0.131	0.284	0.134	0.286
REM	Coherence	Gamma	0.175	0.151	0.163	0.194
REM	Spectral Power	Delta 1	0.241*	0.046	0.244*	0.050
REM	Spectral Power	Delta 2	0.100	0.413	0.100	0.428
REM	Spectral Power	Theta	0.117	0.339	0.127	0.313
REM	Spectral Power	Alpha	0.097	0.426	0.101	0.423
REM	Spectral Power	Sigma	0.117	0.340	0.125	0.322
REM	Spectral Power	Beta	0.106	0.384	0.104	0.410
REM	Spectral Power	Gamma	0.105	0.392	0.092	0.465
		General Development				
N1	Coherence	Delta 1	0.006	0.962	0.019	0.883
N1	Coherence	Delta 2	-0.078	0.523	-0.054	0.667
N1	Coherence	Theta	0.040	0.746	0.066	0.604
N1	Coherence	Alpha	0.096	0.431	0.124	0.323
N1	Coherence	Sigma	0.081	0.511	0.099	0.435

N1	Coherence	Beta	0.113	0.356	0.130	0.304
N1	Coherence	Gamma	0.108	0.375	0.116	0.356
N1	Spectral Power	Delta 1	0.320*	0.007	0.275*	0.027
N1	Spectral Power	Delta 2	0.181	0.137	0.107	0.398
N1	Spectral Power	Theta	0.199	0.101	0.146	0.246
N1	Spectral Power	Alpha	0.138	0.257	0.081	0.519
N1	Spectral Power	Sigma	0.095	0.439	0.038	0.765
N1	Spectral Power	Beta	0.034	0.781	-0.010	0.939
N1	Spectral Power	Gamma	0.092	0.454	0.065	0.605
N2	Coherence	Delta 1	0.031	0.797	0.005	0.970
N2	Coherence	Delta 2	-0.108	0.376	-0.103	0.415
N2	Coherence	Theta	0.015	0.905	-0.002	0.990
N2	Coherence	Alpha	0.084	0.491	0.087	0.489
N2	Coherence	Sigma	0.029	0.815	0.004	0.978
N2	Coherence	Beta	0.236*	0.050	0.243	0.051
N2	Coherence	Gamma	0.258*	0.032	0.266*	0.032
N2	Spectral Power	Delta 1	0.185	0.129	0.166	0.186
N2	Spectral Power	Delta 2	0.154	0.206	0.119	0.346
N2	Spectral Power	Theta	0.154	0.206	0.116	0.357
N2	Spectral Power	Alpha	0.141	0.247	0.125	0.322
N2	Spectral Power	Sigma	-0.046	0.706	-0.060	0.636
N2	Spectral Power	Beta	0.046	0.705	-0.016	0.902
N2	Spectral Power	Gamma	0.133	0.278	0.068	0.589
N3	Coherence	Delta 1	-0.187	0.124	-0.164	0.191
N3	Coherence	Delta 2	-0.139	0.256	-0.096	0.449
N3	Coherence	Theta	-0.087	0.476	-0.059	0.638
N3	Coherence	Alpha	0.006	0.961	0.058	0.644
N3	Coherence	Sigma	-0.066	0.589	0.006	0.964
N3	Coherence	Beta	0.087	0.476	0.132	0.294
N3	Coherence	Gamma	0.127	0.297	0.158	0.208
N3	Spectral Power	Delta 1	0.205	0.092	0.159	0.205
N3	Spectral Power	Delta 2	0.228	0.059	0.167	0.184
N3	Spectral Power	Theta	0.155	0.202	0.098	0.437
N3	Spectral Power	Alpha	0.115	0.348	0.080	0.524
N3	Spectral Power	Sigma	-0.103	0.398	-0.104	0.411
N3	Spectral Power	Beta	0.131	0.283	0.084	0.507
N3	Spectral Power	Gamma	0.162	0.184	0.104	0.410
REM	Coherence	Delta 1	-0.128	0.293	-0.170	0.175
REM	Coherence	Delta 2	-0.193	0.112	-0.239	0.055
REM	Coherence	Theta	-0.074	0.545	-0.091	0.470
REM	Coherence	Alpha	-0.091	0.457	-0.104	0.412
REM	Coherence	Sigma	0.003	0.979	-0.037	0.771
REM	Coherence	Beta	0.098	0.421	0.100	0.428

REM	Coherence	Gamma	0.110	0.367	0.077	0.540
REM	Spectral Power	Delta 1	0.217	0.074	0.209	0.095
REM	Spectral Power	Delta 2	0.126	0.303	0.091	0.472
REM	Spectral Power	Theta	0.175	0.149	0.163	0.195
REM	Spectral Power	Alpha	0.164	0.178	0.128	0.311
REM	Spectral Power	Sigma	0.177	0.145	0.139	0.271
REM	Spectral Power	Beta	0.084	0.492	0.008	0.947
REM	Spectral Power	Gamma	0.123	0.313	0.069	0.583

**coefficients whose p-value <0.05. [1]Partial Spearman's Rank Correlations are corrected for postnatal age of both EEG and Griffiths at 18-months assessments, gestational age, and sex.*

Supplementary Table 7 - Association between Griffiths III quotients and sleep features that are co-associated with household income, n=50.

Griffiths III Measures	Sleep stage	qEEG feature	Frequency band	Partial Spearman's Rank Correlation[1]	
				r	p-value
Subscale B DQ, Language and Communication	N3	Coherence	Theta	0.003	0.985
Subscale C DQ, Eye and Hand Coordination	N3	Coherence	Theta	0.089	0.560
Subscale D DQ, Personal-Social-Emotional	N3	Coherence	Theta	0.208	0.171
GD DQ, General Development	N3	Coherence	Theta	0.076	0.622

[1]Partial Spearman's Rank Correlations are corrected for postnatal age of both EEG and Griffiths at 18-months assessments, gestational age, sex and household income.

Appendix 9 – Supplementary tables from chapter 5

Supplementary Table 1 – Coherence Analysis

Frequency Band	Channels	Median (IQR) Term infants (n=72)		Median (IQR) MLP (n=42)		Median difference (MLP-term)	p-value[a]
NREM Interhemispherical Coherence							
Delta 1	A1-T5 & A2-T6	0.86	(0.85 to 0.88)	0.86	(0.84 to 0.88)	0.001	0.98
	C3-P3 & C4-P4	0.88	(0.86 to 0.89)	0.87	(0.85 to 0.89)	-0.009	0.20
	F3-C3 & F4-C4	0.88	(0.86 to 0.89)	0.87	(0.86 to 0.89)	-0.003	0.75
	F7-A1 & F8-A2	0.89	(0.87 to 0.91)	0.89	(0.88 to 0.91)	0.005	0.34
	F7-T3 & F8-T4	0.85	(0.83 to 0.86)	0.85	(0.83 to 0.87)	0.000	0.80
	Fp1-F3 & Fp2-F4	0.90	(0.89 to 0.92)	0.90	(0.88 to 0.92)	-0.001	0.43
	Fp1-F7 & Fp2-F8	0.88	(0.86 to 0.89)	0.88	(0.86 to 0.90)	0.003	0.86
	P3-O1 & P4-O2	0.90	(0.89 to 0.92)	0.89	(0.86 to 0.90)	-0.013	0.002
	T3-T5 & T4-T6	0.85	(0.83 to 0.87)	0.86	(0.83 to 0.87)	0.010	0.55
	T5-O1 & T6-O2	0.87	(0.85 to 0.88)	0.87	(0.84 to 0.89)	-0.005	0.47
Delta 2	A1-T5 & A2-T6	0.85	(0.84 to 0.86)	0.85	(0.84 to 0.86)	-0.007	0.10
	C3-P3 & C4-P4	0.88	(0.86 to 0.89)	0.87	(0.85 to 0.88)	-0.008	0.07
	F3-C3 & F4-C4	0.89	(0.87 to 0.90)	0.88	(0.86 to 0.89)	-0.004	0.79
	F7-A1 & F8-A2	0.87	(0.86 to 0.88)	0.87	(0.86 to 0.89)	-0.003	0.94
	F7-T3 & F8-T4	0.84	(0.83 to 0.85)	0.84	(0.83 to 0.86)	0.001	0.85
	Fp1-F3 & Fp2-F4	0.90	(0.89 to 0.91)	0.89	(0.87 to 0.92)	-0.006	0.31
	Fp1-F7 & Fp2-F8	0.86	(0.84 to 0.87)	0.85	(0.84 to 0.87)	-0.011	0.08
	P3-O1 & P4-O2	0.88	(0.87 to 0.90)	0.87	(0.85 to 0.88)	-0.017	0.001
	T3-T5 & T4-T6	0.84	(0.83 to 0.85)	0.84	(0.83 to 0.85)	-0.002	0.78
	T5-O1 & T6-O2	0.85	(0.84 to 0.87)	0.85	(0.84 to 0.86)	0.001	0.89
Theta	A1-T5 & A2-T6	0.87	(0.87 to 0.88)	0.87	(0.86 to 0.88)	0.001	0.80
	C3-P3 & C4-P4	0.88	(0.87 to 0.89)	0.88	(0.87 to 0.89)	-0.003	0.81
	F3-C3 & F4-C4	0.88	(0.87 to 0.88)	0.87	(0.87 to 0.88)	-0.001	0.94
	F7-A1 & F8-A2	0.88	(0.87 to 0.88)	0.88	(0.87 to 0.89)	-0.001	0.92
	F7-T3 & F8-T4	0.86	(0.86 to 0.87)	0.87	(0.86 to 0.87)	0.001	0.91
	Fp1-F3 & Fp2-F4	0.87	(0.87 to 0.88)	0.87	(0.86 to 0.88)	-0.001	0.37
	Fp1-F7 & Fp2-F8	0.88	(0.88 to 0.89)	0.88	(0.87 to 0.89)	-0.004	0.21
	P3-O1 & P4-O2	0.89	(0.88 to 0.90)	0.88	(0.87 to 0.89)	-0.009	0.013
	T3-T5 & T4-T6	0.87	(0.86 to 0.87)	0.87	(0.86 to 0.87)	0.002	0.68
	T5-O1 & T6-O2	0.88	(0.87 to 0.89)	0.88	(0.87 to 0.89)	-0.004	0.34
Alpha	A1-T5 & A2-T6	0.88	(0.87 to 0.89)	0.88	(0.87 to 0.89)	0.006	0.25
	C3-P3 & C4-P4	0.88	(0.87 to 0.88)	0.88	(0.87 to 0.89)	0.004	0.45
	F3-C3 & F4-C4	0.88	(0.87 to 0.88)	0.88	(0.87 to 0.89)	0.002	0.38

