Electroencephalographic functional connectivity analysis in preterm infants

Pauliina Yrjölä
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A doctoral thesis completed for the degree of Doctor of Science (Technology) to be defended, with the permission of the Aalto University School of Science, at a public examination held at the lecture hall F239a (Otakaari 3) of the school on 27 October 2023 at 12.00.

This doctoral thesis is conducted jointly at Aalto University (Finland), University of Helsinki (Finland), and Helsinki University Central Hospital (Finland).

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Abstract

Preterm birth coincides with a critical period of brain development and can disturb the early organization of both the structural network and the large-scale functional connections within it, which are imperative for normal neurocognitive development. Despite the well-mapped structural connectome, the development of the functional networks, and the effects of prematurity on them, are poorly understood. Further, recent endeavours to improve neurocritical care of preterm infants via environmental enrichment (EE) strategies exerted in the neonatal intensive care unit (NICU) lack assessment of the direct effects on their therapeutic target, functional brain networks.

Scalp electroencephalography (EEG) provides a method for recording cortical activity with high temporal resolution. Functional cortico-cortical networks may be identified by estimating interareal synchronization between the phase or amplitude attributes of cortical signals from distinct brain regions with measures of functional connectivity (FC). There is extensive research on FC in adults, but studies on neonates are scarce and require specific methods adapted for the physiological and technical requirements of this population.

This Thesis uses FC analysis methods optimized on neonates to study the developing functional networks on preterm populations during the neonatal period. In Study I, prematurity-related changes in FC were investigated at term age, and correlations between functional networks and later neurodevelopmental outcomes were explored. Spectrally and regionally distinct network differences were found between the cohorts, and the correlation of networks to later clinical outcomes was found to be amplified in the preterm cohort. In Study II, the effects of Family Nurture Intervention (FNI), a bedside EE intervention aiming to facilitate the parent-infant emotional contact, on functional networks were assessed at term age. The results showed a clinically relevant change in the FC levels of infants receiving FNI, which even rendered the intervention cohort comparable to a cohort of healthy term-born controls. In Study III, the development of distinct neuronal coupling modes was investigated during the neonatal period from the last trimester to the first weeks after term age. The findings indicated differential development of different coupling modes, providing developmental growth charts which are consistent with established neonatal neurophysiological mechanisms.

The results together give new insight on the functional networks of preterm infants, on their longitudinal development, and on the effect of the endogenous parent-infant contact on network development. This Thesis supports FC measures as a clinically relevant tool with potential for use in both basic scientific research and clinical intervention studies in neonates.

Keywords neonatal EEG, functional connectivity, preterm infants, brain networks

ISSN (printed) 1799-4934 ISSN (pdf) 1799-4942
Location of publisher Helsinki Location of printing Helsinki Year 2023
Pages 124
Tekijä
Pauliina Yrjölä

Väitöskirjan nimi
Elektroenkefalografisen toiminnallisen konnektiviteetin analyysi keskosvauvoilla

Julkaisija
Perustieteiden korkeakoulu

Yksikkö
Neurotieteen ja lääketieteellisen tekniikan laitos

Sarja
Aalto University publication series DOCTORAL THESES 159/2023

Tutkimusala
Lääketieteellinen tekniikka

Käsikirjoituksen pvm
05.07.2023

Väitöspäivä
27.10.2023

Väittelyluvan myöntämispäivä
20.09.2023

Kieli
Englanti

Monografia
Artikkeliväitöskirja

Esseeväitöskirja

Tiivistelmä
Keskoset syntyvät keskellä aivoille kriittistä kehitysvaihetta, ja ennenaikeinen syntymi voi häiritä normaallille neurokognitiiviselle kehitykselle välttämättömien rakenteellisten ja toiminnallisten aivoverkostojen muodostumista. Rakenteellisia aivoverkoja on hyvin kartoitettu, mutta toiminnallisten verkostojen kehitystä, sekä keskosuuden vaikutusta niihin, tunnetaan vielä heikosti. Lisäksi viimeaikaisten teho-osastolle kehitettyjen ympäristöä rikastavien aistiarvokkeiden vaikutusta toiminnallisiin aivoverkkoihin on tutkittu varsin vähän.

