Breathing and Brain Development in Hospitalised Neonates

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Abstract

An immature brain-respiratory control mechanism predisposes the neonate to increased risk of breathing interruptions, including apnoeas, and these may disrupt long-term brain development. Electroencephalography has reliably been used to assess immediate brain function and for prognostication. Given the interactions between the respiratory and nervous systems, including neuro-developmental complications, it is important to identify early the immediate effect of respiratory changes on brain function which might contribute to long-term outcomes. To do this, I conducted a systematic review (**Chapter 2)** summarizing the effect of periods of acute respiratory events and respiratory stimulants on EEG activity in neonates.

Variations exist in individual brain development trajectories modulated by genetics and the environment. These disparities may better explain neonatal apnoea risk than age, supporting personalised care and prognostication. In **Chapter 3**, I assessed the value of brain maturity as a marker to help clinicians identify neonates with abnormal brain development relative to their actual age and inform apnoea treatment decisions.

Finally, painful procedures are associated with apnoeas. Preterms undergo many painful procedures, including retinopathy of prematurity (ROP) screening, which coupled with the immature respiratory mechanisms and a vulnerable brain, increase the risk of complications. Swaddling has been shown to improve responses to painful procedures in neonates. To evaluate the effectiveness of a new swaddling device in reducing physiological instability, I compared changes in vital signs between neonates

swaddled with the new device and neonates receiving standard care during ROP screening (**Chapter 4**).

Overall, this thesis aims to inform neonatal care decisions by offering a comprehensive overview of the immediate interactions between neonatal breathing and brain function. It proposes brain biomarkers to detect the likelihood of apnoea in neonates and provides new insights into the effectiveness of a comfort measure for ROP screening. Ultimately, this thesis advocates for a more personalised approach to care.

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Abbreviations

aEEG	Amplitude-integrated Electroencephalography
AOP	Apnoea of Prematurity
BSID	Bayley Scales of Infant Development
BIO	Binocular Indirect Ophthalmoscopy
BA	Brain Age
BBD	Breathing and Brain Development
CNS	Central Nervous System
CLOCK	Circadian Locomotor Output Cycles Kaput
CI	Confidence Interval
CROSS	Consensus-Based Checklist for Reporting of Survey Studies
cEEG	Conventional Electroencephalography
CINAHL	Cumulative Index of Nursing and Allied Health Literature
ECG	Electrocardiography
EEG	Electroencephalography
EMG	Electromyography
GLMM	Generalized Linear Mixed Model
GA	Gestational Age
IP	Impedance Pneumography
IBI	Inter-Breath Interval
IQR	Interquartile range
JBI	Joanna Briggs Institute Critical Appraisal Tool
JRH	John Radcliffe Hospital

LMEM	Linear Mixed Effects Model
MATLAB	MATrix LABoratory
MAE	Mean Absolute Error
MeSH	Medical Subject Headings
NICU	Neonatal Intensive Care Unit
NIPI	Newborn Infant Pain Investigation
OUH	Oxford University Hospital
PCO_2	Partial Pressure of Carbon dioxide
PMA	Postmenstrual Age
PNA	Postnatal Age
PRISMA	Preferred Reporting Items for Systematic reviews and Meta-Analyses
QR code	Quick Response Code
RCT	Randomised Controlled Trial
ROP	Retinopathy of Prematurity
SAT	Spontaneous Activity Transient
SD	Standard Deviation
SMD	Standardized Mean Differences
SPSS	Statistical Package for the Social Sciences
SpO_2	Oxygen Saturation
UWFI	Ultra-Wide Field Imaging
WHO	World Health Organisation

1

General Introduction

1.1. Thesis motivation and aims

In neonates, both the respiratory and central nervous systems (CNS) are underdeveloped, and in particular, preterm neonates born < 37 completed weeks gestational age (GA; the period between conception and time of birth) are at high risk of breathing disruptions due to the immaturity of their brain-respiratory mechanisms [1]. The synergistic effect of brain and lung immaturity can lead to a severe neonatal respiratory complication known as apnoea, which is potentially life-threatening, causing breathing cessation of variable lengths with or without cardiac compromise [1,2]. The risk of apnoea is greatest in the extremely premature neonate, and virtually all neonates born < 28 weeks GA experience this complication. Apnoea susceptibility reduces with increasing age [3] and by term-corrected age, the risk is approximately 2% [4]. However, apnoea can persist beyond term gestation (≥ 37 completed weeks GA), especially in those born very premature [5]. Additionally, the developing brain of the neonate is more vulnerable to the effect of intermittent hypoxia caused by frequent apnoeas [6]. This can alter brain function, and early impairment of brain activity is known to negatively impact cortical neuronal plasticity and maturity [7,8]. Apnoea, especially when accompanied by desaturations, has been linked with impaired cerebral perfusion, brain injury [6,9–11], and poor neurodevelopmental outcomes later in life [12–14]. While it is challenging to separate the independent effect of apnoea from other

confounders on long-term neurodevelopment, Janvier et al (2004) in their work which focused on apnoea in very low birthweight infants adjusted for gestational age, sex, intrauterine growth restriction, intraventricular haemorrhage, periventricular leukomalacia, steroids, and maternal education. The authors showed that for each additional day with at least one apnoea, there was a significant increase in the odds of neurodevelopmental impairment**.** On follow-up at 3 years, 6% of the cohort had Bayley Scales of Infant Development (BSID) and Psychomotor Development Index scores of <70, which were suggestive of neurodevelopmental impairment. Significant functional delay (Vineland score < 70) was also reported in 17% of the neonates who had high apnoea burden [12]. In another study, intermittent hypoxia (oxygen saturation < 80%) from apnoea lasting ≥ 1 minute in extremely preterm neonates was independently associated with worse neurodevelopmental outcomes, including cognitive and motor impairments at 18 months corrected age. Infants with the highest apnoea burden had a 4 - 6 point decrease in their BSID [13], which corresponds to a small-to-moderate effect size (Cohen's d = 0.3 - 0.4).

Nevertheless, it remains unclear how long respiratory disruptions in the neonate can last without causing changes in brain activity and impairment in long-term brain development. While studies in animals [15–17], neonates [18–20] and adults [21,22] have described the possible interactions between the respiratory and nervous systems, the exact mechanism remains unclear. Key to understanding the potentially cyclical relationship between breathing and brain function is to first examine how age-related physiological respiratory changes impact brain function and how brain activity, in turn, influences breathing; and to compare changes in brain activity during pathological breathing such as apnoea.

Electroencephalography (EEG), a non-invasive, bedside tool is useful in assessing brain neurophysiology and pathology [23–25]. Serial EEG recordings from birth can detect the timing and nature of brain injury, which is promising, providing valuable information on short- and long-term neurodevelopmental outcomes [25]. Depending on the GA at birth, differences in developmental brain architecture, synaptic connectivity [26–28] and EEG activity exist [29,30]. These developmental features are less mature in the preterm compared with term neonate. Given the interaction between the respiratory system and the CNS, directly monitoring brain activity - an indicator of function during respiratory changes can provide a clearer understanding of the temporal connection between brain and respiratory dynamics. It could also be useful for personalised care, aiding clinical decisions by providing valuable insights into the neuroprotective mechanisms of respiratory medications used in apnoea management.

Finally, hospitalised neonates while on admission have many clinically required painful procedures [31]. One such intervention is an eye examination, called retinopathy of prematurity (ROP - a vascular proliferative disease of the retina) screening, which all preterm-born babies undergo. For my thesis, I included ROP screening as a model painful procedure in neonates because it is a routine component of standard care for all babies born prematurely, involving repeated eye examinations. This procedure is known to cause adverse complications including acute behavioural and physiological changes such as significant hypoxaemia and apnoea [32–35]. The combination of multiple invasive ROP screenings, combined with the inherent risk of the neonate to respiratory disturbances and an immature brain that is susceptible to injury from physiological instability makes ROP screening a relevant model for pain research.

Since ROP screening is already a necessary clinical procedure, it makes it a good proxy to ethically investigate neonatal pain and measures that could potentially limit the impact of pain without subjecting newborns to additional invasive procedures solely for research. To minimize procedural pain and its associated adverse physiological responses, numerous pharmacological [36,37] and non-pharmacological [38–41] modalities have been evaluated as comfort measures during ROP screening with varying levels of success. While pharmacological measures may not be ideal for pain control in all instances as they have the additional drawback of causing cardiorespiratory compromise, non-pharmacological approaches are increasingly being used either alone or in combination with other measures to minimize pain responses during ROP screening [42]. Recently, in the neonatal unit of the John Radcliffe Hospital (JRH), a new swaddling device, the Dandle® WRAP stretch, was introduced for babies having ROP screening. Evaluating the effectiveness of the Dandle® WRAP stretch as a comfort measure during the procedure will provide beneficial data on the efficacy of this new swaddling device for reducing physiological responses during preterm eye examinations.

The main aims of this thesis are to:

1. Conduct a systematic review on the immediate effects of normal respiration, acute respiratory events, and the use of respiratory stimulants on EEG activity in neonates 28 - 42 weeks postmenstrual age (PMA; time from conception onwards, calculated as the sum of gestational and postnatal age).
2. Examine how respiratory and apnoea rates change with PMA and EEG-derived brain measures, comparing their effectiveness in determining apnoea risk and as a guide for caffeine treatment in neonates.

3. Assess vital signs changes during a painful procedure (i.e., ROP screening), comparing neonates swaddled with the conventional swaddle and the new specialised Dandle® WRAP stretch, alongside staff opinion on the usefulness of the new swaddling device.

1.2. Thesis structure

This **Chapter (1)** gives a general overview of the thesis motivation and structure, a summary of neonatal respiration and brain development, the effect of apnoea on neurodevelopment and management strategies, and the physiological responses associated with ROP screening. Key concepts about neonatal brain activity monitoring, including methods of brain age and maturity estimation using EEG are described. These will provide the foundation for understanding the subsequent chapters of this thesis.

Chapter 2 details a systematic review collating evidence on the association between neonatal respiratory changes and EEG brain activity. The chapter will provide a comprehensive summary of all empirical studies, research gaps and highlight potential areas for further research on neonatal respiration and EEG activity.

Chapter 3 presents an exploratory study comparing how neonatal respiration co-varies with PMA and EEG-derived brain measures (brain age and maturity: calculated by combining age-specific functional EEG features) and assessing their value in determining apnoea-risk and need for treatment in neonates at high risk of breathing disruptions.

Chapter 4 presents a prospective study investigating whether neonates swaddled with the Dandle® WRAP stretch are more stable during and after the painful ROP screening compared with those swaddled using conventional swaddle. It will also describe the findings from a staff opinion survey on the usefulness of the Dandle® WRAP stretch as a comfort measure in neonates undergoing ROP screening.

Chapter 5 presents an overall summary of this thesis and recommendations for further investigations.

1.3. Personal motivation

As a neonatologist from Africa, my research interests are in neonatal neurological disorders, especially preventive and diagnostic approaches. My career goal is to become an expert in neonatal brain development, using simple brain monitoring tools to research neonatal neurological conditions and contribute to care. This interest stems from my recognition that neonates are particularly vulnerable to brain injury, and from my experiences working in Africa, most of the conditions that affect brain development are usually acquired and avertible.

In most developing countries, medical care is a privilege and the resources for immediate and long-term care, including rehabilitation services are limited. This reality has driven my curiosity to explore ways to effectively assess and monitor neonatal brain function for early detection of abnormalities and prognostication. I was keen on

learning how to conduct and analyse neonatal EEG, as it can provide valuable information on neonatal brain function.

Pursuing a DPhil has been useful in my career journey, learning about different study designs and equipping me with the necessary skills to conduct studies and analyse my results. From the systematic review, I have gained an in-depth understanding of neonatal brain-respiratory dynamics and identified key research gaps to build on in future studies. One important aspect of my career goal is to identify measures that could improve neurodevelopmental outcomes in neonates. By assessing the prognostic value of neonates' 'brain age' compared to actual PMA as a marker of respiratory function, I hope to provide neonatologists with the best metric (brain or chronological age) to better identify neonates at greater risk of breathing disruptions and ultimately lead to an individualised approach to care with improved long-term neurodevelopmental outcomes. Additionally, evaluating the benefits of a new swaddling device as a comfort measure during ROP screening could help reduce the risk of immediate adverse physiologic responses and possible long-term complications. All the works I have done during my DPhil align well with my career goals of learning core research skills, doing EEG, and contributing to science.

The next sections of this chapter will review the relevant background literature that will underpin the subsequent chapters of this thesis.

1.4. Neonatal brain and respiratory system

Brainstem nuclei regulate breathing, primarily by the pre-Bötzinger complex, and these develop rapidly in response to functional adaption as the foetus transitions from

the intrauterine to the extrauterine environment [43,44]. The rate of maturity of the brainstem nuclei is region-specific, maximal in the reticular formation at 34 - 36 weeks and the dorsal vagal region at around 36 - 40 weeks PMA [43]. In general, the brain develops maximally during the first year of life [45] and plays a critical role in controlling respiration [1]. In lamb foetuses, transection of the brainstem interrupts brain-respiratory function leading to abnormal respiratory patterns [46]. The brainstem plays a crucial role in controlling breathing by sending efferent motor signals through the spinal cord to generate distinct respiratory rhythms (pre-inspiratory, inspiratory, post-inspiratory, and late-expiratory) to regulate gas exchange [47]. Oxygen- and carbon dioxide-sensitive chemoreceptors are widely distributed in the CNS from the thalamus to the medulla, and depending on blood gas levels, these chemoreceptors adjust neuronal metabolic demand and activity to either stimulate or depress respiration [48,49]. A mutation of the paired-like homeobox 2B gene has been linked with the occurrence of a congenital central hypoventilation syndrome - a genetic condition associated with life-threatening central apnoeas. The abnormal gene is present in the brainstem region. It causes disintegration of chemoreceptor sensory inputs, poor or absent respiratory responses to hypoxia and hypercapnia, leading to apnoea [50]. Chemoreceptor dysfunction as well as abnormalities in early brain development, therefore, play a key role in the pathophysiology of abnormal respiration including apnoea [51].

The development of the neonatal respiratory system progresses from early gestation (embryonic and foetal period) through to term gestation and continues after delivery into early adulthood (postnatal period) [52]. During each stage, increased morphological and functional specialisation of the respiratory apparatus occurs. The transition from extreme prematurity to term gestation is characterised by a gradual maturation of the

respiratory system through five developmental stages namely embryonic, pseudo glandular, canalicular, saccular and alveolar stages (**Figure** 1.1), Typically, the larger airways (trachea and bronchi) develop at approximately 4 - 6 weeks gestation, followed by the smaller airways (bronchioles) from 5 - 17 weeks. The lungs of the extremely premature born neonate are underdeveloped, consisting mainly of terminal bronchioles (canalicular stage). Between 26 - 36 weeks (saccular stage), the distal airways start to develop and form primitive alveolar saccules. At term gestation, the air-blood interface becomes thinner and more permeable in preparation for gas exchange. Mature alveoli begin to form and multiply rapidly, continuing through to early adulthood (alveolar stage) with further expansion of the gas exchange surface area and capillary networks [52]**.** Functionally, the production of surfactant, a surface tension-lowering phospholipid and protein complex, by the type II pneumocytes begins around 16 - 26 weeks of gestation. However, at this age, the levels are insufficient to support independent respiration; this increases significantly after 28–30 weeks. Other functional respiratory changes such as airway tone and patency, chemoreceptor responses to changes in oxygen and carbon dioxide, and coordination between sucking, swallowing and breathing, which are all underdeveloped in the extremely premature infant, start to improve around 34 - 26 weeks. At birth, premature neonates demonstrate unstable and faster breathing with a prolonged expiratory phase compared with the term neonate. This is a compensatory extrauterine adaptation that facilitates early lung pressure and volume increase, enhanced airway fluid clearance and gas exchange [53]. The frequency of respiratory rate reduces with increasing GA and PMA as postnatal adaptations such as improved lung compliance, coordination of respiratory muscle contraction and chest wall stability develop [54,55].

Therefore, the central and respiratory systems undergo distinct developmental structural and functional changes that are age dependent. The younger the GA, the more the brain and lung dysmaturity, and the greater the risk of respiratory problems.

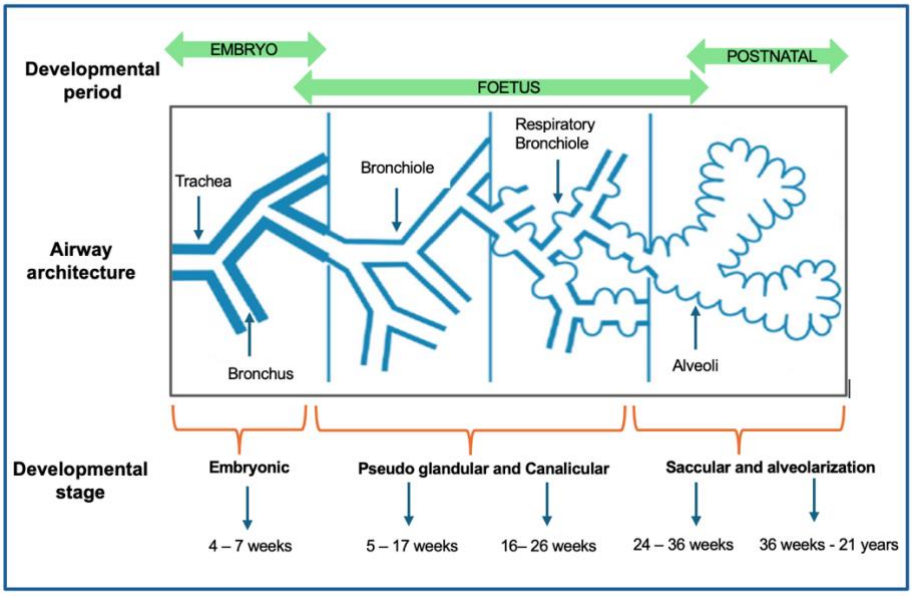

Figure 1.1: Developmental stages of the respiratory system

1.4.1. Determinants of respiratory function

An important factor that determines respiratory function is the GA at birth. Being born early (premature) means the lungs are less developed, respiratory rate is more rapid and unstable than in a full-term neonate [56]. Respiratory movements start around 11 weeks GA and become more regular by term GA [57] coinciding with both morphologic (terminal alveoli) and functional (increasing surfactant production) development. Another factor that affects normal lung development and function is the extent of breathing movement both in-utero and at the time of birth [58]. Rhythmic diaphragmatic contractions in the foetus have been shown to cause the release of growth factors that contribute to lung expansion [59]. By contrast, diminished respiratory movements in

utero result in pulmonary hypoplasia and impaired lung function [60]. Also, damage to the cervical cord and phrenic nerve has been shown to cause atypical lung function leading to abnormal diaphragmatic contraction and impaired respiratory excursions [61,62]. Another factor is the presence of residual lung fluid, which can cause respiratory distress. Adequate foetal lung fluid clearance, facilitated by breathing movements and appropriate balance between production and absorption is necessary for optimal lung growth and function [58]. Still, in the lungs, the presence of surfactant makes the lungs more compliant and expandable. Surfactant levels are higher at term GA, facilitating normal breathing, compared with low levels seen in the preterm. Genetics, environmental factors and stressors such as infection, certain medications, toxins, mechanical ventilation, glucose instability, temperature instability, acid-base imbalance and maternal conditions (for example, poor nutrition, smoking, alcohol and drug ingestion) [58,63], and exposure to painful procedures including ROP screening also influence respiratory function (**Section** 1.7).

1.5. Respiratory and nervous systems interactions

The respiratory and nervous systems are intricately interconnected, with various neural pathways influencing each other. Mild hypoxia stimulates brain function and consequently, respiration; but when extreme, it has an inhibitory effect. Studies in sheep models have shown centrally mediated inhibition of foetal breathing movement as a consequence of hypoxia-induced low-voltage brain activity [46], demonstrating the effect of higher brain input in breathing control. The cortical modulatory effect of breathing acts through the brainstem, central and peripheral chemoreceptors [63]. Neurons in the pons and medulla respond to afferent inputs from oxygen-sensitive peripheral chemoreceptors located in the aortic and carotid bodies as well as carbon

dioxide-sensitive central chemoreceptors located in the brainstem to control respiratory muscle contractions [63]. After birth, the peripheral chemoreceptors mature faster than the central chemoreceptors, highlighting the role of central brain immaturity as a key factor contributing to dysfunctional breathing. Additionally, central chemoreceptor sensitivity to changes in the partial pressure of carbon dioxide (PCO_2) is attenuated in the preterm neonate predisposing them to greater risks of respiratory instability compared with the term neonate [1,51,64]. Thus, changes in breathing mechanics reflect the extent of maturation of both the respiratory and central nervous systems.

The interplay between neonatal respiration and brain activity is especially important in preterm infants because both the respiratory and the central nervous systems are immature, developing, closely influencing each other and are highly interdependent. Dysfunctional breathing, e.g. during apnoeic episodes, can reduce brain oxygenation and potentially affect neural activity with subsequent impairment in long-term neurodevelopment [6,12–14]. On the other hand, dysmature brain activity from thalamocortical immaturity in the premature infant [30] can alter respiratory dynamics by reducing arousal responses to hypoxia and hypercapnia, leading to abnormal breathing [65]. This, therefore, creates a cycle where brain immaturity can worsen breathing patterns, and poor respiration can further compromise brain development. Understanding how these systems interact and what modulates that interaction is essential for improving outcomes in this vulnerable group.

1.6. Effect of apnoea on lungs and brain development

Apnoea of prematurity is a good example of the respiratory manifestation of the immature function of the brain-respiratory control mechanisms. Clinically, it manifests as breathing cessation of variable duration - at least 20 seconds, or shorter with associated depression of cardiac activity and/or cyanosis [2,66]. Depending on whether apnoea is accompanied by breathing efforts, it may be classed as central (absence of both airflow and breathing attempts), obstructive (presence of breathing attempts in the absence of airflow) or mixed (both central and obstructive, with the latter manifesting as a minimum of three obstructive breathing attempts) [67,68]. Over the years, clinicians and researchers have used various apnoea definitions to describe its relevance [2], however, huge variations within and between individuals exist on the implication of significant episodes requiring intervention, the degree of response and/or tolerance to treatment.

While apnoea typically resolves with increasing development of the brain and the lungs (although it can still occur in the term neonate), the ensuing complications from low oxygen levels predispose to lung and brain injury which could lead to more frequent cardio-respiratory events or delay the expected time of symptom resolution. Acutely, apnoea may cause intermittent hypoxia triggering a cascade of multisystem pathology [6]. Respiratory complications, for example, obstructive apnoea-related sleep-disordered breathing [69], bronchopulmonary dysplasia and ventilator-induced lung injury on a background of lung immaturity [69,70], increased airway reactivity disorders and desaturations [71] have been reported. While these problems may not be a direct

consequence of apnoea, they result from secondary induced hypoxia and/or complications arising from oxygen therapy such as barotrauma and free radical-induced lung injury.

The type and severity of apnoea have been linked with neurodevelopmental complications. Obstructive apnoea is associated with higher risks of CNS complications such as intraventricular bleeding and hydrocephalus [67]. Apnoeic episodes, more so when accompanied by peripheral desaturation, have been significantly correlated with the occurrence of ROP [69] and can also considerably lower cerebral oxygen levels exacerbating existing brain injury [9]. A pre-existing brain pathology may predispose to more frequent apnoeic spells. Severe apnoea characterised by longer hospital stay, worse cardiac decompensation (bradycardia and hypoxaemia), delayed resolution of symptoms and the need for prolonged respiratory support have all been correlated with poor cognition, motor delays in infancy and early childhood, and mortality [6,12–14]. To minimize both the immediate and long-term impact of apnoea, clinicians use various preventive and therapeutic approaches in the neonatal unit to manage the condition.

1.6.1. Respiratory stimulants for apnoea management

Aside from general measures like tactile stimulation, kangaroo care, temperature, glucose and haemodynamic control [72], respiratory stimulant medications such as methylxanthines (e.g., caffeine, theophylline) and doxapram are increasingly being used, either prophylactically or therapeutically for apnoea of prematurity management. The mechanism of action of these medications is multifaceted, acting both centrally

on the brain's respiratory centres to increase carbon dioxide and oxygen chemoreceptor sensitivity as well as peripherally, stimulating respiratory muscles contraction [73–76]. Doxapram is currently not routinely recommended for neonatal apnoea management due to the limited evidence on efficacy [77] and safety causing cerebral ischaemia, hypoxaemia [78], and on the long-term, dose- and duration of therapy-related developmental delays [79,80].

Caffeine is the most widely used methylxanthine for apnoea management due to its safety profile and long half-life [81]. There is an intrinsic individual variation in response to caffeine therapy, linked with the circadian locomotor output cycles kaput (CLOCK) gene polymorphism, which confers a positive response in some preterm neonates [82]. Caffeine reduces the risk of complications such as bronchopulmonary dysplasia and persistent patent ductus arteriosus during infancy. It is also neuroprotective, improving the chance of childhood survival without neuro-disability [83,84]. In rat models, caffeine was shown to reduce inflammation, brain atrophy, and ventriculomegaly [85]. It also restores brain activity following hypoxic-ischemic brain injury [86]. In human neonates, some authors postulate that the neuroprotective mechanism of caffeine is indirect via the respiratory pathway, acting by reducing the risk of bronchopulmonary dysplasia and ventilator-associated lung injury and, consequently, brain injury [84]. Other authors found a direct brain stimulatory effect enhancing neuronal activity [87].

1.7. Retinopathy of prematurity screening and associated physiological changes

Prematurity is associated with many debilitating complications including ROP, a preventable vaso-proliferative disease of the developing retina [88,89], which without timely intervention, can cause visual impairment and irreversible blindness from retinal detachment. ROP screening is an uncomfortable and painful procedure[90–93]; and adverse physiological responses similar to those observed during other painful procedures [94–96] have been described. Potential causes of physiological instability during ROP screening include fatigue from the stress of handling, eye manipulations following speculum insertion, and scleral indentation to stabilize or rotate the eyeball [97,98]. Other reasons for abnormal physiological changes during ROP screening could be as a result of the oculo-cardiac reflex, which arise from the sequential stimulation of the trigeminal and vagus nerve during ocular examination, causing transient bradycardia, arrythmias or in severe cases cardiac arrest [99]; pain-induced hormonal changes i.e., elevated cortisol, catecholamines, adrenocorticotrophic hormones and β endorphin levels mainly from the activation of the hypothalamo-pituitary and sympathetic nervous systems, leading to stress metabolic responses e.g. increased heart rate, blood pressure, glucose and lactate levels [100,101]; and digestive system problems such as reflux, abdominal distension, transient ileus, and feeding intolerance [102–104].

During and immediately following ROP screening, neurobehavioral, cardiovascular, and respiratory system related changes have been observed. Neonates demonstrated increased crying and movement activity during the procedure [105], suggesting it might

be distressing. Some authors report significantly elevated pain scores in neonates during ROP screening [90,92,93], including up to 24 hours after the test [93]. An increase in blood pressure after the procedure compared with a period of up to 6 hours before, a drop in oxygen level and diverse heart rate changes (some neonates developing marked tachycardia and others experiencing transient bradycardia) have also been documented [106]. Significant tachycardia with accompanying desaturation was reported in one study only during eye manipulations [32]. Comparing two ROP screening techniques, a study showed a consistent increase in heart rate, blood pressure and desaturation following both methods [107]. Preterm neonates are prone to apnoea [4] and coupled with multiple invasive eye exams, susceptibility to breathing cessation is increased. In a case report [34], severe apnoea and bradycardia were reported in two neonates at 36 weeks corrected age immediately following removal of the eye speculum. In another report, significant apnoea and desaturation occurred up to 48 hours after the procedure compared with before [35]. Gastrointestinal manifestations including feeding intolerance [106,108], abdominal distension [109], gastric haemorrhage [106] and necrotising enterocolitis [104,106,110] have also been observed.

Adverse physiological responses have also been linked to instilling eye drops during ROP screening. Some studies report that mydriatic eye drops (cyclopentolate, phenylephrine and tropicamide) used routinely for pupillary dilatation during the procedure have anticholinergic and alpha-adrenergic effects. These medications are 80% absorbed into the systemic circulation via the cornea, conjunctiva and nasal mucosa causing respiratory and cardiovascular effects such as desaturation [106,108], apnoea [111], bradycardia [108,109,112], labile blood pressure and tachycardia [106]. By

contrast, other studies reported no change in vital signs from the use of mydriatics [32,112–114].

1.8. Monitoring neonatal brain activity with electroencephalography

The Electroencephalogram is an electrical signal originating from neuronal activity and measured using scalp electrodes. EEG is an objective, neurophysiological tool that records the sum of the post-synaptic potentials generated from cortical neurons. It displays the averaged potential as interpretable waves of varying frequencies and amplitudes, reflecting ongoing development of cortical, subcortical, and thalamocortical connections - a direct indication of brain function [24]. EEG is the gold standard for assessing normal and abnormal neuronal function in neonates and can distinguish characteristic developmental patterns depending on the age, behavioural state (i.e. sleep and wakefulness) and physical activity state [30,115].

The age of the neonate mainly determines the temporal and spatial organization of the EEG recording. With increasing gestational age, EEG activity evolves from discontinuous and immature to a more continuous and synchronised pattern typical of term infants [30] (**Figure 1.2**). In the extreme preterm (< 28 weeks), the EEG tracing is predominantly intermittent with low frequency and amplitude, and long inter-burst intervals (≥ 10 - 20 seconds). There's no distinct background symmetry, reactivity or sleep–wake cycle [30,116]. Between 28 - 32 weeks gestational age, activity bursts become more frequent, with increased amplitude and complexity, and decreased inter-burst intervals (approximately 5 - 10 seconds). Delta brushes, considered the hallmark

of early brain development, begin to appear over the central and temporal regions. Sleep–wake cycling becomes discernible [30,117]. Around 32 - 36 weeks gestational age, there is an obvious transition from being asymmetric and discontinuous to a more symmetric, continuous tracing; this is most noticeable during active sleep. The delta brushes reduce in number [30,116]. Finally, at term (≥ 37 weeks gestational age), brain activity becomes continuous, synchronous, and symmetric. Reproducible reactivity is well established during both wakefulness and active sleep. While the delta brushes disappear completely in healthy term infants, new waves - encoches frontales (frontal sharp transients) and sleep spindles appear during quiet sleep, and the sleep–wake cycling becomes more distinct, differentiating between quiet and active sleep states [30,116].

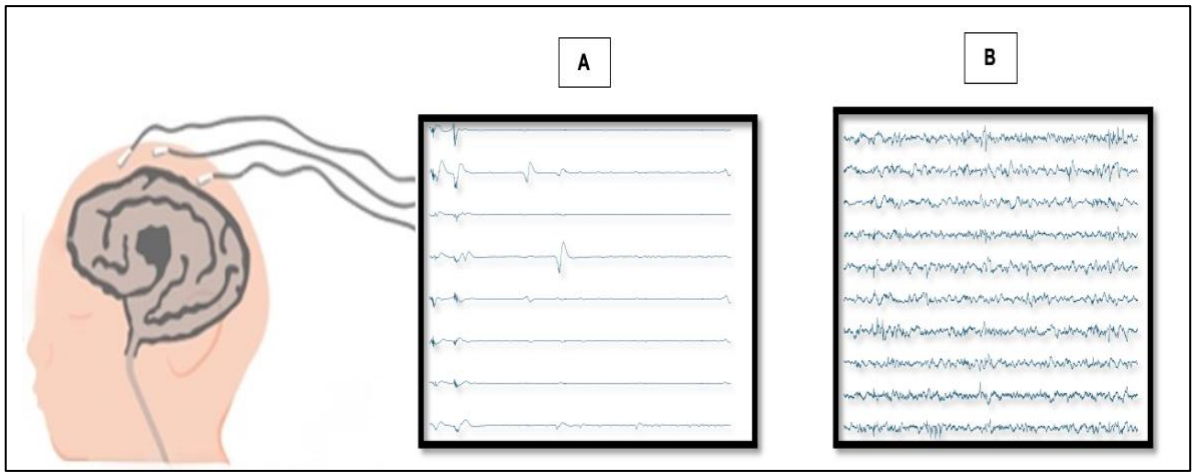

Figure 1.2: Comparison of preterm and term neonatal EEG activity. The preterm EEG (**A**) is discontinuous with bursts of irregular asynchronous electrical activity and is less organised. The term EEG **(B**) is more stable and mature, displaying rhythmic bursts of continuous synchronised activity.

1.8.1. Electroencephalography for assessing brain age and maturity

The clinical usefulness of EEG for prognostication in the neonate following hypoxic-ischaemic encephalopathy, cerebral infection, seizures, drug monitoring for seizure control and anaesthesia depth has been described [24,25,118,119]. Recent studies in both adults [120,121] and children [122–124] have used EEG to accurately predict the biological age of the brain. Similarly, in neonates, brain development measures have been assessed using machine-learning algorithms to provide reliable estimates of the neonates' brain age (the biological/functional age of the brain; it ascertains how 'old' a neonate's brain is by analysing and comparing specific EEG features with data from typical age-related trained models [124,125]) and brain maturity (a standardised measure of the brain's functional development, calculated as the difference between the neonate's brain age and their chronologic age, providing a consistent age reference measure of brain development independent of chronologic age).

While visual assessments have been used to track EEG maturational development, this approach is subjective, time-consuming and requires an expert. Computational EEG methods are more reliable and accurate in estimating functional brain age [126]. In the preterm neonate, automated algorithms combining amplitude, temporal and spatial EEG features have accurately estimated brain maturity better than conventional visual quantification methods [127]. Moreover, studies have shown a correlation between brain age deviations from normal with reduced life expectancy in adults [128] and neuro-disability in neonates [124]. Comparing healthy term and preterm neonates using time-

series sleep EEG data from a single EEG channel, significant age-related maturational differences, more developed in the term neonate, have been demonstrated [129].

Ansari et.al (2024) [124] used a deep convolutional neural network to train and optimise their model to quantify brain age and maturational differences in preterm neonates using resting-state EEG data. They first validated the model on an independent cohort of preterm neonates with normal BSID-II at 24 months (data set 1) using an 8 channel full EEG recording to obtain accurate age predictions with a mean absolute error (MAE) of 0.73 weeks. Next, the model was retrained using a reduced (one) channel and recording duration (20 minutes); the average brain age predictions were comparable to predictions obtained from the full recordings with a MAE of 0.79 weeks. Finally, using two independent data sets, one comprising preterm babies with both normal and abnormal BSID-II at 9 months (data set 2) and another with normal BSID-II (dataset 3), a significant correlation was shown between the magnitude of deviation of the estimated brain age from the actual PMA with long-term neurodevelopmental outcomes and age prediction, respectively (MAE 0.98 weeks in dataset 2 and 1.03 weeks in data set 3). Reducing both the number of EEG channels and the recording duration was highly efficient and accurate in estimating neonatal brain maturational differences. In addition, recent work published during my DPhil with colleagues used sensory-evoked EEG responses to light and touch to accurately predict a neonate's age. A training data set comprising 98 EEG recordings was used to calculate age-weighted average responses, identify, and characterise developmental neurodynamic response functions (four visual and two tactile were generated) using principal component analysis with a MAE of 1.41 weeks in predicting the sensory brain age. This was validated on a separate test data (65 recordings) by fitting their visual and

tactile responses to the neurodynamic response functions generated from the training data set to predict the sensory brain age of the test data (MAE = 1.55 weeks). The sensory brain age was also related to outcome at 2 years using the Bayley-III Scales of Infant Development [125]. EEG-derived brain-based measures can, therefore, be used as early indicators for long-term neurodevelopmental outcomes.

1.9. Summary

This Chapter establishes the complex interaction between the neonatal lung and brain; the physiological impact of apnoea and painful procedures such as ROP screening which preterm neonates routinely undergo; and the usefulness of EEG for brain monitoring. It becomes prudent to explore key ideas to better understand the intricate relationship and individual differences in breathing and brain development. In **Chapter** 2 of this thesis, I report the findings from a systematic review I conducted summarizing existing work up till 2022 on the relationship between neonatal respiration and EEG activity. In **Chapter** 3**,** I hypothesise that EEG-derived brain-based measures might be better predictors of respiratory function compared with chronological age. To identify potential brain biomarkers that might be useful for immediate personalised care and long-term prognostication, I simultaneously recorded EEG brain activity (calculating the brain age and maturity) and vital signs (for respiratory and apnoea rates) and compared the brain-based measures with chronological age to determine which is a more accurate biomarker for respiratory outcomes and the need for caffeine treatment. Lastly, regardless of the cause of physiological changes in preterm neonates undergoing ROP screening, it is important to identify techniques that improve comfort and limit the occurrence of adverse physiological responses. In **Chapter** 4, I

investigated one approach, assessing the usefulness of the Dandle® WRAP stretch swaddle compared with the standard practice of using a conventional swaddle to minimize the risks of ROP-related cardiorespiratory complications and potential long-term effects. This was done by recording and comparing changes in vital signs, alongside gathering staff opinions.

2

The Relationship Between Neonatal Respiration and EEG - Brain Activity: A Systematic Review

2.1. Background

As described in **Chapter** 1, respiratory dynamics in neonates differ according to the GA at birth, and the preterm neonate is more susceptible to irregular breathing and apnoea [53]. With medical and technological advances, an increasing number of extremely premature neonates now survive [131]. As their developing brain is also more vulnerable to the effects of hypoxia, the number of preterm survivors with hypoxic brain injury (some from recurrent and prolonged respiratory disruptions) and neurodevelopmental complications is also rising [132–134]. In animal models, low cerebral oxygen levels alter key enzyme activation and cellular metabolism, compromising neuronal maturation and synapse formation [135]. This hinders the activity of specific

subplate neurons during a critical developmental period resulting in impaired functional connectivity and alteration in brain function [136]. A similar mechanism of hypoxia-induced brain injury has been described in human neonates [137]. Additionally, impaired brain activity early in life is known to alter the development of neurons, increasing the likelihood of brain injury; this was also shown to be predictive of long-term neurological outcomes [7,8]. Therefore, maintaining normal brain function during the neonatal period is key for subsequent healthy brain development.

Changes in cerebral blood flow and oxygenation in the neonate may occur during episodes of acute respiratory changes including apnoea [6,9,10]. While very long periods of oxygen deprivation, such as those observed during hypoxic-ischaemic brain injury, have been associated with abnormal brain function and poor neurologic outcome [138,139], it remains unclear to what extent these periods of low oxygen, including the duration and frequency of episodes, could last without causing significant alterations in brain function. With a simultaneous recording of EEG brain activity and vital signs monitoring to assess breathing dynamics, valuable information about the immediate effect of respiratory changes on brain function can be obtained. This will enhance our understanding of the impact of respiratory events on brain activity as well as identify early any significant respiratory episodes with potential long-term effects on brain development.

Respiratory stimulants are commonly used in the neonatal intensive care unit (NICU) to reduce the incidence of apnoea [74], and they show favourable long-term benefits on neurocognitive outcomes [84]. However, the exact mechanisms underlying the neuroprotective role of respiratory stimulants are not fully understood. To better

comprehend how these medications work, it is important to investigate whether simultaneous changes in breathing and brain activity occur together with their use and how these medications modulate immediate brain function as these could be useful for individualised medicine. Identifying immediate EEG changes in response to respiratory stimulants may provide clinicians with early, useful biomarkers of treatment effectiveness, helping to distinguish neonates who are likely to benefit from continued medication use from those that may require additional support. This could facilitate timely and tailored interventions that ultimately could improve long-term neurodevelopmental outcomes.

In this chapter, I describe a systematic review, collating evidence on the acute effects of respiratory changes (normal and abnormal) and respiratory stimulants on EEG activity in neonates 28 - 42 weeks PMA. This review provides a comprehensive overview of all empirical work on this topic i.e., summarising the immediate association between neonatal brain-respiratory mechanisms, the impact of respiratory stimulants on brain function, and highlighting areas for further research.

2.2. Aim and objectives

This study aims to review the current literature on the relationship between neonatal respiratory changes (i.e., normal versus abnormal), respiratory stimulants and EEG activity. The specific objectives are to summarise:

1. How respiratory and EEG activity vary in neonates across different PMA, comparing normal respiration and periods of acute respiratory events, namely: apnoea, periodic breathing, tachypnoea, bradypnea, sighing, and any breathing pattern such as shallow breathing, hyperpnea or respiratory-related muscle contractions like hiccups.
2. The relationship between the characteristics of acute respiratory events (such as the duration and severity) and EEG-recorded brain function.
3. The immediate effect of medications, specifically respiratory stimulants, used for apnoea management on EEG brain activity.