	F7-A1 & F8-A2	0.87	(0.87 to 0.88)	0.88	(0.87 to 0.89)	0.003	0.33
	F7-T3 & F8-T4	0.87	(0.86 to 0.88)	0.87	(0.86 to 0.88)	0.000	0.78
	Fp1-F3 & Fp2-F4	0.87	(0.87 to 0.88)	0.88	(0.86 to 0.89)	0.001	0.71
	Fp1-F7 & Fp2-F8	0.88	(0.88 to 0.89)	0.88	(0.87 to 0.89)	-0.003	0.29
	P3-O1 & P4-O2	0.89	(0.87 to 0.90)	0.88	(0.87 to 0.89)	-0.012	0.009
	T3-T5 & T4-T6	0.87	(0.86 to 0.87)	0.87	(0.86 to 0.88)	0.000	0.62
	T5-O1 & T6-O2	0.88	(0.87 to 0.89)	0.88	(0.87 to 0.89)	0.000	0.77
	A1-T5 & A2-T6	0.88	(0.87 to 0.91)	0.89	(0.87 to 0.90)	0.007	0.62
	C3-P3 & C4-P4	0.87	(0.86 to 0.89)	0.88	(0.86 to 0.89)	0.006	0.44
	F3-C3 & F4-C4	0.90	(0.88 to 0.91)	0.89	(0.89 to 0.90)	-0.004	0.29
Sigma	F7-A1 & F8-A2	0.87	(0.86 to 0.88)	0.87	(0.85 to 0.88)	0.003	0.70
	F7-T3 & F8-T4	0.87	(0.87 to 0.88)	0.87	(0.86 to 0.88)	-0.005	0.06
	Fp1-F3 & Fp2-F4	0.87	(0.86 to 0.88)	0.87	(0.86 to 0.89)	-0.004	0.92
	Fp1-F7 & Fp2-F8	0.88	(0.87 to 0.90)	0.87	(0.86 to 0.89)	-0.006	0.08
	P3-O1 & P4-O2	0.88	(0.86 to 0.89)	0.86	(0.85 to 0.88)	-0.013	0.002
	T3-T5 & T4-T6	0.86	(0.85 to 0.87)	0.86	(0.85 to 0.87)	0.001	0.77
	T5-O1 & T6-O2	0.86	(0.85 to 0.87)	0.86	(0.85 to 0.87)	0.002	0.74
	A1-T5 & A2-T6	0.94	(0.94 to 0.95)	0.95	(0.94 to 0.96)	0.010	0.002
	C3-P3 & C4-P4	0.93	(0.93 to 0.94)	0.93	(0.93 to 0.94)	0.000	0.82
	F3-C3 & F4-C4	0.94	(0.93 to 0.94)	0.94	(0.93 to 0.94)	-0.001	0.87
	F7-A1 & F8-A2	0.94	(0.93 to 0.95)	0.94	(0.93 to 0.95)	0.000	0.44
Beta	F7-T3 & F8-T4	0.93	(0.93 to 0.94)	0.93	(0.93 to 0.94)	0.001	0.56
	Fp1-F3 & Fp2-F4	0.93	(0.93 to 0.94)	0.93	(0.93 to 0.94)	-0.001	0.61
	Fp1-F7 & Fp2-F8	0.93	(0.93 to 0.94)	0.94	(0.93 to 0.94)	0.002	0.10
	P3-O1 & P4-O2	0.94	(0.94 to 0.95)	0.94	(0.93 to 0.94)	-0.004	0.035
	T3-T5 & T4-T6	0.93	(0.93 to 0.94)	0.93	(0.93 to 0.94)	-0.002	0.20
	T5-O1 & T6-O2	0.94	(0.93 to 0.94)	0.94	(0.93 to 0.94)	0.001	0.32
	A1-T5 & A2-T6	0.94	(0.93 to 0.94)	0.94	(0.94 to 0.95)	0.004	0.047
	C3-P3 & C4-P4	0.93	(0.93 to 0.94)	0.93	(0.93 to 0.94)	0.000	0.94
	F3-C3 & F4-C4	0.93	(0.93 to 0.93)	0.93	(0.93 to 0.94)	0.000	0.43
	F7-A1 & F8-A2	0.94	(0.93 to 0.95)	0.94	(0.93 to 0.95)	0.001	0.14
Gamma	F7-T3 & F8-T4	0.93	(0.93 to 0.93)	0.93	(0.93 to 0.94)	0.001	0.34
	Fp1-F3 & Fp2-F4	0.93	(0.93 to 0.94)	0.93	(0.93 to 0.94)	-0.004	0.10
	Fp1-F7 & Fp2-F8	0.93	(0.93 to 0.94)	0.93	(0.93 to 0.94)	0.001	0.78
	P3-O1 & P4-O2	0.94	(0.94 to 0.95)	0.94	(0.93 to 0.94)	-0.003	0.011
	T3-T5 & T4-T6	0.93	(0.93 to 0.94)	0.93	(0.93 to 0.94)	-0.001	0.56
	T5-O1 & T6-O2	0.94	(0.93 to 0.94)	0.94	(0.93 to 0.94)	0.000	0.84
	NREM Intrahemispherical Coherence						
	A1-T5 & C3-P3	0.85	(0.84 to 0.86)	0.86	(0.84 to 0.87)	0.011	0.20
Delta 1	A1-T5 & T5-O1	0.81	(0.79 to 0.83)	0.81	(0.80 to 0.84)	0.002	0.60
	A2-T6 & C4-P4	0.85	(0.83 to 0.87)	0.86	(0.84 to 0.88)	0.009	0.24
	A2-T6 & T6-O2	0.81	(0.80 to 0.83)	0.81	(0.80 to 0.84)	0.000	0.60