Aivojen sähköistä toimintaa voidaan mitata pinta-aivosähköläävätutkimuksella (elektroenkefalografia, EEG), jonka etuna on erinomainen aikaresoluutio. Toiminnallisia verkostoja voidaan havaita arviomallalla aivoalueiden väälistä synkroniaa eli toiminnallista konnektiviteettia (functional connectivity), jonka laskennassa määritetään eri alueilta mitattujen signaalien vaihetta amplitudipiirteiden yhteneväisyttä. Aiikusväestöllä on tehty runsasta toiminnallisen konnektiviteetin tutkimuksia, mutta vastasyntyneillä tutkimuksia on vain vähän. Tällä populaatiolla on myös lukuisia erityispiirteitä, jotka tulee ottaa huomioon menetelmää käytettäessä.


Väitöskirjan tulokset antavat uutta tietoa keskosten toiminnallista aivoverkostoista, niiden kehityksestä, sekä vanhemman ja keskosen välisen tunnehytynen vaikutuksesta niiden toimintaan. Tulokset tukevat toiminnallista konnektiviteettia klinisesti relevanttina työkaluna, jolla on potentiaalia niin perustutkimuksen kuin klinisten interventioiden vaikuttavuuden tutkimuksessa vastasyntyneillä.

Avainsanat
vastasyntyneiden EEG, toiminnallinen konnektiviteetti, keskiset, aivoverkosto

ISBN (painettu)
978-952-64-1455-3

ISBN (pdf)
978-952-64-1456-0

ISSN (painettu)
1799-4934

ISSN (pdf)
1799-4942

Julkaisupäivä
Helsinki
Painopäivä
Helsinki
Vuosi
2023

Sivumäärä
124

This Thesis work was carried out at BABA Center, Department of Physiology, University of Helsinki in collaboration with Helsinki University Central Hospital and the Department of Neuroscience and Biomedical Engineering, Aalto University. I am grateful for the financial support provided by University of Helsinki, Helsinki University Central Hospital, Jusélius Foundation, and Tietotekniikan ja elektronikan seura.

First, I would like to express my deepest gratitude to my advisors Prof. Sampsa Vanhatalo and Dr. Anton Tokariev. I thank Sampsa for the phenomenal facilitation of my research work, for sharing his unparalleled expertise, and for always reacting immediately to any issues I had. In the same vein, I thank Anton for his unfaltering support and guidance through my PhD, which had a fundamental impact on my work. His ideas, expertise and enthusiasm towards our work always left me greatly inspired after our meetings. I consider it a privilege to work with this exceptional team.

I would like to give my greatest thanks my supervisor and custos, Prof. J. Matias Palva, for sharing his remarkable expertise in functional connectivity analysis and for providing an invaluable link to the university.

I wish to express my gratitude to Dr. Amir Omidvarnia for kindly accepting to be my opponent and for the time and effort put in preparing for it. I also cordially thank Prof. Fabrice Wallois and Prof. Petro Julkunen for their efforts in reviewing this Thesis and for their excellent comments.

I am grateful to my coauthors Prof. Michael M. Myers, Prof. Martha G. Welch, Dr. Nathan Stevenson, and Susanna Stjerna for their invaluable contributions and expertise in our joint publications. I also wish to thank Dr. Pantelis Lioumis for his important role in initiating me into research in the field of neuroscience years ago.

I give my deepest thanks to chief physicists Mika Kortesniemi and Jaana Perkola, who have been key facilitators in combining my early research career and medical physicist residency. Due to their flexible juggling of different work percentages and their ongoing support for my research, they are the very reason for the successful balance between clinical and research work. I also cordially thank all other chief physicists at Helsinki
University Central Hospital for enabling part-time work and research days during my clinical rotation.