2.3. Methods

2.3.1. Study design and duration

This was a systematic review and was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [140]. The review was started in January 2022 and completed in September 2022. A protocol describing the details and conduct of the review was registered in PROSPERO with identification reference CRD42022339873 [141]. The registration was done after completing preliminary searches and piloting the study selection process against the set eligibility criteria (**Section** 2.3.2) but before formal screening was concluded, in line with the PROSPERO guidance for registering systematic reviews [142].

2.3.2. Research framework

This was formulated per the core methods of systematic review design [143], and the framework adopted for the selection of studies to be included in the review is shown in **Table** 2.1**.** All relevant publications that met the eligibility criteria were included.

2.3.3. Exposure and outcome definitions

Apnoea, periodic breathing, tachypnoea, bradypnea, and other breathing patterns, for instance, shallow breathing; sighing and respiratory-related muscle contractions such as hiccups were considered as acute respiratory events. In practice, differences exist in the definition of these measures, hence, any eligible article with a clear methodological description of the exposure of interest was considered. These

exposures were identified as variables of interest following a pilot review (**Section** 2.3.6). Any additional acute respiratory change clearly described in the literature during the formal review were also considered provided the article met the eligibility criteria. However, no further exposure variables were identified during the formal review process.

Table 2.1: Review eligibility criteria

PECOS criteria	Report characteristics	Exclusion criteria
Population: Human neonates between 28 - 42 weeks PMA.	No restriction in the publication year until 2022.	Wrong study population e.g., adults, older children, and non-humans.
Exposure: Any dysfunctional irregular or abnormal respiratory change in neonates.	English only.	In the exposure group, neonates with congenital cardiovascular and central nervous system malformations such as seizures, hypoxic-ischemic encephalopathy, and intraventricular haemorrhage; these can act as confounders during EEG interpretation.
Comparator: neonates with regular or normal respiration.	Articles published in peer-reviewed journals, conference proceedings and grey literature.	In the comparison group, studies that included neonates with respiratory pathologies like pneumonia, bronchiolitis, or any abnormal respiratory event.
Outcome: EEG brain activity.		Studies that used EEG recordings for sleep staging only.
Study design: All primary empirical studies including descriptive, observational, and experimental designs.		Other study designs: Review articles, systematic reviews, commentaries, questionnaires and survey reports.

Abbreviations: Population, Exposure, Comparison, Outcomes and Study (PECOS), postmenstrual age (PMA), electroencephalography (EEG).

The primary outcome measures were any neonatal EEG feature recorded using conventional (cEEG) and/or amplitude-integrated EEG (aEEG) described in relation

to either normal or abnormal respiratory event, or the use of respiratory stimulants like caffeine citrate, doxapram or theophylline. The outcomes considered were:

1. EEG band power: This is calculated using spectral analysis as the total amount of energy in microvolts squared within a specific frequency range. It is classed as absolute (total power within a defined EEG frequency or relative (proportion of the total power within a specific EEG frequency relative to total power across all bands).[144] **Table** 2.2 shows a summary of the relevant neonatal EEG frequency bands [30,145].

Table 2.2: Characteristics of neonatal EEG waveforms

Wave	Frequency (Hz)	Characteristics	Behaviour and activity state	Illustration
Delta	1-3	Highest amplitude and slowest rhythm. Widespread distribution	Dominant wave in neonates, indicative of cortical immaturity. Seen mostly during deep sleep	
Theta	4-7	Very slow rhythm. Diffuse distribution mainly temporal and parietal lobes	Less dominant, increases with brain maturity and during transition between sleep and wakefulness. Represents quiescent cerebral activity and attention development	
Alpha	8-12	Slow rhythm and sinusoidal. Seen in the posterior part of the brain, mostly in the occipital region	Few or absent in neonates. Associated with sensory processing and cortical arousal	
Beta	13-30	Low amplitude and fast rhythm. Common in the frontal and central brain regions	Generally, absent in neonates and linked with the alert state, or during concentration in older children. Touch and contralateral body movement attenuate these oscillations.	
Gamma	30-80	Very fast rhythm and lowest amplitude. Seen in the somatosensory cortex	Limited presence and unclear function in neonates but may reflect early network formation. Observed during stress and extreme brain activity.	

2. EEG frequency: This is the rate of oscillation of the EEG per second, measured in Hz. The average frequency of the full EEG signal, delta, theta, alpha or beta band or 90 % spectral edge frequency will be considered.

3. EEG amplitude: This is how large the voltage fluctuates from the baseline; this will include the minimum, maximum, or average absolute amplitude in the different frequency bands.

4. EEG continuity pattern, inter-burst interval duration, or occurrence of burst suppression patterns.

Like the exposure variables' identification process, additional EEG outcomes noted from any eligible article during the process of formal screening were also considered.

2.3.4. Information sources

Bibliographic databases including Medline, Embase, Global Health and PsycINFO (via Ovid SP), Cumulative Index of Nursing and Allied Health Literature (CINAHL), Cochrane Library, Science Citation Index and Conference Proceeding Citation Index via Web of Science database, and ProQuest for grey literature were used to generate literature. These databases were chosen because they offer a specialised broad coverage identifying the relevant articles for inclusion in the review.

2.3.5. Search strategy

Search strategies were developed in collaboration with a Bodleian healthcare librarian. A comprehensive database-specific approach combining search terms - Medical Subject Headings (MeSH) terms and controlled keywords to reflect the concept of the review was used. During the search, three subject domains namely EEG, breathing and neonate were combined using Boolean operators and truncation to yield precise results. The detailed database-specific search strategy is provided in **Appendix - Section** 7.1.

The first database searches were conducted in January 2022. These were re-run in August 2022 prior to the completion of the review write-up to ensure an updated search result. Finally, a backward citation search of all the articles included in the review was employed for robust literature acquisition.

2.3.6. Piloting of review, article selection, data extraction and management

The articles generated from the different databases were exported as 'ris files', uploaded and de-duplicated on Rayyan® software for systematic reviews [146]. Before the start of the formal screening, the study selection process was piloted on a subset of 20 randomly selected articles against the set inclusion and exclusion criteria to ensure consistency in the screening process by all the reviewers. Once the pilot screening was completed, a formal screening of all the articles followed.

The screening was done in two stages:

1. Titles and abstracts screening to identify potentially relevant papers: This was carried out by two independent reviewers (FU and SM) who were both blinded to each other's decisions based on the selection criteria described (**Table** 2.1). The selection options were either to 'include' for review eligible articles, 'exclude' for review ineligible articles or 'maybe' for articles reviewers were unsure of eligibility. The reasons for each article's exclusion were noted. After this initial stage of article selection, the reviewers were then unblinded to the results in order to assess similarities and differences in the articles selected by each reviewer for eligibility to proceed to the next stage of screening. Discrepancies in article selection and any

paper tagged as 'maybe' were resolved through discussion by the two reviewers. Where there was no consensus, a third reviewer, the arbitrator (CH), made the final inclusion decision.

2. Full-text screening of the eligible articles included from the first stage screening for potential inclusion in the final review synthesis: This followed the same process in (**1**) above, but here, instead of focusing on the title and abstract for decision-making, a detailed scrutiny of the entire text for the relevant information was undertaken. The same two independent reviewers in (**1**) above were involved, and again, disagreements were resolved the same way as above. If an article had the key information required (**Section** 2.3.3), then the paper was included in the final review synthesis and the relevant data was extracted.

The data extracted and summarised from each included article following full-text screening included the author's name; title of journal; publication year; country of study; World Health Organisation region of study; the study period; study design; sample size; age of study participants (GA at birth, PMA or postnatal age at the time of study); neonate category (term or preterm) and sub-population where specified (e.g. neonates with low-birth weight); type of exposure variable (normal or abnormal respiration); detailed description of the comparison group where provided; and the type of respiratory stimulant used (if any). Technical details about the EEG technique such as the type of EEG used to record brain activity i.e., cEEG or aEEG, montage, number of EEG channels, corresponding EEG and respiratory physiology findings were also extracted. No authors were contacted for this review as all the selected articles had details of key information required.

2.3.7. Risk of bias assessment

This was independently performed by two reviewers (FU and CH) who were blinded to each other's responses. The Joanna Briggs Institute (JBI) critical appraisal tool for systematic reviews, which provided a range of checklists for the different study designs was used. The JBI appraisal tool was used to assess the quality of conduct and reporting of the included studies and not for article selection decisions. Each study-specific appraisal checklist had a "yes", "no", "unclear", or "not applicable" option to prespecified questions (**Appendix – Section** 7.2). Discrepancies were resolved using a similar approach described during the article screening process (**Section** 2.3.6).

2.3.8. Data synthesis

A narrative synthesis approach as described by the Centre for Reviews and Dissemination for Systematic Reviews [147,148] was used to summarise all the findings from the individual studies. The review process, detailing the total number of articles generated from the databases and backward citation search, articles screened, and those excluded, was depicted using a PRISMA flow diagram [140]. Tables and charts were used to display the results. Findings were grouped and reported based on the specific review aims, exploring variations in baseline characteristics of the included study population, methods, and differences in exposure and outcome measures. Finally, the strengths and limitations of the review were discussed based on the overall findings, and recommendations including areas for future work proffered.

2.4. Results

A total of 267 articles were identified from the electronic databases and an additional 6 from backward citation search. From the initial electronic database search, 33 duplicates were removed before the start of the screening process; 176 articles were excluded in the first stage of the titles and abstracts screening, and a further 46 papers were excluded during the second stage of the full-text screening (**Figure** 2.1). Overall, 14 distinct articles consisting of a total of 534 eligible neonates were included in the final review synthesis. Most of the included studies (9 of 14, 64.3 %) reported the relationship between apnoea and EEG brain activity while just over a quarter (4 of 14, 28.6 %) investigated the effect of respiratory stimulants on EEG features. Only one article focused on a subset of low birthweight neonates. Bipolar EEG montage was used during most (10 of 14, 71.4%) studies comprising a wide-ranging (1 - 16) number of EEG channels. While most of the articles were from Europe (9 of 14, 64.3%), none were reported from Africa. **Figure** 2.2 shows the demographic summary of the included articles. The exposure definitions and outcome measures evaluated in the included studies were heterogeneous (**Tables** 2.3 - 2.5).

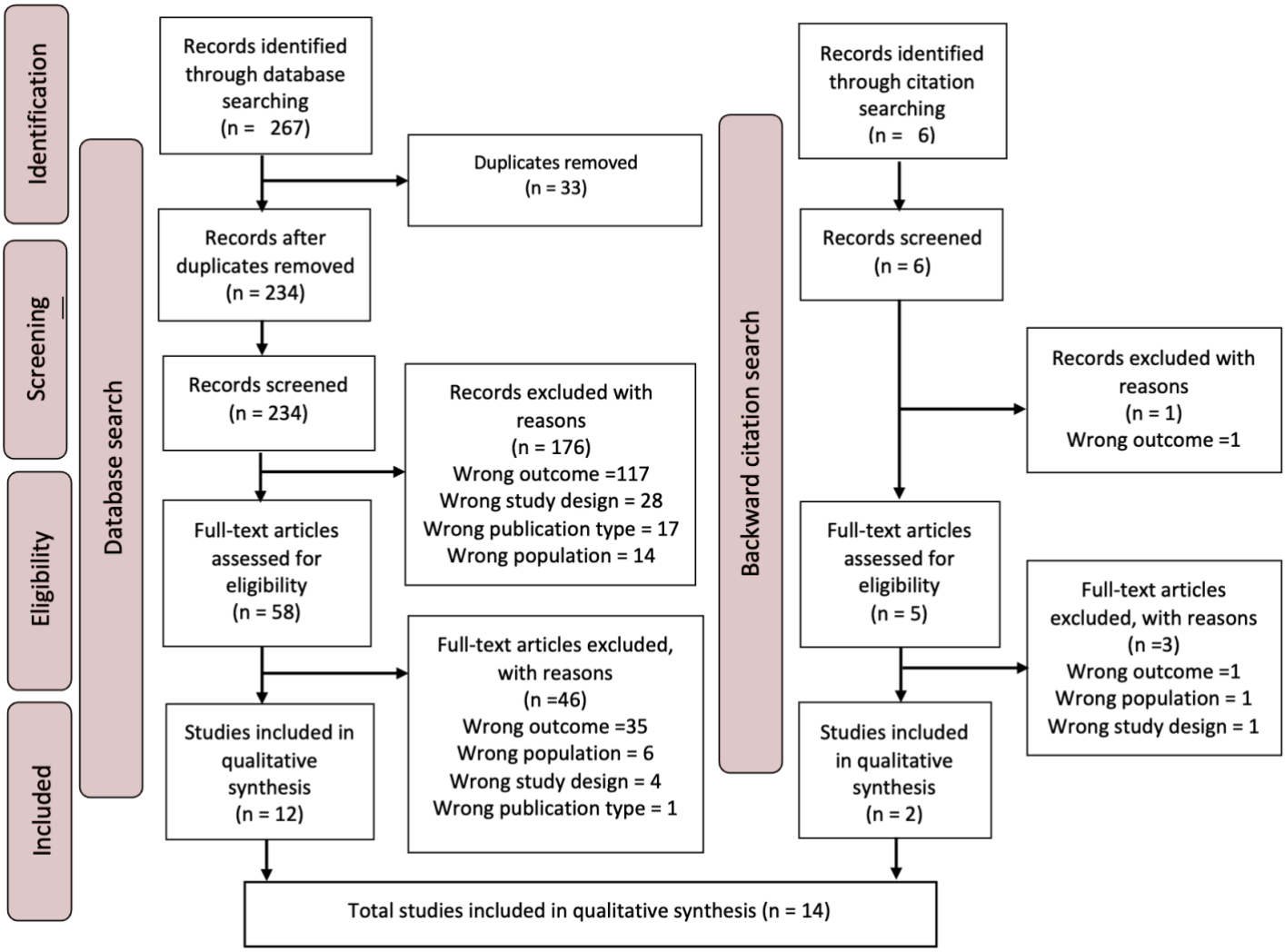

Figure 2.1: PRISMA flow chart. Displaying results generated from the database and backward citation search, articles excluded including the reasons for exclusion and the total number of articles included in the final review synthesis.

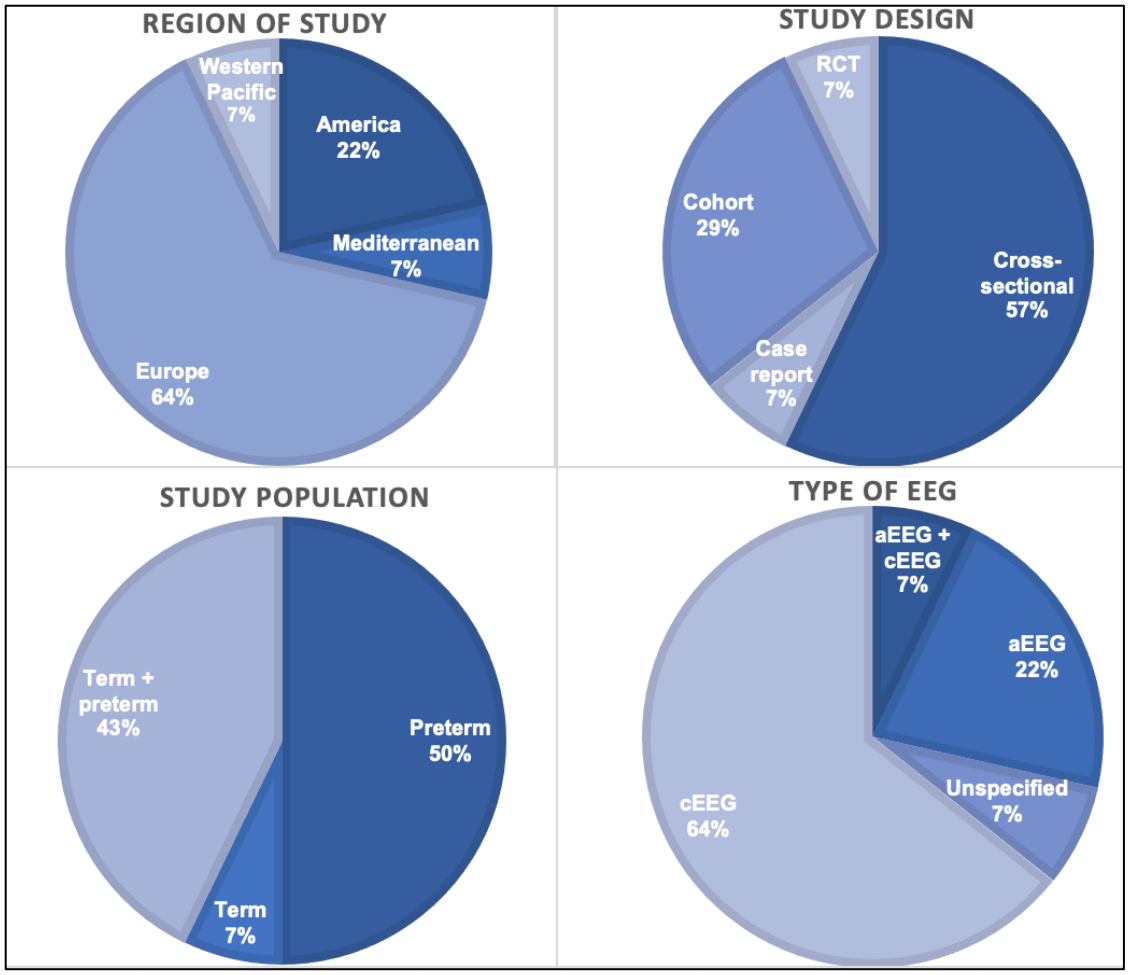

Figure 2.2: Demographic summary of included articles. *Abbreviations: amplitude augmented electroencephalography (aEEG), conventional electroencephalography (cEEG), randomised controlled trial (RCT).*

2.4.1. Studies describing the effect of normal respiration and acute respiratory events on brain activity in neonates

No studies were identified that examined how normal respiration and EEG signals co-varied in neonates across different PMAs.

2.4.1.1. Apnoea and brain activity

Nine articles published between 1973 and 2014 studied the effect of apnoea on EEG brain activity, with two-thirds (6 of 9, 66.7%) reported from Europe (**Table** 2.3). Two of

the articles were published as conference abstracts. While over half (5 of 9, 55.6%) of the papers studied both term and preterm neonates together, only a third (3 of 9, 33.3%) and just over a tenth (1 of 9, 11.1%) exclusively assessed preterm and term neonates, respectively. In most of the studies, each participant was its own control i.e., EEG epochs during periods of normal breathing in the same neonate were compared with periods of apnoeic episodes.

Apnoea definitions varied from as short as 3 seconds to >20 seconds. One paper, a conference abstract, recognised apnoea as the exposure of interest but did not detail its definition and description [18]. The EEG outcomes assessed were diverse, including frequency [149], amplitude [19,20,150,151], continuity [152], burst suppression pattern [152–155]; absolute and relative power [19], magnitude squared coherence function, phase synchronization and nonlinear generalized synchronization [18] changes.

EEG sleep staging was described in all but three [20,151,153] articles. The method of EEG evaluation reported in the papers differed, including the use of expert visual assessment in 3 of 9 studies [150,151,153], the use of more objective quantitative assessment method in another 3 of 9 papers [18–20] and a combination of the two methods in one study [149]. While most studies used statistical tests for inference [18–20,149,155], other studies reported their findings in narrative form using terms like ‘frequently’ or ‘sometimes’ to describe the occurrence of apnoea events [151,152].

No changes in EEG activity were found during approximately 60 - 80% of the observed apnoeic episodes [149,150,153]. Three studies [152,153,155] reported simultaneous EEG suppression during apnoea, two reported widespread amplitude reduction [150,151] and

one showed a reduction in the absolute EEG power [19]. Holthausen et al. (1999) compared preterm neonates based on age groups with term neonates ≥ 41 weeks, describing specific frequency band amplitude (delta and theta) changes during apnoea. They reported a significant amplitude reduction during apnoea in the term neonates; this change, however, was not significant in younger neonates [20]. Lowering of EEG amplitude may indicate a shift from sleep to wakefulness [19] and immature arousal mechanisms in the very preterm may account for the non-significant observation in the younger age group. Similarly, other measures like the magnitude squared coherence and non-linear generalized synchronization index, both measures of functional connectivity, were found to be significantly increased during apnoea, indicating enhanced signal efficiency and network interaction between cortical neurons [18]. Post-apnoea, only one paper [149] showed a significant but variable frequency change (some neonates showing an increase while others a decrease) compared with a control period one minute prior to the onset of the apnoea.

2.4.1.2. Other respiratory events and brain activity

Only one study described the effect of a respiratory event other than apnoea on EEG activity, investigating the effect of hiccups [156]. This was cross-sectional in design and studied 13 term and preterm neonates altogether. However, only results from 10 of the participants were eligible for inclusion in this review (**Table** 2.4). The authors described EEG-evoked brain activity changes during hiccups as three distinct event-related potentials mainly in the central brain regions with maximum amplitudes occurring at 16, 125 and 310 milliseconds post diaphragmatic muscle contraction. This suggests a cortico-sensory connection between the brain and respiratory muscle, with the 3

event-related potentials indicating afferent signals from the diaphragm to the cerebral cortex.

We did not find any eligible study that reported on the other respiratory measures described in **Section** 2.3.3.

2.4.2. Relationship between characteristics of acute respiratory events and EEG activity

Three papers [150,152,153] studied apnoea duration and evaluated the resultant EEG changes. Amplitude reduction and burst suppression were observed during some long and shorter apnoeas. Fenichel et al. (1980) defined long apnoeas as ≥ 20 seconds and short apnoeas as ≤ 19 seconds duration. They reported that 32% of the short apnoeas showed mild amplitude suppression, while more marked amplitude suppression was observed during 6.3% of long apnoeas. A case report by Low et al. (2012) described 2 prolonged apnoeas with a duration of 213 and 376 seconds and less profound apnoeas with a mean duration of 79 seconds. The authors reported complete EEG burst suppression with associated changes in oxygen saturation below 20% in the two prolonged apnoeic episodes requiring resuscitation. These changes, however, were not documented for less profound apnoeas in the same neonate. Deuel (1973) described periodic respirations as < 20 seconds in duration and apnoeic episodes as > 20 seconds. They reported 'frequent' burst suppression at the start of some but not all periodic respiratory pauses and apnoeas (**Table** 2.3).

The type of apnoea was shown to affect EEG frequency. Greater frequency changes were seen during obstructive apnoeas observed in five neonates [149]. The extent of

these changes was found to be dependent on the baseline EEG frequency but, notably, were unrelated to apnoea duration (as previously reported) or sleep state. By contrast, another author, Manas et al. (2014) in a conference abstract reported sleep-state dependent changes in functional brain connectivity (quiet > active sleep) using the magnitude squared coherence and phase synchronization indices. They showed a generalised increase in functional neuronal connectivity with asymmetric inter-hemispheric network signals (left > right hemisphere) during apnoea compared with normal respiration.

2.4.3. Effect of respiratory stimulants on EEG brain activity

Only four of the 14 included articles described EEG changes in preterm neonates following the use of respiratory stimulants, either for therapeutic or prophylactic purposes (**Table** 2.5). Amplitude-integrated EEG was used in all the studies. Only one study was a Randomised Controlled Trial (RCT) comparing caffeine with aminophylline [157]. The other three were cohort studies [87,158,159]. While the effect of caffeine was assessed in all four studies, the dose used was not the same. All four studies were primarily focused on evaluating respiratory stimulant-induced changes in brain activity and, hence, did not provide detailed data on simultaneous respiratory changes during therapy. The studies highlighted respiratory monitoring including oxygen saturation [87,158,159], respiratory rate [159] and apnoea rate [158] recordings but did not describe the measurement techniques, frequency and synchrony of events in relation to the observed EEG features. No respiratory physiology monitoring was described in the RCT by Yang et al. (2019).

The aEEG features assessed in the four papers were changes in continuity pattern, sleep arousal [157,158], amplitude [87,157,159], voltage [157], bandwidth [157], spontaneous activity transient (SAT) rate and interval [159]. SAT is a physiologically immature ultra-slow multiband EEG feature characterised by very low-frequency band (0.1 - 0.5 Hz) activity interspersed with several higher frequency bursts mostly seen in the preterm, and disappears around term; it is a measure of the functional status of neuronal connections [160]. There was a significant increase in continuity pattern [87,157,158], sleep arousal [157,158], SAT rate [159] and lower edge amplitude and boundary voltage values [157] following the use of the respiratory stimulants. However, one study [159] found no changes in EEG amplitude, but instead, a reduction in SAT intervals following caffeine use. In other words, caffeine increases the SAT rate, and this suggests an enhancement of the brains functional network [161]. These changes were greater with caffeine than with aminophylline.

Notably, none of the included studies reported a significant difference in the frequency of apnoea or respiratory rate following stimulant introduction compared with before use. In most instances, respiratory stimulants were administered prophylactically a few hours after birth. Within-subject comparisons from periods 2 hours before up to a maximum of 6 hours after drug administration were evaluated which may not be sufficient to observe any significant differences.

2.4.4. Quality assessment of the included articles

A summary of the adjudged quality for each included article is provided in **Tables** 2.3 – 2.5. A more detailed critical appraisal of the individual papers, using a study design-specific checklist, is provided in **Appendix – Section** 7.2. Of note, three papers

[149,153,156] were well described and comprehensively reported their findings. Other studies lacked detail in key descriptive domains, including clarity in the inclusion criteria, validity, and reliability of their methods. For example, such studies failed to include the methods of measurement and analysis for the exposure and outcome variables. Two of the included articles [18,155] were conference proceedings with limited descriptive information.

Table 2.3: Summary of included studies reporting the effect of apnoea on neonatal EEG features.

Author (year)	Country and WHO region	Study design	Neonate age (weeks)	Sample size	Comparison group	Respiratory and cardiovascular changes definition	EEG methods	Results	Quality assessment/ Comments
Deuel (1973)	USA, America.	Cross-sectional.	Term and preterm (32 - 39 GA, 2 - 8 PNA).	13: 10 with review eligible population inclusion criteria (3 excluded: 2 had GA of 52 and 58 weeks and 1 had apnoeic seizures).	Within and between subjects; during regular period of respiration.	Apnoea: cessation of breathing >20 seconds measured using impedance pneumography. Respiratory pauses: intervals >2 seconds and <20 seconds with no thoracic wall movement. All subjects had ECG recorded. Bradycardia defined as any R-R interval of ≥ 0.6 secs. Oxygen saturation was not recorded.	Montage: At least 4 channels bipolar cEEG (Fp1-C3, Fp2-C4, C3-O1, C4-O2). Outcomes: Degree of continuity and burst suppression pattern. Quantification/ assessment methods used were unclear. Sleep states were differentiated into active (movement on EOG, movement artefact on EEG, observer comment), quiet (no evidence of movement on EOG or EEG), wakefulness (20-secs epoch with eyes opened or when crying).	3 neonates had apnoea. 1 neonate met this review's inclusion criteria. They had 4 apnoeas commencing during quiet sleep and manifesting as simultaneous apnoea and EEG suppression. The initial EEG suppression was no longer than suppression during regular respirations. Bradycardia occurred many secs after apnoea with EEG suppression. All 10 neonates had pauses in respiration (5-20 secs) during quiet sleep (54%) with concomitant bradycardia at the start, middle or end of the pause. The start of some respiratory pauses coincided with a burst suppression pattern. The author does not give the frequency of these changes.	No clear inclusion criteria and little information on subject demographics. Recording methods were clearly described but visually assessed to identify pauses in breathing and EEG changes. No statistical analysis were provided.

Fenichel et al. (1980)	USA, America.	Cross-sectional.	Term and preterm (≤ 38 PMA).	15: 11 neonates had nonconvulsive apnoea and met the inclusion criteria for this review.	Within-subject; before, during and after apnoea.	Apnoea: ≥ 4 secs, measured using a thermocouple taped under the nose in non-intubated neonates, or a piezoelectric transducer on the abdomen in intubated neonates. Heart rate changes measured using ECG and described as a percentage decrease from baseline respiration. Oxygen saturation measured using arterial blood samples 1-5 hours before EEG recording, but it was unclear if measurements were taken during the recording.	Montage: 3 different 6-channel bipolar cEEG montages were used (Fp1-T3, Fp2-T4, Cz-C3, Cz-C4, T3-O1, T4-O2; Fp1-Fp2, Cz-C3, C3-T3, Cz-C4, C4-T4, O1-O2; Fp1-C3, Fp2-C4, C3-O1, C4-O2, O1-T3, O2-T4) Outcomes: visual assessment of EEG amplitude, frequency changes and sleep states. Active sleep was defined as movement on EOG and EMG with decreased tone, continuous EEG, and irregular breathing while the opposite was described as quiet sleep. Any state that did not meet the criteria above was considered as indeterminate sleep.	35 apnoeas were observed in the 11 neonates. 19 episodes were ≤19 secs (10 occurred in active, 5 in quiet and 4 in indeterminate sleep), 6 showed mild amplitude suppression, while 13 had no changes. Likewise, heart rate changes were inconsistent, showing an increase in 4, a decrease in 12 (with a median time change of 2 secs and return to pre-apnoea level within 6 secs) and unchanged in 3 neonates. 16 apnoeas were ≥20 secs (3 in active, 8 in quiet and 5 in indeterminate sleep), only 1 episode showed mild amplitude suppression. Heart rate decreased in all but 1 apnoeic episode with no EEG change. The median time to bradycardia was 3 secs and return to baseline within 22 secs.	Inclusion/exclusion criteria were clearly defined, and methods of recording EEG and apnoea were clear. The definition of apnoea used was not standard. Outcome assessment was visually performed limiting reporting validity and reliability. No statistical analysis was performed.

Bridgers et al. (1985)	USA, America.	Cross-sectional.	Term and preterm (Grouped into <30, 30-33, 34-37 and ≥38 GA).	50: 38 met eligibility criteria (12 had seizure diagnosis independent of apnoeic episodes, treated with antiepileptics during recording). 37 of the 50 neonates had apnoeic episodes during the EEG recording – 10 of these had seizure diagnosis but results were not reported separately for this group.	Within and between subjects; before during and after apnoea.	Apnoea: ≥15 seconds with or without bradycardia (≤90 beats per minute) using monitor alarms and identified by an event marker and nursing log entry. ECG was recorded in 27 neonates and bradycardia was defined as heart rate <90/minute. Oxygen saturation measurements were not described	Montage: 3 channels bipolar cEEG (P3-F3, T3-T4. F4-P4). Outcome: Visual assessment of amplitude. Sleep states were not described.	153 apnoeas were detected. Loss of EEG amplitude was observed during some, but not all episodes. However, the authors do not specify the frequency of amplitude suppression and heart rate changes during apnoeic episodes. No apnoeas were associated with seizure activity on the EEG.	The main aim of the paper was to determine whether apnoeas were related to electrical seizures. Only brief mention was given to EEG changes associated with apnoea. This was based on a subjective visual assessment by 2 raters. No statistical analysis was conducted.
Wulbrand et al. (1994)	Germany, Europe.	Cohort-conference abstract.	Preterm (mean GA 28 weeks range 26.1 to 32.1 weeks, recorded at 36, 40, 44 and 52 weeks)	10.	Within-subject, at 36, 40, 44 and 52 weeks conceptional age.	Apnoea:>10 seconds assessed using nasal airflow and chest wall movement. Augmented breath or sigh (not defined). ECG was recorded but	Montage: Not specified. Outcome: EEG suppression. Quantification/ assessment methods used were unclear. Sleep states were described as N-	Apnoeas followed a sigh in 16 of 106 recorded during REM sleep and in 29 of 43 recorded during N-REM. 99 of the apnoeas were mixed/ obstructive (33 in NREM and 66 in REM sleep). Significant EEG suppression ($p<0.05$) was found during mixed/obstructive N-	This study is a very brief report of a conference abstract. Many details were unclear. The EEG recording techniques and analysis methods were not described. The results were not split by age at recording.

						bradycardia definition and oxygens saturation measures were not specified.	REM and REM sleep; no details define the two sleep states.	REM apnoeas and inactive REM apnoeas. Significant EEG suppression in the 8-13 Hz band in N-REM-sleep apnoeas with an initial sigh occurred ($p<0.05$) in contrast to those without. Apnoea with bradycardia was observed during 11 REM in contrast to 13 N-REM. The occurrence was higher during mixed apnoeas in both sleep states ($p<0.005$). Initial sighs and bradycardia were found in 10 N-REM mixed apnoeas. There was a significant correlation between the occurrence of apnoea preceded by a sigh, bradycardia, and EEG suppression during N-REM.	
Holthausen et al. (1999)	Germany, Europe.	Cross-sectional.	Term and preterm (28-100 GA).	71.	Within-subject; before during and after apnoea.	Apnoea defined as a flat respiratory tracing for ≥ 3 seconds on both nasal airflow and thoracic movement monitors.	Montage: 1 channel bipolar cEEG (C3-C4) Outcomes: mean power, power variance and entropy in 7 frequency bands: sub delta (0±1.5 Hz), delta (1.5±3.5	Subjects were split into three groups: up to 35 weeks, 36±40 weeks and >41-100 weeks GA. A significant reduction in normalised delta and theta amplitude during apnoea compared with before and after apnoea was observed in	Inclusion and exclusion criteria were not clearly defined, and neonate demographics were not presented. The main aim of the paper was to create a model that could predict the neonate

						No heart rate and oxygen saturation measurements were described	Hz), theta (3.5±7.5 Hz), alpha (7.5±13.5 Hz), beta 1 (13.5±19.5 Hz), beta 2 (19.5±25 Hz) and gamma (25±50 Hz) using fast fourier transformation. EEG changes relative to sleep states were not evaluated.	neonates > 41 weeks. Similar, but not statistically significant, changes were observed in the other two age groups	age category from the EEG but changes in amplitude during apnoea were examined. Statistical methods were not clearly defined.
Curzi-Dascalova et al. (2000)	France, Europe.	Cross-sectional.	Preterm (32-33 GA, 2-15 days PNA, 33-34 PMA).	5.	Within-subject; 1 minute before the onset of apnoea and of similar duration as the resultant apnoeic event.	Apnoea: reduction in abdominal respiratory movement amplitude by ≥20% and/or airflow measured using strain gauges and thermistors, respectively, and lasting > 5 secs. R-R intervals on ECG and cardiotocography tracings were recorded to assess heart rate changes. Pulse oximetry was also monitored	Montage: 3 channels bipolar cEEG with at least centro-occipital (C3-O1, C4-O2). Outcomes: Amplitude changes (visually assessed) and frequency changes (assessed both visually and using a computer). Behavioural stages were described visually (using 30-second epochs) as wakefulness (presence of	492 apnoeas were recorded and 27 were >10 seconds. None of the apnoeas were preceded or followed by wakefulness. Only 77 (all during active or indeterminate sleep) were assessed for EEG changes. Others were excluded as they occurred close to other apnoeas, body movements or sighs. Most (62.7%) apnoeas were not associated with amplitude change and these were not significantly different from control periods. Using quantitative analysis, post-apnoea	The definition of apnoea used is not standard (quite short), and with a very small sample size making results difficult to interpret. The study is well described, with a clear explanation of the inclusion criteria, methods of data collection and analysis.

						and compared 1 sec before to 10 secs after an apnoea.	continuous movement or crying; eyes open with or without eye movement), active sleep (continuous EEG pattern, eye movements) quiet sleep (discontinuous EEG, no eye movement) and indeterminate sleep (discrepancy between active and quiet sleep criteria).	frequency changes did not occur in 10.4% of apnoeas, 44.2% of apnoeas showed an increase in frequency and 45.4% showed a decrease. Although the proportion of apnoeas showing a frequency increase was like the control periods, the value of the change observed following an apnoea was significantly different ($p < 0.02$). Regression analysis showed that the normalized percentage of EEG change was significantly dependent on apnoea type (obstructive versus central apnoeas ($p < 0.007$), with no change after central apnoea); amplitude modification ($p < 0.008$), and basal EEG frequency ($p < 0.02$). Frequency change was not related to apnoea duration or sleep state. Most apnoeas (58.9%) were followed by bradycardia (pre- and post-apnoea difference	

								p <0.002), 34.7% by tachycardia and no heart rate changes in 6.7%. For control periods, bradycardia (50.6%) tachycardia (42.9%) and no changes (6.5%) were observed. Mean bradycardia following apnoea was significantly greater compared with controls ($p < 0.03$) and was dependent on apnoea duration ($p < 0.0001$), and baseline heart rate ($p < 0.0007$) but not on sleep state. Nearly half the apnoeas (48%) were followed by desaturation, 17.3% by an increase in saturation (this occurred when the baseline saturation was < 90%) with no change during 37.7% of apnoea episodes. There were no significant differences between the saturation changes following apnoea and control periods. There was no correlation between EEG frequency and cardiorespiratory changes.	

Schramm et al. (2000)	Germany, Europe.	Cross-sectional.	Term and preterm (22-40 GA, mean 34.7 GA, mean 45.2 PMA).	51.	Within-subject; consisting of periods just before and after apnoea; and other apnoea-free phases of active sleep.	Not specified. ECG and pulse oximetry were recorded	Montage: Two channels bipolar cEEG (C3-T3, C4-T4). Outcomes: Absolute power, relative power, median frequency, and peak frequency for sub delta (0.4±1.5 Hz), delta (1.5±3.5 Hz), theta (3.5±7.5 Hz), alpha (7.5±12.5Hz), beta 1 (12.5±19.5 Hz), and beta 2 (19.5±25.0Hz) band using spectral analysis. Sleep states were automatically annotated using a polysomnographic device.	A significant reduction in the mean and standard deviation of absolute EEG power during apnoeas in the sub delta, delta, theta, and alpha frequency bands compared to before and after periods of apnoea and the apnoea-free phases of active sleep. In the beta 1 band, significantly lower absolute power during the apnoea compared with before the apnoea and the apnoea-free periods occurred (not different to after the apnoea). No significant power differences in the beta 2 band were observed. The highest relative reduction of 45% was in the theta band. There were no differences in the means and standard deviations of peak frequencies in all the frequency bands.	No clear description of the exposure variable (e.g., duration of apnoea not specified). It is not clear what statistical test was used or whether all apnoeas were recorded in one study neonate or more evenly distributed across participants. Demographic information was not clearly described. However, outcome measures were well described. No heart rate or saturation changes were described as part of the results. Sleep state comparisons were not detailed, only active sleep states during apnoea-free periods were considered.
Low et al. (2012)	Ireland, Europe.	Case report.	Term (32 GA/38 corrected GA).	1	Within subject; before, during and after the onset of apnoea.	Apnoea: >20 secs, or <20 secs with associated bradycardia (20% below baseline heart	Montage: 4 channels bipolar cEEG (F4-C4, F3-C3, C4-O2, C3-O1).	8 apnoeas were recorded. 6 apnoeas (range 23–119 seconds) had no EEG changes with a mean lowest desaturation of 45% and bradycardia of 99	A clearly described case report.

						rate) or oxygen desaturation (<80%) and measured with neonatal monitors. ECG and oxygen saturation were recorded.	Outcomes: EEG suppression is defined as amplitude reduction below 5μV in all EEG channels for at least 10 secs. This was reviewed and annotated by a neurophysiologist. Sleep states were not described.	beats/min. 2 episodes (213 and 376 secs) required resuscitation and were associated with burst suppression. These were preceded by a rapid drop in heart rate and saturation below 20% with accompanying cyanosis. During recovery, EEG activity returned with improved oxygen saturation above 30 to 40%.	
Mañas S et al. (2014)	Spain, Europe.	Cross-sectional-conference abstract.	Preterm (31-35 weeks corrected GA).	12.	Within-subjects; apnoea compared with a period of normal respiration.	Not defined. Vital signs monitoring not specified.	Montage: 8 electrode cEEG (FP1, FP2, C3, C4, T3, T4, O1, O2) referenced to mastoids. Outcomes: Functional connectivity assessed using MSC and PSI in the delta frequency band (0.5-4 Hz) and NLGSI between all pairs of EEG channels were quantitatively evaluated. Active and quiet sleep states were recognised but	MSC was increased during quiet sleep apnoea compared to normal respiration in the same state ($p < 0.001$). Intra-hemispheric EEG channel pairs exhibited significant differences between normal respiration and apnoea ($p < 0.001$) which was dependent on sleep state ($p < 0.01$), whereas inter-hemispheric EEG channels showed differences regardless of the type of sleep ($p < 0.01$). NLGSI was greater for apnoea than during normal respiration (p	This was a conference abstract with limited details. In particular, the definition of apnoea was not given. The EEG measures assessed are not as commonly used as those used in other studies. It was not clear in what direction the interhemispheric EEG changes were dependent on either active or passive sleep.

							definitions were not provided.	<0.01) irrespective of the sleep state. The PSI during normal respiration was not different from zero while during apnoea, it was significantly different from zero and positive (p <0.01)	

Abbreviations - amplitude augmented electroencephalography (aEEG), conventional electroencephalography (cEEG), electrooculogram (EOG), gestational age (GA), magnitude squared coherence function (MSC), nonlinear generalized synchronization index (NLGSI), non-rapid eye movement (NREM), phase synchronization index (PSI), postmenstrual age (PMA), postnatal age (PNA), rapid eye movement (REM).