	C3-P3 & T5-O1	0.80	(0.78 to 0.81)	0.79	(0.79 to 0.82)	-0.002	0.72
	C4-P4 & T6-O2	0.80	(0.79 to 0.81)	0.80	(0.79 to 0.82)	0.004	0.22
	Fp1-F3 & A1-T5	0.81	(0.80 to 0.83)	0.82	(0.80 to 0.84)	0.005	0.50
	Fp1-F3 & C3-P3	0.83	(0.81 to 0.84)	0.83	(0.81 to 0.85)	0.004	0.58
	Fp1-F3 & T5-O1	0.79	(0.78 to 0.81)	0.79	(0.78 to 0.81)	0.003	0.96
	Fp2-F4 & A2-T6	0.81	(0.80 to 0.83)	0.81	(0.80 to 0.83)	0.000	0.98
	Fp2-F4 & C4-P4	0.83	(0.81 to 0.84)	0.83	(0.80 to 0.85)	0.008	0.80
	Fp2-F4 & T6-O2	0.79	(0.78 to 0.80)	0.80	(0.79 to 0.82)	0.009	0.039
	A1-T5 & C3-P3	0.87	(0.86 to 0.88)	0.88	(0.86 to 0.89)	0.007	0.17
	A1-T5 & T5-O1	0.83	(0.82 to 0.84)	0.82	(0.81 to 0.84)	-0.008	0.07
	A2-T6 & C4-P4	0.87	(0.85 to 0.89)	0.88	(0.86 to 0.89)	0.006	0.27
	A2-T6 & T6-O2	0.83	(0.82 to 0.85)	0.83	(0.82 to 0.85)	0.000	0.81
	C3-P3 & T5-O1	0.81	(0.80 to 0.82)	0.82	(0.80 to 0.83)	0.004	0.40
Delta 2	C4-P4 & T6-O2	0.81	(0.81 to 0.82)	0.81	(0.81 to 0.82)	0.002	0.71
	Fp1-F3 & A1-T5	0.83	(0.82 to 0.85)	0.83	(0.82 to 0.84)	0.000	0.45
	Fp1-F3 & C3-P3	0.85	(0.84 to 0.86)	0.85	(0.84 to 0.86)	-0.004	0.63
	Fp1-F3 & T5-O1	0.81	(0.80 to 0.82)	0.82	(0.81 to 0.82)	0.007	0.14
	Fp2-F4 & A2-T6	0.83	(0.82 to 0.84)	0.83	(0.82 to 0.84)	-0.005	0.11
	Fp2-F4 & C4-P4	0.85	(0.84 to 0.86)	0.86	(0.84 to 0.87)	0.006	0.38
	Fp2-F4 & T6-O2	0.81	(0.80 to 0.81)	0.82	(0.81 to 0.83)	0.007	0.002
	A1-T5 & C3-P3	0.90	(0.89 to 0.91)	0.90	(0.89 to 0.92)	0.004	0.21
	A1-T5 & T5-O1	0.88	(0.87 to 0.90)	0.88	(0.87 to 0.89)	-0.002	0.46
	A2-T6 & C4-P4	0.90	(0.89 to 0.91)	0.90	(0.89 to 0.91)	0.003	0.29
	A2-T6 & T6-O2	0.88	(0.88 to 0.90)	0.88	(0.87 to 0.90)	-0.002	0.85
	C3-P3 & T5-O1	0.86	(0.86 to 0.87)	0.86	(0.86 to 0.87)	0.001	0.30
Theta	C4-P4 & T6-O2	0.87	(0.86 to 0.87)	0.87	(0.86 to 0.87)	0.000	0.54
	Fp1-F3 & A1-T5	0.86	(0.86 to 0.87)	0.87	(0.86 to 0.87)	0.001	0.80
	Fp1-F3 & C3-P3	0.91	(0.90 to 0.92)	0.91	(0.89 to 0.92)	-0.007	0.20
	Fp1-F3 & T5-O1	0.86	(0.85 to 0.87)	0.86	(0.85 to 0.87)	0.000	0.99
	Fp2-F4 & A2-T6	0.87	(0.86 to 0.87)	0.87	(0.86 to 0.88)	0.003	0.40
	Fp2-F4 & C4-P4	0.91	(0.90 to 0.92)	0.91	(0.89 to 0.92)	-0.002	0.50
	Fp2-F4 & T6-O2	0.86	(0.85 to 0.87)	0.86	(0.85 to 0.87)	0.000	0.66
	A1-T5 & C3-P3	0.89	(0.88 to 0.90)	0.89	(0.88 to 0.91)	0.001	0.33
	A1-T5 & T5-O1	0.88	(0.87 to 0.89)	0.88	(0.87 to 0.89)	-0.002	0.98
	A2-T6 & C4-P4	0.89	(0.88 to 0.90)	0.89	(0.88 to 0.90)	0.002	0.39
	A2-T6 & T6-O2	0.88	(0.87 to 0.89)	0.88	(0.87 to 0.90)	-0.003	0.54
	C3-P3 & T5-O1	0.86	(0.86 to 0.87)	0.86	(0.86 to 0.87)	0.000	0.89
Alpha	C4-P4 & T6-O2	0.86	(0.86 to 0.87)	0.86	(0.86 to 0.87)	0.001	0.61
	Fp1-F3 & A1-T5	0.87	(0.86 to 0.87)	0.87	(0.86 to 0.87)	0.003	0.34
	Fp1-F3 & C3-P3	0.90	(0.89 to 0.91)	0.90	(0.88 to 0.91)	-0.003	0.23
	Fp1-F3 & T5-O1	0.86	(0.85 to 0.87)	0.86	(0.85 to 0.87)	0.004	0.18
	Fp2-F4 & A2-T6	0.87	(0.86 to 0.87)	0.86	(0.86 to 0.87)	-0.001	0.60
	Fp2-F4 & C4-P4	0.90	(0.89 to 0.91)	0.90	(0.89 to 0.91)	0.005	0.42

	Fp2-F4 & T6-O2	0.86	(0.86 to 0.87)	0.86	(0.86 to 0.88)	0.004	0.027
	A1-T5 & C3-P3	0.90	(0.88 to 0.92)	0.89	(0.87 to 0.92)	-0.007	0.20
	A1-T5 & T5-O1	0.86	(0.85 to 0.88)	0.86	(0.85 to 0.88)	0.001	0.75
	A2-T6 & C4-P4	0.89	(0.87 to 0.91)	0.89	(0.87 to 0.91)	0.001	1.00
	A2-T6 & T6-O2	0.87	(0.86 to 0.88)	0.86	(0.85 to 0.89)	-0.007	0.32
	C3-P3 & T5-O1	0.85	(0.84 to 0.86)	0.85	(0.84 to 0.86)	-0.003	0.48
Sigma	C4-P4 & T6-O2	0.85	(0.84 to 0.86)	0.85	(0.84 to 0.86)	-0.003	0.52
	Fp1-F3 & A1-T5	0.87	(0.86 to 0.89)	0.86	(0.85 to 0.87)	-0.009	0.009
	Fp1-F3 & C3-P3	0.91	(0.89 to 0.93)	0.90	(0.88 to 0.93)	-0.008	0.16
	Fp1-F3 & T5-O1	0.85	(0.84 to 0.86)	0.85	(0.84 to 0.86)	-0.004	0.18
	Fp2-F4 & A2-T6	0.88	(0.87 to 0.89)	0.88	(0.86 to 0.89)	0.002	0.26
	Fp2-F4 & C4-P4	0.90	(0.88 to 0.92)	0.91	(0.89 to 0.93)	0.005	0.67
	Fp2-F4 & T6-O2	0.85	(0.84 to 0.85)	0.85	(0.84 to 0.86)	0.004	0.13
	A1-T5 & C3-P3	0.94	(0.94 to 0.95)	0.94	(0.94 to 0.95)	0.000	0.83
	A1-T5 & T5-O1	0.94	(0.93 to 0.94)	0.94	(0.93 to 0.95)	0.001	0.81
	A2-T6 & C4-P4	0.94	(0.93 to 0.94)	0.94	(0.93 to 0.95)	0.002	0.38
	A2-T6 & T6-O2	0.94	(0.94 to 0.95)	0.94	(0.93 to 0.95)	-0.001	0.17
	C3-P3 & T5-O1	0.93	(0.92 to 0.93)	0.93	(0.93 to 0.94)	0.001	0.039
Beta	C4-P4 & T6-O2	0.93	(0.93 to 0.93)	0.93	(0.92 to 0.93)	0.001	0.83
	Fp1-F3 & A1-T5	0.93	(0.93 to 0.93)	0.93	(0.93 to 0.93)	0.001	0.50
	Fp1-F3 & C3-P3	0.94	(0.94 to 0.95)	0.94	(0.93 to 0.95)	-0.004	0.16
	Fp1-F3 & T5-O1	0.93	(0.92 to 0.93)	0.93	(0.92 to 0.93)	0.001	0.39
	Fp2-F4 & A2-T6	0.93	(0.93 to 0.93)	0.93	(0.93 to 0.94)	0.003	0.019
	Fp2-F4 & C4-P4	0.94	(0.93 to 0.94)	0.94	(0.93 to 0.95)	0.001	0.41
	Fp2-F4 & T6-O2	0.93	(0.93 to 0.93)	0.93	(0.92 to 0.93)	0.001	0.95
	A1-T5 & C3-P3	0.94	(0.93 to 0.94)	0.94	(0.93 to 0.94)	0.000	0.87
	A1-T5 & T5-O1	0.94	(0.94 to 0.95)	0.94	(0.94 to 0.95)	0.001	0.95
	A2-T6 & C4-P4	0.94	(0.93 to 0.94)	0.94	(0.93 to 0.94)	0.001	0.52
	A2-T6 & T6-O2	0.94	(0.94 to 0.95)	0.94	(0.93 to 0.95)	-0.003	0.11
	C3-P3 & T5-O1	0.93	(0.93 to 0.93)	0.93	(0.92 to 0.93)	0.002	0.29
Gamma	C4-P4 & T6-O2	0.93	(0.92 to 0.93)	0.93	(0.92 to 0.93)	0.001	0.84
	Fp1-F3 & A1-T5	0.93	(0.93 to 0.93)	0.93	(0.93 to 0.94)	0.004	0.028
	Fp1-F3 & C3-P3	0.93	(0.93 to 0.94)	0.93	(0.93 to 0.94)	-0.001	0.90
	Fp1-F3 & T5-O1	0.93	(0.92 to 0.93)	0.93	(0.93 to 0.93)	0.000	0.87
	Fp2-F4 & A2-T6	0.93	(0.93 to 0.93)	0.93	(0.93 to 0.94)	0.001	0.42
	Fp2-F4 & C4-P4	0.93	(0.93 to 0.94)	0.93	(0.93 to 0.94)	0.001	0.74
	Fp2-F4 & T6-O2	0.93	(0.93 to 0.93)	0.93	(0.92 to 0.93)	0.001	0.68
	REM Interhemispherical Coherence						
	A1-T5 & A2-T6	0.87	(0.85 to 0.90)	0.88	(0.85 to 0.90)	0.009	0.64
Delta 1	C3-P3 & C4-P4	0.91	(0.89 to 0.93)	0.90	(0.88 to 0.93)	-0.010	0.50
	F3-C3 & F4-C4	0.89	(0.87 to 0.91)	0.89	(0.87 to 0.91)	-0.006	0.34
	F7-A1 & F8-A2	0.91	(0.87 to 0.93)	0.90	(0.86 to 0.93)	-0.010	0.39