I wish to thank my wonderful colleagues at BABA Center: Sami Auno, Saeed Montazeri, Amirreza Asayesh, Mohammad Al-Sa’d, Sofie de Sena, Sebastian König, Elisa Taylor, and Manu Airaksinen. Thank you for the support, for Friday coffee, for volley ball, and for our “team building” activities. To the same extent, I sincerely thank my fellow resident medical physicists: Emmi Kirjanen, Satu Inkinen, Katri Nousiainen, Henna Kavaluus, Juuso Ikola, Jenna Tarvonen, and Pauliina Petrow, amongst others. Thank you for being such brilliant colleagues, for sharing the highs and lows of residency, and for all the after works. Finally, I thank all medical physicists at Helsinki University Central Hospital whom I have had the pleasure to work with and learn from during these years.

My heartfelt gratitude goes to my invaluable friends. I thank my university friends for all the support and joy since the beginning our unforgettable Teekkari years in Otaniemi. I thank my high school friends for maintaining such a close-knit haven, and especially for our (numerous) rendezvous at Fazer café. Ultimately, I thank Eeva-Leena Karihtala for two decades of friendship, trust, and Fabulous Ideas.

Finally, I thank my parents, Eila and Kari, and my sister Elina for the immense support you have always given, not the least during the past years. This Thesis would not have been possible without you.

Helsinki, September 22, 2023,

Pauliina Yrjölä
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List of Publications

This thesis consists of an overview and of the following publications which are referred to in the text by their Roman numerals.


Author’s Contribution

Publication I: “Phase-based cortical synchrony is affected by prematurity”

PY, AT, SV, and JMP conceptualized the study. PY performed the analyses and visualized the results. AT and SS collated the combination scores from neurocognitive assessments. AT and SV supervised EEG analyses. All authors contributed to the writing, editing, and proofing of the manuscript.

Publication II: “Facilitating early parent-infant emotional connection improves cortical networks in preterm infants”

Conceptualization and coordination of interventions and related data collection were performed by MGW and MMM. Data collection of the healthy term cohort was coordinated by SV. EEG analyses were conceptualized by PY, AT, SV, and NJS. Network analyses and visualization of results were performed by PY. Local maturation analysis was performed by NJS. AT and SV supervised EEG analyses. The original draft of the manuscript was written by PY. All authors contributed to the editing and finalization of the manuscript.

Publication III: “Neuronal coupling modes show differential development in the early cortical activity networks of human newborns”

Conceptualization of the study was performed by all authors. PY performed the analyses and visualized the results. AT and SV supervised analyses. PY wrote the original draft of the manuscript. All authors contributed to the editing and finalization of the manuscript.
Abbreviations

AAC  amplitude-amplitude correlation
ADHD  attention deficit/hyperactivity disorder
ASD  autism spectrum disorder
AP  action potential
AS  active sleep
BIC  Bayesian information criterion
BOLD  blood-oxygen-level-dependent
CA  conceptional age
CC  correlation coefficient
CFS  cross-frequency phase synchrony
CP  cerebral palsy
d-PAC  distant phase-amplitude coupling
dwPLI  debiased weighted PLI
EE  environmental enrichment
ECG  electrocardiography
EEG  electroencephalography
EPSP  excitatory post-synaptic potential
FC  functional connectivity
Fc  central frequency
Abbreviations