Table 2.4: Summary of included studies reporting the effect of other respiratory changes on neonatal EEG features

Author (year)	Country and WHO region	Study design	Neonate category and age	Sample size	Comparison group	Respiratory and cardiovascular change definition	EEG methods	Results	Quality assessment/Comments
Whitehead et al. (2019)	United Kingdom, Europe.	Cross-sectional.	Term and preterm (23-41 weeks GA, 30-42 weeks PMA, 2-78 days PNA).	13: 10 met the review inclusion criteria (2 had germinal matrix intraventricular haemorrhage while one neonate had ventriculomegaly).	Within-subject event-free period.	Hiccups: diaphragm contractions were recorded with a movement transducer attached to the trunk. ECG and oxygen saturation measurements were done. No cut-offs for bradycardia and desaturation were described.	Montage: 16-18 channel referential cEEG (F7, F8, F3, F4, Cz, C3, C4, T7, T8, P7, P8, O1, O2, CPz, CP3, CP4, TP9, TP10) with reference at Fz, re-referenced to common average for analysis. Outcomes: ERP were quantitatively assessed using grand averages, individual subject traces and scalp maps. Both wakefulness and active sleep were characterised by movement, irregular	A total of 1316 hiccups were recorded. Hiccups elicited 3 distinct ERPs compared to baseline. ERP1 comprised of front-central-temporal negativity, with positivity most prominent across the posterior region at -49 to 35 ms (GFP peak latency: 16 ms). ERP2 showed central and posterior negativity, with positivity most prominent across the anterior and bi-temporal regions at 91 to 150 ms (GFP peak latency: 125 ms), while ERP3 showed central and posterior positivity, with negativity most prominent bi-temporally at 223 to 913 ms (GFP peak latency: 310 ms). There were no correlations between	An exploratory study identifying neonates from an EEG research database who had hiccups during the recording. Methods are clearly described.

							breathing, and continuous low-voltage EEG while quiet sleep was defined by the absence of movement, regular respiration, and a fluctuating EEG amplitude.	the ERPs and neonate-corrected gestational age. There was no significant difference in heart rate and oxygen saturation changes before and after hiccup bouts.	

Abbreviations: electromyography (EMG), event-related potential (ERP), gestational age (GA), Global Field Power (GFP), non-rapid eye movement (NREM), postmenstrual age (PMA).

Table 2.5: Summary of included studies reporting the effect of respiratory stimulants used for apnoea on neonatal EEG features.

Author (year)	Country and WHO region	Study design	Neonate category	Sample size	Comparison groups exposure and definition	Respiratory stimulant and vitals measurement	EEG methods	Results	Quality assessment/ Comments
Supcun et al. (2010)	Germany, Europe.	Cohort.	Preterm (<34 weeks GA, median GA 29 weeks (range, 24-33 weeks); 2 (range 1-14) days PNA).	51: 20 intubated.	Within-subject comparison; preterm neonates scheduled to receive the first dose of caffeine for prophylaxis or treatment of apnoea were compared from 2 hours before until 2 hours after the loading dose of caffeine. Apnoea was defined as oxygen saturation < 80% for at least 5 seconds; assessment method unspecified.	Caffeine, intravenous loading dose 10 mg/kg body weight. Heart rate and arterial oxygen saturation were recorded. Details about methods of measurement are not described.	Montage: 2 channels bipolar aEEG (C3-P3 and C4-P4). Outcomes: EEG continuity assessed visually as continuous (minimum amplitude > 5 μV, maximum amplitude 10-50 μV) or discontinuous (minimum amplitude variable but < 5 μV, maximum amplitude > 10 μV) in 10-minute epochs. Quantitatively, one-minute average values for the maximum, minimum, and mean amplitudes were calculated. No sleep staging was considered	After caffeine administration there was a significant increase in the percentage of aEEG continuity ($p<0.001$), and the maximum ($p<0.001$), minimum ($p<0.001$) and mean ($p<0.001$) amplitudes compared to before use. These changes persisted throughout the 2-hour recording after caffeine administration, with no differences between the first 30 minutes after caffeine administration and the 30 minutes at the end of the 2-hour window. There were no significant changes in heart rate or oxygen saturation pre- and post-caffeine administration. In the 31 spontaneously breathing neonates, there was no difference in the number of apnoeic episodes between the 2 observation periods.	A very brief report, an exploratory study for a limited time range, but EEG measures are described in detail.
Hassanein et al. (2015)	Egypt, Eastern Mediterranean.	Cohort.	Preterm (<34 weeks GA).	33: only 20 cases were relevant	Within-subject comparison; Caffeine group received the first	Caffeine, intravenous loading dose	Montage: Single aEEG biparietal channel recorded to assess changes in response	There was a statistically significant increase in aEEG continuity ($p=0.002$) 30 minutes after caffeine	This study compared neonates given caffeine for

				to the review, 13 control groups were not relevant and were only studied for follow-up comparison.	dose of caffeine for prophylaxis or treatment of apnoea (recorded for 1 hour before, during and 2 hours after caffeine administration. Apnoea definition and measurement method not specified.	20 mg/kg body weight. Continuous cardiorespiratory monitoring was done but methods were not specified	to caffeine and 9 channel cEEG (Fp1, Fp2, C3, C4, T3, T4, O1, O2, and Cz, reference not given) recorded at 36 weeks. Outcomes: aEEG continuity was assessed visually and defined as continuous with minimum amplitude >5 μV and maximum amplitude of 10–50 μV; or discontinuous with variable minimum amplitude < 5 μV and maximum amplitude >10 μV). cEEG was used to identify seizures and sleep arousal if they occurred. Sleep state differentiation into 6 stages (quiet, active, drowsy, quiet alert; active alert, crying) was considered but findings were not described based on the classification.	administration (51.96% ± 34.30%) compared to before (33.33% ± 30.05%) in the caffeine-treated group. Arousability significantly increased after caffeine administration, with more babies showing EEG features indicative of alertness compared with sleep ($p< 0.001$). No significant difference in apnoea occurrence with caffeine administration. Also, caffeine significantly increased heart rate ($p = 0.0001$), mean arterial blood pressure ($p = 0.0001$), and oxygen saturation ($p = 0.003$) compared with period before administration. At 36 weeks, aEEG score was significantly higher in the caffeine group (10.11±1.62) compared to the control group (6.85±1.77, $p<0.001$), whereas conventional EEG background cerebral activity grade and electrographic seizure activity showed no significant difference between both the two groups ($p=0.091$, 0.38).	clinical reasons with those born at a similar age who were not given caffeine, either as it was not clinically necessary or because parents did not consent to treatment. There may be differences between the two groups. Half of the caffeine group was lost to follow-up at 36 weeks, but reasons are not given.

Dix et al. (2018)	The Netherlands, Europe.	Cohort.	Preterm (<32 weeks GA).	32.	Within-subject comparison; neonates receiving their first dose of caffeine therapy prescribed by the treating neonatologist. Periods compared were before caffeine therapy and 6 hours after therapy started. Physiological parameters were not defined but measurement described.	Caffeine intravenous loading dose 10 mg/kg body weight. Patient monitors were used for physiology monitoring.	Montage: 2 channels aEEG, electrode positions not specified Outcomes: SAT rate and ISI were calculated from the raw EEG; minimum, mean, and maximum amplitudes of aEEG. Sleep staging was not described.	There was a significant increase in SAT rate by 0.33/min per week GA ($p < 0.001$), and a decrease in ISI length of 0.42 s per week ($p < 0.001$) but no change with caffeine intake. Maximum, mean, and minimum amplitude did not change significantly after caffeine intake but were all significantly associated with GA. There was no significant difference in respiratory rate and oxygen saturation, but the heart rate increased following caffeine administration over time.	EEG results were not reported in detail e.g., no values or plots given for SAT rate and ISI length before compared with after caffeine start. The analysis methods, such as how SATS were identified were not clearly specified.
Yang et al. (2019)	China, Western Pacific	RCT.	Low birth weight preterm enrolled in the caffeine group (mean 32.56 ± 2.35 weeks GA) and the control group who	212 (106 per group).	Between subject comparisons; neonates with apnoea treated with caffeine citrate were compared with controls that received aminophylline. Recording time frames were not indicated by the authors.	Caffeine citrate (intravenous loading dose 20 mg/kg, then maintenance dose 5 mg/kg every 24 hours) or aminophylline (intravenous loading dose 10 mg/kg, then maintenance dose 2 mg/kg	Montage: aEEG electrode positions not specified. Outcomes: Sleep arousal cycle, graphic continuity, lower edge amplitude value, aEEG continuity voltage, periodic occurrence rate narrowband voltage, and bandwidth were evaluated using	There were significant differences in all the EEG outcomes between the caffeine and aminophylline groups before treatment. Sleep arousal cycle ($p=0.029$), graphic continuity ($p=0.017$), lower edge amplitude value ($p=0.047$), continuous voltage positive rate ($p=0.011$), sleep-wake cycle ($p= 0.042$), and lower boundary voltage ($p=0.007$) were significantly higher in the caffeine group than in the aminophylline group. All measures increased	Many details were not reported in the paper, specifically relevant for this review, for example, the time points at which the EEG measurements were taken, the equipment used, definition of apnoea. Treatment allocation concealment,

			received aminophylline (33.02 ± 1.98 weeks GA).		Apnoea definition and measurement not defined.	every 12 hours). No physiology monitoring was reported.	defined scoring systems. Quantification method was not specified. No sleep state categorisation was reported.	in both groups after treatment compared with before, but no statistical analysis was performed (as the main outcome of the trial was the comparison between groups). aEEG detection bandwidth and narrow-band upper boundary voltage decreased after treatment, with significantly lower values in the caffeine group (p=0.020, 0.032 respectively).	blinding (treatment and outcome assessment), the method used (reliability) for outcome assessment and deviations from standard RCT design were not specified. No correction for multiple comparisons were used and no measure was pre-selected as the primary outcome.

Abbreviations: gestational age (GA), inter SAT interval (ISI), postmenstrual age (PMA), randomised controlled trial (RCT), spontaneous activity transients (SATs).

2.5. Discussion

This systematic review shows that only a small number of studies (14) were conducted up until August 2022 which investigated the relationship between neonatal respiration and EEG changes. The exposure and outcome descriptions were heterogeneous. Studies reporting apnoea-related EEG changes were the commonest and the findings during and after apnoeic episodes were non-uniform, including burst suppression and amplitude reduction occurring in some but not all apnoeas. A reduction in EEG power and amplitude is suggestive of a shift in arousal from sleep to wakefulness [19] and when occurring during an apnoea, may signify an adaptive mechanism increasing alertness, possibly initiating body movement to stimulate breathing and limit the impact of apnoea. Interestingly, no change in brain activity from baseline was observed during some apnoeic episodes. The factors that drive these differences are still unclear but may be linked with the characteristics of apnoea such as the duration, type of apnoea and sleep state. Other factors like the frequency and severity of episodes, including accompanying adverse cardiovascular changes like desaturation and bradycardia, may also be contributory. However, these have not been detailed from the review. Only one study investigated another respiratory event other than apnoea (hiccups) and showed the occurrence of simultaneous EEG event-related potentials, suggesting a link between respiratory events and cortical responses. We did not identify any study that looked at how normal respiration and EEG signals co-vary in neonates by age. Respiratory control is dependent on neonatal age, and the preterm is more likely to experience significant adverse respiratory events [4]. Exploring this relationship is important to better understand the link between breathing and brain function. Finally, respiratory stimulants enhanced EEG continuity, sleep arousal, lower edge amplitude

and boundary voltages; and decreased SAT interval bandwidth and narrow-band upper boundary voltage. These features put together may imply an enhancement in brain activity and CNS arousal following respiratory stimulant use.

During apnoea, EEG amplitude was generally reduced or fluctuating, suggesting weaker brain signals [19,149–151]. These changes are more pronounced in neonates with central apnoea**,** pointing to a CNS origin rather than a peripheral dysfunction. EEG power, a measure of the signal strength at a given frequency, was also attenuated in the low-frequency bands (delta/theta), often during or preceding apnoeic events, suggesting reduced cortical synchrony and a compromised functional capacity [19]. Importantly, a discontinuous EEG and burst suppression pattern, reflective of severe cortical depression or encephalopathy, has been identified in some apnoeic neonates [152–155], suggesting a link between impaired respiratory drive and disrupted cortical activity. Only one study [18] provided insight into functional connectivity changes associated with apnoea by employing advanced signal processing methods. They showed that during apnoeic events, there were significant reductions in the (1) magnitude-squared coherence**,** which measures linear correlations between EEG signals across different frequency bands, suggesting disrupted inter-regional communication (2) Phase synchronization index, which assesses the synchrony of neural oscillations between different brain regions, implying weakened temporal coordination of brain networks and finally, (3) Nonlinear generalized synchronization**,** a reflection of the strength and complexity of brain connectivity measures, indicating broader dysregulation in neural coupling mechanisms. These findings put together indicate a weakened functional coupling between cortical or subcortical regions, disrupted information integration, especially in networks involved in respiratory control

and arousal and a less resilient brain network, where different regions are no longer communicating in a coordinated way.

Generally, EEG recordings show age-dependent maturational features, ranging from a relatively discontinuous dysmature recording in the extremely preterm to a more continuous pattern in the term neonate [30]. Correspondingly, it might be that age-related brain activity differences may also exist during apnoea. Only one study measured apnoea-related EEG changes in relation to age, observing a significant amplitude reduction in term neonates > 41 weeks only but not in the younger age groups [20]. While the study may have been underpowered to observe a significant effect in the latter age ranges, the finding may simply reflect the immature brain function and poor apnoea adaptive responses of the preterm neonate.

EEG features like suppression pattern (low amplitude EEG alternating with bursts of high voltage activity), persistent amplitude and frequency reductions denote attenuated neuronal discharge or in extreme cases following profound brain damage, may indicate electrocerebral inactivity [119]. The inconsistency in EEG suppression following apnoeic episodes observed in the included studies (i.e., suppression occurred in only 20–40% of neonates while most (60-80%) showed no EEG findings) can be explained by several interrelated factors including apnoea-related (e.g., severity, duration) and infant characteristics (e.g., gestational age, cerebral vulnerability). Methodologically, the heterogeneous nature of apnoea definitions/severity, and the accuracy of the different apnoea detection methods may account for the inconsistencies in the findings. Physiologically, not all apnoeas cause significant hypoxia or cardiovascular instability, which are triggers for cortical

suppression. Studies such as Curzi-Dascalova et al. (2000) and Deuel (1973) reported considerable variability in physiological consequences following apnoea, with many episodes causing minimal or no disruption to the vital signs. Similarly, Fenichel et al. (1980) found that only a subset of apnoeas elicited bradycardia or marked autonomic changes—factors that may be necessary to provoke EEG abnormalities. While some studies only included preterm neonates, others studied, in addition, term babies. Cerebral response to apnoea differs by neonatal age, with the immature cortex likely exhibiting inconsistent responses due to developing neurovascular coupling and thalamo-cortical connectivity [19]. Moreover, variability in cerebral autoregulation or oxygen reserve capacity, which is greater in the term compared with the preterm, could protect against cortical dysfunction in many cases. Only in neonates with lower physiological resilience, e.g., the preterm or in more severe apnoeic events, might cortical suppression emerge, as seen in an isolated case report [153] and other studies [151,155].

The relationship between burst suppression and apnoea in neonates tends to be bidirectional. Primarily, EEG burst suppression from impaired brain function, as seen in neonates with an underlying CNS pathology, e.g., hypoxic ischemic encephalopathy, is more likely to result in apnoea, especially prolonged or severe ones. Less commonly, apnoea with accompanying desaturation and bradycardia can also cause hypoxic cortical depression, especially in the vulnerable neonate, leading to EEG suppression, and in extreme or repeated episodes, contribute to a burst suppression-like pattern (e.g., in hypoxic-ischemic injury). To disentangle this reciprocal relationship, only studies that included neonates with normal CNS findings were included in the review. While some studies have shown EEG suppression during

apnoea episodes [152–155], which might suggest brain inactivity, this could also be a neuroprotective energy conservation mechanism limiting potential brain injury [153]. In the long-term, apnoea has been associated with poor neurodevelopmental outcomes [12–14,162], however, it remains unclear what duration of apnoea-related brain inactivity could cause brain injury, or whether poor outcomes occur solely as a result of the impaired cerebral oxygenation that accompanies some apnoea episodes [163]. Some of the included studies showed variable heart rate and oxygen desaturation changes during episodes of apnoea [149,150,152], and others did not monitor vital signs during EEG recording [18,20] or describe EEG features in relation to physiology changes [19,151]. Repeated and prolonged apnoeas are known to cause cerebral hypoperfusion and dysfunction [163], and the finding in one case report [153] that extremely low level of oxygen saturation during prolonged apnoeas resulting in burst suppression pattern suggest a complex interaction between apnoea, physiological changes and brain activity. Following these findings from this systematic review, our research group and collaborators in Leuven further investigated respiratory-brain networks exploring phase-amplitude coupling between respiratory and brain signals. We found that EEG frequencies between 1 – 8 Hz were significantly coupled with impedance pneumography respiratory signal in the 1Hz frequency in a top-down control. The stronger the coupling metric, the lesser the likelihood of apnoea occurring. Further work is needed to investigate the relationship between specific apnoea characteristics and EEG changes [164].

We found four studies that looked at the influence of respiratory stimulants on neonatal brain activity. In all the studies, the effect of respiratory stimulants was evaluated following the start of the medications (and in most cases, it was given prophylactically)

and not with specific apnoea episodes. Moreover, the studies did not explore detailed simultaneous interactions between EEG and cardiovascular physiology such as oxygen saturation and heart rate changes. Only one study [157], an RCT, compared the effect of caffeine and aminophylline during apnoea on EEG features, and reported significantly enhanced EEG cortical activity with caffeine compared with aminophylline. This observation is perhaps related to the mechanism of action of caffeine, being a neuroprotective, non-selective adenosine receptor blocker preventing apoptosis and increasing cerebral oxygen metabolism, reducing oxidative stress and inflammation [165,166] more than aminophylline. Further work is needed to ascertain whether these changes are consistent during an apnoea and if they are dose- or age-related to help drive individualised care. None of the studies linked short-term EEG changes with later neurodevelopmental outcomes.

This review was limited by the small number of studies, many of which had small sample sizes. Two papers were case reports [153,154] with known study design limitations [167], another two were conference proceedings [18,155] with limited information, and three [150–152] were published up to four decades ago so findings may be outdated due to technological advancements. We found diverse exposure variable definitions, EEG outcome measures, and study designs (the vast majority were descriptive studies), thus, making study comparison and generalisation of relevant results difficult. Several studies [150–152] used visual EEG assessment (in part as they were from the 1980s or before) and while this is the clinical gold standard, it can be highly subjective and may lack reproducibility. Statistical analysis was lacking in several studies, and when used, no correction for multiple comparisons was done. Some studies used few EEG channels which would result in important loss of spatial information. While reduced

EEG montage has been shown to have high sensitivity and specificity, at least three channels are required for the differentiation of EEG artefacts, normal transients and abnormalities, especially for unilateral cortical involvement [168,169]. Conventional apnoea detection by observation as used in some studies may be inaccurate due to human error in identifying apnoeic episodes.

A strength of our review is that it systematically identified and reviewed all studies conducted globally (English only) relating EEG activity with neonatal respiratory changes, and importantly, apnoea, a common and avertible cause of neurologic disability. To the best of my knowledge, no similar review has summarised the existing data on this topic.

3

Breathing and Brain Activity in Neonates: Biomarkers for Personalised Caffeine Treatment

This chapter has been submitted for publication in Nature Communications and is currently under the third round of review. I am a first author on the manuscript [170]. The work has been restructured to fit a thesis format and the content of this chapter is my own.

3.1. Background

From the systematic review in **Chapter** 2, no studies investigated the relationship between normal breathing and brain activity across different PMAs. Exploring this relationship is crucial to enhancing our understanding of the normal brain-respiratory physiology of neonates. While brain activity is vital for brain development and respiratory function, key interactions between brain maturity and breathing mechanics remain unexplored. For instance, why some neonates have fewer, shorter, or earlier resolution of apnoea symptoms, and vice versa, than others is incompletely understood. Although neonatal age has been proposed as a possible factor for these differences, it does not explain why these variations exist even in neonates of the same

GA or PMA. It may be that other intrinsic factors, for example, intrinsic individual differences in brain function and development are responsible for driving respiration regardless of age. Henderson-Smart et al. (1983) proposed that apnoea is related to underlying immature brain-respiratory control mechanisms and showed that the occurrence of apnoea in preterm neonates was significantly correlated with delayed brainstem conduction times using auditory evoked potentials [171]. Additionally, hospitalised neonates are exposed to pathological conditions e.g. infections, environmental stress [145], medications [172,173], and painful procedures [174]; and these have been shown to alter brain function, which contributes to the regulation of breathing. Therefore, the control of respiration in the neonate is unlikely to be best explained by age alone, but rather by other factors, and these relationships need to be explored further.

While neonates with more spontaneous cortical activity early in life have been shown to have faster brain growth [175], impaired brain function has been linked with altered neuronal plasticity causing brain immaturity [7,8] and predisposing to acute respiratory complications like apnoea. The effectiveness of the respiratory control mechanism in the neonate is, thus, dependent on brain development. EEG machine-learning algorithms [122–124] have accurately been used to predict biological biomarkers of brain health and function (brain age and maturity – **Section** 1.8.1). In contrast to chronological age (a linear measure of time), neonatal brain age (a biological (true) age of the brain relative to the actual PMA) and brain maturity (a standardised measure of the brain's functional development calculated as the difference between brain age and PMA) are functional markers of whether an individual's brain is comparatively more or less mature than their actual chronological age [127,176,177]. These brain-based

measures are modulated by genetics and environmental factors including disease [122,178,179]. Therefore, detecting deviations from the normal brain developmental trajectory using measures like brain age and maturity could be more valuable for prognostication. Little is known about the immediate effect of brain age and maturity on respiratory dynamics at different PMAs. To the best of my knowledge, no authors have assessed the association between brain age and/or brain maturity with physiological (normal) and pathological (apnoea) respiration. I propose that EEG-derived brain-based measures might be better predictors of respiratory function than the current chronological age paradigm because, in practice, individual variations exist in respiratory dynamics including apnoea frequency, severity, age at onset and symptom resolution [3]. Therefore, relying solely on chronological age, a linear 'ticking clock', instead of the more individualised brain-based measures as predictors of respiratory changes may provide an imprecise indication of respiratory function. Since brain age and brain maturity are influenced by both intrinsic and extrinsic factors and provide a more direct metric for assessing brain function, they could be better metrics than chronological age in explaining individual differences in normal respiration and complications such as apnoea.

Caffeine is the drug of choice for apnoea prevention and treatment. Prescription guidelines are based on weight and GA at birth, which do not directly relate to the primary underlying cause of apnoea i.e., immaturity of brain-respiratory control mechanisms [165]. Despite the guidelines, widespread institutional variations exist in management protocols e.g., when to start and stop caffeine therapy. Prophylactic caffeine is prescribed for all extremely premature neonates but not for all late preterm neonates, who are also susceptible to apnoea. Most babies born < 32 weeks GA

receive caffeine irrespective of whether they develop apnoea or not and it is discontinued at approximately 34 weeks. In some centres, caffeine is often continued until the neonates are > 34 weeks (up to 36 weeks corrected GA), are apnoea-free for at least 8 days or at the discretion of the clinician. Determining caffeine requirement based on age alone and the non-uniform practice is far from ideal, placing neonates at significant risks including drug side effects from prolonged therapy or undertreatment when caffeine is discontinued too early causing apnoea to reoccur. Moreover, treatment guidelines do not consider individual variation in response to therapy. Similar to inherent individual differences in apnoea risk, response to caffeine therapy also varies [82]. Consequently, the need to optimise caffeine treatment guidelines, using a more personalised approach based on appropriate risk-stratification measures, for example, using brain age and maturity instead of chronological age and weight measures to standardise treatment protocols is necessary.

In this Chapter, I will compare how EEG brain-based measures (brain age and maturity) and PMA affect respiration and apnoea frequency (both assessed using continuous vital signs monitoring). I will also assess, in preterm neonates, the relationship between EEG-derived brain measures, the need for caffeine treatment and the subsequent risk of respiratory instability following caffeine discontinuation. I will use validated machine-learning models - resting-state [124] and sensory-evoked response [125] algorithms - to assess brain age and brain maturity, and a neonatal-specific respiratory algorithm [180] to assess respiration and apnoea rates in neonates.

3.2. Objectives

The specific objectives of this study are to:

1. Identify how respiratory and apnoea rates change with PMA, brain age and maturity.
2. Compare PMA, brain age and brain maturity in determining the risk of apnoea in preterm neonates.
3. Determine the association between PMA and brain maturity to guide caffeine treatment for apnoea of prematurity.

3.2.1. Hypothesis

Brain age and maturity are more accurate determinants of neonatal respiratory changes i.e., physiological (normal) and pathological (apnoea) breathing than PMA; and especially in the preterm with increased susceptibility to apnoea, brain maturity can inform the need for caffeine treatment and more effectively guide clinical decisions on when to stop therapy better than PMA.

3.3. Methods

This chapter details the approach used to investigate the specific research objectives of this sub-study. Participants were recruited based on 2 study designs:

1) A cross-sectional design where both term and preterm neonates were recruited and studied on one test occasion.
2) An ongoing longitudinal cohort design comprising mainly preterm neonates studied multiple times from the time of recruitment up until discharge or withdrawal from the study.

3.3.1. Study site, population, and eligibility criteria

This study was conducted at the Neonatal Intensive Care Unit (NICU) and postnatal ward of the John Radcliffe Hospital (JRH), Oxford, United Kingdom. EEG features are poorly correlated in the extremely preterm neonate, and become well established by 28 weeks GA) [30]; thus for interpretability, the study population comprised neonates 28 weeks to 42 weeks PMA. All clinically stable neonates whose mothers were aged > 16 years and with written informed consent were included. Participants were excluded if they were receiving opiates at the time of recruitment, had congenital malformations, CNS abnormalities (hypoxic ischaemic encephalopathy; grade III or IV intraventricular haemorrhage) or history of maternal substance abuse during pregnancy. All these were potential confounders that could affect result interpretation. Outpatients or neonates without parental consent were also excluded.

3.3.2. Ethics approval and consent

The study with participants enrolled under the cross-sectional design was approved under the Newborn Infant Pain Investigation (NIPI) ethics (reference: 12/SC/0447) while the cohort design was approved as the Breathing and Brain Development (BBD) study (ethics reference: 19/LO/1085). Prior to recruitment, informed and written parental or caregiver consent was obtained. The studies were conducted according to the standards set by the Declaration of Helsinki and Good Clinical Practice guidelines.

3.3.3. Recruitment and data collection process

This was an exploratory study. A convenience sampling technique [181] was used to recruit every eligible neonate, targeting a minimum sample size of 70 neonates, comprising 10 participants per 2-week interval (i.e., 28 - 29 weeks, 30 - 31 weeks etc) to strengthen sample representativeness and generalisability.

The NICU and postnatal unit handover sheets at the JRH were screened for eligible participants. Families were approached, provided with information and a leaflet about the study, and at least an hour to decide on participation. Information such as GA (based on the best estimate between last menstrual period and ultrasound assessment in the first trimester. The last menstrual period was the primary method of assessing the GA unless there was significant (>7 days) deviation between reported LMP and ultrasound assessment at which point the ultrasound date was used), PMA, sex, ethnicity, Apgar scores, mode of delivery, need for resuscitation at birth, birth weight, weight at test occasion, presence of infection, jaundice , respiratory distress, medications received, patent ductus arteriosus and persistent pulmonary hypertension (both diagnosed using echocardiography done by the clinical team), and type of

respiratory support at the time of the study i.e., whether on oxygen therapy (high flow or low flow) or not on oxygen (spontaneous ventilation in air) were obtained from the clinical notes. These were recorded on a demographic paediatric neuroimaging group database. For neonates that were studied multiple times, these details were updated following each test occasion.

3.3.4. Test occasion recordings

At least two members of the research team were involved during the study. Each test occasion consisted of simultaneous resting-state EEG, sensory-evoked responses to experimental stimuli (visual and tactile) and vital signs recording. Electromyography (EMG) was also recorded to detect movement artefacts during the EEG recording [30]. On the day of the study, before the start of data collection, the nursing team were approached regarding the general condition and clinical stability of the participant, and the study was planned according to recommendations from the nurse looking after the neonate. Studies were usually planned preferably after a feed when the neonate is settled. Test occasions did not interfere with the clinical management of enrolled participants and all recordings were carried out in the NICU or postnatal ward by the bedside, outside clinical procedures, care, and cuddle times.

3.3.4.1. Electroencephalographic recording of brain activity

A multi-channel resting-state EEG was recorded from DC to 400Hz using the CURRYscan 7 neuroimaging suite (Neuroscan SynAmps2) at a sampling rate of 2000Hz. Disposable Ag/AgCl cup EEG electrodes (Ambu Neuroline, Ballerup, Denmark) were used for the recording, placed at eight positions (Cz, CPz, C3, C4,

Oz, FCz, T3, and T4) on the neonate's scalp (Figure 3.1A) according to the standardized international 10 - 20 electrode placement system [182]. The typical neonatal montage is often a modified or reduced montage that uses the standardised 10 - 20 electrode placement system with a minimum of 8 - 10 electrodes. This is due to the relatively small infant head size and for clinical practicality. The reduced montage is simple and ideal for most neonatal EEG research as the electrodes cover the midline, lateral and temporal brain regions, and consequently, record neuronal activity in the underlying primary motor cortex and sensorimotor cortex including any event related potential [183,184]. For this study, a referential montage (reference electrode - Fz) was used which is ideal for showing large amplitude waves, such as the delta waves commonly present in neonates, or for identifying generalized brain dysfunction. It allows for better measurement of signal amplitude as all channels share the same reference (Fz) [185]. FPz was used as the ground electrode and is essential to limit surrounding electrical interference from affecting the quality of the recording.

To record the EEG brain activity, first, scalp measurements were made to appropriately identify electrode placement positions. Next, these positions were lightly marked with an 'easy wipe-off' water-soluble marker and the underlying skin prepped by gently massaging the scalp in a circular motion with a cotton bud covered in a preparation gel - NeuroPeP gel. This approach removed any residual oil on the skin, increased scalp blood flow for optimal signal and ensured good electrode contact to allow for good impedance of no more than 5 -10 kΩ during the recording (**Figure** 3.1B). Once all the electrode positions were marked and prepped, the EEG electrodes were attached to the neonate's scalp using a conductive paste (Elefix EEG paste, Nihon

Kohden) and secured with tape (**Figure** 3.1C). Equipment setup and electrode placement took approximately 10 - 20 minutes.

At the start of each recording, approximately 10 background annotations were time-locked on the EEG and vital signs monitor using a push button device connected with the PiNe box [186] to synchronize the EEG and vital signs data and facilitate analysis. EEG was recorded for approximately 1 - 2 hours. Neonates recruited under the NIPI ethics were studied once, while those studied under the ongoing BBD ethics had recordings approximately once a week till discharge.

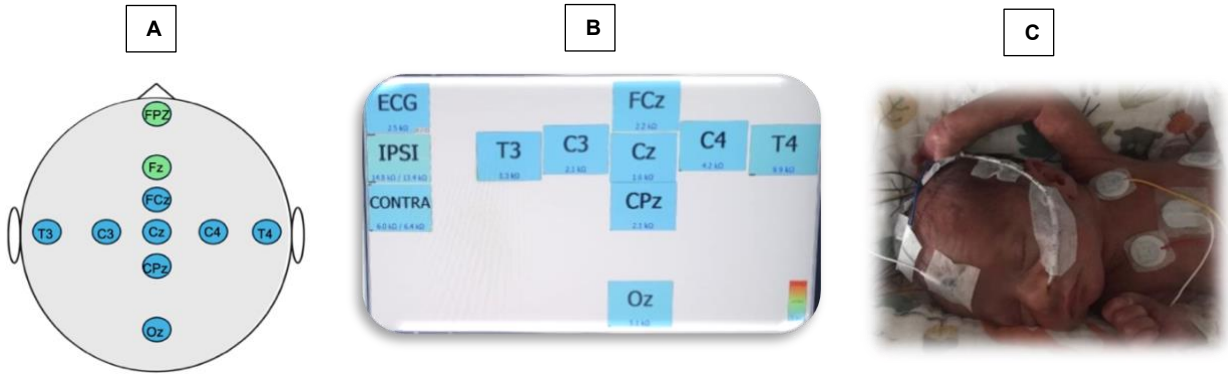

Figure 3.1: EEG montage, impedances, and electrode placement. An illustration of 8-channel scalp electrode placement is shown in blue, and FPz and Fz as the ground and reference electrodes are shown in green, respectively (**A**). Example of an impedance check showing values ranging between 1-9 kΩ before the start of an EEG recording **(B**). Photo of a neonate during a test occasion, showing EEG electrodes on the scalp and chest electrodes for vital signs monitoring (**C**) - Image used with parental permission).

3.3.4.2. Recording sensory-evoked responses

These comprised visual- and tactile-evoked responses. These sensory-evoked potentials reflect electrical activity in the thalamocortical tract, which are distinct responses from the baseline activity that measures ongoing background/resting-state brain function. Like the background activity, sensory function could potentially be

affected by respiratory instability [187]. Moreover, abnormal respiration (e.g., apnoea) may have varying effects across brain systems, i.e., disrupt global cortical responses (resting-state EEG) in a different way from the sensory system (sensory-evoked potentials). While tactile-evoked potentials are functionally linked to arousal and brainstem mechanisms involved in respiratory control (in apnoeic neonates, tactile stimulation can trigger arousal and restart breathing), visual-evoked potentials reflect the development of the visual sensory pathways including the visual cortex, which by proxy, is indicative of the occipital lobe function as it's the visual processing part of the brain. Therefore, including the sensory-evoked potentials in the brain age quantification will provide a complementary and comprehensive estimate of brain maturity and likely improve the robustness of the model.

To elicit the visual responses, a light-emitting diode flashlight (Lifelines photic stimulator) placed 30 cm from the neonates' direct visual field was used to generate brief white light flashes (4Hz frequency) of variable intensity (max 10). Ten successive flashes at least 10 seconds apart (or longer, if the neonate was unsettled to allow the baby time to calm down) were manually triggered to produce the responses, mostly on the Oz electrode channel of the EEG recording. The visual testing parameters were selected based on the findings from previous studies relevant to neonatal cortical visual processing, safety, and stimulus reliability [125,188–190]. Flashing light is a common technique used to elicit visual evoked responses in neonates as it is suited for the limited neonatal vision. Babies respond to the brief white light flashes, and the technique doesn't require participant cooperation[125,188–190]. The frequency of 4 Hz and interval of 10 seconds allows for adequate cortical recovery between flashes while minimising desensitisation or adaptation. While varying the light intensity ensures

stimulus response across different behavioural states (e.g., sleep or awake), the maximum intensity of 10 protects against overstimulation, thus adhering to safety standards.

To generate the tactile responses, a modified tendon hammer (impedance head integrated with a calibrated force transducer between the tip and handle of the tendon hammer to time-lock and measure the force of the touch stimuli applied [191]) was used to lightly tap the heel of the neonate. At least 10 gentle touch stimulations were applied on the heel of the foot when the neonate was settled, 10 seconds apart (or longer if the neonate was actively moving). Responses were observed mainly at the Cz EEG electrode channel. Gentle tactile stimulation (tapping) using the modified tendon hammer is commonly used in neonatal studies to activate the somatosensory system without causing pain or discomfort as it closely mimics natural touch[191–193].

Both the sensory-evoked potentials were elicited over approximately 3 - 5 minutes and were recorded either at the beginning of the test occasion immediately following the 10 background EEG annotations or at the end of the resting-state EEG recording. These were automatically annotated and time-locked on the EEG recordings.

3.3.4.3. Vital signs recording

Each neonate admitted in the NICU had continuous vital signs monitoring as the standard of care. A Philips monitor (IntelliVue MX800 patient monitor, Philips) was used for the recordings. Three electrocardiogram (ECG) electrodes, recording at a frequency of 250Hz, were applied to the neonate's chest. These were used to measure

the heart rate (at frequency 1Hz) and respiratory rate from an impedance pneumography (IP) signal (at frequency 62.5Hz). A second ECG recording from a single chest electrode was also recorded and displayed on the EEG monitor to allow for the detection of any electrical heart activity artefacts that could interfere with the EEG recording [30]. A saturation probe on the foot or hand of the neonate generated pulse oximetry plethysmography waveforms at a frequency of 125Hz and measured the oxygen saturation (SpO_2) at a rate of 1Hz. These data were continuously downloaded from the Phillips monitor at a sampling rate of 0.97Hz via ethernet, using an electronic data capture software (iXtrend) on a laptop.

Babies in the NICU who were studied on multiple test occasions had their vital signs data continuously downloaded until the time of discharge or when the neonate was clinically off vital signs monitoring, while those studied on one test occasion had their data accessed only once during EEG data acquisition (duration 1 - 2 hours). Similarly, the neonates in the postnatal ward also had their vital signs recorded only once during the EEG study. **Figure** 3.2 shows the equipment set-up and consumables used during each test occasion.

3.3.5. Data analysis

The EEG and vital signs data generated were exported from the acquisition computers on to a laptop using an encrypted hard drive. Annotations were standardised prior to further processing and analysis on MATLAB (ver. 2022b; MathWorks Inc., Natick, USA). Analysis on MATLAB was carried out with the support of a postdoctoral fellow, who has experience using MATLAB programming software. They provided guidance on how to use the group's EEG and vital signs analysis codes stored on the Gitlab

repository (https://gitlab.com) and supervised the writing of specific analysis codes for this project. Some exploratory analyses were done using Microsoft Excel (Version 16.86, 2024).

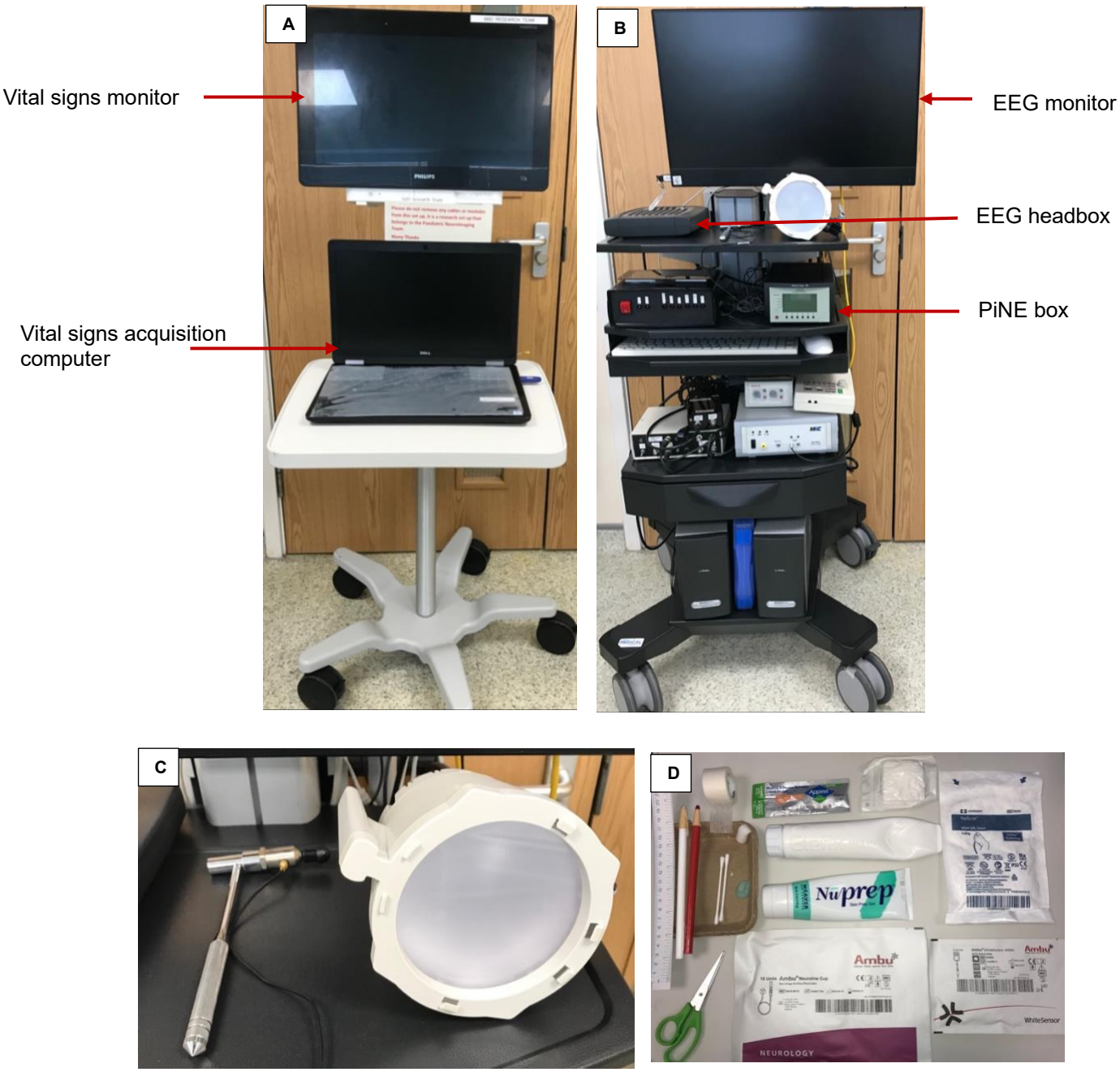

Figure 3.2: Study equipment and consumables. Vital signs acquisition set showing a Phillips monitor with a laptop (**A**). EEG equipment setup with a monitor, headbox and PiNEbox, (**B**). Close-up of tendon hammer and photic stimulator for tactile and visual stimulations, respectively (**C**). Study consumables (**D**).