	F7-T3 & F8-T4	0.86	(0.84 to 0.88)	0.86	(0.83 to 0.88)	-0.007	0.39
	Fp1-F3 & Fp2-F4	0.91	(0.90 to 0.94)	0.91	(0.89 to 0.94)	-0.002	0.83
	Fp1-F7 & Fp2-F8	0.88	(0.86 to 0.90)	0.88	(0.86 to 0.90)	0.004	0.51
	P3-O1 & P4-O2	0.91	(0.87 to 0.93)	0.89	(0.85 to 0.91)	-0.014	0.029
	T3-T5 & T4-T6	0.87	(0.85 to 0.88)	0.88	(0.85 to 0.90)	0.015	0.033
	T5-O1 & T6-O2	0.89	(0.85 to 0.91)	0.87	(0.84 to 0.91)	-0.017	0.38
	A1-T5 & A2-T6	0.88	(0.86 to 0.89)	0.88	(0.86 to 0.90)	0.005	0.76
	C3-P3 & C4-P4	0.93	(0.91 to 0.95)	0.93	(0.90 to 0.94)	-0.004	0.29
	F3-C3 & F4-C4	0.90	(0.87 to 0.92)	0.90	(0.87 to 0.91)	-0.007	0.31
	F7-A1 & F8-A2	0.90	(0.88 to 0.91)	0.89	(0.87 to 0.92)	-0.006	0.56
Delta 2	F7-T3 & F8-T4	0.86	(0.84 to 0.88)	0.87	(0.85 to 0.89)	0.008	0.15
	Fp1-F3 & Fp2-F4	0.91	(0.89 to 0.93)	0.91	(0.90 to 0.94)	-0.002	0.69
	Fp1-F7 & Fp2-F8	0.87	(0.85 to 0.90)	0.89	(0.87 to 0.90)	0.017	0.050
	P3-O1 & P4-O2	0.89	(0.86 to 0.92)	0.88	(0.86 to 0.91)	-0.015	0.30
	T3-T5 & T4-T6	0.87	(0.86 to 0.89)	0.88	(0.86 to 0.90)	0.003	0.38
	T5-O1 & T6-O2	0.88	(0.85 to 0.91)	0.88	(0.85 to 0.90)	-0.004	0.28
	A1-T5 & A2-T6	0.90	(0.89 to 0.91)	0.91	(0.89 to 0.93)	0.008	0.43
	C3-P3 & C4-P4	0.93	(0.91 to 0.95)	0.94	(0.91 to 0.95)	0.005	0.91
	F3-C3 & F4-C4	0.89	(0.88 to 0.91)	0.89	(0.87 to 0.91)	-0.005	0.16
	F7-A1 & F8-A2	0.91	(0.90 to 0.92)	0.91	(0.89 to 0.92)	-0.002	0.54
Theta	F7-T3 & F8-T4	0.89	(0.87 to 0.91)	0.88	(0.87 to 0.90)	-0.005	0.50
	Fp1-F3 & Fp2-F4	0.89	(0.88 to 0.91)	0.89	(0.88 to 0.91)	-0.002	0.81
	Fp1-F7 & Fp2-F8	0.90	(0.88 to 0.91)	0.90	(0.88 to 0.92)	0.000	0.49
	P3-O1 & P4-O2	0.90	(0.88 to 0.91)	0.89	(0.88 to 0.92)	-0.006	0.47
	T3-T5 & T4-T6	0.89	(0.88 to 0.91)	0.88	(0.87 to 0.92)	-0.004	0.90
	T5-O1 & T6-O2	0.90	(0.88 to 0.92)	0.89	(0.87 to 0.91)	-0.005	0.24
	A1-T5 & A2-T6	0.90	(0.88 to 0.91)	0.90	(0.89 to 0.92)	0.003	0.10
	C3-P3 & C4-P4	0.91	(0.89 to 0.93)	0.91	(0.89 to 0.93)	-0.003	0.94
	F3-C3 & F4-C4	0.88	(0.87 to 0.90)	0.89	(0.88 to 0.90)	0.003	0.54
	F7-A1 & F8-A2	0.89	(0.88 to 0.91)	0.89	(0.87 to 0.91)	0.005	0.52
Alpha	F7-T3 & F8-T4	0.88	(0.87 to 0.89)	0.88	(0.87 to 0.89)	0.001	0.74
	Fp1-F3 & Fp2-F4	0.89	(0.87 to 0.91)	0.89	(0.88 to 0.91)	0.002	0.64
	Fp1-F7 & Fp2-F8	0.89	(0.87 to 0.90)	0.90	(0.88 to 0.91)	0.010	0.15
	P3-O1 & P4-O2	0.89	(0.88 to 0.91)	0.89	(0.87 to 0.90)	-0.009	0.12
	T3-T5 & T4-T6	0.88	(0.87 to 0.90)	0.88	(0.87 to 0.90)	-0.003	0.58
	T5-O1 & T6-O2	0.89	(0.88 to 0.91)	0.89	(0.87 to 0.91)	0.003	0.77
	A1-T5 & A2-T6	0.87	(0.86 to 0.90)	0.90	(0.87 to 0.92)	0.029	0.003
	C3-P3 & C4-P4	0.88	(0.87 to 0.90)	0.89	(0.87 to 0.91)	0.004	0.29
	F3-C3 & F4-C4	0.86	(0.85 to 0.88)	0.86	(0.84 to 0.87)	-0.008	0.11
Sigma	F7-A1 & F8-A2	0.88	(0.85 to 0.90)	0.87	(0.85 to 0.90)	-0.002	0.91
	F7-T3 & F8-T4	0.86	(0.85 to 0.88)	0.86	(0.84 to 0.88)	0.000	0.59
	Fp1-F3 & Fp2-F4	0.87	(0.86 to 0.89)	0.89	(0.87 to 0.90)	0.015	0.003
	Fp1-F7 & Fp2-F8	0.86	(0.85 to 0.88)	0.87	(0.85 to 0.89)	0.008	0.26