**FBA**  functional brain age

**fMRI**  functional magnetic resonance imaging

**fNIRS**  functional near-infrared spectroscopy

**GA**  gestational age

**GABA**  γ-aminobutyric acid

**GM**  grey matter

**GMDS**  Griffiths Mental Developmental Scales

**HNNE**  Hammersmith Neonatal Neurological Examination

**ICA**  independent component analysis

**ICM**  intrinsic coupling modes

**IMC**  imaginary part of coherence

**IPSP**  inhibitory post-synaptic potential

**IVH**  intraventricular hemorrhage

**LMM**  linear mixed-effects model

**l-PAC**  local phase-amplitude coupling

**MSC**  magnitude squared coherence

**MEG**  magnetoencephalography

**MRI**  magnetic resonance imaging

**NICU**  neonatal intensive care unit

**oCC**  orthogonalized correlation coefficient

**PAC**  phase-amplitude coupling

**PLI**  phase-lag index

**PLV**  phase-locking value

**PPC**  phase-phase correlation

**PS**  phase synchrony

**PSP**  postsynaptic potential

**PVL**  periventricular leukomalacia

**QS**  quiet sleep
**Abbreviations**

**REM**  rapid eye movement

**SAT**  spontaneous activity transient

**SD**  standard deviation

**SC**  structural connectivity

**TEA**  term-equivalent age

**wPLI**  weighted phase-lag index

**WM**  white matter

**WMI**  white matter injury
1. Introduction

Interrupting approximately 10% of all births globally, preterm birth constitutes a major risk factor of lifelong neurocognitive compromise and a key challenge in neonatal medicine (Johnson and Marlow, 2017; Organization, 2012; Saigal and Doyle, 2008). Preterm infants are exposed to an *ex utero* environment during the critical period of the third trimester when the brain is engaged in profuse activity-dependent development of both structural and functional networks (Luhmann et al., 2016; Molnár et al., 2020). It is during this time window that the first connections are established between the thalamus and the transient cortical subplate zone, a temporary structure that underlies the immature cerebral cortex and has a fundamental role in mediating the initial thalamocortical connections (Kostović and Judaš, 2010; Lagercrantz et al., 2010; McConnell et al., 1989). Even minor alterations to spontaneous brain activity patterns during this period can induce significant changes in functional networks and may underlie the development of neurocognitive disorders (Molnár et al., 2019, 2020). Thus, it is essential to understand the early development of these functional networks, and how pathological conditions, such as prematurity, can affect them.

During the last trimester, preterm infants are exposed to the unnatural environment of the neonatal intensive care unit (NICU), which constitutes of an abundance of distinct sensory stimuli (Beltrán et al., 2021; Vitale et al., 2021). Endeavours to advance neurocritical care have focused on implementing different approaches of environmental enrichment (EE) in the NICU (Beltrán et al., 2021; Filippa et al., 2020; Guzzetta et al., 2009; Haslbeck et al., 2020; Lordier et al., 2019; Mclean et al., 2021; Moore et al., 2016; Vitale et al., 2021; Welch et al., 2012). However, further evidence-based development of these EE strategies calls for studies assessing the direct effects of these interventions on their target, functional brain networks.

Cortical activity of neuronal populations may be recorded using scalp electroencephalography (EEG), which provides a measure of direct brain activity with excellent temporal resolution. The early cortical activity of
preterm infants is characterized by alternating periods of transient bursting and relative quiescence, which gradually transition into continuous adult-like oscillations during the final months of gestation and the first weeks of life (Vanhatalo and Kaila, 2006). This period is also the time of rapid development of temporal communication between distinct brain regions, giving rise to the establishment of functional networks (Wallois et al., 2021).

Functional networks can be quantitatively characterized with measures of functional connectivity (FC), which capture the interareal temporal correlations between phase or amplitude attributes in time series signals of brain function (Bastos and Schoffelen, 2016; Palva and Palva, 2012). FC encompasses multiple modes of intrinsic coupling. Phase-phase correlations (PPCs) provide the temporally most accurate measure of communication via subsecond correlations in activity between neuronal populations. On the contrary, amplitude-amplitude correlations (AACs) capture gross neuronal synchrony over multiple seconds. Cross-frequency interactions may be estimated by phase-amplitude correlations (PACs), which quantify relationships between a low-frequency carrier wave and the amplitude envelope of a higher-frequency oscillation.

FC is often associated uniquely with studies of the functional connectome, estimated by measures of hemodynamic response with functional magnetic resonance imaging (fMRI). However, the relationship between neuronal activity and blood oxygen levels is not straightforward in neonates, whose vascular system is still immature and adopts multiple strategies of hemodynamic response (Nourhashemi et al., 2020). Furthermore, the neurovascular coupling delays inherent to fMRI recordings (Allievi et al., 2016) preclude the possibility to investigate coupling modes of accurate subsecond timing. For these reasons, accurate estimation of FC in the neonatal population is most readily achievable using direct measures of brain activity with high temporal resolution, such as EEG or magnetoencephalography (MEG). The technical limitations of FC include artificial connectivity brought about by linear mixing and common unobserved sources. While both of these challenges can be reduced with signal processing measures, the challenge of spurious interactions, which always arise in the vicinity of true connections, remains in all FC studies. This must therefore be taken into account in the interpretation of FC results, as must the fact that the commonly used pairwise FC comparisons remain a simplification of the complex human brain networks.