3.3.5.1. Calculating respiratory and apnoea rates from impedance pneumography signal

Respiratory and apnoea rates (dependent/ response variables) were calculated from the inter-breath interval (IBI) generated from the vital signs monitor IP signal. IBI is the time between successive breaths (**Figure** 3.3) and it is a precise and reliable indicator of respiratory variability in neonates [180]. Individual breaths were identified using a validated neonatal apnoea detection algorithm developed by Adjei et al. (2021); and were used to calculate the respiratory and apnoea rates. While conventional monitor-derived respiratory and apnoea rate detection were validated using adult data [194,195] and are prone to signal interference errors e.g. from movement, the algorithm used to calculate IBI for respiratory and apnoea rate in this study is neonatal-specific and has the added advantage of removing any period of shallow breathing >20 seconds duration incorrectly identified as a true apnoea, thus, allowing for a more accurate identification of respiratory events [180]. The respiratory rate was expressed as the median number of breaths per minute. Median was used as IBI were not constant, changing in length over time and didn't follow a normal distribution. Apnoea rates were calculated as the number of IBIs >15 seconds per hour [196]. This apnoea cut-off was chosen because studies have shown that negative cardiovascular events can occur during shorter respiratory pauses [197] less than the conventional 20 seconds cut-off used in clinical practice. Episodes of desaturation were also noted and defined as oxygen saturation levels of < 80%.

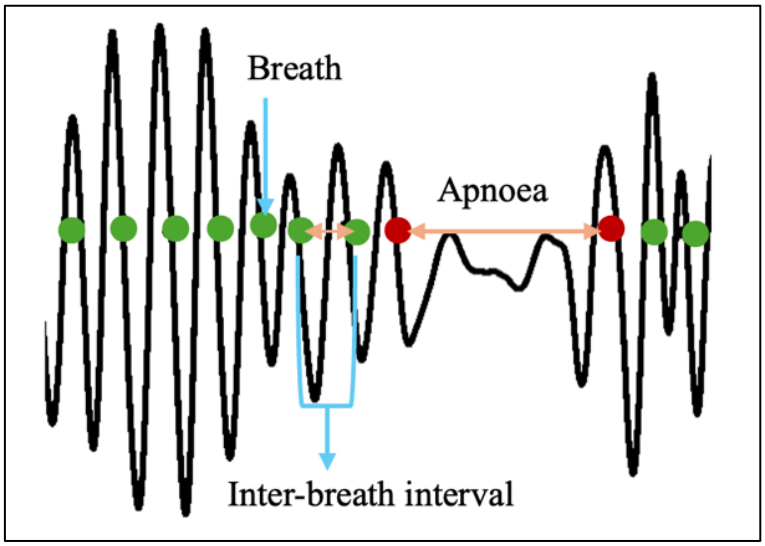

Figure 3.3: A schematic of an impedance pneumography signal. Each green dot illustrates the start of a breath. An inter-breath interval is shown as the time interval between successive breaths (short orange arrow) or two green dots while an apnoea, a period of breathing cessation is shown as the time interval between the two red dots (long orange arrow).

3.3.5.2. Calculating brain age and maturity from EEG

First, the resting-state EEG data was pre-processed using Brainstorm (version 3) [198] and EEGLAB (version 2022.1) [199] toolboxes. A high-pass filter at 0.1 Hz, a low-pass filter at 30 Hz and a notch filter of 50Hz (to filter out electrical interference from the mains supply) were used to remove frequency ranges outside the filter limits. This was then followed by brain age calculation for each EEG test occasion using two machine-learning algorithms: **(1)** a resting-state model [124] that uses functional EEG features [30,115] between bipolar channels C3 and C4. Data was first down-sampled to 64Hz and the first 20-minute EEG epoch in each recording with no visual or tactile responses was used to predict the resting-state brain age (BA). This ensured consistency across all recordings and was shown to be a reliable approach with comparable results to brain age estimations using the entire EEG recording (See more details in Section 1.8.1 and Ansari et al. 2024 [124]) and **(2)** a sensory model [125] that uses a minimum of 5 epochs of evoked potential to assess visual (Oz channel) and tactile (Cz channel)

sensory maturation and predict the sensory BA. In this model, the generated data was first epoched around -0.5 seconds before and 1.0 to 1.5 seconds after the time of stimulus onset. This was then baseline-corrected by subtracting the mean amplitude of the baseline period (time window between -0.5 secs to the onset of stimuli), artefacts were excluded manually, and responses fitted on to neurodynamic response functions to predict the sensory BA (See more details in **Section** 1.8.1 and Zandvoort et al. 2024 [125]). **Figure** 3.4 shows an illustration of sensory-evoked responses grouped according to PMA.

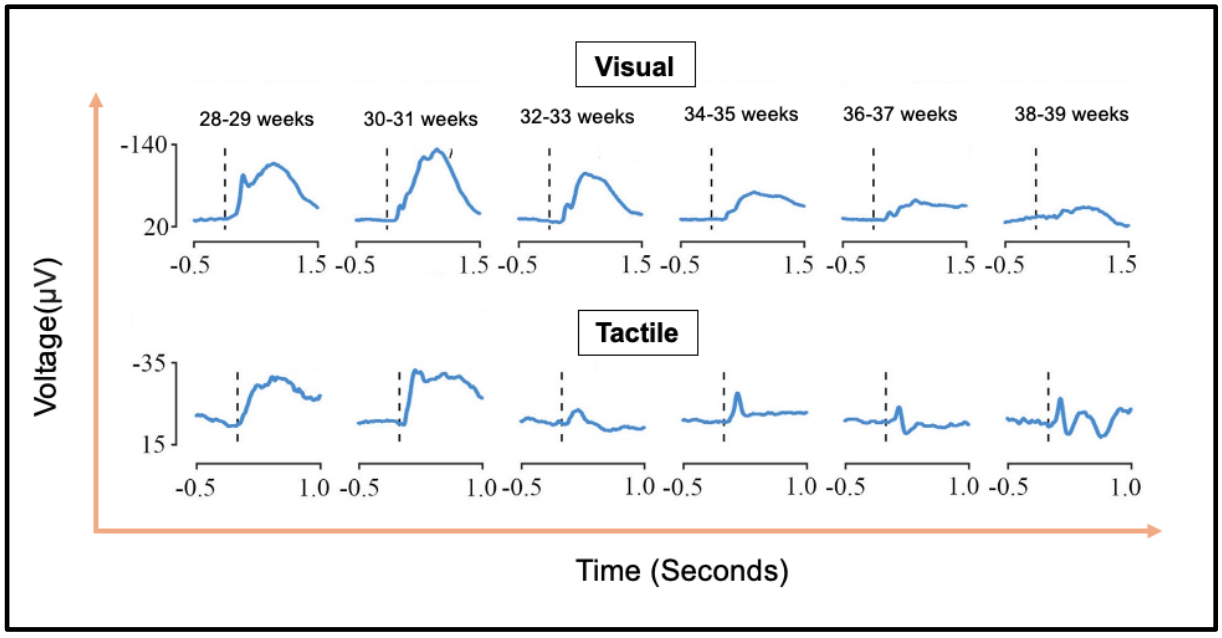

Figure 3.4: Sensory-evoked EEG responses according to age. The average evoked responses by the type of stimuli applied (visual and tactile) are grouped by two weeks postmenstrual age intervals. The dotted vertical lines indicate the time (zero) when the corresponding stimuli were applied. The baseline period is from -0.5 seconds to the start of the dotted line when the stimuli were applied. Responses were elicited immediately after the onset of the stimuli, shown as age-dependant deflections of different magnitudes lasting approximately 1.5 seconds and 1 second for the visual and tactile responses, respectively. Figure adapted from Zandvoort et al. 2024 [125].

Preterm babies show higher-magnitude neural responses to both visual and tactile stimuli because they have immature inhibitory systems, heightened thalamocortical excitability, and reduced sensory gating [200,201]. The gamma-aminobutyric acid

inhibitory pathway and thalamocortical connections are underdeveloped, cortical neurons are less differentiated, and the brain's ability to filter out sensory inputs is unrefined compared to a term neonate. Responses to simple stimuli are, thus, more pronounced and appear larger in amplitude, reflecting the preterm brain's increased excitability and immaturity rather than functional development.

The resting state and sensory models were combined to calculate the average brain age relative to the neonate's PMA. Where insufficient data is found, for instance, < 20 minutes resting state EEG recording or < 5 epochs for either the visual- or tactile-evoked responses, then only one brain age estimation algorithm (either the resting-state or sensory-evoked) was used to calculate the brain age. As brain models generally estimate brain age with systematic prediction errors relative to PMA i.e., overestimate for the very preterm and underestimate for the older term neonate [202], bias-correction was done by fitting a linear model between the calculated brain age and PMA. PMA was then predicted from this linear model and the model bias was defined as the difference between the actual PMA and predicted PMA (so that PMA and brain maturity are completely uncorrelated). The calculated brain age was subsequently corrected for the model prediction error by subtracting the model bias from the brain age. This age-bias adjustment is a standard approach in brain age modelling [202,203].

Brain maturity was calculated from the brain age (the difference between the neonates' brain age and their PMA) expressed as a value ranging from negative, through zero to positive. A brain maturity of zero (0) indicates a brain maturity that is appropriate or

equal to the actual PMA, while a score of > 0 and < 0 indicate a more developed and less developed brain maturity relative to the PMA, respectively.

3.3.5.3. Confounding variables selection

Participants with only one EEG recording had approximately 1 - 2 hours of vital signs data while those with multiple EEG recordings had continuous vital signs monitoring from the time of enrolment up to the point of discharge or the time when a clinical decision was made to stop vitals monitoring. As such, depending on the number of EEG test occasions participants had, the vital signs data length recorded per participant varied considerably. Using the common-cause approach for confounder selection [204], vital signs data length was, thus, adjusted for in the analysis. Additional confounders such as infection which is known to predispose to apnoea (defined as an abnormal white blood cell count of >20 x 10^9/L or <5 x 10^9/L, or elevated C-reactive protein of >10mg/L or a positive blood culture), and the type of respiratory support at the time of the test (high flow, low flow, or no supplementary oxygen support) which could affect the rate of respiration were also adjusted for. Covariates such as sex, GA, ethnicity, weight and Apgar scores were not controlled for as they acted mostly as mediators and instrumental variables [204] and were, thus, not appropriate to be adjusted for as confounders.

3.3.5.4. Statistical analysis

The recruited participants were grouped by 2-weeks intervals according to their PMA at the time of the test occasion. The age distribution was displayed as a histogram. Demographic information such as GA, PMA, birthweight sex, ethnicity, mode of

delivery, Apgar scores, need for active resuscitation at birth, and presence of medical conditions such as respiratory distress syndrome, persistent pulmonary hypertension, patent ductus arteriosus, jaundice and infection were documented. Other relevant information such as the level of care, need for respiratory support and medications received on the day of the test occasion were also noted. These demographic data were displayed in a table, summarised as frequencies and percentages. Quantitative data such as GA, PMA, birthweight, brain age, Apgar scores, brain maturity, resting-state BA and sensory BA, apnoea, respiratory and desaturation rates were assessed for normality using the Kolmogorov-Smirnov test. Parametric data were summarised as means and standard deviations (SD) while non-parametric data were summarized as median ± interquartile range (IQR). To determine the direction, strength of association and possible collinearity between key predictor/independent variables such as brain age, brain maturity, resting-state BA, sensory BA and PMA, correlation coefficient with the corresponding 95% confidence intervals (CI) were evaluated. Pearson's correlation coefficient was used for parametric data, while the Spearman-rank coefficient of correlation was used for non-parametric data [205]. Results were displayed using scatter plots with a line of best fit indicating the direction of the relation. The significance level for all analyses conducted was set at an alpha of 5%.

Change in respiratory and apnoea rates with PMA, brain age and maturity: To provide a general descriptive overview of how IBIs (a measure of respiration) change with PMA, brain age and brain maturity, different metrics such as the median (± IQR), mean (± SD) and proportion of IBIs of varying lengths i.e., > 2, 3, 5, 10, 15 and 20 seconds were calculated. These were depicted using scatterplots. Different proportions of IBI cut-offs were chosen to explore the wide-ranging variability in respiratory dynamics by age and brain maturity. Absolute respiratory and apnoea rates

were then calculated (**Section** 3.3.5.1) as these metrics are more intuitive than IBI descriptions and commonly used by clinicians for medical decisions. The relationships between apnoea and respiratory rate by PMA, brain age and brain maturity were evaluated using Linear Mixed Effects Models (LMEM). These models allow for both random (subject) and fixed (confounding) factor adjustments [206]. Participants were included as a random factor in the model while data length, infection status and type of respiratory support were included as fixed factors. This approach allowed for the repeated measures, as recorded in some neonates, and confounders (**Section** 3.3.5.3) to be accounted for.

PMA, brain age and brain maturity in determining the risk of apnoea in preterm neonates: To confirm the relationship of apnoea with PMA, brain age and maturity in a population of neonates who are more susceptible to apnoea, similar respiratory and apnoea rate analysis methods as above was used. Here, only a subset of the entire recruited participants i.e., preterm neonates were included in the analysis.

Brain maturity as a guide for caffeine treatment in apnoea of prematurity: To support treatment decisions and determine the association between brain maturity and age at caffeine discontinuation, preterm neonates who had their brain maturity assessed two weeks prior to caffeine discontinuation were evaluated. Brain maturity but not brain age (because the latter is collinear with PMA, having the age factor as part of the metric) was correlated with the neonate PMA at caffeine discontinuation. Here, only infection status was included as a confounder as all the participants included for this sub-analysis had continuous vital signs monitoring and hence, similar recording lengths. They were not on respiratory support, so this variable was also not

included as confounds. Additionally, an exploratory analysis was performed comparing brain maturity, grouped as mature (maturity score ≥ 0) and immature (maturity score < 0), and the frequency of apnoea and desaturations in the week after discontinuation of caffeine. Results were reported as percentages, mean and standard deviation; and depicted using boxplots. No statistical tests of significance were estimated due to the limited population (participants with brain maturity assessment prior to caffeine discontinuation and longitudinal vital signs recording for a week after caffeine was stopped) and the frequency of outcomes of interest (apnoea and desaturation) observed.

3.4. Results

A total of 138 eligible neonates were recruited. One hundred and twenty-one participants (92%), including term and preterm babies were studied on a total of 205 separate EEG test occasions (**Figure** 3.5). During my DPhil, I prospectively studied 79 of the 121 (65.3%) participants on 130 EEG test occasions between January 2022 and December 2023. Forty-two (34.7%) of these participants were studied between February 2018 and November 2021 by members of the paediatric neuroimaging team before the start of my DPhil recruitment; these data were collected using a similar study protocol in this chapter and, hence, were included in the analysis. The GA at birth of the recruited participants ranged between 23 weeks + 0 days to 41 weeks + 6 days. The distribution by PMA at the time of the EEG test occasion grouped by 2 week intervals is shown in **Figure** 3.6. There were fewer participants recruited for the age group 28 - 29 weeks (n = 13) and 40 - 41 weeks (n = 11). This was because the younger the age at the time of the test, the more likely the neonates were to meet an exclusion criterion, such as being on mechanical ventilation, receiving morphine, or being clinically unstable, which prevented participation in the study. By contrast, older term neonates (i.e., those at 40 - 41 weeks PMA) were typically on the postnatal ward and more likely to be discharged, resulting in lower recruitment counts for this age group. Many of the participants (90, 74.4%) had a single EEG test occasion recording, while 31 (25.6%) were studied multiple times. The majority were males (n =72, 59.5%) with a Male: Female ratio = 1.5:1, born preterm (n = 95, 78.5%) and white (n = 57, 47.1%; n = 39 [32.2%] had no ethnicity information recorded at the time of recruitment as the ethics approval for data collected between 2018 and 2021 did not allow for such

information to be documented). **Table** 3.1 shows a summary of participant demographic information.

The total vital signs data recording length was approximately 8,035 hours, equivalent to 335 days of continuous recording. The average duration of EEG data recorded was 5,938 ± 2,420 minutes (mean ± SD). Of the 205 individual test recordings, most (n = 159, 77.5%) had both their resting-state and sensory BA data used for brain age calculation. By contrast, about a quarter (n = 44, 21.5%) had only resting-state EEG data, and two (1.0%) participants had only sensory-evoked potentials recorded. Of the quantitative variables, only PMA, brain age, brain maturity and respiratory rate were normally distributed. Results of the normality test are shown in **Appendix - Table** 7.5**.**

To evaluate the relationships between EEG-derived brain measures and PMA, correlations were assessed to determine the strength, direction, and linearity of associations. Using Spearman rank's coefficient of correlation, sensory BA showed a significant positive and moderate correlation with resting-state BA ($\rho = 0.63$, $p = 0.01$, 95% CI = 0.54, 0.71) and PMA ($\rho = 0.59$, $p = 0.01$, 95% CI = 0.57, 0.73) **Figure** 3.7A and B. Similarly, PMA demonstrated a positive and stronger correlation with resting-state BA and brain age (average of resting-state BA and sensory BA) (**Figure** 3.7C**,** D). On the other hand, brain maturity (calculated as the difference between brain age and PMA) shows no correlation with PMA, with an almost flat trend line ($r = 0.016$, $p = 0.82$, 95% CI = -0.12, 0.15, **Figure** 3.7E). Brain maturity is only weakly correlated to brain age ($r = 0.33$, $p = 0.01$, 95% CI = 0.20, 0.44, **Figure** 3.7F). These findings, thus, support the assertion that brain maturity, a measure of functional brain development, is independent of the age metric which is a feature of both PMA and brain age.

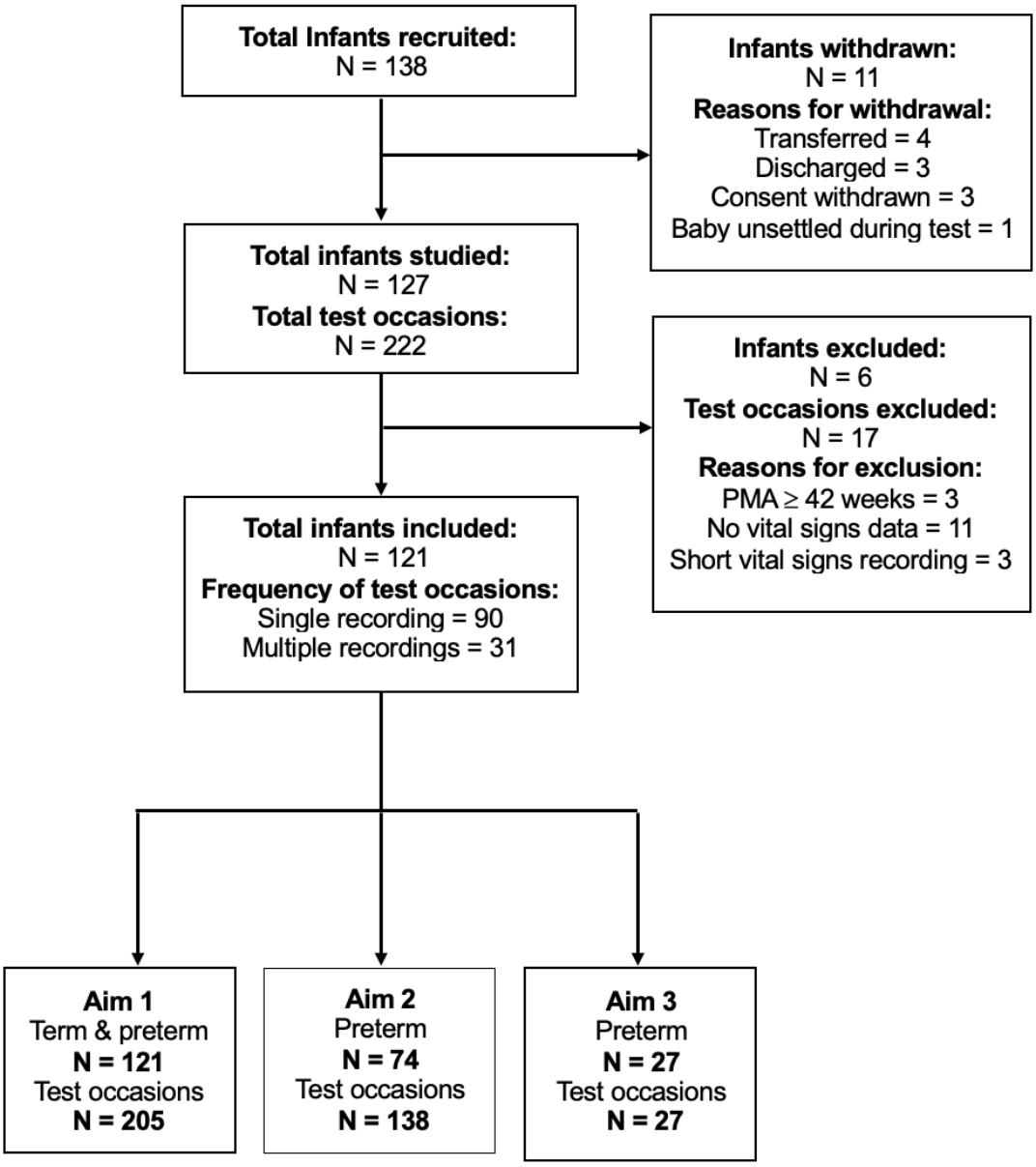

Figure 3.5: Recruitment flow-chart grouped by study aims

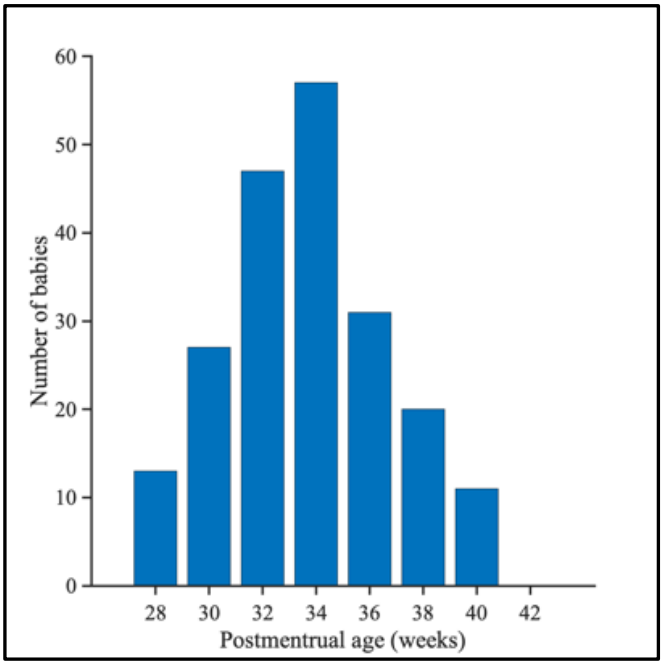

Figure 3.6: Distribution of neonates by postmenstrual age at test occasion

Table 3.1: Participants' descriptive characteristics by study aim

Factors	**Aim 1 (N = 121 babies; 205 test occasions)**	**Aim 2 (N = 74 babies; 138 test occasions)**	**Aim 3 (N = 27, babies; 27 test occasions)**
Age (weeks) [&]			
Gestational age	31.5 (23.5-41.7)	31.0 (23.6-36.6)	29.7 (26.9-32.6)
Postmenstrual age at recording	34.5 (28.0-41.8)	34.0 (31.0-36.9)	33.3 (31.7-35.4)
Birthweight (g)	1688 (530-5000)	1573 (630-4525)	1312 (760-2120)
Sex			
Females	49 (40.5)	25 (33.8)	8 (29.6)
Males	72 (59.5)	49 (66.2)	19 (70.4)
Mode of delivery			
Normal vaginal	32 (26.4)	13 (17.6)	2 (7.4)
Breech vaginal	0 (0)	2 (2.7)	0 (0)
Assisted vaginal	6 (5.0)	4 (5.4)	2 (7.4)
Elective Caesarean section	11 (9.1)	8 (10.8)	0 (0)
Emergency Caesarean section	72 (59.5)	47 (63.5)	23 (85.2)
Apgar scores*			
Apgar at 1 minute	7 (5-9)	7 (5-9)	7 (6-8)
Apgar at 10 minutes	10 (9-10)	10 (9-10)	10 (10-10)
Ethnicity			
White	57 (47.1)	26 (35.1)	13 (48.2)
Asian	11 (9.1)	5 (6.8)	3 (11.1)
Black	3 (2.5)	1 (1.4)	1 (3.7)
Mixed	8 (6.6)	6 (8.1)	2 (7.4)
Other	3 (2.5)	1 (1.4)	0 (0)
Information not recorded	39 (32.2)	35 (47.3)	8 (29.6)
Resuscitation at birth			
Yes	104 (86.0)	55 (74.3)	21 (77.8)
No	17 (14.0)	19 (25.7)	6 (22.2)

Respiratory distress syndrome			
Ongoing condition	72 (35.1)	47 (34.1)	11 (40.7)
Past treated	76 (37.1)	57 (41.3)	10 (37.0)
No	57 (27.8)	34 (24.6)	6 (22.2)
Persistent pulmonary hypertension			
Ongoing condition	1 (0.5)	0 (0)	0 (0)
Past treated	6 (2.9)	4 (2.9)	1 (3.7)
No	198 (96.6)	134 (97.1)	26 (96.3)
Patent ductus arteriosus			
Ongoing condition	15 (7.3)	9 (6.5)	2 (7.4)
Past treated	9 (4.4)	7 (5.1)	1 (3.7)
No	181 (88.3)	122 (88.4)	24 (88.9)
Jaundice			
Ongoing condition	9 (4.4)	6 (4.3)	0 (0)
Past treated	99 (48.3)	78 (56.5)	14 (51.9)
No	97 (47.3)	54 (39.1)	13 (48.1)
Infection			
Past treated	66 (32.2)	60 (43.5)	9 (33.3)
Suspected	82 (40.0)	17 (12.3)	9 (33.3)
None	57 (27.8)	61 (44.2)	9 (33.3)
Level of care			
Intensive Therapy Unit	18 (8.8)	6 (4.3)	0 (0)
High Dependency Unit	89 (43.4)	65 (47.1)	13 (48.2)
Low Dependency Unit	71(34.6)	59 (42.8)	12 (44.4)
Postnatal care	24 (11.7)	5 (3.6)	0 (0)
Information not recorded	3 (1.5)	3 (2.2)	2 (7.4)
Respiratory support			
High flow therapy	58 (28.3)	35 (25.4)	7 (25.9)
Low flow therapy	17 (8.3)	14 (10.1)	5 (18.5)
Spontaneous ventilation	130 (63.4)	89 (64.5)	15 (55.6)
Medications on the day of EEG recording			
Vitamins and minerals	137 (66.9)	112 (81.2)	25 (92.6)
Probiotics	74 (36.1)	59 (42.8)	24 (88.9)
Breastmilk fortifier	63 (30.7)	56 40.6)	17 (63.0)
Caffeine	90 (43.9)	71 (51.4)	24 (88.9)
Antibiotics	23 (11.2)	6 (4.3)	1 (3.7)
Anti-reflux	6 (2.9)	6 (4.3)	0 (0)
Paracetamol	5 (2.4)	2 (1.4)	0 (0)
Budesonide	6 (2.9)	6 (4.3)	1 (3.7)
Nystatin	3 (1.5)	1 (0.7)	0 (0)
Diuretics	3 (1.5)	1 (0.7)	0 (0)
No medication	36 (17.6)	16 (11.6)	1 (3.7)

Reported values are number (%), [&]mean (range), and *median (interquartile range). All demographic details are computed per recording (aim 1 = 205, aim 2=138, aim 3 =27) except ethnicity, Apgar scores, need for resuscitation at birth, gestational age (which are provided per neonate, aim 1 = 121, aim 2 = 74, aim 3 = 27). Antibiotics include: benzylpenicillin, gentamicin, co-amoxiclav, cefotaxime, chloramphenicol, and amoxicillin; vitamins and minerals include: abidec, calcium, phosphate, vitamin D, folic acid, and iron; diuretics include: chlorothiazide and spironolactone; anti-reflux include: gaviscon and omeprazole.

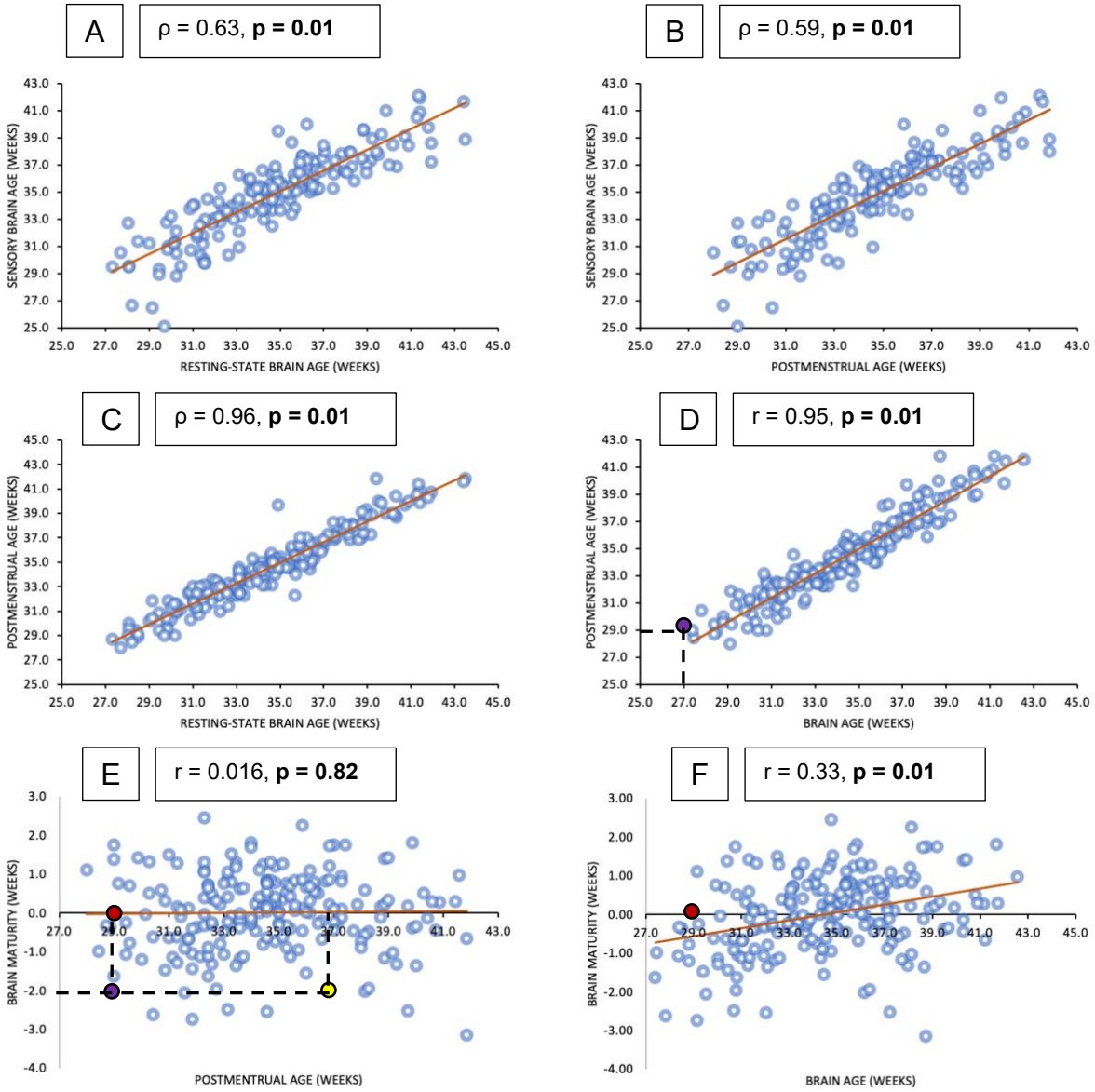

Figure 3.7: Scatter plots showing the association between EEG-derived brain metrics and age. Correlation between resting-state and sensory brain age (**A**), postmenstrual age (PMA): and sensory (**B**) and resting-state (**C**) and brain age (**D**). The orange line indicates the line of best fit and its positive (**A, B, C, D**) signifying an increasing trend with advancing age. Each blue dot represents a separate test occasion. The associations between brain maturity (BM) and PMA (**E**) and brain age - BA (**F**) were less strong with greater variability compared with the more compact distributions in A, B, C and D. Each blue dot above the line of best fit indicates an advanced BM relative to PMA (**E**), BA (**F**) and vice versa. E.g., in **D** the purple dot neonate has a PMA of 29 weeks but an immature BA of 27 weeks. The same purple neonate in **E** has a BM of - 2 suggesting a 2-week immature deviation relative to their actual PMA of 29 weeks. Another yellow dot neonate at 37 weeks PMA has its BM at 35 weeks. While the yellow dot neonate is older than the purple dot neonate, they both have a brain maturity of -2**.** In **E** and **F**, red dot neonate has brain maturity 0 at PMA/ BA of 29 weeks, indicating a BM equal to their PMA or BA. *Abbreviations: p-value (p), Pearson's correlation* coefficient *(r) and Spearman rank's correlation coefficient (ρ).*

3.4.1. Effect of PMA, brain age and maturity on neonatal respiration

3.4.1.1. Overview of inter-breath interval changes by PMA, brain age and maturity

This section provides an initial comparative description of different IBI metrics by PMA, brain age and brain maturity to assess basic respiratory pattern distribution and variability with advancing PMA and brain-derived measures. With increasing PMA and brain age, the median IBI and IQR also increase, indicating longer or deeper breaths, and wider intervals or gaps between individual breaths with aging, respectively (**Figure** 3.8 **-** Panels A and B**)**. Median and IQR were chosen for a better representation because the IBI data generated were non-parametric. On the other hand, brain maturity had little or no impact on the average (median) IBI and its distribution (IQR) which are indicative of physiological (normal) respiration and variability. IBI skewness and kurtosis (**Figure** 3.8 - Panels C and D**)**, both indicate the occurrence of outlier respiratory events (e.g., longer IBIs such as apnoea) and decrease with advancing PMA, brain age and brain maturity. This suggests that the younger the neonate and less mature the brain is, the greater the likelihood for longer IBIs and consequently, apnoea.

Although the IBI distribution is non-parametric, examining IBI changes in relation to changes in PMA, brain age, and brain maturity using other common descriptive measures such as the mean and standard deviation revealed a similar trend to those observed with the medians and IQRs **(Figure** 3.9). Scatter plots of additional descriptive IBI measures comparing the distribution of various IBI proportions (>2, 3,

5, 10, 15 and 20 seconds) by PMA, brain age and brain maturity are shown in **Figure** 3.10 and **Figure** 3.11**.** While both PMA and brain age show a relatively steeper trend line for short IBI proportions (>2, 3 and 5 seconds) with advancing PMA and brain age than brain maturity (**Figure** 3.10) this pattern was less pronounced for longer proportions (**Figure** 3.11) The proportions of long IBIs (>10, 15 and 20 seconds) occurred less frequently than short IBIs. Of note, only brain maturity displayed a decreasing trend in the frequency of long IBI proportions as brain maturity increased from negative to positive values; this is more obvious for proportions > 15 and 20 seconds (**Figure** 3.11) suggesting that brain maturity might be related to the occurrence of pathological respiration i.e., the likelihood of long IBIs decrease as the brain matures.

In general, findngs from this section show that increasing PMA and brain age appear to positively influence the average duration (median and mean), variability (IQR and SD) and frequency of occurrence of short IBI proportions. The scatter plot for extreme IBIs, assessed by the skewness and kurtosis decreased with advancing PMA, brain age and maturity. However, only the brain maturity plots for long IBI proportions showed a decreasing pattern. To clarify the findings from this section, statistical tests for significance will be applied to further assess these relationships in **Sections** 3.4.1.3 3.4.1.4 and 3.4.1.5 below. For simplicity, more conventional respiratory terminologies i.e., respiratory rate and apnoea rate, which are the standard for quantifying respiratory changes in clinical practice instead of IBIs, will be used for subsequent respiratory descriptions (**Section** 3.3.5.1 describes how these measures were calculated from the IBIs).

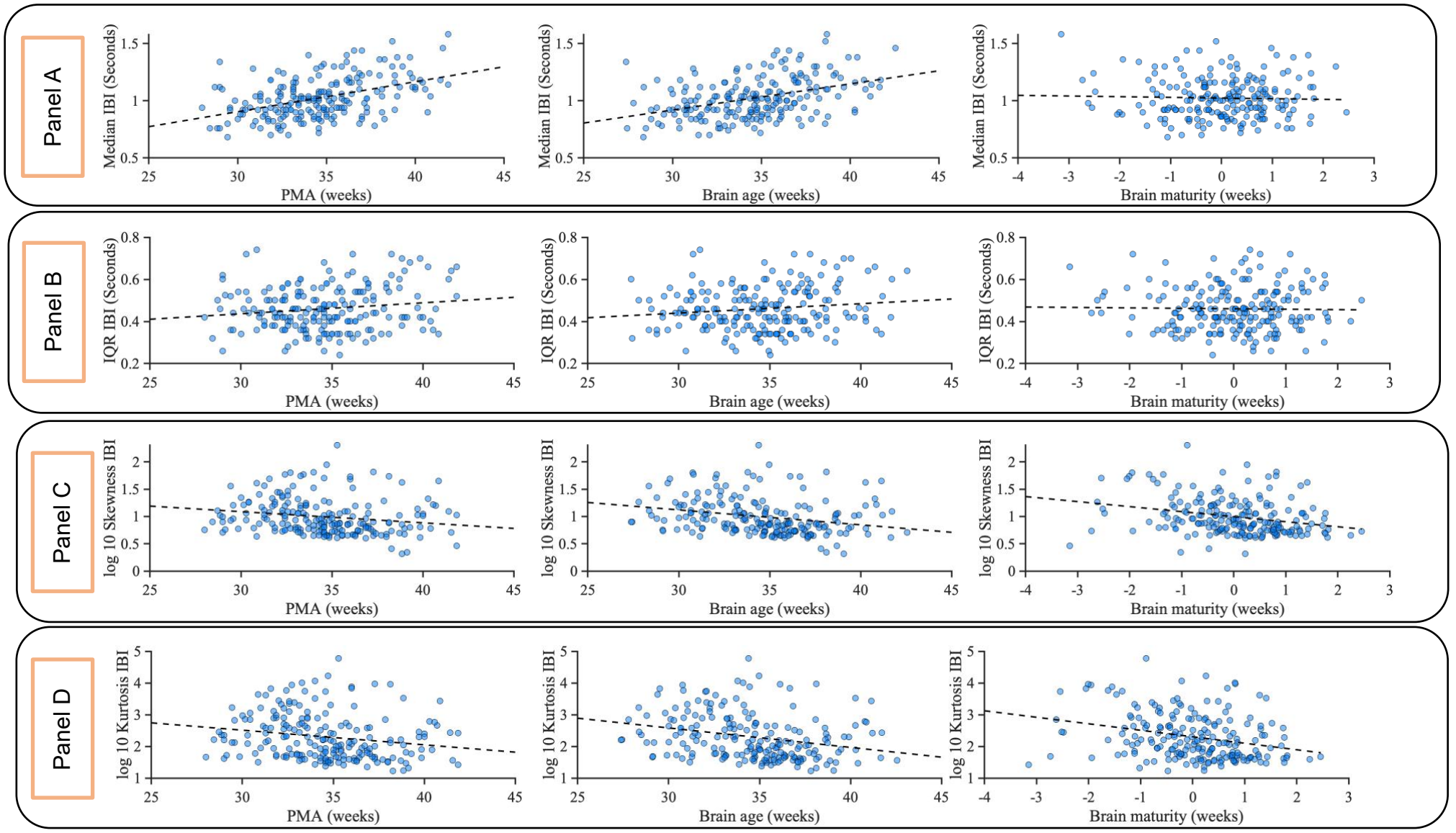

Figure 3.8: Comparison of inter-breath interval distribution. The change in the median inter-breath interval (IBI) - **Panel A**, variability (**Panel B**), skewness (**Panel C**) and kurtosis (**Panel D**) with advancing postmenstrual age (PMA), brain age and brain maturity is shown. The dotted lines are the lines of best fit indicating the direction of change with advancing age and brain maturity. Increasing PMA and brain age have a more positive effect on median IBI than brain maturity (**Panel A**). A similar change in trend is shown in the distribution of IBI inter-quartile range - IQR (**Panel B**). Both skewness (**Panel C**) and kurtosis (**Panel D**) represent the occurrence of extreme IBIs, and the trend line slopes down from left to right suggesting a decrease in the occurrence of extreme IBIs, for example, apnoea, with both ageing and brain maturity.