Beta	P3-O1 & P4-O2	0.88	(0.86 to 0.89)	0.87	(0.85 to 0.88)	-0.005	0.34
	T3-T5 & T4-T6	0.86	(0.85 to 0.88)	0.87	(0.85 to 0.88)	0.007	0.90
	T5-O1 & T6-O2	0.87	(0.85 to 0.89)	0.86	(0.84 to 0.90)	-0.004	0.63
	A1-T5 & A2-T6	0.95	(0.94 to 0.96)	0.96	(0.94 to 0.97)	0.010	0.010
	C3-P3 & C4-P4	0.94	(0.93 to 0.95)	0.94	(0.93 to 0.95)	-0.005	0.13
	F3-C3 & F4-C4	0.94	(0.93 to 0.95)	0.94	(0.93 to 0.95)	0.000	0.58
	F7-A1 & F8-A2	0.94	(0.93 to 0.95)	0.95	(0.93 to 0.96)	0.004	0.55
	F7-T3 & F8-T4	0.94	(0.93 to 0.95)	0.94	(0.93 to 0.94)	-0.002	0.18
	Fp1-F3 & Fp2-F4	0.94	(0.93 to 0.95)	0.95	(0.94 to 0.95)	0.006	0.029
	Fp1-F7 & Fp2-F8	0.94	(0.93 to 0.95)	0.94	(0.93 to 0.95)	0.000	0.79
	P3-O1 & P4-O2	0.94	(0.94 to 0.95)	0.94	(0.93 to 0.95)	-0.003	0.07
	T3-T5 & T4-T6	0.94	(0.93 to 0.95)	0.94	(0.93 to 0.95)	0.001	0.21
	T5-O1 & T6-O2	0.94	(0.93 to 0.94)	0.94	(0.93 to 0.95)	0.002	0.27
	A1-T5 & A2-T6	0.94	(0.94 to 0.95)	0.95	(0.94 to 0.96)	0.006	0.12
	C3-P3 & C4-P4	0.93	(0.93 to 0.94)	0.94	(0.93 to 0.94)	0.001	0.47
	F3-C3 & F4-C4	0.94	(0.93 to 0.94)	0.93	(0.93 to 0.95)	-0.003	0.53
Gamma	F7-A1 & F8-A2	0.94	(0.93 to 0.95)	0.94	(0.94 to 0.95)	0.005	0.19
	F7-T3 & F8-T4	0.94	(0.93 to 0.95)	0.94	(0.93 to 0.94)	-0.003	0.10
	Fp1-F3 & Fp2-F4	0.94	(0.93 to 0.95)	0.95	(0.93 to 0.95)	0.007	0.16
	Fp1-F7 & Fp2-F8	0.94	(0.94 to 0.95)	0.94	(0.94 to 0.95)	0.000	0.71
	P3-O1 & P4-O2	0.94	(0.93 to 0.95)	0.94	(0.93 to 0.95)	-0.002	0.28
	T3-T5 & T4-T6	0.94	(0.93 to 0.94)	0.93	(0.93 to 0.94)	-0.005	0.18
	T5-O1 & T6-O2	0.94	(0.93 to 0.94)	0.94	(0.92 to 0.95)	0.000	0.82
	REM Intrahemispherical Coherence						
Delta 1	A1-T5 & C3-P3	0.88	(0.86 to 0.89)	0.89	(0.87 to 0.92)	0.014	0.034
	A1-T5 & T5-O1	0.84	(0.81 to 0.87)	0.84	(0.80 to 0.87)	0.000	0.74
	A2-T6 & C4-P4	0.87	(0.85 to 0.90)	0.87	(0.84 to 0.90)	0.001	0.70
	A2-T6 & T6-O2	0.84	(0.82 to 0.87)	0.83	(0.81 to 0.85)	-0.016	0.028
	C3-P3 & T5-O1	0.82	(0.79 to 0.84)	0.82	(0.79 to 0.84)	0.005	0.70
	C4-P4 & T6-O2	0.82	(0.79 to 0.85)	0.82	(0.80 to 0.84)	0.000	0.86
	Fp1-F3 & A1-T5	0.81	(0.79 to 0.84)	0.82	(0.80 to 0.85)	0.009	0.09
	Fp1-F3 & C3-P3	0.84	(0.82 to 0.86)	0.83	(0.81 to 0.86)	-0.005	0.50
	Fp1-F3 & T5-O1	0.81	(0.79 to 0.84)	0.81	(0.79 to 0.84)	0.002	0.79
	Fp2-F4 & A2-T6	0.81	(0.79 to 0.84)	0.83	(0.79 to 0.85)	0.014	0.19
	Fp2-F4 & C4-P4	0.84	(0.82 to 0.86)	0.85	(0.82 to 0.87)	0.004	0.37
	Fp2-F4 & T6-O2	0.80	(0.41 to 0.82)	0.81	(0.39 to 0.85)	0.012	0.43
Delta 2	A1-T5 & C3-P3	0.91	(0.89 to 0.92)	0.93	(0.91 to 0.94)	0.020	0.002
	A1-T5 & T5-O1	0.85	(0.82 to 0.88)	0.86	(0.83 to 0.88)	0.007	0.60
	A2-T6 & C4-P4	0.91	(0.89 to 0.92)	0.92	(0.89 to 0.94)	0.015	0.036
	A2-T6 & T6-O2	0.86	(0.83 to 0.88)	0.84	(0.82 to 0.86)	-0.019	0.06
	C3-P3 & T5-O1	0.85	(0.83 to 0.87)	0.84	(0.82 to 0.86)	-0.007	0.69
	C4-P4 & T6-O2	0.85	(0.82 to 0.87)	0.84	(0.82 to 0.86)	-0.013	0.13