This Thesis uses EEG-based FC analysis to study multiple aspects of functional brain networks in preterm populations. **Study I** identifies prematurity-related changes in FC by comparing the functional brain networks of preterm infants and term-born controls at term age, as well as the association of FC strength to neurocognitive outcomes. **Study II** explores the effects of a multimodal EE intervention on preterm functional
brain networks at term age, finding significant network differences in the intervention group, which link to later clinical outcomes and render the intervention cohort comparable to term-born controls. Finally, Study III characterizes the longitudinal development of distinct coupling modes through the neonatal period from 33–45 weeks of conceptional age (CA). Altogether, the current Thesis provides a meticulous exploration of the adversities, development, and improvement of functional brain networks in preterm infants.
2. Literature review

2.1 Electrophysiological signals of the human brain

The human brain operates as a centralized control system, receiving both external and internal input, integrating received information, and transmitting orders via neuronal pathways. Information is transmitted by electric signaling. The electric activity of the brain gives rise to weak measurable electric potentials (1–100 µV) and magnetic fields (100–500 fT) on the scalp (Hari and Puce, 2017).

Measurable electrophysiological signals arise primarily from the cerebral cortex, the 2–7 mm thick outer layer of grey matter (GM) in the largest structure of the human brain, the cerebrum (Soinila and Kaste, 2015). The cortex is a strongly gyrated and organized structure of six anatomically distinct layers (I–VI), revealed by histological staining (Kandel et al., 2021). The regions of the cerebral cortex are classified by function into sensory, motor, and association areas. Sensory information from the body is relayed to these specialized cortical areas via the thalamus, a hub-like nucleus of GM with projections to all cortical regions (Soinila and Kaste, 2015). Beneath the cortex is the white matter (WM), which houses the axonal pathways of neurons linking cortical areas. These axons are often sheathed with myelin, hence providing the distinct colour and name for the structure (Kandel et al., 2021).

Electric signalling is initiated by disruptions of the cell membrane potential. Cell membranes maintain a resting potential of approximately -60 mV by actively transferring an abundance of Na\(^+\) and Cl\(^-\) ions to the extracellular space and K\(^+\) ions to the intracellular space (the respective extra-/intracellular concentrations for these ions are 140/20 mmol, 120/20 mmol, and 5/140 mmol) (Hari and Puce, 2017). Action potentials (APs; amplitude 100 mV, duration 1–2 ms) cause transient increases of membrane permeability to Na\(^+\) ions, inducing a propagating depolarization of the cell membrane through the neuron, followed by a repolarization via active
transfer of ions. APs operate on an all-or-none principle, i.e. generate always a maximal response after a stimulation surpassing a threshold.

Information is transferred between neurons via neurotransmitters, which are emitted to the synaptic cleft as a result of the arriving AP and bind to ion channels in the postsynaptic membrane. This increases the permeability of the postsynaptic membrane to specific ions, thus generating postsynaptic potentials (PSPs). Excitatory neurotransmitters, such as glutamate, induce a depolarization (increased permeability to \( \text{Na}^+ \) or \( \text{K}^+ \)) to the postsynaptic membrane, resulting in an excitatory PSP (EPSP; amplitude 10 mV, duration 10–30 ms), whereas inhibitory neurotransmitters, such as \( \gamma \)-aminobutyric acid (GABA), induce a hyperpolarization (increased permeability to \( \text{Cl}^- \)) leading to an inhibitory PSP (IPSP; amplitude 10 mV, duration 80–100 ms). PSPs spread electrically through the conducting intracellular space as primary currents. Leakages in the cell membrane induce return currents, which flow passively back through the extracellular space.