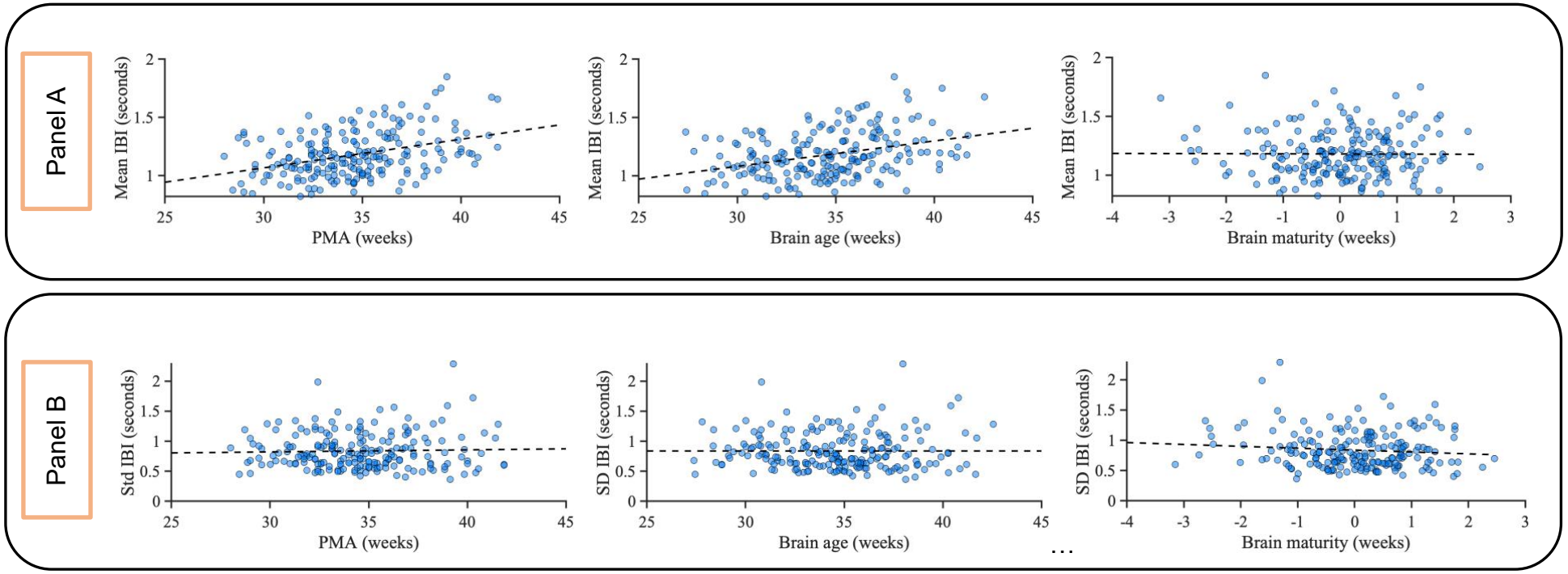

Figure 3.9: Distribution of mean and standard deviation of inter-breath interval by postmenstrual age, brain age and maturity. Increasing postmenstrual age (PMA) and brain age cause a similar positive increase in the mean inter-breath interval (IBI) more than brain maturity (**Panel A**). While the effects of PMA and brain age on IBI variability (standard deviation - SD) is less obvious, brain maturity has a slight decreasing trend (**Panel B**).

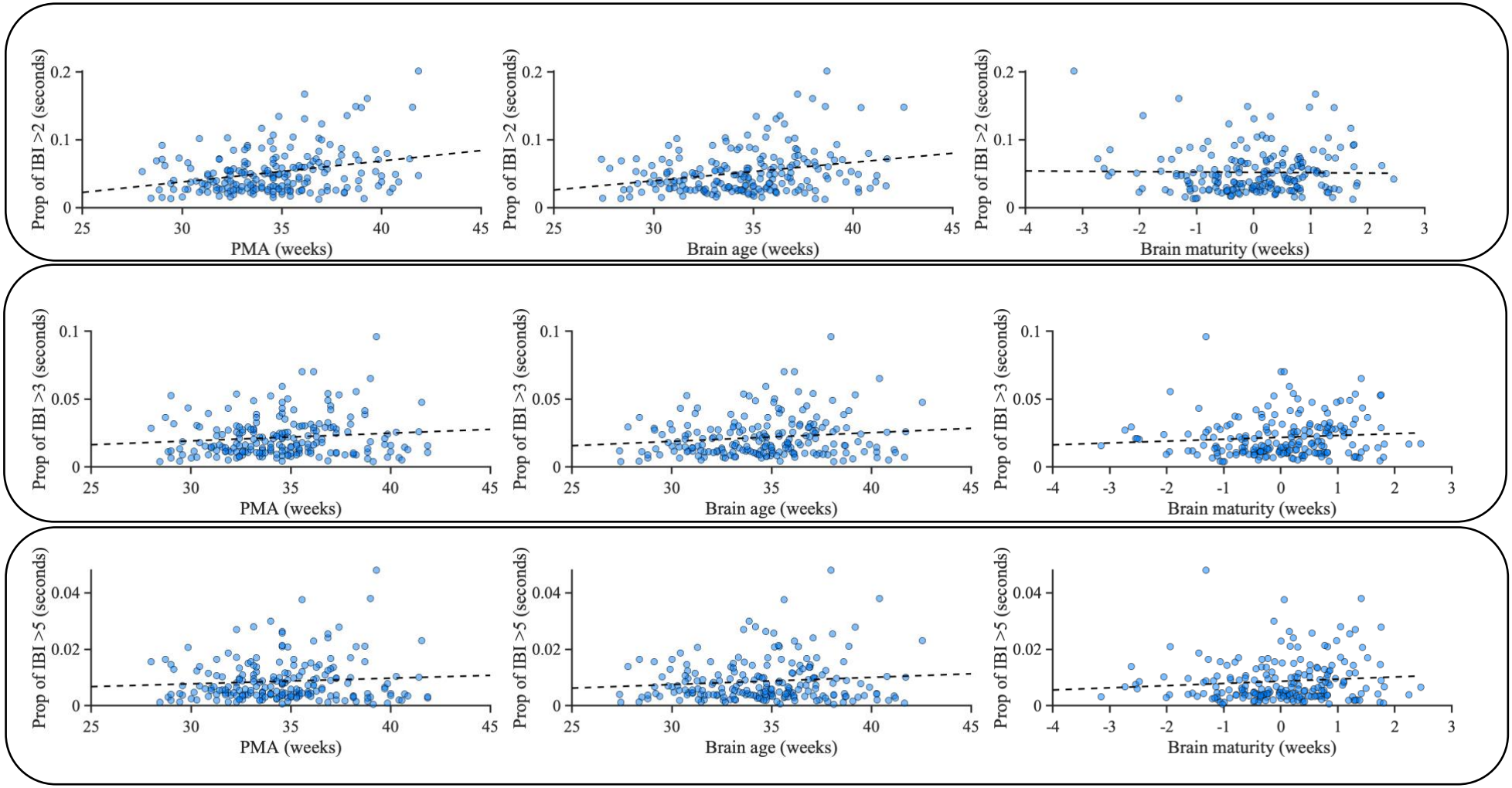

Figure 3.10: Distribution of short inter-breath interval proportions by postmenstrual age, brain age and maturity. *Abbreviations: Inter-breath interval (IBI) and postmenstrual age (PMA).*

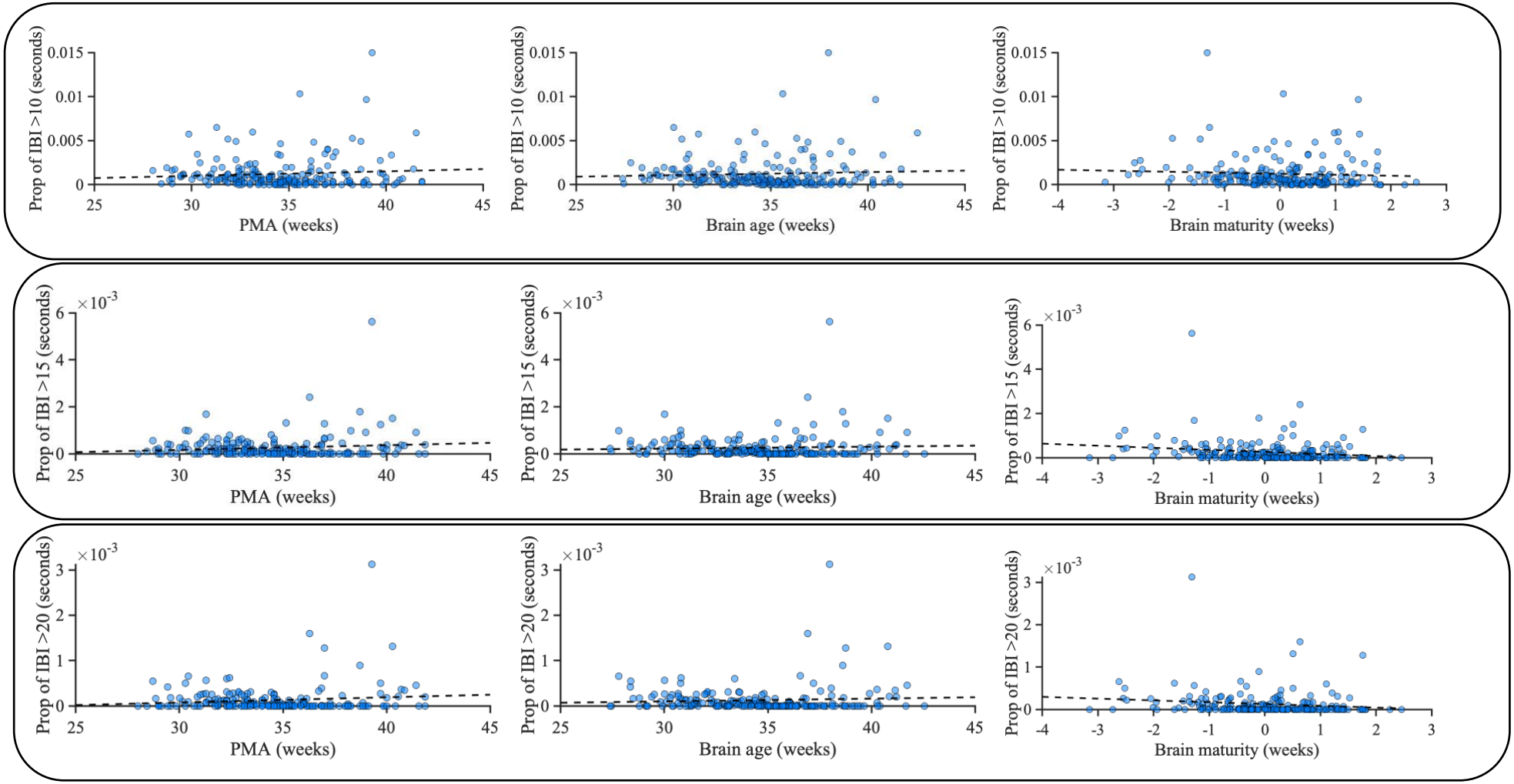

Figure 3.11: **Distribution of long inter-breath interval proportions by postmenstrual age, brain age and maturity**. Only brain maturity shows a decreasing trend in the occurrence of IBI. *Abbreviations: Inter-breath interval (IBI) and postmenstrual age (PMA).*

3.4.1.2. Correlation between caffiene and postmenstrual age

In **Figure** 3.12, younger neonates were more likely to be receiving caffeine compared to older neonates. Point-biserial correlation analysis revealed a significant negative association between PMA and caffeine use (r = -0.67, p = 0.01; 95% CI: -0.74 to -0.59). Given this strong association and the resulting collinearity between caffeine exposure and PMA, adjusting for caffeine in subsequent models would be inappropriate, as it will yield biased estimates.

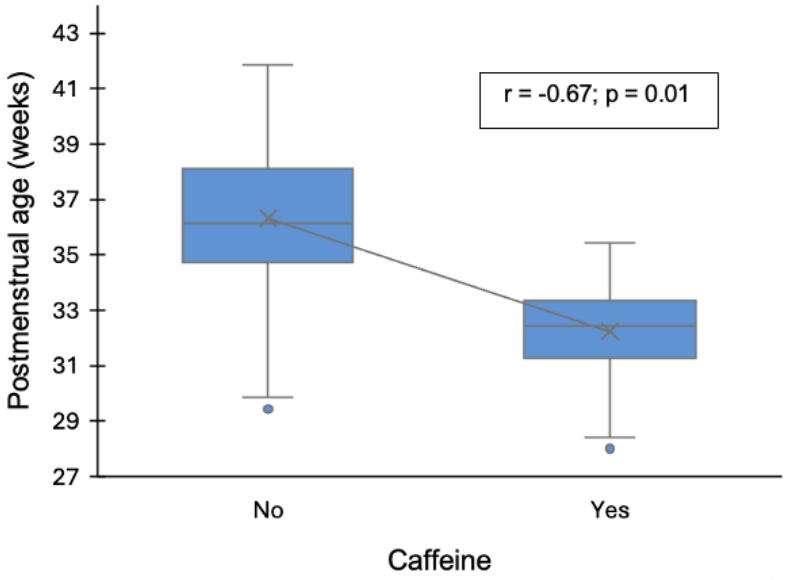

Figure 3.12: Simple boxplot comparing median PMA by caffeine exposure. The horizontal lines in the middle of the boxplots represent the median, and the upper and lower box limits indicate the interquartile ranges. The error bars indicate the 95% confidence intervals. The blue circled data points represent extreme outliers.

3.4.1.3. Effect of postmenstrual age on respiratory and apnoea rates

Using LMEM analysis to compare changes in respiratory and apnoea rates by PMA (28 - 41 weeks), there was a significant decrease in the respiratory rate per minute with increasing PMA (β = -0.88, p= 0.01, 95% CI = -1.27, -0.31). This suggests that the more preterm a newborn is, the higher the respiratory rate - **Figure** 3.13A**.** By

contrast, apnoea rates were not significantly affected by advancing PMA (β = -0.05, p = 0.09, 95% CI = -0.01, 0.095) suggesting that PMA did not affect the frequency of apnoeas - **Figure** 3.13B.

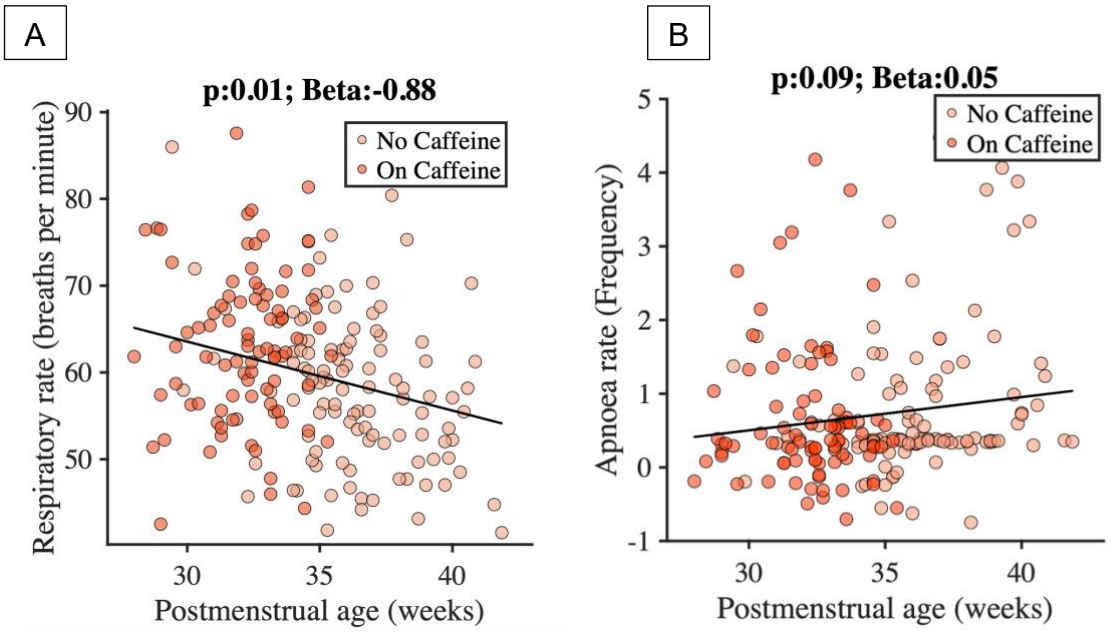

Figure 3.13: Effect of postmenstrual age on neonatal respiration. Increasing postmenstrual age significantly reduces respiratory (A) but not apnoea rates (B) in neonates 28- 41 weeks. Each orange dot indicates an individual test occasion (121 neonates were studied on 205 test occasions); neonates on caffeine are shown as dark orange dots and those not on caffeine as the light orange dots. The black lines are the mean fit of the linear model. Adjusted for data length, infection, and respiratory support**.**

Overall, there were 31 (25.6%) term neonates included in the study (Male: Female ratio = 1.1:1). Of these, 15 (48.4%) did not experience any apnoea. These infants had a median respiratory rate of 52 breaths per minute (range: 38 – 71 breaths per minute). All were breathing spontaneously in air, except for one neonate who was receiving low-flow oxygen therapy due to ongoing respiratory distress and suspected infection (but not on antibiotics) while in the high dependency unit.

Sixteen (51.6%) of the term neonates (Male: Female ratio = 1:1) experienced apnoea. They had a median respiratory rate of 51 breaths per minute (range: 39 – 67 breaths per minute), and a median apnoea rate of 1 per hour (range: 0.1 – 4.1 per hour). Six were admitted to the high dependency unit, eight were in the postnatal ward, and one was in the intensive therapy unit. Twelve (75%) had suspected infection (11 of whom were on antibiotics), three had ongoing respiratory distress, and one had persistent pulmonary hypertension. Three infants were receiving high-flow oxygen therapy, while the remaining were not receiving any oxygen support at the time of testing.

3.4.1.4. Effect of brain age on respiratory and apnoea rates

First, the effects of resting-state and sensory brain age on respiratory and apnoea rate were independently assessed (**Figure** 3.14 and **Figure** 3.15). For a comprehensive approach, these two brain metrics were averaged to generate the combined brain age and the association between the latter and the outcomes were evaluated (**Figure** 3.16). Similar to the effect of PMA on respiratory parameters described in **Sections** 3.4.1.1 and 3.4.1.2, the resting-state (β = -0.58, p = 0.01, 95% CI = -0.93, -0.11**;** **Figure** 3.14A), sensory (β = -0.77, p = 0.02, 95% CI = -1.06, -0.09**;** Figure 3.15A) and combined brain ages (β = -0.66, p = 0.01, 95% CI = -1.05, -0.14**;** **Figure** 3.16A) were inversely related to respiratory rate, and these findings was also significant. The associations between all three measures with apnoea, however, did not reach a significant level (**Figure** 3.14B, **Figure** 3.15B, and **Figure** 3.16B)

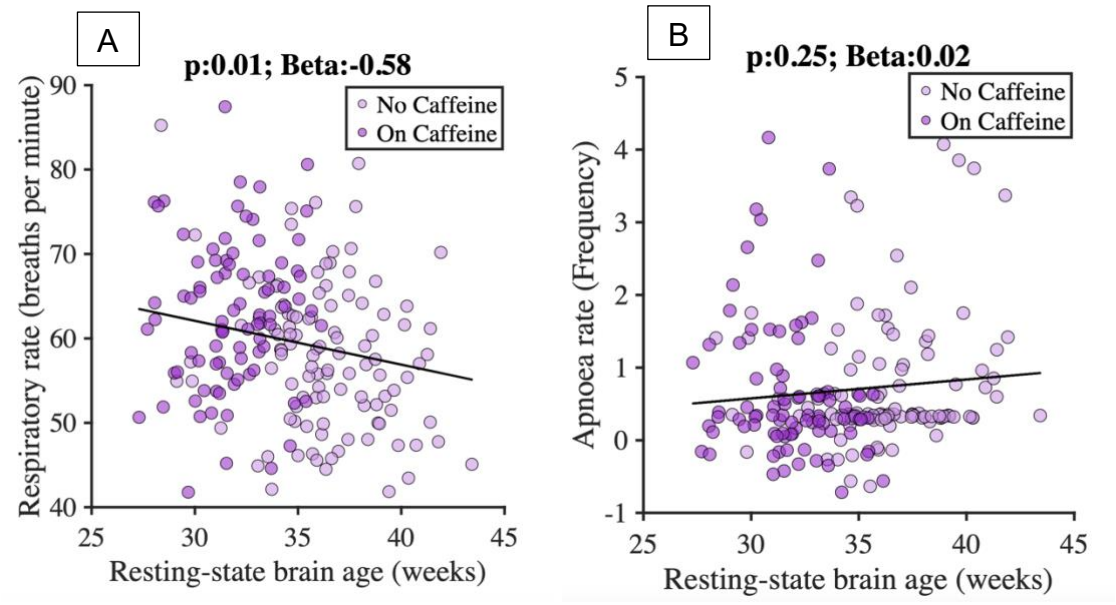

Figure 3.14 : Effect of resting-state brain age on neonatal respiration. Increasing resting-brain age significantly reduces respiratory (A) but not apnoea rates (B) in neonates 28 - 41 weeks postmenstrual age. Each dot indicates an individual test occasion (121 neonates were studied on 205 test occasions); neonates on caffeine are shown as dark purple dots and those not on caffeine as the light purple dots. The black line is the mean fit of the linear model. Adjusted for data length, infection, respiratory support.

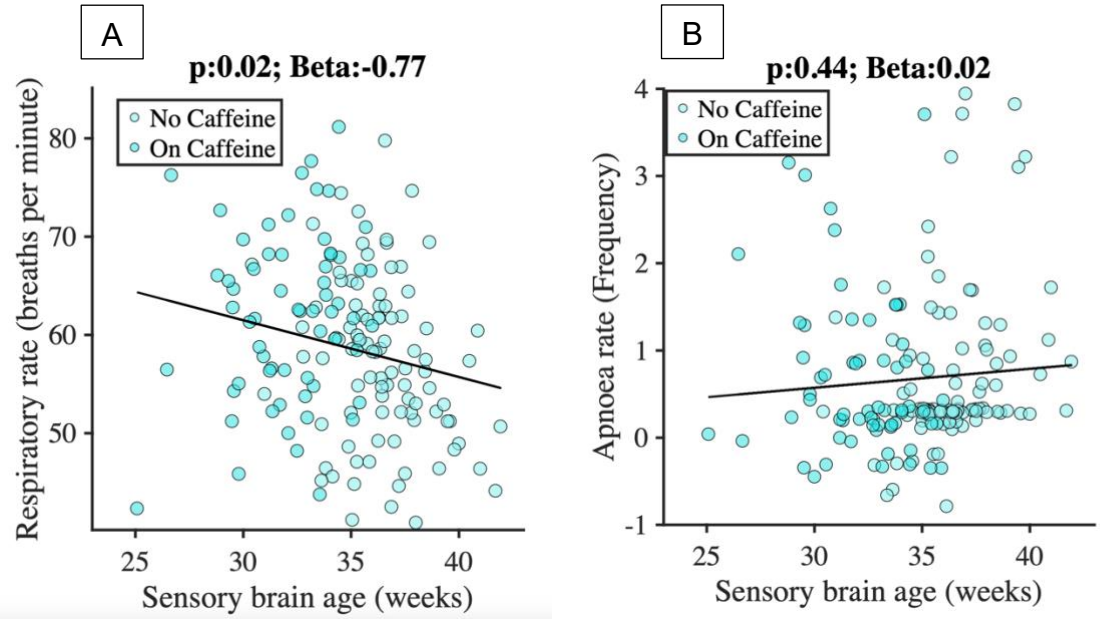

Figure 3.15 : Effect of sensory brain age on neonatal respiration. Increasing sensory brain age significantly reduces respiratory (A) but not apnoea rates (B) in neonates 28 - 41 weeks postmenstrual age. Each dot indicates an individual test occasion (121 neonates were studied on 205 test occasions); neonates on caffeine are shown as dark turquoise dots and those not on caffeine as the light turquoise dots. The black line is the mean fit of the linear model. Adjusted for data length, infection, respiratory support.

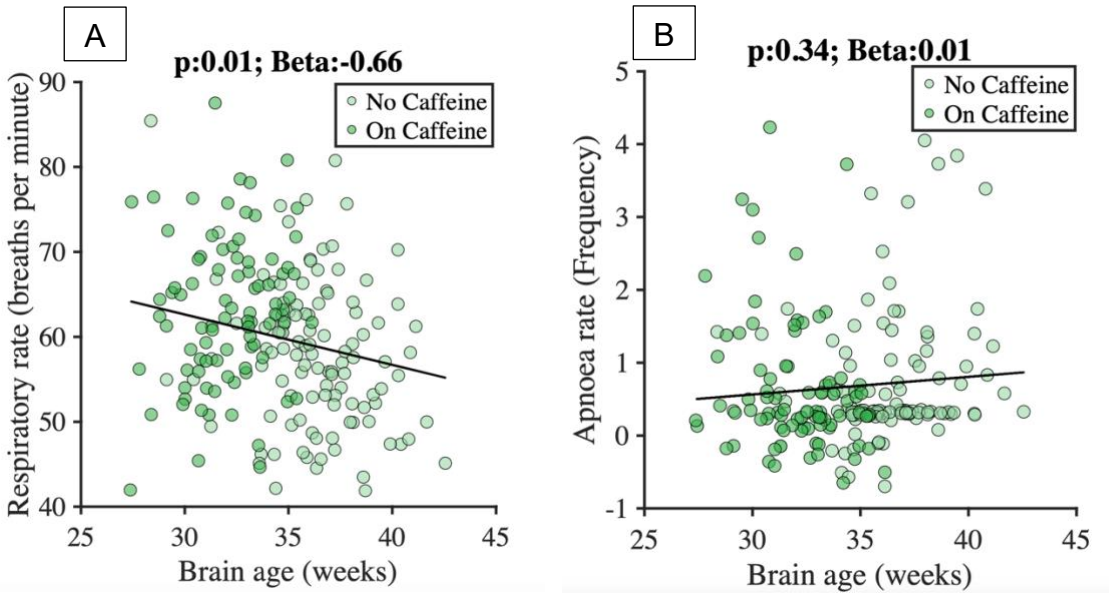

Figure 3.16: Effect of combined brain age on neonatal respiration. Increasing brain age significantly reduces respiratory (A) but not apnoea rates (B) in neonates 28 - 41 weeks postmenstrual age. Each dot indicates an individual test occasion (121 neonates were studied on 205 test occasions); neonates on caffeine are shown as dark green dots and those not on caffeine as the light green dots. The black line is the mean fit of the linear model. Adjusted for data length, infection, respiratory support.

3.4.1.5. Effect of brain maturity on respiratory and apnoea rates

Brain maturity (a standardised measure of functional brain development, calculated as the difference between brain age and PMA) in contrast to both PMA and brain age, better explains the occurrence of apnoea rates i.e., the less mature the brain, the higher the apnoea rate and vice versa (β = -0.17, p = 0.02, 95% CI = -0.33, -0.02; **Figure** 3.17A). It is the only measure that shows a decreasing apnoea rate trend with advancing brain maturity. Additionally, brain maturity showed a significant relationship with respiratory rate (β = 1.37, p = 0.26, 95% CI = 0.47, 2.88; **Figure** 3.17B).

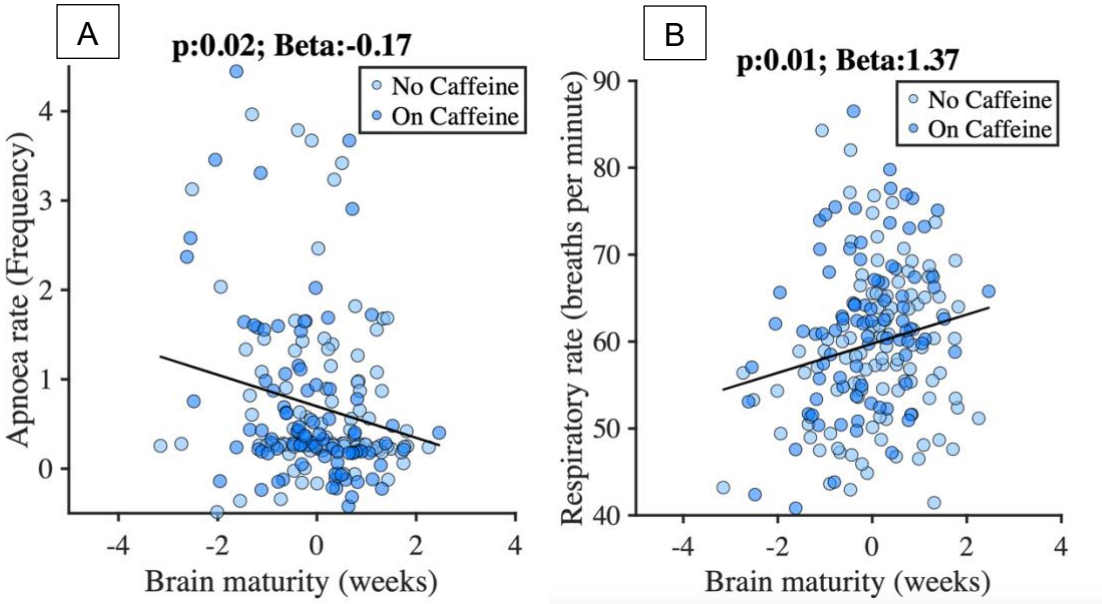

Figure 3.17: Effect of brain maturity on neonatal respiration. Apnoea rate decreases significantly with increasing brain maturity (A). There was no significant relationship between respiratory rate and brain maturity (B). Each blue dot indicates an individual test occasion (121 neonates were studied on 205 test occasions); neonates on caffeine are shown as dark blue dots and those not on caffeine as the light blue dots. The black line is the mean fit of the linear model. Adjusted for data length, infection, respiratory support. Negative values indicate the brain maturity is less than the corresponding neonate's postmenstrual age and vice versa. A value of zero (0) signifies a brain maturity that is equal to the corresponding neonate's postmenstrual age.

Overall, more infants had advanced brain maturity (n = 103, 50.2%) compared to those with brain immaturity (n = 90, 43.9%). Only 12 neonates (5.9%) had a brain maturity equal to their PMA. There were no notable differences in the distribution of basic characteristics among neonates when grouped by their level of brain maturity (**Table** 3.2).

Table 3.2: Distribution of infant characteristics by brain maturity

Variable	Advanced (N = 103)	Immature (N = 90)	Normal (N =12)
Age			
Gestational age (weeks)	31.8 (±3.8)	31.4 (4.2)	29.5 (2.8)
Postmenstrual age (weeks)	34.8 (2.8)	34.3 (3.3)	33.9 (1.9)
Age category			
Preterm	90 (87.4)	72 (80.0)	12 (100)
Term	13 (12.6)	18 (20)	0 (0)
Sex			
Female	36 (35.0)	36 (40.0)	2 (16.7)
Male	67 (65.0)	54 (60.0)	10 (83.3)
Mode of delivery			
Elective C-Section	8 (7.8)	4 (4.4)	0 (0)
Emergency Section	69 (67.0)	61(67.8)	9 (75.0)
Vaginal	18 (17.5)	22 (24.4)	2 (16.7)
Assisted vaginal	8 (7.8)	3 (3.3)	1 (8.3)
Birth resuscitation			
No	22 (21.4)	18 (20)	2 (16.7)
Yes	81 (78.6)	72 (80)	10 (83.3)
Infection			
None	14 (13.6)	9 (10.0)	1 (8.3)
Suspected	43 (41.7)	36 (40.0)	3 (25.0)
Treated	29 (28.2)	30 (33.3)	7 (58.30)
Respiratory support			
High flow therapy	26(25.2)	28 (31.1)	3 (25.0)
Low flow therapy	7 (6.8)	7 (7.8)	3 (25.0)
Spontaneous breathing	67 (65.0)	54 (60.0)	5 (50.0)
Caffeine			
No	64 (62.1)	45 (50)	7 (58.3)
Yes	39 (37.9)	45 (50)	5 (41.7)

3.4.2. Comparing PMA and brain maturity as markers of apnoea in preterm neonates

Given that apnoea is significantly related to brain maturity, to further explore this relationship in a cohort that are more predisposed to apnoea, only preterm neonates were included for this analysis. The participants comprised 74 preterm neonates, ranging from 23 + 6 weeks to 36 + 6 weeks GA (PMA between 31 and 36 + 6 weeks at the time of test occasion) and tested on 138 occasions **Figure** 3.5 and **Table** 3.1**.** This PMA range, with a lower limit of 31 weeks, was selected to investigate whether

brain maturity can be used to assess the need for caffeine treatment. Unlike the extreme and very preterm neonates, not all moderate or late preterms will experience apnoea, and the decision regarding when to start caffeine treatment in these older premature neonates is often debated. Preterms < 32 weeks are invariably commenced on caffeine due to a greater likelihood for apnoea than older preterms, but at 32 weeks, decisions are more unclear. For this reason, including neonates from 31 weeks, slightly younger than the age of treatment decision controversy is appropriate to support clinical decisions on the need for treatment at this age.

To assess whether brain maturity is indeed a better predictor of apnoea than PMA, the analysis comparing PMA and brain maturity as determinants of apnoea rate was replicated in the 74 preterm neonates. While apnoea rate decreased with increasing PMA, the rate of decline was not significant ($\beta = -0.04$, $p = 0.58$, 95% CI = -0.16; 0.09). Apnoea rate was, however, significantly associated with brain maturity ($\beta = -0.22$, $p = 0.02$, 95% CI = -0.41; -0.03 - **Figure** 3.18).

Still, in this cohort (**Figure** 3.19), respiratory rate was significantly reduced with increasing PMA ($\beta = -1.87$, $p = 0.01$, 95% CI = -2.81; -0.93). Brain maturity, on the other hand, did not have a significant effect on respiratory rate ($\beta = 1.17$, $p = 0.18$, 95% CI = -0.54; 2.88).

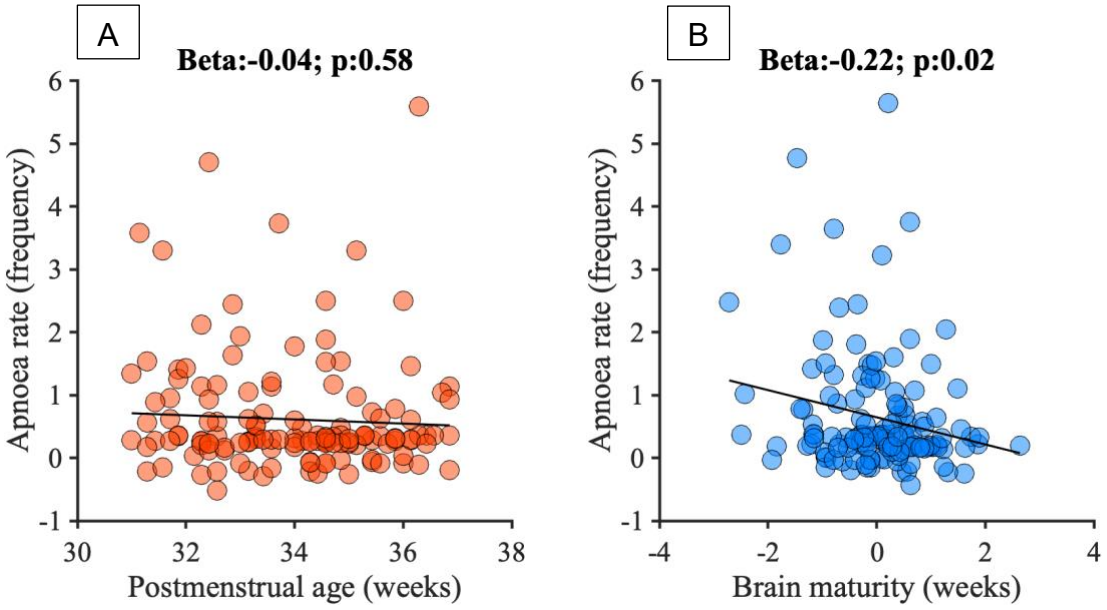

Figure 3.18: Relationship between apnoea rate, postmenstrual age, and brain maturity. Brain maturity (**B**) better explains the apnoea rate than postmenstrual age (**A**). Each dot indicates an individual test occasion (74 neonates were studied on 138 test occasions). The black line is the mean fit of the linear model. Adjusted for data length, infection, and respiratory support. For the brain maturity axis, the negative values indicate the brain maturity is less than the corresponding neonate's postmenstrual age and vice versa. A value of zero (0) signify a brain maturity that is equal to the corresponding neonate's postmenstrual age.

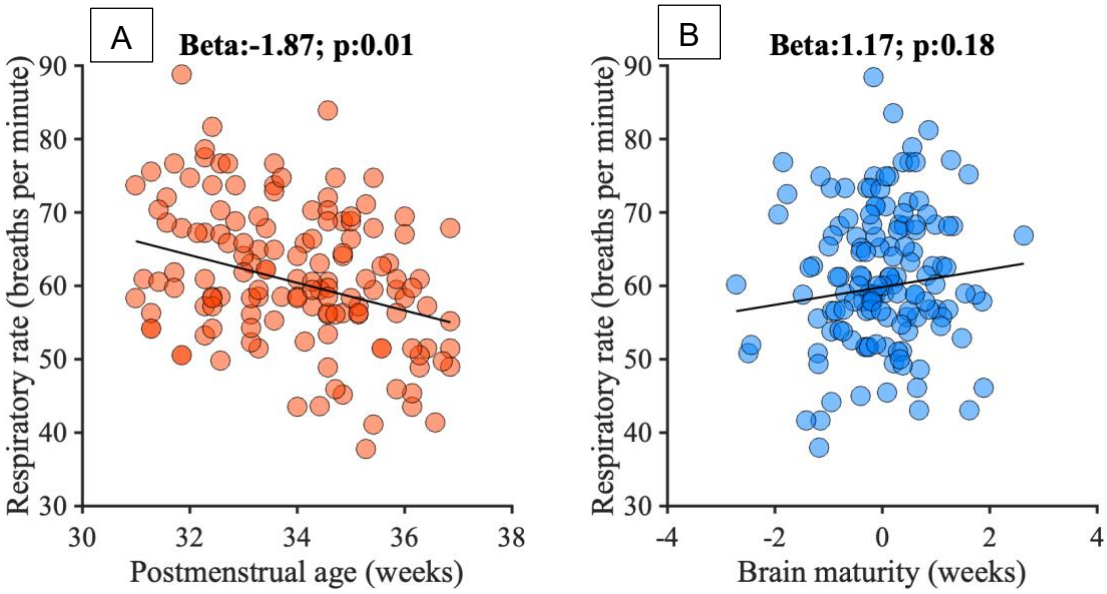

Figure 3.19: Relationship between respiratory rate, postmenstrual age, and brain maturity. Postmenstrual age better explains respiratory rate (**A**) than brain maturity (**B**). Each dot indicates an individual test occasion (74 neonates were studied on 138 test occasions). The black line is the mean fit of the linear model. Adjusted for data length, infection, respiratory support. For the brain maturity axis, the negative values indicate the brain maturity is less than the corresponding neonate's postmenstrual age and vice versa. A value of zero (0) signify a brain maturity that is equal to the corresponding neonate's postmenstrual age.