	Fp1-F3 & A1-T5	0.83	(0.81 to 0.84)	0.83	(0.80 to 0.85)	0.003	0.65
	Fp1-F3 & C3-P3	0.87	(0.86 to 0.89)	0.88	(0.85 to 0.91)	0.004	0.51
	Fp1-F3 & T5-O1	0.82	(0.81 to 0.84)	0.82	(0.81 to 0.84)	0.004	0.68
	Fp2-F4 & A2-T6	0.83	(0.82 to 0.85)	0.83	(0.82 to 0.86)	0.005	0.66
	Fp2-F4 & C4-P4	0.88	(0.86 to 0.91)	0.87	(0.86 to 0.89)	-0.006	0.40
	Fp2-F4 & T6-O2	0.82	(0.80 to 0.84)	0.82	(0.80 to 0.83)	-0.009	0.16
	A1-T5 & C3-P3	0.93	(0.92 to 0.94)	0.94	(0.92 to 0.95)	0.002	0.56
	A1-T5 & T5-O1	0.90	(0.88 to 0.91)	0.88	(0.87 to 0.91)	-0.014	0.11
	A2-T6 & C4-P4	0.93	(0.92 to 0.94)	0.93	(0.92 to 0.95)	0.002	0.24
	A2-T6 & T6-O2	0.90	(0.88 to 0.92)	0.88	(0.87 to 0.90)	-0.015	0.008
	C3-P3 & T5-O1	0.88	(0.86 to 0.89)	0.87	(0.86 to 0.88)	-0.008	0.07
Theta	C4-P4 & T6-O2	0.88	(0.87 to 0.89)	0.88	(0.86 to 0.89)	-0.007	0.17
	Fp1-F3 & A1-T5	0.88	(0.86 to 0.89)	0.88	(0.86 to 0.90)	-0.001	0.88
	Fp1-F3 & C3-P3	0.93	(0.92 to 0.94)	0.93	(0.91 to 0.94)	0.000	0.91
	Fp1-F3 & T5-O1	0.87	(0.86 to 0.88)	0.87	(0.85 to 0.88)	-0.002	0.46
	Fp2-F4 & A2-T6	0.89	(0.87 to 0.90)	0.88	(0.86 to 0.89)	-0.011	0.023
	Fp2-F4 & C4-P4	0.93	(0.91 to 0.94)	0.92	(0.91 to 0.94)	-0.007	0.30
	Fp2-F4 & T6-O2	0.87	(0.86 to 0.88)	0.87	(0.86 to 0.89)	-0.002	0.98
	A1-T5 & C3-P3	0.91	(0.89 to 0.92)	0.91	(0.89 to 0.93)	-0.002	0.92
	A1-T5 & T5-O1	0.89	(0.88 to 0.90)	0.88	(0.86 to 0.90)	-0.006	0.06
	A2-T6 & C4-P4	0.90	(0.89 to 0.92)	0.91	(0.89 to 0.92)	0.007	0.60
	A2-T6 & T6-O2	0.89	(0.87 to 0.91)	0.88	(0.87 to 0.90)	-0.007	0.19
	C3-P3 & T5-O1	0.87	(0.86 to 0.88)	0.87	(0.85 to 0.88)	-0.002	0.53
Alpha	C4-P4 & T6-O2	0.87	(0.86 to 0.88)	0.87	(0.85 to 0.88)	-0.001	0.38
	Fp1-F3 & A1-T5	0.87	(0.86 to 0.88)	0.88	(0.86 to 0.89)	0.006	0.22
	Fp1-F3 & C3-P3	0.91	(0.89 to 0.92)	0.90	(0.89 to 0.93)	-0.003	0.83
	Fp1-F3 & T5-O1	0.87	(0.86 to 0.88)	0.87	(0.86 to 0.89)	0.007	0.27
	Fp2-F4 & A2-T6	0.87	(0.86 to 0.89)	0.87	(0.86 to 0.89)	0.003	0.66
	Fp2-F4 & C4-P4	0.90	(0.89 to 0.92)	0.90	(0.89 to 0.92)	0.001	0.62
	Fp2-F4 & T6-O2	0.87	(0.85 to 0.88)	0.88	(0.85 to 0.89)	0.009	0.16
	A1-T5 & C3-P3	0.88	(0.86 to 0.89)	0.88	(0.86 to 0.89)	0.004	0.41
	A1-T5 & T5-O1	0.87	(0.85 to 0.88)	0.87	(0.85 to 0.88)	-0.004	0.51
	A2-T6 & C4-P4	0.87	(0.86 to 0.89)	0.88	(0.86 to 0.89)	0.004	0.45
	A2-T6 & T6-O2	0.87	(0.85 to 0.89)	0.87	(0.85 to 0.89)	0.002	0.71
	C3-P3 & T5-O1	0.85	(0.84 to 0.86)	0.85	(0.84 to 0.86)	0.002	0.41
Sigma	C4-P4 & T6-O2	0.85	(0.83 to 0.87)	0.86	(0.84 to 0.87)	0.004	0.14
	Fp1-F3 & A1-T5	0.86	(0.84 to 0.87)	0.86	(0.85 to 0.87)	0.001	0.94
	Fp1-F3 & C3-P3	0.88	(0.86 to 0.89)	0.88	(0.86 to 0.89)	-0.003	0.45
	Fp1-F3 & T5-O1	0.85	(0.84 to 0.87)	0.85	(0.84 to 0.87)	-0.002	0.86
	Fp2-F4 & A2-T6	0.85	(0.83 to 0.87)	0.86	(0.84 to 0.89)	0.012	0.009
	Fp2-F4 & C4-P4	0.88	(0.86 to 0.89)	0.87	(0.86 to 0.89)	-0.011	0.45
	Fp2-F4 & T6-O2	0.85	(0.83 to 0.87)	0.85	(0.84 to 0.88)	0.001	0.46
Beta	A1-T5 & C3-P3	0.94	(0.94 to 0.95)	0.94	(0.93 to 0.94)	-0.003	0.08

	A1-T5 & T5-O1	0.94	(0.93 to 0.95)	0.94	(0.93 to 0.95)	-0.007	0.19
	A2-T6 & C4-P4	0.94	(0.93 to 0.95)	0.94	(0.93 to 0.95)	0.001	0.62
	A2-T6 & T6-O2	0.95	(0.94 to 0.96)	0.95	(0.94 to 0.96)	0.001	0.51
	C3-P3 & T5-O1	0.93	(0.93 to 0.94)	0.93	(0.93 to 0.94)	0.003	0.22
	C4-P4 & T6-O2	0.93	(0.92 to 0.94)	0.93	(0.93 to 0.94)	0.001	0.09
	Fp1-F3 & A1-T5	0.94	(0.93 to 0.94)	0.94	(0.92 to 0.94)	0.000	0.85
	Fp1-F3 & C3-P3	0.94	(0.94 to 0.95)	0.94	(0.93 to 0.95)	-0.004	0.18
	Fp1-F3 & T5-O1	0.93	(0.92 to 0.94)	0.94	(0.93 to 0.94)	0.006	0.037
	Fp2-F4 & A2-T6	0.93	(0.93 to 0.94)	0.94	(0.93 to 0.94)	0.002	0.32
	Fp2-F4 & C4-P4	0.94	(0.93 to 0.95)	0.94	(0.93 to 0.95)	-0.002	0.93
	Fp2-F4 & T6-O2	0.93	(0.93 to 0.94)	0.93	(0.92 to 0.94)	-0.001	0.48
	A1-T5 & C3-P3	0.94	(0.93 to 0.95)	0.94	(0.93 to 0.95)	0.003	0.26
	A1-T5 & T5-O1	0.95	(0.94 to 0.96)	0.95	(0.94 to 0.96)	-0.004	0.27
	A2-T6 & C4-P4	0.94	(0.93 to 0.94)	0.94	(0.93 to 0.95)	-0.001	0.89
	A2-T6 & T6-O2	0.95	(0.95 to 0.96)	0.95	(0.94 to 0.96)	-0.008	0.005
	C3-P3 & T5-O1	0.93	(0.92 to 0.94)	0.93	(0.92 to 0.94)	0.002	0.81
Gamma	C4-P4 & T6-O2	0.93	(0.93 to 0.94)	0.93	(0.92 to 0.94)	-0.004	0.23
	Fp1-F3 & A1-T5	0.93	(0.92 to 0.94)	0.93	(0.93 to 0.94)	-0.001	0.45
	Fp1-F3 & C3-P3	0.94	(0.93 to 0.94)	0.93	(0.93 to 0.94)	-0.005	0.51
	Fp1-F3 & T5-O1	0.93	(0.92 to 0.94)	0.93	(0.92 to 0.94)	-0.001	0.84
	Fp2-F4 & A2-T6	0.94	(0.93 to 0.94)	0.93	(0.92 to 0.94)	-0.003	0.43
	Fp2-F4 & C4-P4	0.94	(0.93 to 0.94)	0.94	(0.93 to 0.95)	0.002	0.23
	Fp2-F4 & T6-O2	0.93	(0.92 to 0.94)	0.94	(0.93 to 0.94)	0.008	0.023

[d]From Mann-Whitney U Test

Supplementary Table 2a - Unadjusted analysis of the correlation between sleep parameters and neurodevelopmental outcome at 4 and 18 months, respectively, n=108