3.4.3. Association between PMA and brain maturity as a guide for caffeine treatment in preterm neonates

To evaluate the relationship between brain maturity and the timing of caffeine discontinuation in order to inform treatment decisions, preterm neonates whose EEG was recorded prior to the cessation of caffeine were included in this analysis. Most of the neonates did not have their EEG-recorded on the day caffeine was stopped, and thus, for consistency, only a sub-set of 27 preterm neonates who had their EEG recorded (and brain maturity calculated) within two weeks prior to the discontinuation of caffeine were included in this analysis. The PMA at which caffeine was stopped was negatively correlated with brain maturity (ρ = - 0.28, p = 0.14; 95% CI = -0.61; 0.09; **Figure** 3.20). While the finding did not reach significance, in the age range studied, the negative correlation coefficient indicates an inverse relationship between brain maturity and the age at which the clinical decision to stop caffeine was made, i.e., neonates with a mature brain relative to their PMA were likely to come off caffeine earlier and vice versa.

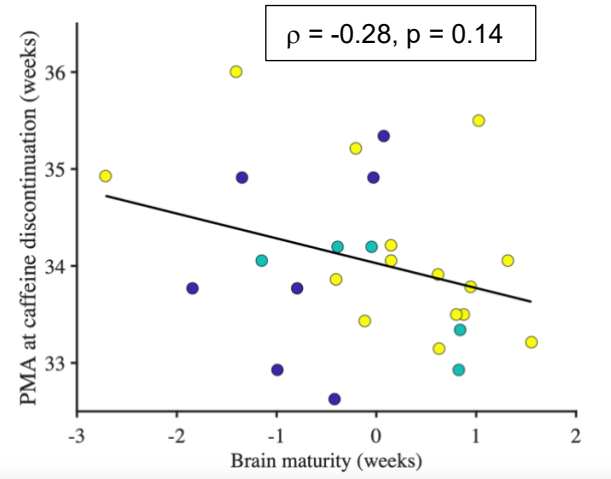

Figure 3.20: Correlation between postmenstrual age at caffeine discontinuation and brain maturity. The negative values on the brain maturity axis indicate the brain maturity is less than the corresponding neonate's postmenstrual age and vice versa. A value of zero (0) signify a brain maturity that is equal to the corresponding neonate's postmenstrual age. Model adjusted for infection and mode of ventilation (circle colours: yellow = spontaneous ventilation in air, turquoise = low flow oxygen therapy and purple = high flow oxygen therapy).

In a further exploratory hypothesis-generating analysis (**Figure** 3.21), participants were grouped by their brain maturity and the rates of apnoea in 11 preterm neonates and desaturation in 17 preterm neonates who had continuous vital signs monitoring in the seven days following treatment discontinuation were compared. Participants with relatively mature brain activity had fewer apnoeas (60% less, 0.41 ± 0.40 apnoeas/hour - mean ± SD) compared with neonates with immature brain activity (1.02 ± 0.60 - mean ± SD); as well as fewer desaturations (42% less, 0.26 ± 0.38 desaturations/hour - mean ± SD) relative to neonates with immature brain activity (0.45 ± 0.33 (mean ± SD)).

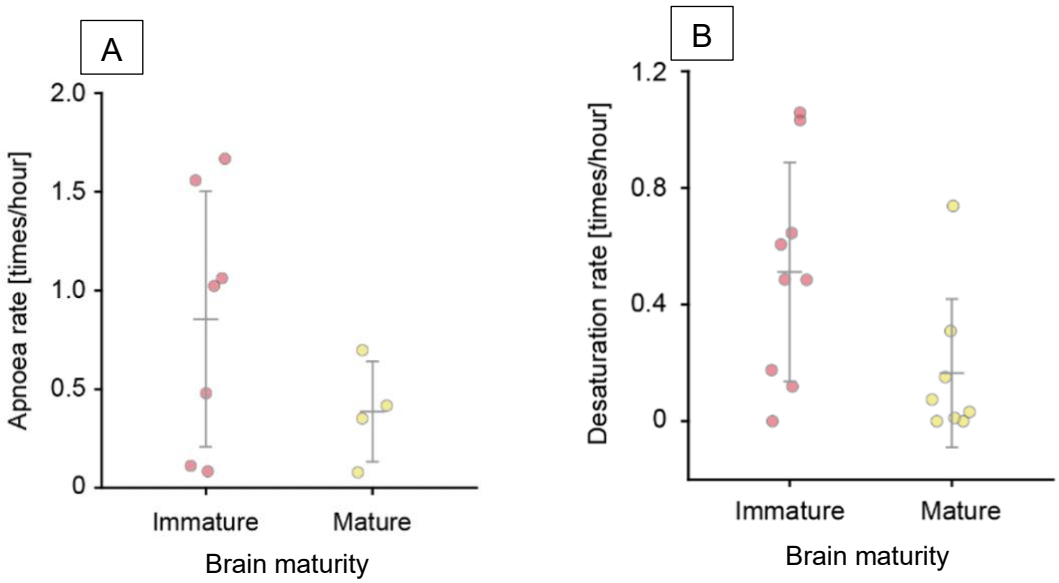

Figure 3.21: Box-plots comparing the distribution of apnoea and desaturation rate in neonates by brain maturity. Neonates with brain maturity values of < zero (0) were classed as immature while values ≥ 0 were classed as mature.

3.5. Discussion

In this Chapter, I have shown that while PMA and brain age are related to respiratory rate in neonates between 28 - 41 weeks PMA, apnoea rate is significantly dependent on brain maturity (a standardised measure of functional brain development). While brain maturity showed a significant association with respiratory rate (**Figure** 3.17B), the direction of the the relationship i.e., increasing brain maturity causing an increase in breathing rate is not biologically plausible. We would expect respiratory rate to decrease with advancing brain maturity as shown by PMA and brain age in **Figure** 3.13A and Figure 3.16A, respectively reflecting improved respiratory control and stability with aging. Evaluating further the true impact of brain maturity on apnoea rate in a population of preterm neonates, who are generally more prone to apnoea than term neonates, it remains that brain maturity is still a better indicator of apnoea than PMA. Age is a continuous progressive metric and both PMA and brain age (Brain age = Brain maturity + PMA) are linked to time. Brain maturity (Brain maturity = Brain age - PMA), on the other hand, is primarily influenced by genetics and environmental factors and is poorly correlated with age (**Figure** 3.7 E, F). As such, it makes sense that brain maturity may better explain inherent individual susceptibility to apnoea than PMA alone considering the differences in apnoea risk among neonates even of the same age. For the treatment of apnoea of prematurity with caffeine, this study provides initial evidence to suggest that brain maturity might be negatively correlated with the PMA at caffeine discontinuation and also relates to the risk of apnoea and desaturation after cessation of caffeine treatment. This finding could be useful in personalised care guiding the need for caffeine therapy decisions in preterm neonates.

The control of breathing in neonates is complex, maintained by an immature respiratory system and an underdeveloped brain regulatory mechanism [1]. Functional and anatomical changes in the respiratory system occur after birth, including an exponential increase in the number of alveoli, upregulation of surfactant production, improved compliance of the chest wall, and respiratory muscles. Additionally, there is maturation of protective laryngeal and respiratory reflexes, along with enhanced chemoreceptor sensitivity [1,55,64,207]. Normal ventilatory responses are triggered by changes in blood gas levels via the action of peripheral and central chemoreceptors. Peripheral chemoreceptors, however, mature more rapidly than central chemoreceptors in the brain [1] and are activated by a rise in the partial pressure of oxygen with the first breath at the time of birth, setting a cascade for a range of developmental changes. This developmental resetting is typically accomplished within 48 hours, further enhanced by concurrent mechanical changes that occur in the respiratory system [208]. The relatively mature peripheral chemoreceptors can easily detect hypoxic changes and respond automatically by stimulating the brainstem to increase the respiratory rate and maintain normal oxygen levels. As gestational age at birth increases or as the neonate matures postnatally, peripheral chemoreceptor function improves much more rapidly than central chemoreceptors to support optimal breathing. These changes could, therefore, explain the findings from this study of a significant association between age and respiratory rate.

Brain volume, structural and functional connectivity reflect the extent of brain development and are contingent on both intrinsic (genetic) and extrinsic (environmental) factors [27,209–211]. The risk of respiratory complications such as apnoea is related to the extent of brain development. Therefore, relying on direct brain

estimates such as brain maturity, a marker of brain development, instead of chronological age to determine the likelihood of apnoea might be more reasonable for prognostication. In this thesis, the resting-state and sensory-evoked EEG measures relative to the actual PMA of the neonate at the time of EEG recording were combined to calculate the neonate's brain age. Brain maturity (also known as brain age delta or brain age gap) is a standardized adaptation of brain age which excludes the effect of PMA. While brain age provides distinct information on individual brain chronology, brain maturity provides a population-based average metric [202] much like the z-scores. For example, in **Figure** 3.7D, a 29-week PMA neonate (purple dot) has immature brain age of 27 weeks relative to their PMA which is a brain maturity of -2 (**Figure** 3.7E**)**, suggesting an immature deviation of their brain development by 2 weeks from the normal developmental trajectory. The interpretation of brain maturity on immediate clinical significance should, however, be in the context of the pathology. Relating the example above to apnoea, the probability of the 29 week preterm (purple dot) with a brain maturity of - 2 (27 weeks) having apnoeas is more than that of a 37-week term neonate (yellow dot) with the same brain maturity (-2, brain age of 35 weeks) (Figure 3.7E**)**. This is supported by the findings in **Figure** 3.17 where apnoea risk was significantly higher in neonates with lower brain maturity compared to neonates with a higher brain maturity. The emphasis on the interpretation of brain maturity is as a standardised metric identifying deviations away from the normal brain trajectory at group level i.e., relative to other neonates of the same PMA. Importantly, the usefulness of brain maturity has been reported when assessing neurodevelopment outcomes in neonates, exhibiting better discriminant ability in classifying neonates as normal or with severe abnormality later in life [123,124]. While several validated measures are currently used to track physical growth e.g., the modified Ballard chart for

gestational age assessment at birth, weight, and length charts; using brain age or maturity measures for evaluating neurological outcomes in neonates is not yet standard practice, and having this may potentially change management guidelines including prognostication.

Apnoea susceptibility is linked with genetics [212,213], exacerbated by disease, blunted chemoreceptor hypercapnic responses and reflex respiratory inhibition from diminished neuromuscular control of upper airway patency [1,73]. Depending on the severity of an episode, hypercapnia and later hypoxia ensue. Hypercapnia is the main trigger for the central chemoreceptors causing reflex upregulation of breathing rhythmogenesis, however, this response is diminished and less efficient at birth, suggesting immaturity of central brain-respiratory mechanisms [1,64]. This theory is supported by the findings from this study of a significant relationship between apnoea and brain immaturity. Moreover, preterm neonates cannot handle increases in respiratory demand [51] and reports have shown that their central chemoreceptor function is less efficient than term neonates, increasing their apnoea risk [73,208,214]. Although peripheral chemoreceptors develop earlier than central receptors and respond effectively to hypoxia, the central processing of afferent signals from the periphery relies on the state of brain function, which is immature in the neonate. Therefore, using brain-based measures rather than chronological age could provide a more accurate approach to predicting apnoea susceptibility.

The resolution of apnoea and the ability to maintain normal respiration over time is a key developmental milestone for many premature neonates. Caffeine is the main pharmacologic treatment for apnoea [74] and the World Health Organisation (WHO)

recommends starting therapy in neonates born < 32 weeks GA [215]. This decision, however, may vary depending on local clinical guidelines; and neonates could receive caffeine irrespective of whether they develop apnoea or not based on the age recommendation guidelines. Likewise, the duration of therapy is variable [75,216,217], usually at the discretion of the clinician and guided by the physiological stability of the neonate. In this study, we found the PMA at caffeine discontinuation was highly variable, with caffeine stopped in some very preterm neonates despite their relative brain immaturity and vice versa (**Figure** 3.20) This study found a negative correlation between the age at caffeine discontinuation and brain maturity, in other words, neonates with more mature brains tended to stop caffeine treatment earlier than those with less developed brains. The decision to stop caffeine was made by clinicians, who were unaware of the neonate's EEG-derived brain measures. While this finding was not statistically significant, most likely due to the small sample size for this analysis (n = 27), the possible clinical implication of these findings, especially for neonatologists who typically rely on age to predict apnoea risk and caffeine requirement, is a shift in practice from this standard to a more objective, neonate-specific measure based on brain maturity. Utilizing brain maturity for clinical decisions may better account for individual variations in apnoea rates than age alone, leading to improved practices. Moreover, evaluating neonatal brain maturity can easily be incorporated into routine clinical practice because EEG monitoring is a simple, bedside test and maturity assessment requires just 20 minutes of recorded data for estimation.

In a further exploratory assessment, neonates with immature brain who had their caffeine stopped early continued to have apnoea and desaturations. Stopping caffeine before the neonate is ready to be weaned off, compounded by a lack of accurate

biomarkers to guide treatment decisions exposes preterm neonates to additional risks when respiratory instability re-occurs. One study found caffeine therapy was stopped too early and was subsequently recommenced in 10% of preterms between 33 - 35 weeks [218]. Although caffeine is relatively safe with a wide therapeutic index and long half-life, it has side effects [217,219], can lead to toxicity [220] and drug interactions [221]. Given the numerous uncertainties surrounding apnoea risks and treatment, it becomes prudent to utilise key brain-based determinants that can help caffeine treatment optimisation and clinical decision-making. An individualised approach to care will reduce the likelihood of exposing preterm neonates to prolonged caffeine therapy with potential negative side effects or risks associated with early discontinuation. There is a need for further work using a larger cohort to explore this relationship as the analysis for this sub-aim was exploratory.

A limitation of this study was using convenience sampling for participant selection. Although this is a non-probability sampling method [181], the potential for sampling bias was mitigated by having strict study eligibility criteria, recruiting a large sample with diverse participant representation including term and preterm neonates managed at different levels of care (i.e., intensive care, low and high dependency unit as well as on the postnatal unit) and using robust statistical methods - linear mixed effects models [206] - for analysis. In particular, the sample size for aim 3 was small due to the limited number of neonates that had simultaneous brain maturity assessment and continuous vital signs recording for a week post caffeine discontinuation (n = 27). To ensure appropriate reporting from the limited observations, correlation analysis was used to assess the association between PMA at caffeine discontinuation and brain maturity. Although correlation does not imply causation, it assesses potential biological

plausibility [222] and this is especially useful in hypothesis generation. Finally, only exploratory analysis was used to compare apnoea and desaturation frequencies based on brain maturity. While the results were not tested by statistical inference due to the limited observations, these interesting preliminary findings can be used for sample calculation in future studies. A post hoc sample size estimation using G*Power 3.1 based on the apnoea rates observed, with an effect size of 1.19, alpha of 0.05, and power of 0.95 calculated a total sample size of 34 preterm neonates for future apnoea studies.

This work has several methodological strengths. Validated neonatal-specific tools were used to assess both brain- and respiratory-derived measures, ensuring accurate quantification. Considerable measurement variability has been reported during neonatal breath detection even when using the same evaluation method [223]. Neonatologists often rely on bedside clinical assessments counting respiratory rate and observing for pauses in breathing, which are highly subjective. Although automated devices such as vital signs and apnoea monitors can detect respiratory changes in neonates, these are prone to error [224,225]. The widely used vital signs devices in most NICUs were developed using adult algorithms for signal detection [194,195], thus making them less reliable in neonates. On the other hand, assessing IBI as a marker of respiratory variability in neonates has been shown to be a precise and reliable indicator of respiratory changes [180] with better apnoea predictive value at a certain time window before the onset of breathing cessation [226]. Adjei et al. (2021) showed that with the IBI assessment algorithm, 88% of true apnoeas detected by the algorithm were not recorded in clinical notes. This method was used to reliably quantify IBI changes and subsequently calculate the respiratory and apnoea rates for this

study. These methods are less likely to produce variability (i.e., the same recording will detect the same number of breaths every time). For brain age and maturity assessment, previous works used multiple EEG channels and long recording durations [123,227] to estimate the trajectory of brain development. The validated algorithm for brain age estimations [124] used in this current study required only 20 minutes of EEG recording from 2 channels, thus, making it less cumbersome and potentially easy to adopt in a busy neonatal unit to support quick clinical decisions. Additionally, both the resting-state and sensory models used in this study have a good brain age predictive accuracy with a mean estimation error of approximately 1 week [124,125], compared with visual expert assessment with up to two weeks error [126]. Combining resting-state and sensory models to assess brain age and maturity is likely to increase the accuracy and validity of the EEG-derived brain measures in this study as it incorporates both baseline and sensory data.

4

Efficacy of the Dandle® WRAP Stretch Swaddle in Minimising Physiology Changes During Retinopathy of Prematurity Screening: A Comparative Study and Staff Opinion Survey

This chapter has been published in Paediatric Research. I am joint first co-author on the manuscript [228]. The work has been restructured to better fit a thesis format and the content of this chapter is my own.

4.1. Background

Hospital procedures can be painful and distressing, leading to adverse physiological responses [94–96] including apnoea. One such invasive procedure is retinopathy of prematurity (ROP) screening. Global estimates report approximately 185,000 preterm-born neonates develop some degree of ROP [229], with wide-ranging regional differences in incidence between 10 - 73% [230–237]. A recent meta-analysis reported that

7.5% of preterm neonates with ROP develop severe visual impairment [238] and it is the commonest cause of childhood blindness affecting approximately 32,000 survivors [229]. Data from low- and middle-income countries are limited [239] due to non-reporting, lack of existing screening protocols, and low preterm survival rates. A higher incidence of ROP is observed in the extremely preterm and low birth weight neonate exposed to critically high oxygen levels [88,240,241].

Although ROP treatment is required in <10% of affected preterms [242], multiple eye screenings are recommended in at-risk neonates to ensure early diagnosis, proper disease staging and timely treatment. Variations exist in the timing of the first eye examination, depending on the GA at birth, weight and centre-specific screening protocol [89,91]. The UK screening guidelines recommend that all babies with a birth weight of <1501g or born at GA < 31 weeks should be screened between 31 + 0 and 31 + 6 weeks PMA, or at four weeks postnatal age (PNA), whichever is later. Preterm neonates born ≥ 31 weeks GA (here, without birthweight consideration), should have their first screening between 36 + 0 and 36 + 6 weeks PMA, or at four weeks PNA, whichever is earlier [97]. The procedure is repeated at 1 - 3 weeks intervals based on disease progression and severity [89,97]. The physiological complications of ROP screening are described in **Section** 1.7**.**

Ophthalmologists diagnose ROP using different screening modalities to visualize the retinal vasculature [243]. The procedure requires a speculum to be inserted into the eye to keep the eyelids open and eye drops instilled to dilate the pupils for optimal visualisation and analgesic effect. No sedation or general anaesthesia is required during the procedure [243,244]. The conventional ROP screening method is binocular

indirect ophthalmoscopy (BIO), where the neonate is positioned supine and a scleral indenter is used to rotate and stabilize the eyeball during the procedure [98]. A newer method, the ultra-wide field imaging (UWFI) with Optos®, is a non-contact laser scanning technique. Here, the neonate is held upright with the legs straddling the examiner's forearm ('flying baby' position) and the head stabilized towards the Optos® device for retinal image capture – it generates high-resolution images within 1 - 3 minutes. UWFI is the initial ROP screening method, adopted in January 2021, at the neonatal unit of the JRH.

To date, no single pain management strategy has been reported to effectively control or prevent pain and/or discomfort during ROP screening [245]. While the efficacy of analgesia in improving pain responses during the procedure has been reported [36,245], drug side effects including CNS, respiratory and gastrointestinal complications limit routine use. Non-pharmacological methods have also been evaluated with varying degrees of success [37–42,245–247]. One method is swaddling, where the neonate is wrapped in a blanket to simulate in-utero foetal position. It improves sleep, reduces pain-related physiological responses and is self-comforting [248]. During ROP screening, swaddling has been shown to significantly reduce pain responses when combined with other non-pharmacological modalities like sucrose, non-nutritive sucking [42] and breastmilk [40], though, this is not a consistent finding [41,246,247]. Differences in swaddling effectiveness may be due to variations in the swaddling techniques [249], e.g., neonate limb position, tightness, duration and type of fabric used for swaddling.

Reducing physiological instability during ROP screening in preterm infants is clinically essential as they are highly vulnerable to it due to the immaturity of their respiratory,

cardiovascular, and central nervous systems. ROP screening is a stressful and painful procedure that can cause adverse autonomic disturbances (**Section** 1.7). These complications, when recurrent and prolonged, can have long-lasting consequences on brain structure and function as well as later life pain sensitivity and stress response [174]. Therefore, exploring ways to limit the cumulative impact of this procedural pain is crucial for optimising neurological outcomes. The neonatal unit at the JRH implemented a guideline change in September 2022 to use a specially designed stretchable wrap, the Dandle® WRAP stretch [250], for swaddling as a comfort measure during ROP screening. The reason for this change was that the Dandle® WRAP stretch would make it easier to contain neonates during the procedure, improve their comfort level and facilitate the procedure. This wrap is made from a stretchable material, keeping the neonate's legs in a flexed easy recoil midline position. It is fitted with Velcro for secure holding enabling optimal upper body swaddle position and free lower limbs during the procedure. Conventional swaddling with a muslin, used during ROP screening before the guideline change was difficult to implement during UWFI - the ROP technique used at the JRH, as the neonates' legs need to be free for safe positioning. A muslin wrap easily comes undone and, thus, containment becomes suboptimal during the procedure.

During ROP screening, ophthalmologists work closely with the nurses to perform the procedure and provide continuing care post-procedure. Nursing staff have good knowledge about patient safety and experience recognising patient needs. To assess the acceptance and effectiveness of the newly introduced Dandle® WRAP stretch for patient comfort during ROP screening, it becomes important to consider healthcare professionals' opinions about the effectiveness of this new device. Further to the

guideline change, the NICU team wanted to assess staff perceptions about the newly introduced Dandle® WRAP stretch swaddle, and whether further training was required based on self-assessment.

This Chapter describes two studies: (**1**) The effect of a specialised swaddle, the Dandle® WRAP stretch, compared with a conventional swaddle (control) on vital signs changes in hospitalised neonates undergoing the ROP screening; and (**2**) A healthcare professionals' survey of practices and opinions about the usefulness of the Dandle® WRAP stretch during ROP screening.

4.2. Aim and objectives

This study aims to compare physiological instability from ROP screening (pre- and post-procedure) in neonates before and after the guideline change implementing the use of the Dandle® WRAP stretch as a comfort measure and assess staff perceptions about the newly introduced swaddle.

All comparisons are between Dandle® WRAP stretch and conventional swaddle.

The specific objectives are to:

Study 1:

1. Compare immediate changes in physiology (heart rate, oxygen saturation and respiratory dynamics) in the 15 minutes after the start of the ROP screening with the baseline period before the start of the procedure.
2. Evaluate longer time changes in physiology by comparing the baseline period with a period of 12 hours after ROP screening.

Study 2:

1. To assess staff perceptions of the neonates' comfort level when swaddled with a Dandle® WRAP stretch during ROP screening.
2. To assess staff opinions on their confidence level and ease of using the Dandle® WRAP stretch during ROP screening.
3. To identify staff swaddling method preference for neonates undergoing ROP screening, including their perceptions of the benefits and drawbacks of using the Dandle® WRAP stretch.

4.2.1.1. Hypotheses

Study 1: The specialised Dandle® WRAP stretch is more effective than the conventional swaddle in minimizing adverse changes in physiology (heart rate, oxygen saturation, respiratory changes) in the short (15 minutes) and longer time (12 hours) periods post-ROP screening.

Study 2: Staff will prefer using the Dandle® WRAP stretch and find it easier to use compared with conventional swaddle during ROP screening.

4.3. Methods

4.3.1. Study site, design, and population

The studies were conducted at the NICU of the JRH. It was a mixed methods design comprising an exploratory, prospective cohort study conducted between May and December 2022 and involving preterm-born neonates undergoing ROP screening according to the UK guidelines (**Study 1**); and a qualitative survey of staff opinion and practices conducted between September and December 2022 involving NICU nurses and ophthalmologists engaged in ROP screening and post-procedure care (**Study 2**).

For **Study 1**, the exclusion criteria included outpatients (as they are unlikely to stay in the hospital beyond the immediate post-procedure observation period), neonates on mechanical ventilation and receiving medications like morphine (due to the potential for cardiorespiratory depression, which could act as a confounder), maternal opiate use during pregnancy and lack of parental consent.

For **Study 2,** nurses and doctors that did not directly participate in ROP screening or care for neonates post-procedure were not eligible to take part in the survey.

4.3.2. Sampling method and sample size

A convenience sampling was used, targeting every consecutive neonate due their ROP screening and all eligible NICU staff.

Study 1: This was an exploratory study; hence, no power calculation was performed. The target sample size was to include at least 15 test occasions before the guideline change (implemented on 05/09/2022) and a similar number after the change for a comparison of the effect of the new swaddle (Dandle® WRAP stretch) on physiological changes during ROP screening. This was chosen based on feasibility and practical constraints, i.e., short data collection window, with only three months to recruit and study participants undergoing ROP screening using the standard guidelines before the introduction of the Dandle® WRAP in September 2022. Also, ROP screening is done only once a week in the NICU with an average of 2–3 procedures per test day. Based on the assumption that approximately 50% of families would consent, the target was to study at least one baby per week before the guideline change and a similar number of participants after the introduction of the Dandle® WRAP.

Study 2: The target was to include all staff involved in doing ROP screening, pre- and post-procedural care.

4.3.3. Ethics approval and consent

Study 1: The study was approved by the National Research Ethics Service as part of a wider study titled Newborn Infant Pain Investigation (reference: 12/SC/0447). Families of eligible participants were approached, and informed and written parental consent was obtained before recruitment.

Study 2: The Joint Research Office study classification group at Oxford University Hospitals (OUH) was contacted via email and it was determined that study 2 was a service evaluation based on the Department of Health's UK Policy Framework for Health and Social Care Research [251]. As such, it did not require a research ethics

review. The activity was registered via Ulysses with the OUH trust clinical audit team as a local audit (number: 8476).

Both studies were carried out in accordance with the standards set by the Declaration of Helsinki and Good Clinical Practice guidelines.

4.3.4. Study variables and survey questionnaire description

Study 1: Neonates swaddled with the Dandle® WRAP stretch during the ROP screening were classed as the Dandle® WRAP stretch group. Neonates screened for ROP using the standard protocol (conventional swaddle) before the guideline change and those studied without the Dandle® WRAP stretch (when unavailable) after the guideline change were classified as the control group.

For immediate outcome comparisons, a 15-minute period after the start of ROP screening was chosen because preliminary assessment of the physiological data collected showed the duration (from start to finish) of the procedure was approximately 10 minutes (range = 1.5 - 10.6 min, mean 4.6 minutes). Immediate physiological responses returned to baseline levels within 15-minutes after the start of the procedure (**Figure** 4.1). Baseline values were calculated as the mean outcome value ± two standard deviations (SD) from 5 minutes just before the start of ROP screening.

The outcomes assessed were:

1. Presence of bradycardia - heart rate < 100 beats per minute for 15 seconds.
2. Presence of tachycardia - heart rate > 200 beats per minute for 15 seconds.
3. Presence of desaturation - oxygen saturation <80% for at least 10 seconds.

4. Average increase in heart rate above baseline - average area under the heart rate curve above the baseline ± two SD divided by time (i.e., 15 minutes) after the start of screening.
5. Average decrease in heart rate below baseline - average area under the heart rate curve below the baseline plus ± two SD divided by time (i.e., 15 minutes) after the start of screening.
6. Average increase in respiratory rate above baseline - average area under the respiratory rate curve above the baseline mean ± two SD divided by time.
7. Average decrease in oxygen saturation below baseline - the average area above the oxygen saturation curve that is below the baseline mean ± two standard deviations divided by time.
8. Minimum (lowest) oxygen saturation.
9. Maximum (highest) heart rate.
10. Maximum (highest) respiratory rate.

For the calculation of heart rate and respiratory rate above the baseline, if the observed change was below the baseline threshold, then the average increase was taken as zero (0). Similarly, if the oxygen saturation and heart rate did not go below the baseline, the average decrease was taken as 0.

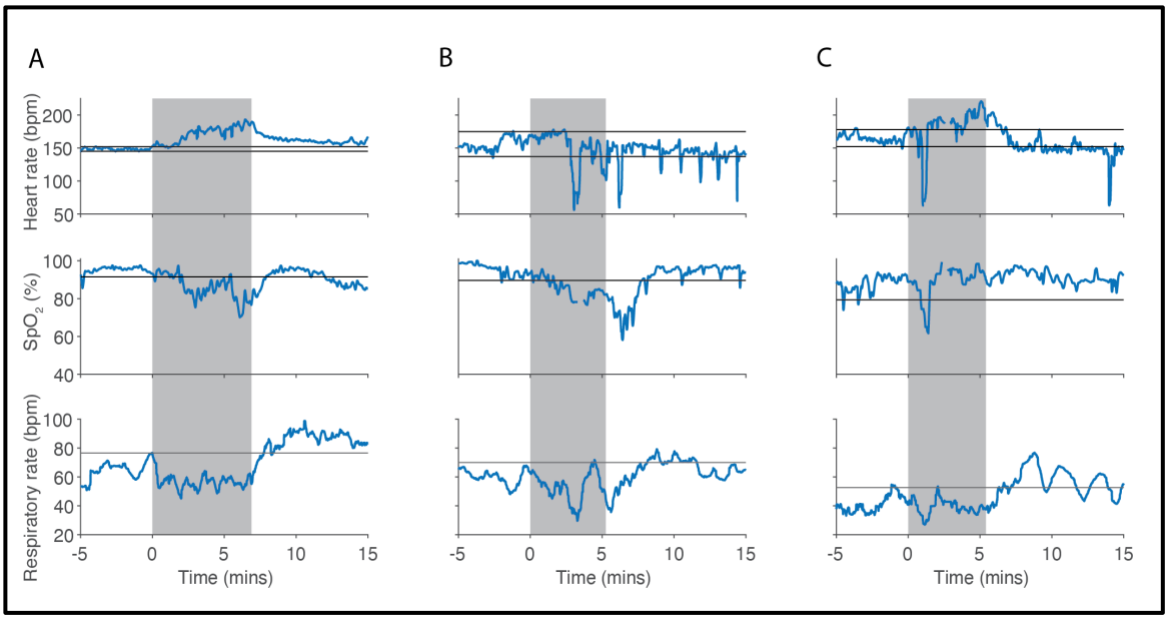

Figure 4.1: Examples of vital signs recording from 5 minutes before to 15 minutes after ROP screening in three neonates (A, B, C). The start of the procedure is shown by time = 0, and the procedure duration is shown by the shaded grey areas. For the heart rate plots, the two horizontal black lines indicate the infants' mean plus (top line) and minus (bottom line) the standard deviation of their 5-minute baseline (pre-ROP screen) values. For the oxygen saturation (SPO_2) plots, the single horizontal line represents the infants' mean minus the standard deviation, while for the respiratory rate figure, it represents the infants' mean plus the standard deviation of their 5-minute pre-ROP screen values. (A) A neonate whose heart rate increases, and SpO_2 and respiratory rate decrease during the examination. (B) A neonate with multiple bradycardias during and after ROP screening and a significant drop in SpO_2. (C) A neonate with immediate bradycardia at the start of screening followed by tachycardia. After an immediate desaturation, their oxygen saturation then recovers and remains within the baseline range for the rest of the 15 minutes. Respiratory rate decreased during the procedure, with a gradual return to normal almost immediately post-procedure.

For the longer time window (12 hours before and after ROP screening), the absolute frequencies of events (bradycardia, tachycardia, oxygen desaturation and apnoea – **Section** 3.3.5.1 for apnoea description) were assessed. Any episodes occurring within a minute of a preceding event were considered as a single episode. Twelve hours post procedure was selected for practical considerations (1) To minimize the burden of research on families. Monitoring was commenced 12 hours before the ROP screening. To match this pre-procedure time, a similar recording duration was used post-procedure. (2) Analysis of the data beyond 12 hours after the procedure showed no significant differences in any of the outcomes between the groups.

The immediate and long-term outcomes were selected based on previous studies showing significant changes in the measures during ROP screening [32,34,35,106,107]. For a comprehensive overview, not limited to average changes as described in previous works, the outcome measure descriptions in this study were expanded to include different quantification methods such as frequency of events, minimum and maximum changes. Additionally, clinically relevant physiological variables, i.e., other respiratory changes (not just apnoea), were included for a broader understanding of ROP screening impact on respiration.

Study 2 (Survey questionnaire): A web-based questionnaire created on Microsoft Forms specifically designed for the study was developed from expert opinion including inputs from two neonatologists, two ophthalmologists, a neonatal nurse, and an academic at the JRH. This was piloted on 4 participants (2 doctors and 2 NICU nurses) to ensure it was easy to understand, obtain feedback on the survey interface usability and accessibility across different operating systems and browsers. The questionnaire was revised for clarity from the pilot study feedback, adding more descriptive terms and questions tailored for nurses who took over the care of patients with the Dandle® WRAP stretch already on but had no experience themselves putting it on.

The questionnaire comprised 13 questions including close-ended multiple-choice, Likert scale and open-ended opinion questions (**Appendix – Section** 7.4**)**. A description of the staff role defining the cadre of the respondents was enquired. A five-opinion-based Likert-type questions - how settled/ comfortable babies were when swaddled with the Dandle® WRAP stretch (ranging between very comfortable to very

uncomfortable), and the ease of using the Dandle® WRAP stretch (from very easy to very difficult) - were asked. These were included to provide information about the perception of staff on the usefulness and practicability of using the Dandle® WRAP stretch as a comfort measure during the procedure. Also, participants were asked about their swaddling preference method i.e., conventional swaddling with muslin or the Dandle® WRAP stretch, and the reason for choice; whether staff would use the Dandle® WRAP stretch regularly if each neonate had their own and to provide a reason if they would rather not use it. These questions provided insight about staff preference i.e., compared the new (Dandle® WRAP stretch) and old (muslin) swaddling methods and the likelihood of continued use of the Dandle® WRAP stretch. Using open-ended questions, respondents were asked to provide suggestions/recommendations that could support or improve the use of Dandle® WRAP stretch as well as drawbacks, if any, that could militate against continued use of the Dandle® WRAP stretch during ROP screening.

4.3.5. Recruitment and data collection

Study 1: Every week, all eligible neonates were identified from an electronic ROP screening database and families were approached for consent before the procedure. Relevant demographic information such as sex, GA, PMA, birth weight, weight at test occasion, mode of ventilation and duration of the procedure were collected. Each neonate had continuous vital signs monitoring as the standard of care. Each test occasion comprised of vital signs recording (**Section** 3.3.4.3**)** before, during and after the scheduled ROP screening. Data acquisition was started at least 12 hours before and continued for at least 12 hours after the procedure.

Non-contact ultra-widefield imaging [244] with a scanning laser ophthalmoscope, also known as the Optos Panoramic 200Tx imaging system (Optos PLC, Dunfermline, Scotland, UK), was used for ROP screening in all neonates. The procedure was carried out by ophthalmologists and supported by the nurse looking after the neonate. Mydriatic eye drops (0.5% tropicamide and 2.5% phenylephrine) were instilled as standard practice approximately 1 hour before the procedure and local topical anaesthetic (proxymetacaine) eyedrops was also instilled immediately before the insertion of the eye speculum. Neonates were swaddled 2 hours before the procedure. **Figure** 4.2A shows a neonate swaddled with the Dandle® WRAP stretch. Just before the eye exam, the neonates' legs were unwrapped (with upper body swaddle still in place) to allow for holding in the required flying position for the procedure (**Figure** 4.2B-C).

At the start of the ROP screening, a researcher pressed a pre-specified key on the laptop connected to the vital signs monitor to mark the beginning of the procedure (immediately before the start of handling of the neonate) and again at the end of the examination when the speculum was removed, and the neonate placed back in its cot with the legs rewrapped in the swaddle (to indicate the end of the procedure). Swaddling was left in place for 2 hours after the screening. The eye examination lasts between 2 - 6 minutes for both eyes [243]. The time when the Dandle® WRAP stretch was put on and taken off was noted by the nurses looking after the neonate and was dependent on the clinical team's decision.

Study 2: Participants were identified via the NICU nursing and ophthalmology emailing list at the JRH and sent an online link to the survey questionnaire. A quick

response (QR) code was also made available on notice boards within the NICU for staff to scan and access the forms on their phones while at work. (**Appendix – Section** 7.4). Reminder emails were sent once every 2 weeks and followed up by a word-of-mouth approach.

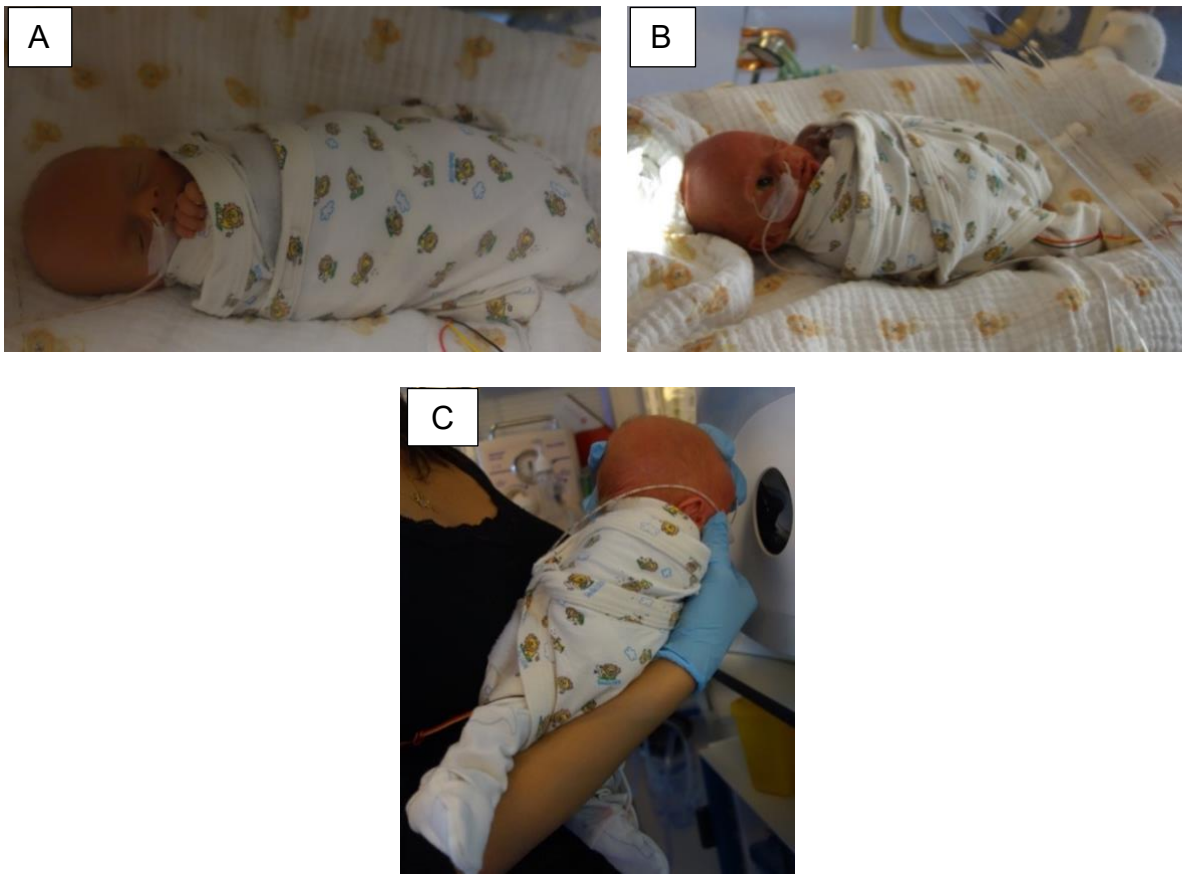

Figure 4.2: Photo of a neonate during ROP screening. Neonate swaddled with Dandle® WRAP stretch (A), showing the eyelid speculum in situ (B), flying baby position with upper body swaddle and free lower limbs during retinal image capture with non-contact ultrawide field imaging (C). Images used with parental consent.

4.3.6. Data analysis

Study 1: Vital signs data was imported onto MATLAB (ver. 9.12.0, R2022a; MathWorks Inc., Natick, USA) for analysis. Custom-made scripts written by another member of the research team were used to calculate the outcome measures described

in **Section** 4.3.4. The apnoea and respiratory rate were calculated from the inter-breath intervals (**Section** 3.3.5.1). The results generated from MATLAB were exported onto an excel sheet and statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) statistics for Macintosh (Version 28.0. Armonk, NY: IBM Corp).

Missing data were excluded, and analysis was conducted using pairwise deletion function which calculates the test statistic using all cases with non-missing responses for a particular variable, hence, reducing data loss. Pre-procedure, one heart rate and four oxygen saturation values were unusable (see 'missing' in **Table** 4.1 and 'n' in **Table** 4.2), while post procedure, six respiratory rate values were not included in the 15 minutes analysis (see 'n' in **Table** 4.2). These were due to noisy or unrecorded data, most likely from the electrodes coming off the infant's chest wall within 15 minutes before, during or after the procedure. There were no missing data in the 12-hour analysis time window. Shapiro Wilk test was used to assess data normality and variables with p value > 0.05 were considered as normally distributed data (**Appendix - Table** 7.6). Normally distributed quantitative variables (weight at test occasion, baseline mean heart rate, baseline mean saturation, duration of ROP screening, maximum heart rate) were summarized as mean ± standard deviation (SD) while skewed data (GA at birth, PMA at test occasion, minimum saturation, average heart rate above and below baseline, absolute numbers of desaturation, bradycardia, tachycardia, and apnoea) were summarized as medians and interquartile ranges (IQRs). Categorical variables were summarized as frequencies and percentages. Figures (boxplots, simple dot plots and dual axis plots) and tables were used to display the results.

To assess the balance of baseline covariates between the Dandle® WRAP stretch and control groups, standardized mean differences (SMDs) were calculated.

For continuous variables, the SMD was calculated as:

$$d = \frac{(\bar{x}_D - \bar{x}_c)}{\sqrt{\frac{s_D^2 - s_C^2}{2}}}$$

For categorical variables, the SMD was calculated using the formula:

$$d = \frac{p_D - p_C}{\sqrt{\frac{p_D(1-p_O) + p_C(1-p_C)}{2}}}$$

Where $\bar{x}$ denotes the sample mean, s is the standard deviation and p is the proportion/prevalence in the Dandle® WRAP stretch group (D) and the control group (C). A cut-off SMD of >10% (>0.1) was considered a covariate imbalance between the groups.[252]

Some participants required more than one ROP screening (these were done at least one week apart) and, therefore, had repeated measures that were not independent (i.e., measurements were more likely to be similar than if they were an independent sample). For this reason, mixed models, which allow for the effect of fixed factors (predictor variables of interest presumed to have the same effect across subjects and/or the outcome) and random factors (predictor variables that are a subset/sample from all possible levels resulting in between subgroup variability; included to control for non-independence of the observations) to be accounted for [253,254] were used to compare the short and long-term outcomes between swaddling methods. For quantitative outcomes (absolute heart rate, respiratory and oxygen saturation variables), a Linear Mixed Effects Model (LMEM) was used for analysis, while for categorical outcomes (presence of tachycardia, bradycardia, desaturation, and

apnoea) analysis, a Generalized Linear Mixed Model (GLMM) methods for logistic regression was used. To run the LMEM, first, each quantitative outcome variable (**Section** 4.3.4) was included as a dependent factor and the type of swaddling method as an independent factor. Next, GA, PMA, birthweight, weight at the time of procedure, sex, type of respiratory support (high flow, low flow, or no oxygen) during the procedure and baseline mean of the corresponding physiological variable and/or number of corresponding event pre-procedure were included as fixed factors in the models to adjust for any effect. Each study participant was included as a random factor in the analysis to account for the non-independence of the sample arising from repeated measurements in some of the participants. The main effects were evaluated based on variance estimates for random intercept and slope. GLMM for the qualitative outcome variables were run similar to the LMEM approach but here a binary logistic regression model function was used for the analysis.

Confounding variables, i.e. the fixed factors, selection was based on the disjunctive causal criterion which considers variables that affect either the exposure, outcome or both; and does not include factors considered as mediator, instrumental or collider variables as confounders. [204] The level of significance was set at $p \leq 0.05$. Multiple comparisons were adjusted for using the Holm-Bonferroni method [255] to account for the 14 outcome variables assessed, resulting in a significance threshold of $p \leq 0.0036$. All p-values are reported unadjusted, and significance level is judged relative to this 0.0036 threshold.

Study 2: Responses from the Microsoft forms were exported onto an excel sheet and analysed. Missing data were excluded from the result synthesis. Findings were

reported based on the Consensus-Based Checklist for Reporting of Survey Studies (CROSS) [256]. Results were summarized as counts and percentages and depicted using charts and a word cloud. Direct respondents' quotes were also used to describe representative opinions and comments/suggestions.

4.4. Results

Study 1: A total of 32 preterm-born neonates were recruited and 29 were studied on 44 separate test occasions (23 with the Dandle® WRAP stretch and 21 without the Dandle® WRAP stretch). Of the 44 test occasions recorded, 13 vital signs recording duration was less than 12 hours and, thus, were excluded from the longer (12 hours) time window analysis (**Figure** 4.3). Also, 13 neonates had multiple ROP screenings: eight were studied on two separate occasions, four on three occasions and one baby four times. All screenings were done at least one week apart. Three neonates had group cross-over and were studied once each as part of the Dandle® WRAP stretch group and on another test occasion, without the Dandle® WRAP stretch as part of the control group.

Using the Shapiro Wilk test for normality, age and weight at test occasion, baseline mean heart rate, oxygen saturation and respiratory rate, maximum heart rate and duration of ROP screen were normally distributed (**Appendix - Table** 7.6). The demographic characteristics of participants are shown in **Table** 4.1. There was a covariate imbalance in the GA at birth, birth weight, Apgar scores, baseline mean heart rate, PMA, and duration of ROP screening between the groups as shown by a SMD of > 0.1.

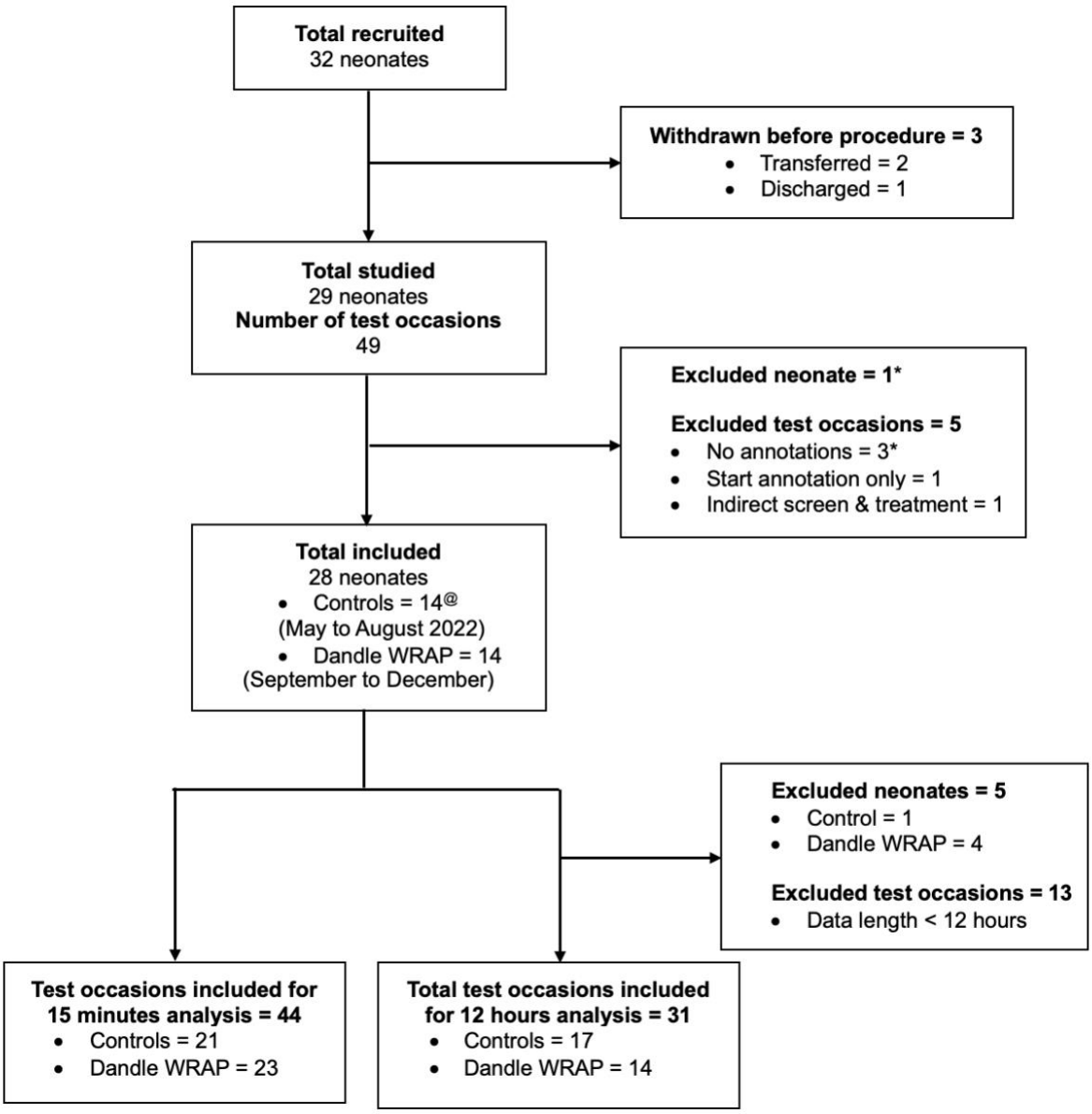

Figure 4.3: Recruitment flow chart. Controls comprise neonates screened for retinopathy of prematurity using the standard protocol (conventional swaddle) before the guideline change implementing the use of the Dandle® WRAP stretch and any neonate after the guideline change studied without the Dandle® WRAP stretch when unavailable at the time of the procedure. @ One neonate was studied as a control after the guideline change due to unavailability of an appropriate sized Dandle® WRAP at the time of the study. *Excluded neonate was among the three test occasions with no annotations on their vital signs recording.

Table 4.1: Participants' descriptive characteristics by intervention group

Variables	**Dandle® WRAP (n=23)**	**Controls (n = 21)**	**Standardised difference**
Gender [a]			
Female	8 (34.8%)	9 (45.0%)	0.13
Male	15 (65.2%)	11 (55.0%)	0.13
Mode of ventilation [a]			
Self-ventilating	3 (13.0%)	4 (19.0%)	0.13
Low-flow oxygen therapy	5 (21.7%)	4 (19.0%)	0.06
High-flow oxygen therapy	15 (65.2%)	13 (62.0%)	0.06
Gestational age at birth in weeks [b]	28.3 (5.4)	26.9 (3.4)	**0.42***
Birth weight in grams [b]	720.0 (594.0)	820.0 (356)	**0.40***
Weight at test in grams [c]	1928.9(±579.3)	1570.7 (510.4)	**0.90**
Postmenstrual age at test in weeks [c]	36.2 (±2.9)	34.4 (±3.1)	**0.82***
Baseline mean heart rate in bpm [c]	150.2 (±16.4) (missing =1)	156.4 (±14.2)	**0.50***
Baseline mean oxygen saturation in % [c]	94.5 (±2.8) (missing =1)	94.8 (±3.8) (missing =3)	0.08
Baseline mean respiratory rate in cpm [c]	56.4 (±14.0)	61.8 (± 15.0)	0.03
Duration of ROP screening in minutes [c]	4.7 (±2.1)	5.3 (±2.0)	**0.29**

Results provided as [a] frequency (percentage), [b] median (interquartile range), [c] mean (± standard deviation). * denote variables with a standardized mean difference of >0.1 signifying a covariate imbalance between the groups. *Abbreviations: number of observations (n), retinopathy of prematurity (ROP), beats per minute (bpm), cycles per minute (cpm).*

4.4.1. Comparing immediate physiology changes 15 minutes post-ROP screening in neonates with the Dandle® WRAP stretch versus controls

Figure 4.4 shows the frequency of occurrence of bradycardia, tachycardia, and desaturation by exposure group. Overall, in the 15 minutes time window post-ROP

screening, only 2/43 (4.7%) neonates had bradycardia, 7/43 (16.3%) had tachycardia and 10/40 (25.0%) developed desaturation.

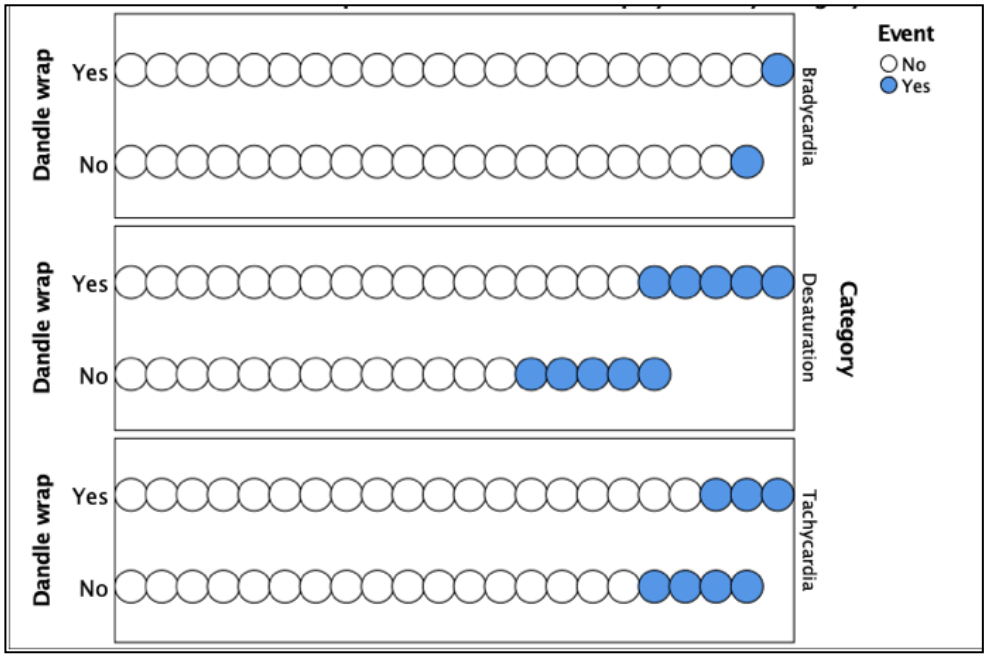

Figure 4.4: Simple dot plot of the frequencies of physiological events by group. Each blue dot indicates the occurrence of an event while a white dot indicates event non-occurrence in the corresponding group. Missing data were excluded from the analysis, and this accounts for the differences in the total observations across the groups.

Figure 4.5 shows a comparison of the changes in vital signs (heart rate, oxygen saturation and respiratory rate) between the Dandle® WRAP stretch and control group from 5 minutes before (baseline) to 15 minutes after ROP screening. In the baseline period, both groups show similar patterns in heart rate and oxygen saturations within ± 2 SD from the mean. From the start of ROP screening to approximately 5 minutes after the procedure, there was a consistent rise in the heart rate, and a drop in both oxygen saturation and the respiratory rate, followed by a gradual return to normal.

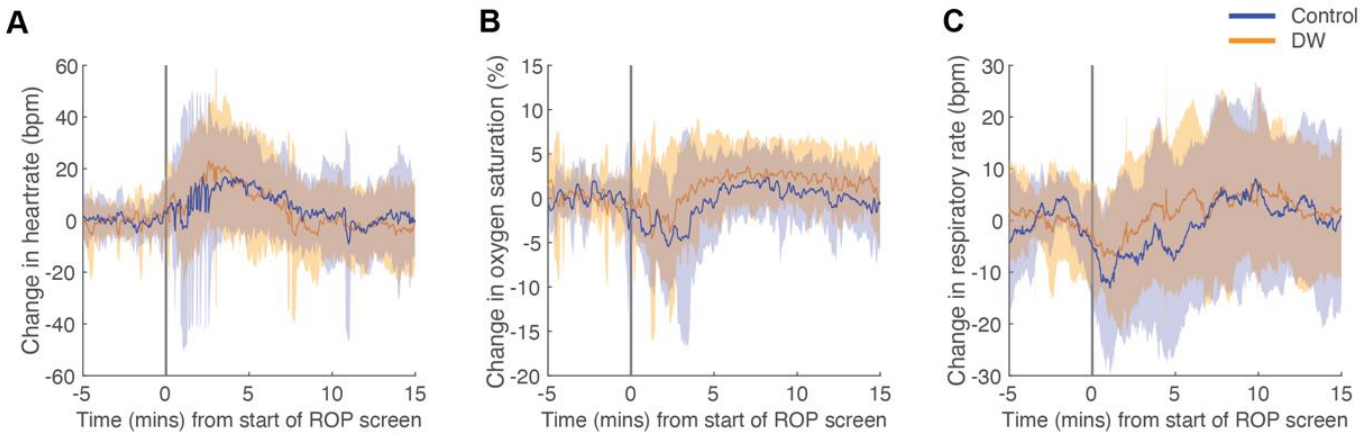

Figure 4.5: Comparison of physiology changes during ROP screening by group. Changes in heart rate (**A**), oxygen saturation (**B**) and respiratory rate (**C**) in response to ROP screening by intervention shown from 5 minutes pre-procedure to 15 minutes post-procedure. Striking physiology changes are noted to occur within 5 minutes after the test. The blue and orange solid lines represent the mean of the control and Dandle® WRAP stretch (DW) group and the corresponding shaded areas represent the standard deviation. Post-procedure values are baseline corrected by subtracting the pre-procedure mean values. The grey vertical line indicates the start of ROP screening coinciding with when the neonate was first touched.

Using the GLMM, there were no differences in the occurrence of desaturation (β = 1.39, unadjusted p = 0.39, 95% CI: -1.85, 4.62), bradycardia (β = 0.27, unadjusted p = 0.87, 95% CI:-3.04, 3.58) and tachycardia (β = 3.83, unadjusted p = 0.19, 95% CI:-1.93 - 9.59) in the Dandle® WRAP stretch group compared with the controls (**Table** 4.2). By contrast, maximum heart rate (β = 8.05, unadjusted p = 0.043, 95% CI: 0.26, 15.8) and average heart rate increase above baseline (β = 3.73, unadjusted p = 0.045, 95% CI: 0.09, 7.37) were significantly higher by approximately 8 bpm and 4 bpm, respectively in the controls relative to the Dandle® WRAP stretch group in the 15 minutes after the procedure. Interestingly, the baseline mean heart rate before the start of the procedure in the Dandle® WRAP stretch group (approximately 150 ± 17 bpm) was lower than in the controls (approximately 156 ± 14 bpm) before the start of the procedure, suggesting that the Dandle® WRAP stretch might be more comforting for babies. However, the average time (± SD) for the heart rate to return to normal

following the procedure was slightly longer for the Dandle® WRAP stretch group (1.32 minutes ± 3.0 SD) than the controls (1.0 minute ± 2.1 SD). Following adjustment for multiple comparisons using the Holm Bonferroni method, the observed differences in the maximum heart rate and average heart rate above baseline between the groups did not reach significance based on an $\alpha = 0.0036$. Of note, the minimum saturation post-procedure was lower in the control group compared with the Dandle® WRAP stretch group by approximately 5% ($\beta = -5.48$, unadjusted p = 0.20, 95% CI: -14.0, 3.01). Similar lower patterns in the average saturation, maximum respiratory rate and average respiratory rate above the baseline level were observed in the controls relative to the Dandle® WRAP stretch group as shown by the corresponding β coefficients (**Table** 4.2). These however were less critical at less than 1% and 1 breath per minute, respectively.

Figure 4.6 shows the boxplots of the various qualitative outcome variables in the 15 minutes post-procedure. Desaturation often follows or occurs at the same time as changes in respiratory rate, particularly a decrease in breathing. However, during a painful procedure like ROP screening, there may be an initial increase in breathing as part of a stressful pain response, followed by a decrease, and in severe cases like apnoea, this could result in significant desaturation. In **Figure** 4.6, a preterm at 36 weeks PMA (24 weeks GA) on low-flow oxygen had a maximum respiratory rate of 136 breaths per minute (mean baseline of 36 breaths per minute); this initial high respiratory rate was most likely a stress response. Their minimum saturation was 91%, a 3% drop from their mean baseline. A second neonate (GA 23 weeks, PMA 39 weeks) on high flow oxygen therapy had the lowest minimum desaturation of 46% (mean baseline 95%). However, their respiratory rate data was unrecorded due to artefact-

related signal loss and would likely have been accompanied by a simultaneous decline in respiratory rate as seen in some of the infants.

Table 4.2: Linear mixed model analysis comparing outcomes 15 mins post-ROP screening by group

Outcomes	Dandle® WRAP stretch	Controls	β (95% CI)	p-value
Presence of desaturation[§,a] (n=40)	5 (22.7%)	5 (27.8%)	1.39 (-1.85, 4.62)	0.39
Presence of bradycardia[§,b] (n=43)	1 (4.5%)	1 (4.8%)	0.27 (-3.04, 3.58)	0.87
Presence of tachycardia[§,b] (n=43)	3 (13.6%)	4 (19.0%)	3.83 (-1.93, 9.59)	0.19
Maximum HR (bpm)[*,b] (n=43)	188.27 ± 15	193.05 ± 15	8.05 (0.26, 15.8)	**0.043**
Average HR above baseline (bpm)[*,b] (n=43)	1.13 ± 4.67	1.99 ± 6.25	3.73 (0.09, 7.37)	**0.045**
Average HR below baseline (bpm)[*,b] (n=43)	0.52 ±2.05	0.38 ± 0.79	-0.01 (-1.78, 1.76)	0.99
Minimum oxygen saturation (%)[*,a] (n=40)	81.75 ± 19.1	79.10 ± 11.5	-5.48 (-14.0, 3.01)	0.20
Average oxygen saturation below baseline (%)[*,a] (n=40)	0.11± 0.58	0.41 ± 1.07	0.44 (-0.15, 1.03)	0.14
Maximum RR (bpm)[*,c] (n=38)	80.19 ±14.53	81.22 ± 22.10	-0.10 (-13.12,13.31)	0.99
Average RR above baseline (bpm)[*,c] (n=38)	0.11 ± 1.74	0.20 ± 3.5	-0.93 (-3.43, 1.56)	0.45

Analysis using [§]generalised linear mixed model and *linear mixed effects model and controlling for gender, ventilation mode, birthweight, weight at test occasion, gestational age, age at test occasion; [a] baseline saturation, [b] baseline heart rate and [c] baseline respiration. All β- coefficients are calculated with the Dandle® WRAP stretch group as a reference group. Positive β values indicate a higher event risk by a factor of the coefficient in the control group, and vice versa. All results are provided in median (± interquartile range) except maximum heart rate (mean ± standard deviation); and presence of desaturation, bradycardia and tachycardia which are reported as counts (within group percentages). Missing data was excluded from the analysis. Significance level when correcting for multiple comparisons set at α **= 0.0036** using the holm Bonferroni method. The bold p-values are the significant unadjusted values. *Abbreviations: heart rate (HR), beats per minute (bpm), percentage (%), beta coefficient (β).*

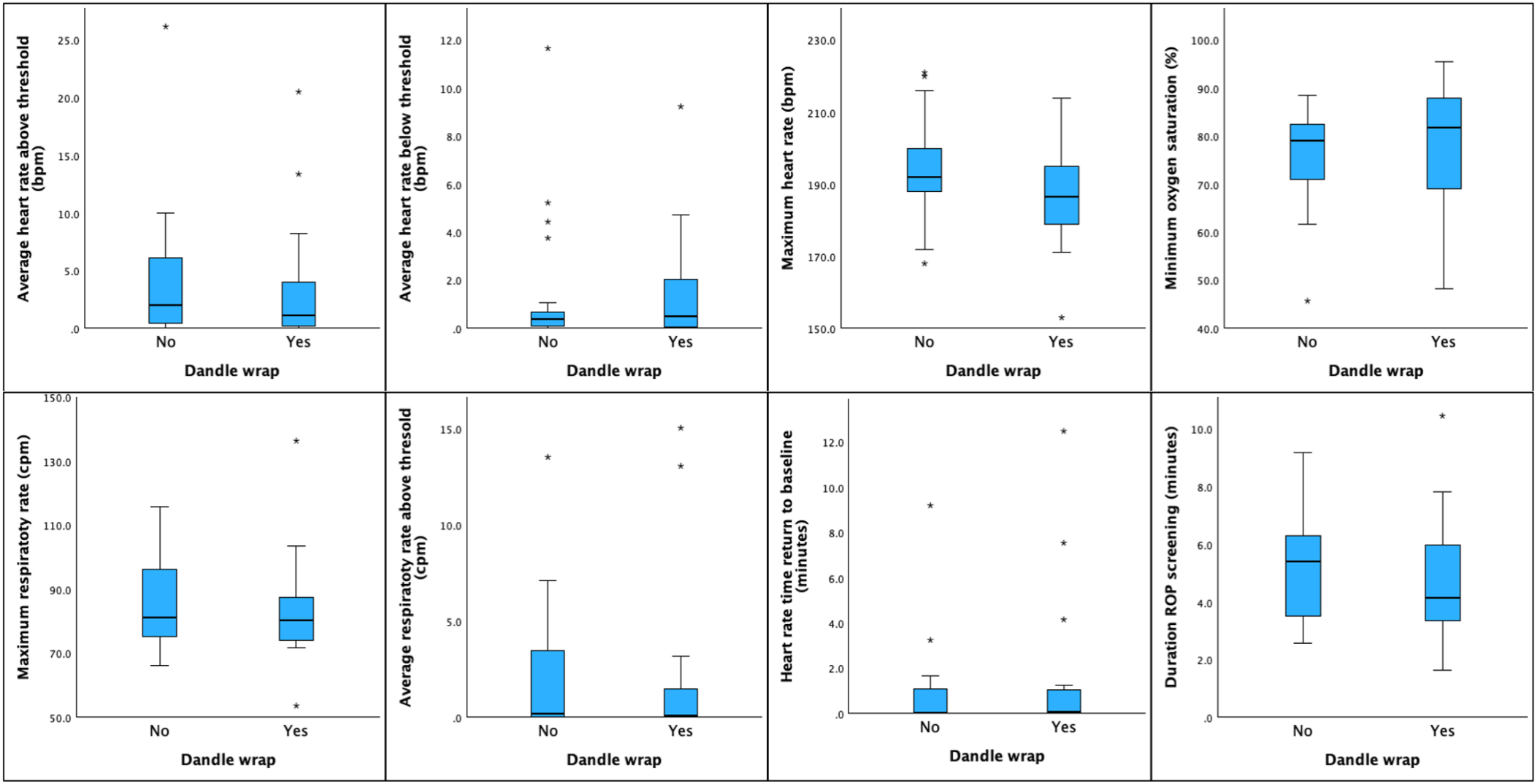

Figure 4.6: Simple boxplots comparing median outcomes by group. Comparison of physiological metrics between the two groups measured in the 15 minutes following the start of ROP screening. The horizontal lines in the middle of the boxplots represent the median, and the upper and lower box limits indicate the interquartile ranges. The error bars indicate the 95% confidence intervals. Asterix (*) in the plot shows extreme outliers. *Abbreviation – beats per minute (bpm), cycles per minute (cpm)*

4.4.2. Comparing physiology changes 12 hours after ROP screening in neonates with the Dandle® WRAP stretch versus controls

Of the 28 neonates studied, only 23 neonates (Dandle® WRAP = 10 babies, Controls = 13 babies) had good quality 12 hours of vital signs recorded post-ROP screening. These 23 neonates had multiple recordings on 31 different test occasions i.e., 14 with the Dandle® WRAP stretch and 17 without the wrap (**Figure** 4.3). **Table** 4.3 shows a summary of the physiological outcomes (desaturation, bradycardia, tachycardia, and apnoea) between the two groups.

Table 4.3: Comparative summary of outcomes 12 hours pre- and post-ROP screening by group

		Dandle® WRAP stretch (n = 14)		**Controls (n = 17)**	
Outcomes	**Number of events**	**Pre-ROP screening**	**Post-ROP screening**	**Pre-ROP screening**	**Post-ROP screening**
Desaturation	Yes	9	11	13	11
	No	5	3	4	6
	Mean ± SD	4.2 ± 3.60	5.7± 5.76	5.12 ± 7.17	4.29 ± 4.74
	Maximum	9	20	24	14
Bradycardia	Yes	7	8	8	7
	No	7	6	9	10
	Mean ± SD	1.43 ± 2.56	1.43 ± 1.70	1.47± 3.00	1.59 ± 2.85
	Maximum (bpm)	9	5	12	10
Tachycardia	Yes	4	4	9	9
	No	10	10	8	8
	Mean ± SD	1.36 ± 3.10	0.71 ± 1.49	1.24 ± 2.20	1.94 ± 2.75
	Maximum (bpm)	10	5	9	8
Apnoea	Yes	8	7	9	10
	No	6	7	8	7
	Mean ± SD	1.07 ± 2.06	1.29 ± 1.49	2.47 ± 3.08	2.94 ± 3.99
	Maximum	8	4	10	13

Pre-procedure, all physiological parameters were higher in the controls than in the Dandle® WRAP group. While these differences were only marginal for bradycardia and apnoea, they were notably higher for tachycardia and desaturation. The Dandle® WRAP group were swaddled at least two hours before the start of the procedure. Ergonomically, the wrap is designed to support a flexed, midline posture that mimics the womb, promoting more stable behavioural states like sleep and self-regulation. It therefore likely has a calming effect on the neonate, reducing autonomic instability as indicated by the lower frequency of tachycardia and desaturation in those swaddled with the Dandle® WRAP. Additionally, there were differences in both gestational and postmenstrual ages between the two groups, with the control group being younger, as shown by the imbalance in the covariates (**Table** 4.1) By virtue of their age, the controls are, therefore, more predisposed to physiological instability. These variables were adjusted for in subsequent analyses to assess the true impact of the two swaddling methods on physiological instability.

Using LMEM analysis, only the frequency of occurrence of tachycardia (β = 1.84, unadjusted p = 0.05, 95% CI: -0.04, 3.73) was significantly different between the Dandle® WRAP stretch group and controls **(Table** 4.4). However, after controlling for multiple comparisons using the Holm Bonferroni method at a critical p value of 0.0036, the difference failed to reach significance.

Table 4.4: Generalised linear mixed model analysis comparing outcome frequencies 12 hours post-ROP screening by group.

Outcomes	Dandle® WRAP (n = 14)	Controls (n = 17)	β (95% CI)	p-value
Tachycardia	0.71 ± 1.49	1.94 ±2.75	1.84 (-0.04, 3.73)	**0.05**
Bradycardia	1.43 ± 2.56	1.52 ± 2.85	-0.09 (-1.64, 1.47)	0.91
Desaturation	5.71 ± 5.76	4.29 ± 4.74	-1.49 (-5.50, 2.52)	0.45
Apnoea	1.29 ± 1.49	2.94 ± 3.99	0.65 (-1.46, 2.76)	0.53

Results are provided in mean and standard deviation. Models were adjusted for sex, ventilation mode, birthweight, weight at test occasion, age at test occasion, gestational age, and the number of the corresponding events pre-procedure. All β - coefficients are calculated with the Dandle® WRAP stretch group as a reference group. Positive β - coefficients indicate higher event risk by a factor of the coefficient in the control group, and vice versa. The bold p-value is a significant unadjusted value and significance level when correcting for multiple comparisons is set at α **= 0.0036** using the Holm Bonferroni method.

Study 2:

A total of 173 participants were invited via email (response rate: 13.9%, comprising 5 (20.8%) ophthalmologists and 19 (79.2%) nurses - **Figure** 4.7). All but three nurses had used the Dandle® WRAP stretch during ROP screening. Of these three, one had only used it as a comfort measure during another procedure, while the other two had no prior experience with it. As a result, the two with no experience were excluded from the analysis, leaving 22 responses for evaluation. The majority of the respondents (21 of 22; 95.5%) were taught how to put on the Dandle® WRAP stretch.

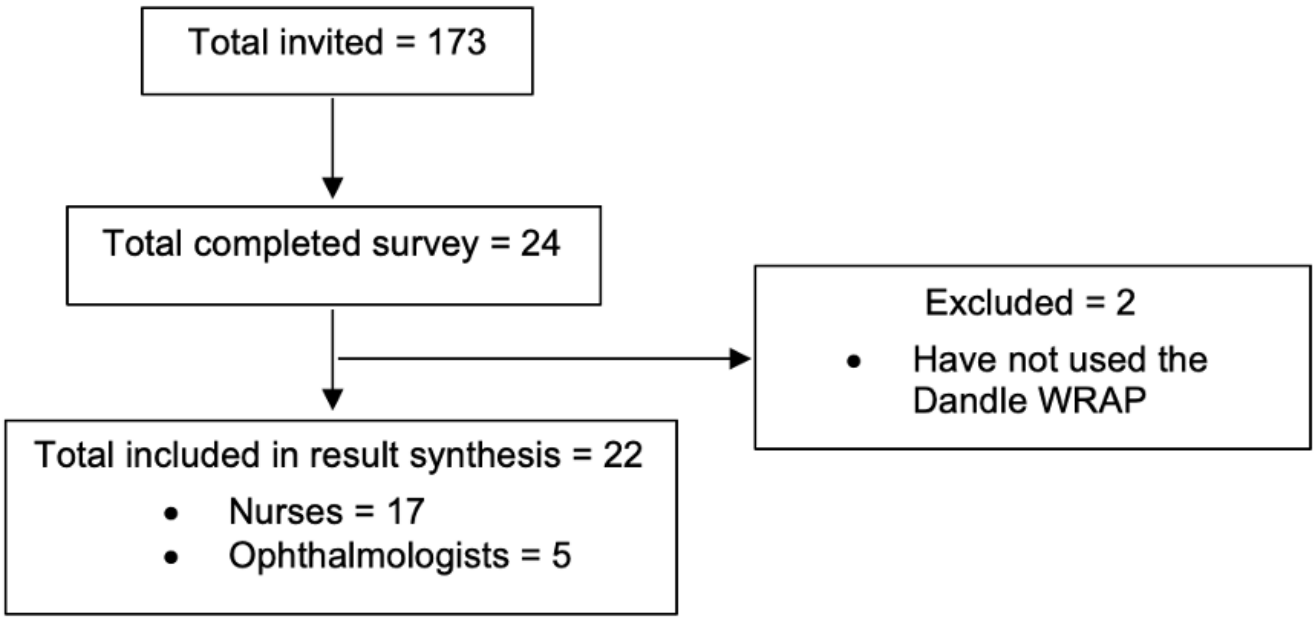

Figure 4.7: Flowchart of survey respondents

4.4.3. Staff perceptions on neonate's comfort level and ease of using the Dandle® WRAP stretch compared with conventional swaddle

Over half (13 of 22, 59.1%) of the respondents believed the Dandle® WRAP stretch was easy to use, were confident explaining to families about the wrap and felt babies were very comfortable in it. None of the respondents felt the Dandle® WRAP stretch was difficult to use in any way, were unconfident or believed babies were uncomfortable when swaddled with the Dandle® WRAP stretch (**Figure** 4.8).

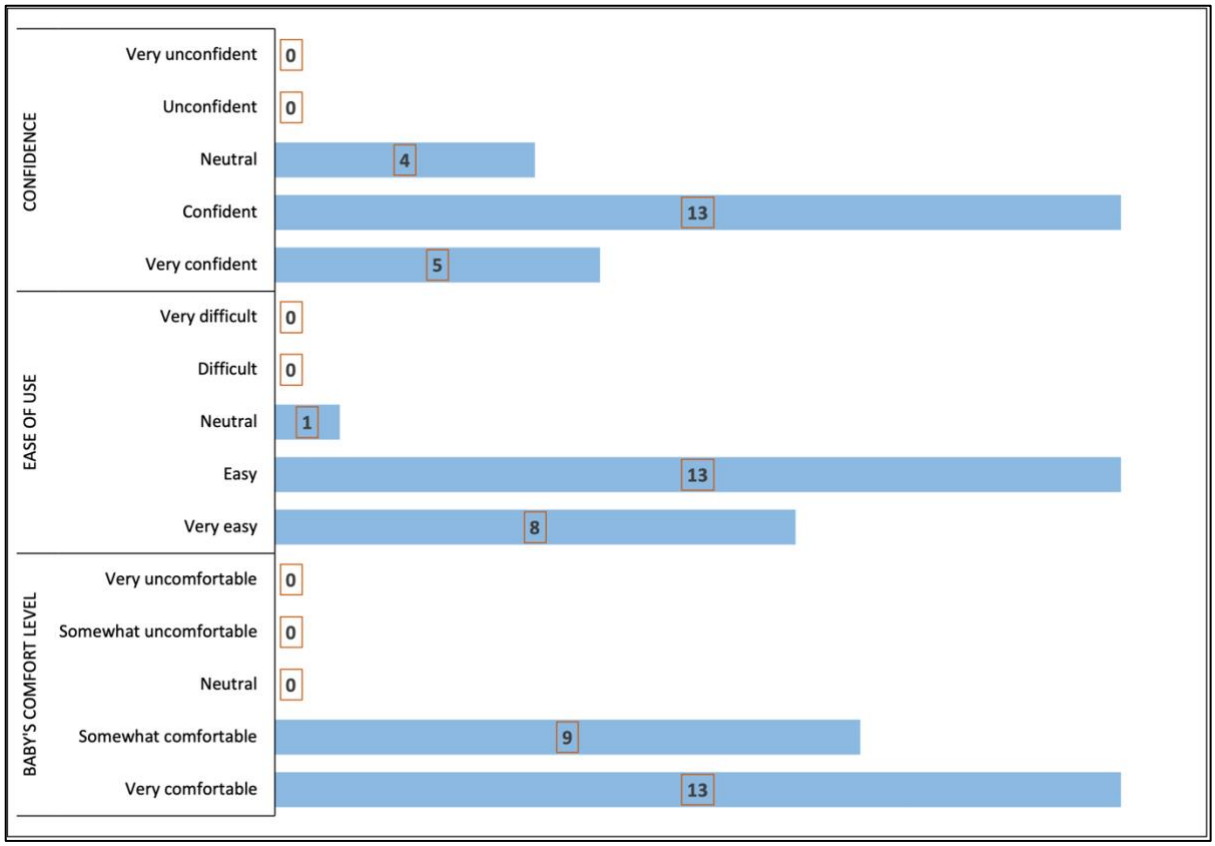

Figure 4.8: Staff opinion about baby's comfort level, ease and confidence using the Dandle® WRAP stretch. Frequencies are displayed in the red boxes.

4.4.4. Staff preferences regarding swaddling methods

When respondents were asked about their preference between the Dandle® WRAP stretch and conventional swaddling with muslin for ROP screening, almost all (21 of 22, 95.4%) preferred the Dandle® WRAP stretch. **Figure** 4.9 shows a word cloud of why respondents prefer to use the Dandle® WRAP stretch over conventional swaddle during ROP screening. Only one (4.6%) respondent preferred to use conventional swaddle and the reason given was *"Both are easy. Muslin is larger for covering baby when compared to dandle".*

Figure 4.9: Word cloud of reasons staff prefer using the Dandle® WRAP stretch over conventional swaddle during ROP screening. The font size represents the frequency of responses (the larger the font, the more often the phrase was used by respondents and vice versa).

The majority (15 of 22, 68.2%) of respondents, of which 11 of 17 (64.7%) were nurses and 4 of 5 (80%) were doctors, said they would use the Dandle® WRAP stretch regularly. By contrast, less than a tenth (2 of 22, 9.1%) of the respondents said they would not use the Dandle® WRAP stretch routinely for care if each baby had their own, with one saying - "*it would depend on the neonate's condition"* while the other felt *"it would affect their neural development"*. Just under a quarter (5 of 22, 22.7%) of the respondents (4 nurses and 1 ophthalmologist) said they would only 'sometimes' use the Dandle® WRAP stretch and the reasons given were:

"Some babies don't seem to settle with wrap and some bigger babies get quite hot".
"I think babies need to move freely in a cot".
"Depends on the babies' condition".
"It doesn't prove useful".
"I don't often attend the ROP round anymore".