	Frequency Bands	Channels	Neurodevelopmental assessment at 18 months (Griffiths III)											
			Subscale A		Subscale B		Subscale C		Subscale D		Subscale E		General development	
			r_s	p-value[a]	r_s	p-value[a]	r_s	p-value[a]	r_s	p-value[a]	r_s	p-value[a]	r_s	p-value[a]
Macrostructure														
Duration of N2			0.004	0.97	-0.12	0.21	-0.06	0.55	-0.14	0.15	0.13	0.17	-0.02	0.80
Spectral power														
NREM Absolute	Delta 1		0.05	0.62	0.11	0.26	0.06	0.54	0.26	0.007*	0.19	0.053	0.14	0.14
NREM Absolute	Delta 2		0.010	0.92	0.20	0.036*	0.04	0.65	0.25	0.008*	0.16	0.098	0.19	0.053
Coherence														
NREM Interhemispherical														
	Delta 1	P3-O1 & P4-O2	0.03	0.73	0.02	0.84	0.04	0.66	-0.006	0.95	0.19	0.051	0.07	0.47
	Delta 2	P3-O1 & P4-O2	0.06	0.52	0.04	0.72	0.04	0.65	-0.02	0.83	0.19	0.045*	0.08	0.43
	Theta	P3-O1 & P4-O2	0.006	0.95	0.007	0.94	0.01	0.91	-0.010	0.92	0.15	0.12	0.04	0.66
	Alpha	P3-O1 & P4-O2	0.003	0.98	-0.08	0.42	0.01	0.89	-0.09	0.35	0.08	0.39	0.008	0.93
	Sigma	P3-O1 & P4-O2	0.11	0.27	0.04	0.70	0.09	0.34	-0.03	0.75	0.09	0.37	0.09	0.34
	Beta	P3-O1 & P4-O2	0.13	0.17	-0.02	0.81	0.12	0.23	-0.07	0.49	0.18	0.063	0.10	0.33
	Beta	A1-T5 & A2-T6	-0.06	0.56	-0.05	0.58	-0.09	0.33	-0.08	0.41	-0.005	0.96		
	Gamma	P3-O1 & P4-O2	0.19	0.049*	0.05	0.63	0.09	0.38	-0.001	0.99	0.26	0.007*	0.20	0.037*
	Gamma	A1-T5 & A2-T6	-0.01	0.89	0.04	0.70	-0.005	0.96	-0.003	0.98	-0.04	0.72	-0.03	0.76
NREM Intrahemispherical														
	Delta 1	Fp2-F4 & T6-O2	0.01	0.91	0.10	0.29	-0.03	0.74	-0.04	0.68	-0.17	0.085	-0.05	0.62
	Delta 2	Fp2-F4 & T6-O2	-0.09	0.36	-0.04	0.66	-0.14	0.15	-0.13	0.17	-0.19	0.055	-0.12	0.23
	Alpha	Fp2-F4 & T6-O2	-0.12	0.21	0.04	0.70	-0.14	0.15	-0.07	0.45	-0.19	0.053	-0.05	0.61
	Sigma	Fp1-F3 & A1-T5	-0.03	0.77	-0.02	0.85	0.03	0.79	0.08	0.40	0.08	0.40	0.03	0.76
	Beta	C3-P3 & T5-O1	-0.008	0.94	0.08	0.40	0.07	0.51	0.10	0.30	-0.05	0.60	0.02	0.85
	Beta	Fp2-F4 & A2-T6	0.08	0.39	-0.04	0.65	-0.08	0.42	-0.16	0.11	-0.05	0.62	-0.01	0.91
	Gamma	Fp1-F3 & A1-T5	-0.19	0.046*	-0.07	0.46	-0.10	0.33	-0.07	0.45	-0.005	0.96	-0.14	0.15
REM Interhemispherical														
	Delta 1	P3-O1 & P4-O2	-0.001	0.99	0.14	0.15	0.15	0.13	0.08	0.42	0.26	0.006*	0.12	0.24
	Delta 1	T3-T5 & T4-T6	-0.09	0.37	-0.19	0.053	-0.23	0.017*	-0.33	<0.001*	-0.06	0.58	-0.24	0.014*
	Delta 2	Fp1-F7 & Fp2-F8	-0.01	0.90	0.05	0.64	-0.02	0.83	0.02	0.88	-0.04	0.65	-0.03	0.77
	Sigma	Fp1-F3 & Fp2-F4	-0.07	0.49	-0.10	0.32	-0.17	0.084	-0.11	0.25	-0.08	0.40	-0.15	0.13
	Sigma	A1-T5 & A2-T6	-0.13	0.19	-0.04	0.66	-0.18	0.07	-0.16	0.11	0.02	0.80	-0.15	0.12
	Beta	Fp1-F3 & Fp2-F4	-0.17	0.086	-0.06	0.53	-0.16	0.10	-0.22	0.026*	-0.04	0.71	-0.18	0.066

	Beta	A1-T5 & A2-T6	0.02	0.87	0.02	0.87	0.06	0.54	0.04	0.68	0.13	0.20	0.01	0.89
REM Intrahemispherical														
	Delta 1	A2-T6 & T6-O2	-0.05	0.64	-0.03	0.75	-0.04	0.68	-0.10	0.31	-0.03	0.79	-0.13	0.18
	Delta 1	A1-T5 & C3-P3	0.12	0.24	0.03	0.77	-0.02	0.84	-0.10	0.29	-0.15	0.13	-0.04	0.66
	Delta 2	A2-T6 & C4-P4	0.02	0.87	0.02	0.84	-0.02	0.88	-0.09	0.35	-0.17	0.083	-0.05	0.64
	Delta 2	A1-T5 & C3-P3	-0.05	0.62	-0.06	0.52	-0.14	0.16	-0.20	0.034*	-0.37	<0.001*	-0.21	0.031*
	Theta	A2-T6 & T6-O2	0.02	0.82	-0.05	0.60	-0.001	0.99	-0.14	0.15	0.02	0.81	-0.08	0.39
	Theta	Fp2-F4 & A2-T6	0.08	0.41	0.02	0.82	0.02	0.84	0.13	0.18	0.09	0.38	0.08	0.39
	Sigma	Fp2-F4 & A2-T6	-0.02	0.85	0.04	0.65	-0.05	0.60	-0.14	0.16	-0.15	0.11	-0.07	0.47
	Beta	Fp1-F3 & T5-O1	-0.004	0.97	0.06	0.53	-0.13	0.19	0.07	0.48	-0.14	0.15	-0.05	0.59
	Gamma	A2-T6 & T6-O2	-0.03	0.75	0.004	0.97	0.002	0.99	-0.05	0.63	-0.03	0.72	-0.10	0.32
	Gamma	Fp2-F4 & T6-O2	0.02	0.84	-0.09	0.38	-0.19	0.047*	-0.22	0.022*	-0.23	0.016*	-0.21	0.029*

Statistically significant differences ($p < 0.05$) marked with an asterisk. [a]Results from Spearman's correlation (r_s).

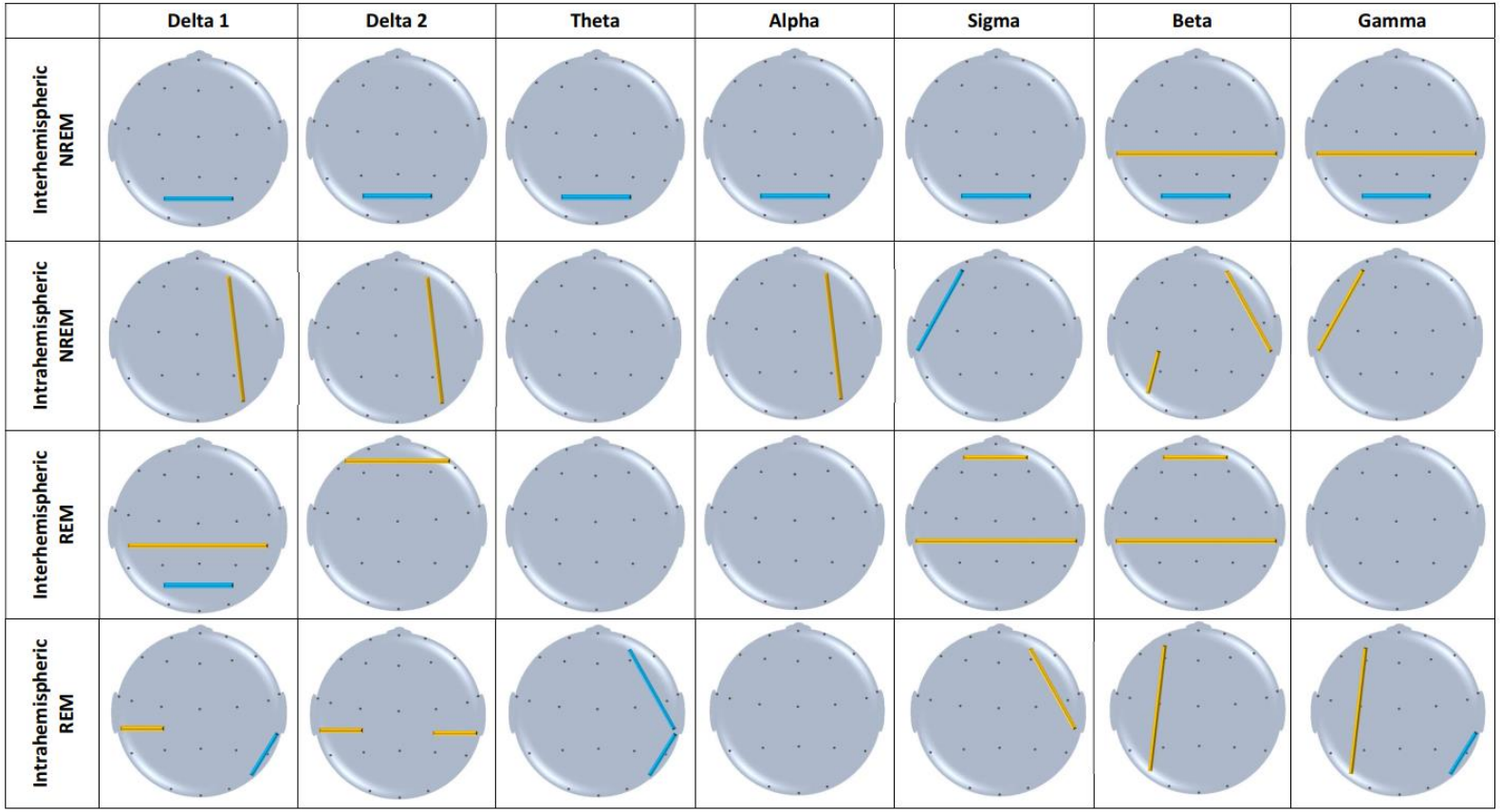
Supplementary Table 2b - Correlation between sleep parameters and neurodevelopmental outcome at 4 and 18 months, respectively, adjusting for sex and multiple births, n=108.