4.4.5. Perceived benefits and drawbacks of Dandle® WRAP stretch

Most (21 of 22, 95.5%) of the respondents believe there were no disadvantages to using the Dandle® WRAP stretch during ROP screening. Just one (4.5%) participant said the downside was "*if babies are not already in the wrap/poorly swaddled then it adds time to examination; also, not confident on how to adjust the wrap".* This respondent was an ophthalmologist, had never been trained to put on the Dandle® WRAP stretch and was not involved with patient care pre- or post-procedure. Interestingly, this same respondent believed that *"babies were somewhat comfortable with more secure wrap of arms for UWF imaging and easy".*

Whilst 14 of 22 (63.6%) provided no comments about the Dandle® WRAP stretch, of those that offered feedback (8 of 22, 36.4%), all were positive responses saying:

"The material is really good, and it's helpful most of the time".
"Very effective!"
"Love them!"
"I have only used it a couple of times but did think it worked very well".

and suggested recommendations and comments like:

"We need more supply".
"It's a bit tricky on babies with a cannula on the foot".
"I find them easy to use, but occasionally, struggle to find one to fit a bigger baby".
"I like to use it. But it's less in number".

4.5. Discussion

In this chapter, I assessed the impact of the specialised Dandle® WRAP stretch on physiological responses in neonates undergoing ROP screening, as well as staff preferences and perceptions regarding its usefulness during the procedure. Whilst the Dandle® WRAP stretch did not significantly alter the occurrence of physiological events compared with conventional swaddle in the immediate and longer time windows after adjusting for multiple comparisons, there was a noticeable trend towards lesser episodes of tachycardia in the 15 minutes (maximum heart rate - unadjusted p = 0.043 and average heart rate above baseline - unadjusted p = 0.045) and 12 hours (frequency of tachycardia - unadjusted p = 0.05) following ROP screening. Importantly, the Dandle® WRAP stretch did not appear to cause more adverse vital signs changes post-procedure. From the survey, almost all the staff preferred using the Dandle® WRAP over conventional swaddling with muslin during ROP screening, felt it was simple to use, and babies were very comfortable in it.

Study 1

ROP screening is an invasive painful procedure, it is uncomfortable and has been associated with changes in vital signs including hypoxaemia, apnoea, and heart rate variability [90–93]. In one report [35], physiological instability occurred as far as 48 hours following the procedure. Adverse physiological changes are known to affect long-term neonatal brain development [12–14]. As with any clinically-required painful procedure [257] including ROP screening, the goal is to minimize pain and make the experience as pleasant as possible for the neonate. During ROP screening and other painful procedures, neonates are unable to communicate their discomfort, making effective

pain management both complex and challenging [258]. While a combination of pharmacological and non-pharmacological pain management approaches in neonates have been described [258,259], cardiorespiratory side effects could result from medication side effects. This limits the routine use of systemic pain relief medications for procedural pain and, thus, neonates may still experience discomfort during procedures. Non-pharmacological measures are increasingly being used and often recommended for reducing pain responses in neonates [260]. Swaddling has been shown to improve pain responses and oxygen saturation after a heel lance [261] and when used alongside other measures, it also proved useful during ROP screening [42]. Although no statistically significant differences were found between the Dandle® WRAP stretch and control groups during ROP screening, the observed trend of lower heart rates both immediately and in the longer period after the procedure suggests that the Dandle® WRAP stretch may help maintain better heart rate responses and serve as a comfort measure during ROP screening. Furthermore, the Dandle® WRAP stretch did not result in worse outcomes compared to the conventional swaddle.

In the 15 minutes post-procedure, there was a propensity for lower levels of heart rate increases in neonates swaddled with the Dandle® WRAP stretch. Although neonates in both groups developed tachycardia, the control group had a tendency for higher rates, with the maximum and average heart rates above baseline approximately 8 beats per minute and 4 beats per minute higher than the Dandle® WRAP stretch group. This may suggest that neonates swaddled with the Dandle® WRAP stretch had relatively better cardiovascular stability in the immediate post-procedure period. Moreover, the baseline mean heart rate and respiratory rates before the start of ROP screening were lower by approximately 6 beats per minute and 5 breaths per minute,

respectively, in the Dandle® WRAP stretch group compared with controls (**Table** 4.1). While the babies swaddled with the Dandle® WRAP had a relatively higher PMA (36.2 ± 2.9 weeks) compared to the controls (34.3 ± 3.1 weeks) and were therefore likely to be more physiologically resilient, PMA at the time of the procedure was adjusted for in the model comparing the effects of the two swaddling techniques. This adjustment controlled for the natural influence of maturation on cardiovascular function, thereby supporting the inference that the more stable cardiac responses observed in the intervention group were attributable to the swaddling method rather than age differences. Because neonates were swaddled with the Dandle® WRAP stretch 2 hours prior to the procedure, this may imply that the Dandle® WRAP stretch, in general, has a calming and settling effect on the neonate, hence, accounting for the lower mean baseline values. Mean oxygen saturation levels were comparable in both groups in the baseline period. Both groups, i.e., neonates swaddled with the Dandle® WRAP stretch and the controls, experienced a drop in oxygen saturation level immediately following the start of procedure and this was greater in the controls with a minimum saturation of 79% as opposed to 82% in neonates swaddled with the Dandle® WRAP stretch. The minimum oxygen saturation after correcting for possible confounders was 5% lower in the control group (**Table** 4.2 and **Figure** 4.5).

In the longer time (12 hours) window, before the procedure (**Table** 4.3), it is noteworthy that neonates swaddled with the Dandle® WRAP stretch had fewer adverse physiological events than the controls, further indicating that babies were more comfortable and settled in the Dandle® WRAP stretch prior to the procedure. Post-ROP screening, while the frequency of desaturation was comparable between the groups, the Dandle® WRAP stretch group experienced slightly more bradycardias and

fewer tachycardias and apnoeas (**Table** 4.3). These findings, however, were not significant (**Table** 4.4). It is essential to note that before the start of the procedure, the lower body swaddling of the neonates in the Dandle® WRAP stretch is removed to allow for optimal positioning in the flying baby position, while upper body containment is left in place. The additional handling for adequate positioning in the Dandle® WRAP stretch group, which was not done for the control group, may account for the marginal observed differences in the frequency of post-procedure events.

A possible explanation for the lack of statistical significance may be because this was an exploratory study and likely underpowered. The interventions being compared in the two groups i.e., conventional versus Dandle® WRAP stretch share some similarities, with very subtle differences in fabric design and the effectiveness of containment. For example, the Dandle® WRAP material is stretchy which allows for easy movement and recoil of limbs back to the neonate's preferred comfort position while its Velcro fastening ensures a more secure fit, maintaining the upper body swaddle throughout the procedure. By contrast, the conventional swaddle fabric is cotton and can easily come undone as it has no secure Velcro, thus, leaving the baby without proper swaddling during the ROP screening. Both interventions might have comparable mechanisms of effect, as such, impact differences might be too small to detect with the number of neonates studied. Although the outcomes of interests were not significantly different between the studied groups, on average, the duration of ROP screening for the control group was longer than the Dandle® WRAP stretch group by 36 seconds. Maintaining upper body swaddling in the Dandle® WRAP stretch group may have resulted in less neonate movement and interruptions for optimal positioning during the procedure, hence, reducing the procedure time.

Generalized inference about the effect of the Dandle® WRAP stretch on physiologic stability during ROP screening might be limited due to the relatively small sample size and frequency of outcomes observed. Based on the findings from this study (mean and SD of episodes of tachycardia 12 hours post-ROP screening in both groups), post-hoc power calculations with G*power 3.1, using an effect size of 0.56, alpha of 0.05 and power of 0.80 determined a total sample size of 104 babies for future studies to detect a true effect. A randomised controlled trial design would have been the ideal study design to determine these effects, but randomisation was not possible, as the study was time sensitive, and based on pre- and post-guideline implementation. At study inception, all eligible neonates before the guideline change to use the Dandle® WRAP stretch for ROP screening were recruited. For comparability, a similar number of neonates to match the control group were studied post-guideline implementation. Although the standardised mean differences in infant age and weight at birth at the time of the ROP screening between the two study groups were unbalanced (**Table** 4.1), these were controlled for in the analysis, improving the generalisation of results. Whilst this study was not designed to look at the effect of ROP screening on physiological responses, our findings of vital signs changes in both study groups undergoing ROP screening are consistent with previous reports that the procedure causes physiological instability. To the best of my knowledge, no study looked at the usefulness and comparative efficacy of the Dandle® WRAP stretch as a comfort measure during ROP screening.

Study 2

The critical role staff play in looking after neonates in the NICU cannot be overemphasized. Nurses are the focal clinical care providers at the NICU, working in

close contact with the neonates daily. They provide continuous care, advocate for best practices, and have first-hand knowledge and experience about measures that potentially improve patient care and comfort levels. For this reason, the opinions of staff directly involved with patient care and management are invaluable in guiding clinical decisions. Because the Dandle® WRAP stretch was a new swaddling device introduced for use at the NICU for babies as a comfort measure during procedures including ROP screening, it is essential to gather unbiased expert opinions based on daily practice regarding its effectiveness to inform care decisions.

While the Dandle® WRAP stretch and conventional swaddling with muslin resulted in similar physiological responses during ROP screening (study 1), staff feedback indicates that the Dandle® WRAP stretch is more efficient and practical for use during the ROP screening. Staff found it easy to use, were confident putting it on and talking to families about it, and importantly, reported babies were more comfortable wrapped in it compared with the conventional swaddle. It is likely that the nature of the fabric, with its added Velcro for a secure hold during handling or when the neonate moves, makes the uninterrupted snug containment comfortable for babies, and preferable to staff over conventional swaddling with muslin. Respondents' feedback like "*The material is really good, and it's helpful most of the time", "Very effective!", "Love them*!" indicates the Dandle® WRAP stretch is acceptable by nursing standards. Only one nurse preferred conventional swaddling to the Dandle® WRAP stretch as they felt the "*muslin is larger*". The Dandle® WRAP stretch does come in different sizes based on neonatal weight, ranging from extra small to double extra-large. During the study period, finding the appropriately sized wrap for all babies was not always possible due to limited numbers, especially during times when the wraps were sent to the laundry.

This issue of low supply was confirmed through some respondents' feedback saying, "*We need more supply*", "*I find them easy to use, but occasionally, struggle to find one to fit a bigger baby*" and "*I like to use it. But it's less in number*". Increasing the Dandle® WRAP stretch supply and in various sizes too will go a long way in addressing shortages.

Only one respondent, an ophthalmologist, thought that the downside of using the Dandle® WRAP stretch was adding to the procedure time if babies were not already swaddled in it. Of note, from study 1, the duration of ROP screening (starting from when the neonate is first touched to the end of the procedure when the neonate is placed back in the cot and lower body swaddle put back in place for the Dandle® WRAP stretch group) was shorter in the neonates swaddled with the Dandle® WRAP stretch compared with controls. This may reasonably be explained by the fact that upper body swaddle is maintained with the Dandle® WRAP stretch while in the flying baby position, keeping the neonates' arms out of the examination field and making the procedure easier and faster. As babies are more comfortable in the Dandle® WRAP stretch, as reported by the majority of the survey respondents, it is conceivable that they are more settled during the procedure, again explaining the shorter procedure times for the Dandle® WRAP stretch group.

A potential limitation of this survey was the response rate of 13.9%. Typically, online response rates are around 44% [262]. Possible reasons for the limited responses in this survey were attributed to changes in staff shift patterns and unit rotations, and new staff who may not have used the Dandle® WRAP stretch before and were on the mailing list. Sending survey requests to more participants does not change

respondents' response rate - higher responses were better observed with a smaller cohort [262] - and perhaps for this survey, targeting staff involved only during ROP screening rather than using a staff emailing list would have generated higher response rates. A strength of this survey was the questionnaire was developed in collaboration with expert specialists and was pretested. This ensured standardised and relevant questions were formulated improving the reliability and consistency of the responses

5

Thesis Summary and Recommendations

5.1. Summary of the main results

This thesis details the association between breathing and brain development in hospitalised neonates using EEG-recorded brain activity. To examine this relationship, inter-related studies exploring four study designs were conducted, namely: a systematic review, a cross-sectional and cohort design, and a staff survey. In summary:

1. From the systematic review in **Chapter** 2, only a few studies evaluated the immediate impact of neonatal respiratory changes on EEG activity. No studies were identified that assessed how normal respiration affects brain activity across different ages; most of the identified literature focused on apnoea-related EEG changes. These findings, though inconsistent, were suggestive of reduced brain activity during apnoeic episodes. There was a paucity of studies assessing the effect of apnoea characteristics such as frequency and duration on immediate brain function. Finally, the review shows that respiratory stimulants used for the management of apnoea enhanced brain activity, with caffeine having a greater effect than aminophylline.

2. From **Chapter** 3, the potential usefulness of brain maturity (a standardised EEG-derived measure of functional brain development; Brain maturity = Brain age - PMA) in determining neonatal apnoea risk better than PMA is promising. This Chapter also provides initial evidence to infer that the age at which caffeine is stopped is negatively correlated with brain maturity, and that physiological instability after caffeine therapy discontinuation might be related to brain maturity. These findings could be valuable in guiding the need for caffeine therapy decisions in preterm neonates. Using brain maturity as a marker of respiratory function could provide an objective unified metric to identify neonates at greatest risk of apnoea and consequently, those who will benefit the most from caffeine treatment. PMA and brain age (an EEG-derived measure of biological brain age) are better related to normal respiration instead of apnoea in neonates.

3. As a comfort measure during a painful procedure (ROP screening with ultrawide field imaging-based method), the Dandle® WRAP stretch swaddle when compared with the conventional swaddle did not significantly alter the physiological responses during the procedure both in the short- and longer periods (**Chapter** 4). Importantly, the Dandle® WRAP stretch did not cause more adverse physiological changes during the procedure, and in fact, neonates had lower baseline vital signs before the procedure and a trend towards better heart rate responses in the immediate (15 minutes) period following ROP screening and 12 hours later. The Dandle® WRAP stretch facilitated handling and the speed of imaging neonates. The design of the Dandle® WRAP stretch also makes it convenient, acceptable, and ideal to use regularly by staff for babies undergoing ROP screening.

While I did not formally collect data on parents' views regarding the studies in **Chapters** 3 and 4 of my thesis, nearly all the families I approached were positive and keen to participate. Many families were proud to help advance scientific knowledge that could improve the care of premature and sick babies in the future. They saw participation as a way of "giving back", providing a sense of purpose during an emotionally difficult time in the NICU. The fact that both the EEG and the Dandle® WRAP were non-invasive and involved observational monitoring was a major reason for acceptance. Some families consented to being contacted in the future for any potential research and were looking forward to reading the outcome of the projects when published.

The few families that declined to take part were often rooted in emotional or practical challenges of having their baby handled for research purposes. I've had parents say their babies (mostly the very premature) "had been handled quite a lot" during clinically required procedures and did not want to take part in the research. A mother in the postnatal ward said the EEG wires were a trigger for her, bringing back difficult memories. Some families had concerns about the time it took to record the EEG and didn't want it to interfere with family time.

Finally, some parents who had signed up withdrew consent before the study as they were discharged the day the study was scheduled and did not want to be delayed going home. Other reasons for withdrawal were parental anxiety - the evening before the study, one baby developed a fever, and another had hypoglycaemia. One baby was unsettled during the EEG recording. A joint decision was made by the research team and the family to withdraw the babies.

5.2. Study limitations

From the systematic review (**Chapter** 2), the eligible literature included in the synthesis were few, with relatively small sample sizes. The EEG measures assessed were heterogeneous, and the respiratory variables had inconsistent definitions, for example, what constitutes apnoea of clinical significance, apnoea characteristics such as duration and the implication of accompanying vital signs changes on brain function. This, thus, made study comparison and generalisation of relevant results difficult. Additionally, no respiratory stimulant studies investigated simultaneous interactions between EEG and vital signs changes (i.e., heart rate, oxygen saturation or respiration) and how the medications alter EEG during apnoea episodes. They only investigated EEG changes in relation to the start of drug therapy, and in most instances, the medications were given prophylactically. None of the studies linked short-term EEG changes with later neurodevelopmental outcomes.

Chapters 3 and 4 were single-centre exploratory studies with limited external validity; however, they form the basis for future research, including informing power calculations for subsequent studies. Participant recruitment was based on a non-probability feasibility sampling. While it is known that probability sampling methods improve sample representativeness, convenience sampling is also a scientifically acceptable approach depending on the specific characteristics of the study design. For example, in the ROP screening study comparing two swaddling techniques, the time before the guideline change to use the Dandle® WRAP was short, and to allow for a valid comparison, there was a need to match participant numbers before and after the guideline change. A randomised controlled trial design would have been the ideal study design; nevertheless, for practical purposes, considering the timelines, this

was not feasible. As such, a convenience sampling, while not as rigorous as probability-based methods, was appropriate to optimise the recruitment target. For the sub-aim analysis in Chapter three, looking at the relationship between PMA and brain maturity as a guide for caffeine treatment in preterm infants, the sample size was small, based on only a subset of neonates (27) that had simultaneous EEG recording before caffeine discontinuation and subsequent longitudinal vital signs monitoring after the cessation of therapy. Participants with the observed outcomes of interest, i.e., the number of observed apnoeas and desaturations, were also few. These results should therefore be replicated in a prospective study before definitive conclusions can be drawn. Nevertheless, the work provides promising initial evidence that physiological instability after discontinuation of caffeine might be related to brain maturity.

Of the total EEGs recorded (n = 205), the majority (n = 183, 89.3%) had optimal impedance of no more than 5 – 10 kΩ. Only 19 (9.2%) had impedance values >10 kΩ, while 3 (1.5%) had no information recorded. While the relatively high impedance from a few of the data could potentially increase the likelihood of a high signal-to-noise ratio in those recordings, the affected electrodes were not limited to the central electrodes analysed, i.e., Cz, C3, C4, Oz. Additionally, the sensory and resting-state models used for brain age calculations are designed to detect and exclude EEG epochs with significant artefacts and are therefore unlikely to bias the results.

5.3. Future directions

A consensus for a clear definition of apnoea and consistent EEG methods needs to be adopted for a valid comparison of EEG-related neonatal respiratory changes. Using

unified metrics such as the validated EEG brain-based measures that integrate multiple EEG features, could provide a standardised assessment of overall brain function and may be a valuable direction for future research. In this thesis, an apnoea definition of >15 seconds was chosen to allow for the inclusion of shorter apnoeas of potential clinical relevance - studies have shown that respiratory pauses < 20 seconds can cause bradycardia, desaturation and suppression of EEG brain activity [196,197]. This could also be a valuable approach for future research. Also, the studies included in the systematic review assessing the effect of respiratory stimulants for apnoea management on brain activity mainly compared short periods before, during and after the start of therapy which were usually given prophylactically, unrelated to apnoea episodes. Further investigation into the effect of caffeine on brain function and physiological changes during apnoea episodes as well as longitudinally assessing the long-term effect of drug therapy on both cardio-respiratory function and neurodevelopment is essential for understanding how respiratory stimulants affect brain function. This research will contribute to the development of individualised drug dosing strategies in neonates.

While changes in vital signs such as oxygen saturation and blood pressure can alter the interpretation of the EEG recording [263], It remains unclear whether immature respiratory function in the neonate drives brain activity or if it is the underdeveloped brain at birth that makes babies more susceptible to frequent respiratory disruptions. Studies looking at simultaneous EEG and vital signs changes to determine brain-respiratory interactions could provide useful insights into understanding early respiratory-brain networks and the cyclical relationship between the brain and lungs - whether brain signals lead to respiration or vice versa. There is also a need for more

studies to look at the relationship between apnoea characteristics (duration, changes in heart rate and oxygen desaturation) as well as other factors such as sleep-staging, medications, and co-morbidities, and how these relate to brain function. From the scatter plots exploring various IBI thresholds in Chapter 3, the frequency of longer IBIs i.e., particularly those proportions greater than 10 seconds (**Figure** 3.11) were lower compared to shorter IBIs (**Figure** 3.10). While no statistical tests were applied, the fewer data points for longer IBI proportions may suggest that potential IBI cut points could be shifting towards abnormal thresholds, pointing towards pathology. Further studies are needed to specifically explore the distribution of IBIs in relation to PMA and brain maturity, as well as to define critical thresholds. Understanding these associations will enhance our comprehension of respiratory physiology in general, leading to a better understanding of the clinical impact of apnoea on brain function, and whether apnoea risk can be predicted based on baseline physiological changes.

The findings from this thesis that brain maturity determines apnoea rate better than PMA i.e., the occurrence of fewer apnoeas in neonates with higher brain maturity calls for larger multicentre prospective studies to be conducted and possibly, the need to routinely assess brain maturity in neonates at risk of apnoea to aid clinical decisions. The questions as to whether caffeine dose requirement can be predicted based on neonatal characteristics, or the need for adjusted caffeine dosing to justify therapy and identify neonates that will benefit most from individualised care are important areas for future work. Brain maturity measures could also be used to develop ‘brain growth charts’ as treatment guidelines and monitor neonatal outcomes. It would also be interesting to explore the immediate influence of common early life neonatal conditions which could impact brain development, such as severe jaundice, sepsis, hypoxic-

ischaemic encephalopathy, congenital heart disease etc, determine longitudinally how these conditions change the trajectory of brain maturity and whether brain maturity could be used to predict later neurodevelopmental outcomes in neonates with these pathologies.

Finally, a randomized controlled trial with a larger sample size is needed to accurately assess the true effect of the Dandle® WRAP stretch swaddle compared to the conventional swaddle on physiological changes in neonates. For future studies, post-hoc sample size estimation based on the findings from **Chapter** 4 of this thesis suggests that 52 babies in each group (Dandle® WRAP stretch and conventional swaddle) would be required to reliably detect an effect on outcomes and provide evidence to guide practice.

5.4. Conclusions

This thesis presents the first systematic summary of previous literature describing the impact of neonatal respiration on brain activity using EEG - the effect of apnoea on immediate brain function is inconsistent and respiratory stimulants enhance brain activity. The two exploratory studies document novel observations. EEG-derived brain maturity provides a more accurate explanation of apnoea in neonates than PMA. The Dandle® WRAP stretch compared with conventional swaddle did not cause adverse changes in vital signs post-ROP screening and staff had positive feedback on its usefulness during the procedure, making it practical as a comfort measure for preterm neonates during ROP screening. Adopting a personalised approach to care, tailoring

interventions according to individual developmental and physiological requirements will enhance the overall quality of neonatal care.

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Appendices

7.1. Systematic review database search strategies

Step-by-step search strategy for Embase via Ovid

1 exp electroencephalography/ or (EEG or electroencephalogra* or EEG pattern or EEG maturation or EEG brain activity or brain activity or brain maturation or brain development or brain dysmaturity or brain wave* or background EEG or stimul* evok* EEG or interburst interval* or inter burst interval*).mp.

2 exp Neonate, Newborn/ or (neonat* or newbor* or new-bor* or term or full term or full-term or fullterm or premat* or baby or babies or neonate*).mp.

3 exp Respiration/ or (breath* or respirat* or apno* or apne* or breath* cess* or stop breath* or breath* hold* or period* breath* or inter breath interval* or interbreath interval*).mp.

4 1 and 2 and 3

5 limit 4 to english language

6 limit 5 to human

7 limit 6 to neonate <to one year>

Step-by-step search strategy for MEDLINE via Ovid

1 exp Electroencephalography/ or (EEG or electroencephalogra* or brain activity or brain maturation or brain development or brain dysmaturity or brain wave* or interburst interval* or inter burst interval*).mp.

2 exp Neonate, Newborn/ or (neonat* or newbor* or new-bor* or term or full term or full-term or fullterm or premat* or baby or babies or neonate*).mp.

3 exp Respiration/ or (breath* or respirat* or apno* or apne* or inter breath interval* or interbreath interval*).mp.

4 1 and 2 and 3

5 limit 4 to (english language and humans)

6 limit 5 to "newborn neonate (birth to 1 month)"

Step-by-step search strategy for PsycINFO via Ovid

1 exp electroencephalography/ or (EEG or electroencephalogra* or EEG pattern or EEG maturation or EEG brain activity or brain activity or brain maturation or brain development or brain dysmaturity or brain wave* or background EEG or stimul* evok* EEG or interburst interval* or inter burst interval*).mp.

2 exp Neonate, Newborn/ or (neonat* or newbor* or new-bor* or term or full term or full-term or fullterm or premat* or baby or babies or neonate*).mp.

3 exp Respiration/ or (breath* or respirat* or apno* or apne* or breath* cess* or stop breath* or breath* hold* or period* breath* or inter breath interval* or interbreath interval*).mp.

4 1 and 2 and 3

5 limit 4 to english language

6 limit 5 to human

7 limit 6 to 120 neonatal <birth to age 1 mo>

Step-by-step search strategy for Global health via Ovid

1 exp electroencephalography/ or (EEG or electroencephalogra* or EEG pattern or EEG maturation or EEG brain activity or brain activity or brain maturation or brain development or brain dysmaturity or brain wave* or background EEG or stimul* evok* EEG or interburst interval* or inter burst interval*).mp.

2 exp Neonate, Newborn/ or (neonat* or newbor* or new-bor* or term or full term or full-term or fullterm or premat* or baby or babies or neonate*).mp.

3 exp Respiration/ or (breath* or respirat* or apno* or apne* or breath* cess* or stop breath* or breath* hold* or period* breath* or inter breath interval* or interbreath interval*).mp.

4 1 and 2 and 3

5 limit 4 to english language

Step-by-step search strategy for CINAHL via EBSCOhost

S1 (MH "Electroencephalography") or EEG OR electroencephalogra* OR brain activity OR brain maturation OR brain development OR brain dysmaturity OR brain wave* OR interburst interval* OR inter burst interval*

S2 (MH "Neonate, Newborn +") or neonat* OR newbor* OR new-bor* OR term OR full term OR full-term OR fullterm OR premat* OR baby OR babies OR neonate*
S3 (MH "Respiration+") or breath* OR respirat* OR apno* OR apne* OR inter breath interval* OR interbreath interval*
S4 S1 and S2 and S3
S5 limit S4 to english
S6 limit S5 to neonate, newborn: birth-1 month

Step-by-step search strategy for Web of Science

1 TS=(EEG OR electroencephalogra* OR brain activity OR brain maturation OR brain development OR brain dysmaturity OR brain wave* OR interburst interval* OR inter burst interval*)
2 TS=(neonat* OR newbor* OR new-bor* OR term OR full term OR full-term OR fullterm OR premat* OR baby OR babies OR neonate*)
3 TS=(breath* OR respirat* OR apno* OR apne* OR inter breath interval* OR interbreath interval*)
4 1 and 2 and 3 and English

Step-by-step search strategy for ProQuest

((EEG OR electroencephalogra* OR 'brain activity' OR 'brain maturation' OR 'brain development' OR 'brain dysmaturity' OR 'brain wave*' OR 'interburst interval*' OR 'inter burst interval*') AND (neonat* OR newbor* OR new-bor* OR term OR 'full term' OR full-term OR fullterm OR premat* OR baby OR babies OR neonate*) AND (breath* OR respirat* OR apno* OR apne* OR 'inter breath interval*' OR 'interbreath interval*'))

Step-by-step search strategy for Cochrane Library

1 MeSH descriptor: [Electroencephalography] explode all trees
2 (EEG OR electroencephalogra* OR brain activity OR brain maturation OR brain development OR brain dysmaturity OR brain wave* OR interburst interval* OR inter burst interval*)
3 MeSH descriptor: [Neonate, Newborn] explode all trees
4 (neonat* OR newbor* OR new-bor* OR term OR full term OR full-term OR fullterm OR premat* OR baby OR babies OR neonate*)

5 MeSH descriptor: [Respiration] explode all trees

6 (breath* OR respirat* OR apno* OR apne* OR inter breath interval* OR interbreath interval*)

7 1 OR 2

8 3 OR 4

9 5 OR 6

10 7 AND 8 AND 9

7.2. Joanna Briggs Institute critical appraisal checklists

Table 7.1: Checklist of the included case report studies

Variables assessed	**Low et. al (2012)**
Were patient's demographic characteristics clearly described?	Yes (excluding race)
Was the patient's history clearly described and presented as a timeline?	Yes
Was the current clinical condition of the patient on presentation clearly described?	Yes
Were diagnostic tests or assessment methods and the results clearly described?	Yes
Was the intervention(s) or treatment procedure(s) clearly described?	Not applicable
Was the post-intervention clinical condition clearly described?	Not applicable
Were adverse events (harms) or unanticipated events identified and described?	Not applicable
Does the case report provide takeaway lessons?	Yes

Table 7.2: Checklist of the included cohort studies

Variables assessed	Hassanein et al. (2015)	Dix et al. (2018)	Supcun et al. (2010)	Wulbrand et al. (1994)
Were the two groups similar and recruited from the same population?	Yes: Within subject comparison.	Yes: Within subject comparison.	Yes: Within subject comparison.	Yes: Within subject comparison.
Were the exposures measured similarly to assign people to both exposed and unexposed groups?	Not Applicable.	Not Applicable.	Not Applicable.	Not Applicable.
Was the exposure measured in a valid and reliable way?	Unclear: Apnoea measured clinically but assessment method and definition not specified. Caffeine given according to clinical practice.	Unclear: Physiological parameters measured using patient monitor, but no definition of apnoea given.	Unclear: Apnoea definition given, but assessment method not specified.	Yes: Airway occlusion, respiration measured using bands
Were confounding factors identified?	Yes: Exclusion criteria specified.	Yes: Age was considered.	Yes: Six neonates excluded for artefacts or high EEG impedance.	No.
Were strategies to deal with confounding factors stated?	Yes.	Yes.	Yes.	No.
Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)?	Not applicable: Outcome measure is EEG change.	Not applicable: Outcome measure is EEG change.	Not applicable: Outcome measure is EEG change	Not applicable: Outcome measure is EEG change
Were the outcomes measured in a valid and reliable way?	Unclear: aEEG methods clearly described, however, EEG assessment was visual.	Yes.	Yes.	Yes.
Was the follow up time reported and sufficient to be long enough for outcomes to occur?	Unclear: Follow-up was for two hours after caffeine administration. Half-life of caffeine is much longer.	Unclear: Follow-up was for six hours after caffeine administration. Half-life of caffeine much longer.	Unclear: Follow-up for two hours after caffeine administration. Half-life of caffeine much longer.	Yes.
Was follow up complete, and if not, were the reasons of loss to follow up described and explored?	Yes: Loss to follow up of caffeine group at 36 weeks not clearly reported, but this was not relevant for our review.	Yes.	Yes.	Yes.
Were strategies to address incomplete follow up utilized?	Not applicable.	Not applicable	Not applicable.	Not applicable.
Was appropriate statistical analysis used?	Yes, but no adjustment for multiple comparisons.	Yes, but no adjustment for multiple comparisons.	Yes, but no adjustment for multiple comparisons.	Yes.

Table 7.3:Checklist of the included cross-sectional studies

Variables assessed	Deule (1973)	Fenichel et al. (1979)	Bridgers (1985)	Holthausen et al. (1999)	Curzi-Dascalova et al. (2000)	Schramm et al. (2000)	Mañas et al. (2014)	Wulbrand et al. (1998)	Whitehead et al. (2019)
Were the criteria for inclusion in the sample clearly defined?	No.	Yes.	Yes.	No: Inclusion and exclusion criteria not specified	Yes.	Yes.	No: Conference abstract; many details were unreported including subject inclusion and exclusion criteria	No: Conference abstract; many details, including subject inclusion and exclusion criteria were not documented.	Yes.
Were the study subjects and the setting described in detail?	Unclear: No detailed description of background and medical history.	Yes.	Unclear: Demographic information not detailed	No: No detailed subject description.	Yes	Unclear: Limited information given.	Unclear: Limited detail given	Unclear: Limited detail given.	Yes.
Was the exposure measured in a valid and reliable way?	Yes.	Yes: Apnoea definition not standard but duration described as 4 seconds. Assessment method was clear.	Yes.	Yes.	Yes.	Unclear: Device name given, and it automatically detects events, but detection algorithm unclear. No detail on average number of events per neonate or whether all neonates experienced apnoeas.	Unclear: No detail given on recording method or definition of apnoea.	Yes.	Yes.
Were objective, standard criteria used for measurement of the condition?	Yes.	Unclear: Apnoea description (4 secs) short and not standard Measurement	Yes.	Yes.	Yes.	Unclear: Duration of apnoea not specified.	Unclear: No detail given on recording method or definition of apnoea.	Yes.	Yes.

		method clearly defined.							
Were confounding factors identified?	Yes: Identified seizures, noted in one neonate.	Yes: Groups split according to convulsive and non-convulsive apnoeas clearly.	No: Seizure activity discussed but results not separated or adjusted	No.	Yes: Excluded apnoeas close to body movements.	No.	Yes: Sleep state investigated	Yes: Sleep state was investigated	Yes: Age and germinal matrix haemorrhage considered. Also discussed that findings unlikely to be caused by hiccup artefact on EEG.
Were strategies to deal with confounding factors stated?	No.	Yes: Subgroup reporting.	No.	No.	Yes.	No.	Yes: Results split according to sleep state.	Yes: Results were split according to sleep state, although detail of results is missing as this was an abstract.	Yes: Investigated single subject averages and the strength of the ERP was unrelated to age.
Were the outcomes measured in a valid and reliable way?	Unclear: EEG outcome assessed visually.	Unclear: Visual assessment of EEG.	Unclear: EEG visually assessed by 2 raters. Mainly focused on seizure detection rather than overall EEG changes.	Yes: But few details provided on EEG recording method.	Yes: EEG changes evaluated qualitatively by trained expert and using algorithm.	Yes: Outcome measures were clearly described.	Yes.	Unclear: No detail for EEG methods or how EEG suppression was defined.	Yes.
Was appropriate statistical analysis used?	No: Statistical analysis not conducted.	No: Statistical analysis not conducted.	No: Statistical analysis not conducted.	No: Authors stated findings as 'significant' in 1 group but did not indicate what statistical tests were used or the outcome of the test.	Yes: Statistics clearly reported.	Unclear: Statistical analysis with 'one-way analysis' performed but the exact test was not specified.	Unclear: Statistical test not specified.	Unclear: Appropriate statistics seem to have been used but unclear as little detail given of the measures assessed.	Yes.

Table 7.4: Checklist of the included randomised controlled trials study

Variables assessed	**Yang et al. (2019)**
Was true randomization used for assignment of participants to treatment groups?	Unclear: Authors reported random table was method used, but further details were lacking.
Was allocation to treatment groups concealed?	No.
Were treatment groups similar at the baseline?	Yes.
Were participants blind to treatment assignment? *	Unclear: Participants were neonates; unclear whether parents were blinded
Were those delivering treatment blind to treatment assignment?	No: No information mentioned about blinding.
Were outcomes assessors blind to treatment assignment?	No.
Were treatment groups treated identically other than the intervention of interest?	Yes: Control group received aminophylline instead of caffeine.
Was follow-up complete and if not, were differences between groups in terms of their follow-up adequately described and analysed?	Yes.
Were participants analysed in the groups to which they were randomized?	Unclear: The authors did not specify.
Were outcomes measured in the same way for treatment groups?	Yes.
Were outcomes measured in a reliable way?	Unclear: EEG outcome methods unclear, quantification method unclear, and authors did not specify when measures were taken, what equipment was used etc.
Was appropriate statistical analysis used?	Yes, but no correction for multiple comparisons or baseline adjustment
Was the trial design appropriate, and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial?	Unclear: Limited information described.

7.3. Normality tests

Table 7.5: Kolmogorov-Smirnov test of normality

Variables	**Statistics**	**Degree of freedom**	**p value**
Gestational age	0.152	205	0.01
Post menstrual age	0.044	205	**0.20**
Birth weight	0.213	205	0.01
1 minute APGAR score	0.120	199	0.01
10 minutes APGAR score	0.447	198	0.01
Sensory brain age	0.500	205	0.01
Resting-state brain age	0.500	205	0.01
Combined brain age	0.031	205	**0.20**
Brain maturity	0.038	205	**0.20**
Respiratory rate	0.058	205	**0.093**
Apnoea rate	0.243	205	0.01
Desaturation	0.351	205	0.01

The Bold p values >0.05 indicate a normally distributed data.

Table 7.6: Shapiro-Wilk test of normality

Variables	Statistics	Degree of freedom	p-value
Gestational age	0.897	44	0.01
Age at test occasion	0.949	44	0.05
Weight at test occasion	0.953	44	**0.07**
Birthweight	0.917	44	0.004
Baseline mean heart rate	0.974	43	**0.44**
Baseline mean saturation	0.969	40	**0.34**
Baseline mean respiratory rate	0.968	39	**0.32**
Average saturation	0.731	40	0.01
Minimum saturation	0.913	40	0.005
Maximum heart rate	0.976	43	**0.51**
Heart rate time return to baseline	0.498	44	0.01
Average heart rate above threshold	0.689	43	0.01
Average heart rate below threshold	0.641	43	0.01
Average respiratory rate above threshold	0.573	38	0.01
Number of desaturations pre-procedure	0.773	31	0.01
Number of desaturations post-procedure	0.860	31	0.01
Number of bradycardias pre-procedure	0.578	31	0.01
Number of bradycardias post-procedure	0.698	31	0.01
Number of tachycardias pre-procedure	0.551	31	0.01
Number of tachycardias post-procedure	0.658	31	0.01
Duration ROP screen (mins)	0.956	44	**0.09**

The bold p-values >0.05 indicate a normally distributed data. *Abbreviation: Retinopathy of Prematurity (ROP)*

7.4. Survey questionnaire

Staff questionnaire: Using the Dandle® WRAP stretch swaddle during ROP screen.

Dear Staff,

Kindly take a few minutes to provide us with valuable feedback about your usage of the Dandle® WRAP stretch. The answers to these questions will help us to assess the wraps/swaddling technique to improve patient care.

If you have any questions about the survey, please contact Michelle or Fatima.

If you want any more information about swaddling or Dandle® WRAP stretch, please talk to senior nurses or Michelle (ROP lead sister).

Thank you for all your help and support.

Michelle Clee

Email: Michelle.Clee@ouh.nhs.uk

Fatima Usman

Email: fatima.usman@paediatrics.ox.ac.uk

Paediatric Neuroimaging Research Group

Department of Paediatrics

University of Oxford

Please respond as appropriate

Free text questions are indicated, multiple choice options enabled one box to be selected):

1. What is your job role? (Free text)

2. In your opinion, how settled/ comfortable are babies when swaddled with the Dandle® WRAP stretch?
[ ] Very comfortable
[ ] Somewhat comfortable
[ ] Neither comfortable nor uncomfortable
[ ] Somewhat uncomfortable
[ ] Very uncomfortable

3. How easy was the wrap to use?
[ ] Very easy
[ ] Easy
[ ] Neither easy nor difficult
[ ] Difficult
[ ] Very difficult

4. Would you prefer to use the Dandle® WRAP stretch or swaddling with a muslin?
[ ] Dandle® WRAP stretch [ ] Muslin swaddle

Why? (Free text)

5. Would you use the Dandle® WRAP stretch regularly if each baby had their own?
[ ] Yes [ ] Sometimes [ ] No
If you answered 'No' or 'Sometimes' to question 5, why not? (Free text)

6. Do you feel there are any drawbacks to using the Dandle® WRAP stretch?
[ ] Yes [ ] No
If you answered 'yes', please provide details. (Free text)

7. Please provide any further comments or suggestions you have on the use of Dandle® WRAP stretch for ROP screening.

8. Have you ever used the Dandle® WRAP stretch during ROP screening?
[ ] Yes [ ] No
If no, why not? (Free text)

9. Have you used the Dandle® WRAP stretch for other procedures or comfort care?
[ ] Yes [ ] No
If 'No', could you tell us why you haven't used it?
[ ] Don't know about the Dandle® WRAP stretch
[ ] Don't feel confident using the Dandle® WRAP stretch
[ ] I don't look after babies needing ROP screen
[ ] I don't think it was suitable for any of the babies

[ ] I haven't been taught how to use the Dandle® WRAP stretch

10. Have you been taught how to use the Dandle® WRAP stretch?
[ ] Yes [ ] No

11. How confident do you feel about explaining to families why we swaddle or use the Dandle® WRAP stretch?
[ ] Very confident
[ ] Confident
[ ] Neither confident nor unconfident
[ ] Unconfident
[ ] Very unconfident

12. In your opinion, which method of ROP screen do you prefer babies to have?
[ ] Optos Screen (Retinal photos using the big camera/ out of bed procedure)
[ ] Indirect screen (using the headset/in-bed procedures)
[ ] No preference

13. Could you tell us why you prefer the ROP method you chose in question 12 or if no preference, why? (Free text)

Survey QR code