	Frequency Bands	Channels	Neurodevelopmental assessment at 18 months (Griffiths III)											
			Subscale A		Subscale B		Subscale C		Subscale D		Subscale E		General development	
			r_s	p-value[a]	r_s	p-value[a]	r_s	p-value[a]	r_s	p-value[a]	r_s	p-value[a]	r_s	p-value[a]
Macrostructure														
Duration of N2			-0.008	0.93	-0.12	0.23	-0.09	0.36	-0.14	0.15	0.10	0.29	-0.03	0.78
Spectral power														
NREM Absolute	Delta 1		0.05	0.59	0.09	0.35	0.07	0.51	0.25	0.009*	0.21	0.028*	0.14	0.15
NREM Absolute	Delta 2		0.02	0.87	0.18	0.064	0.05	0.61	0.25	0.011*	0.20	0.036*	0.19	0.058
Coherence														
NREM Interhemispherical														
	Delta 1	P3-O1 & P4-O2	0.03	0.77	0.02	0.87	0.03	0.74	-0.009	0.93	0.18	0.064	0.07	0.51
	Delta 2	P3-O1 & P4-O2	0.06	0.54	0.06	0.57	0.05	0.63	-0.01	0.92	0.18	0.058	0.09	0.37
	Theta	P3-O1 & P4-O2	0.01	0.91	0.05	0.59	0.04	0.70	0.02	0.87	0.15	0.13	0.07	0.45
	Alpha	P3-O1 & P4-O2	0.001	0.99	-0.07	0.47	0.02	0.88	-0.09	0.38	0.08	0.44	0.01	0.89
	Sigma	P3-O1 & P4-O2	0.10	0.32	0.04	0.71	0.07	0.51	-0.04	0.72	0.06	0.56	0.08	0.40
	Beta	P3-O1 & P4-O2	0.13	0.19	-0.02	0.84	0.10	0.29	-0.07	0.49	0.16	0.10	0.10	0.33
	Beta	A1-T5 & A2-T6	-0.05	0.63	-0.009	0.93	-0.06	0.54	-0.06	0.58	0.003	0.97	-0.08	0.43
	Gamma	P3-O1 & P4-O2	0.19	0.054	0.06	0.54	0.08	0.41	0.004	0.97	0.25	0.011*	0.21	0.030*
	Gamma	A1-T5 & A2-T6	-0.02	0.86	0.03	0.74	-0.02	0.88	-0.007	0.94	-0.04	0.68	-0.04	0.70
NREM Intrahemispherical														
	Delta 1	Fp2-F4 & T6-O2	0.005	0.96	0.09	0.35	-0.06	0.57	-0.05	0.60	-0.18	0.067	-0.07	0.50
	Delta 2	Fp2-F4 & T6-O2	-0.09	0.36	-0.07	0.46	-0.15	0.12	-0.16	0.11	-0.17	0.075	-0.14	0.17
	Alpha	Fp2-F4 & T6-O2	-0.13	0.20	0.02	0.81	-0.16	0.11	-0.08	0.40	-0.19	0.053	-0.06	0.53
	Sigma	Fp1-F3 & A1-T5	-0.04	0.67	-0.02	0.81	-0.005	0.96	0.08	0.39	0.05	0.59	0.02	0.86
	Beta	C3-P3 & T5-O1	-0.002	0.98	0.07	0.45	0.08	0.43	0.10	0.32	-0.03	0.74	0.02	0.87
	Beta	Fp2-F4 & A2-T6	0.11	0.27	0.04	0.71	-0.007	0.95	-0.11	0.25	-0.03	0.79	0.06	0.57
	Gamma	Fp1-F3 & A1-T5	-0.20	0.042*	-0.09	0.35	-0.12	0.24	-0.08	0.39	-0.009	0.93	-0.16	0.11
REM Interhemispherical														
	Delta 1	P3-O1 & P4-O2	-0.02	0.88	0.14	0.17	0.12	0.23	0.07	0.46	0.24	0.013*	0.10	0.32
	Delta 1	T3-T5 & T4-T6	-0.09	0.38	-0.18	0.065	-0.23	0.019*	-0.33	0.001*	-0.05	0.58	-0.23	0.017*
	Delta 2	Fp1-F7 & Fp2-F8	-0.006	0.95	0.04	0.68	-0.01	0.91	0.01	0.91	-0.03	0.78	-0.03	0.78
	Sigma	Fp1-F3 & Fp2-F4	-0.06	0.56	-0.07	0.51	-0.14	0.17	-0.09	0.35	-0.07	0.48	-0.12	0.22
	Sigma	A1-T5 & A2-T6	-0.12	0.23	-0.01	0.89	-0.15	0.13	-0.14	0.15	0.04	0.69	-0.13	0.18

	Beta	Fp1-F3 & Fp2-F4	-0.15	0.12	-0.003	0.97	-0.11	0.25	-0.19	0.056	-0.02	0.81	-0.14	0.16
	Beta	A1-T5 & A2-T6	0.03	0.80	0.04	0.71	0.09	0.37	0.05	0.60	0.14	0.15	0.03	0.74
REM Intrahemispherical														
	Delta 1	A2-T6 & T6-O2	-0.06	0.56	-0.06	0.57	-0.08	0.45	-0.12	0.24	-0.05	0.65	-0.16	0.10
	Delta 1	A1-T5 & C3-P3	0.11	0.28	-0.002	0.99	-0.06	0.56	-0.12	0.21	-0.17	0.088	-0.07	0.46
	Delta 2	A2-T6 & C4-P4	0.01	0.90	0.02	0.81	-0.02	0.81	-0.09	0.35	-0.18	0.065	-0.05	0.61
	Delta 2	A1-T5 & C3-P3	-0.06	0.58	-0.11	0.25	-0.17	0.080	-0.24	0.014*	-0.38	<0.001*	-0.25	0.010*
	Theta	A2-T6 & T6-O2	0.02	0.88	-0.03	0.73	-0.02	0.88	-0.14	0.16	-0.005	0.96	-0.09	0.38
	Theta	Fp2-F4 & A2-T6	0.07	0.50	-0.004	0.97	-0.02	0.84	0.12	0.22	0.06	0.52	0.06	0.55
	Sigma	Fp2-F4 & A2-T6	-0.01	0.92	0.04	0.69	-0.04	0.71	-0.15	0.14	-0.14	0.17	-0.07	0.48
	Beta	Fp1-F3 & T5-O1	0.001	0.99	0.07	0.45	-0.12	0.23	0.08	0.44	-0.13	0.18	-0.05	0.65
	Gamma	A2-T6 & T6-O2	-0.04	0.71	-0.001	0.99	-0.01	0.91	-0.05	0.61	-0.05	0.65	-0.11	0.28
	Gamma	Fp2-F4 & T6-O2	0.03	0.80	-0.08	0.42	-0.19	0.056	-0.22	0.025*	-0.23	0.019*	-0.21	0.033*

Statistically significant differences ($p < 0.05$) marked with an asterisk. [a]Results from Partial Spearman's correlation (r_s).

Appendix 10 – Supplementary figure from chapter 5



Supplementary Figure 1 - Coherence differences. Orange and blue lines correspond to higher and lower median coherence in the MLP group, respectively