### 2.2 Electroencephalography

EEG is a measurement technique for recording neurophysiological signals from the brain, discovered by Richard Caton in 1875 with first human recordings performed by Hans Berger in 1924. EEG measures primarily signals from synchronized PSPs of perpendicularly oriented pyramidal cell populations in the cortex (Kandel et al., 2021). PSPs give rise to extracellular voltages, which can be modelled as dipoles, consisting of a positively charged source and negatively charged sink. Electric fields spread instantaneously via volume conduction and may thus be recorded from the scalp. EEG is sensitive to both radial currents, generated in gyri, and tangential currents, generated in sulci (Hari and Puce, 2017). EEG has an excellent temporal resolution, dependent on the sampling frequency, and temporal accuracy due to instantaneous field spread. On the contrary, the spatial resolution is moderate and dependent on the number of electrodes: with only a couple of electrodes, an order of cubic centimeters is achievable, while with high-density recordings and appropriate source localisation techniques, a spatial resolution of cubic millimeters may be reached (Cohen, 2014).

Non-invasive EEG is measured by electrodes distributed on the scalp surface, most commonly using 19 electrodes placed according to the international 10–20 system (Jasper, 1958); however, the number of electrodes used may vary from just a couple to hundreds of electrodes. The electrode impedances are kept as low and equal to each other as possible (typically \(< 10 \, \text{k}\Omega \)), conventionally using a conducting medium. Recording is performed between pairs of electrodes (bipolar) or between each electrode and a refer-
ence (monopolar/referential). Differential amplifiers are used to amplify the captured potential difference while rejecting the common mode signal, which arises from environmental noise or physiological signals from other parts of the body. The impedance of the amplifier should be considerably larger than the electrode impedances (typically > 250 $M\Omega$). EEG may be displayed in different derivations, referred to as montages, which may be independent from the recording montage. Bipolar montages display the potential difference between pairs of electrodes in either longitudinal or transverse directions, while monopolar montages present the difference between each electrode and a reference, which may be a predefined electrode (vertex, mastoid or linked mastoids), common average, or Laplacian (weighted average between neighbour electrodes). The number of recordings electrodes and choice of montage strongly affect the appearance of EEG signals (waveforms and amplitudes) (Acharya and Acharya, 2019), the spatial resolution achievable (Cohen, 2014; Tokariev et al., 2016a), and the accuracy of connectivity analysis (Tokariev et al., 2016a).

Localisation of recorded sensor-level activity to the actual generator (source) level, i.e. source reconstruction, is a classical challenge of EEG studies. Namely, the activity of a single source is recorded in multiple electrodes, and each electrode is sensitive to activity of multiple sources. The head model, i.e. solution of the forward problem, defines how each source contributes to measured sensor-level signals. A primitive head model is a sphere with conductivity values specified for three layers: scalp, skull, and intracranial volume. However, more accurate source localisation is achieved with head models generated from segmenting anatomical images. The modelling of current flow through the known head geometry is a well-defined problem and commonly solved with numerical techniques such as the finite element method (FEM) or boundary element method (BEM) (Michel and Brunet, 2019; Michel and He, 2017). The solution of the inverse problem, i.e., the distribution of source currents which generate the recorded sensor-level data, cannot be uniquely determined, but may be estimated with constraints set by physiology (Hari and Puce, 2017). Popular source modelling techniques include minimum norm imaging and its modifications (e.g. low resolution electromagnetic tomography, LORETA, and dynamic statistical parametric mapping, dSPM), beamforming and dipole modelling (Michel and Brunet, 2019; Michel and He, 2017).

Neonatal EEG recordings have features specific from adult EEG. Structurally, the thinner skull, its higher conductivity, and the lack of mature connections between pyramidal populations make neonatal EEG much more focal than that of adults (Odabaee et al., 2014; Wallois et al., 2021), requiring a dense array of recording electrodes (Tokariev et al., 2016a). Furthermore, the smoother cortical surface prior to gyration leads to a larger portion of radially oriented dipoles and thus a denser measurable field (Wallois et al., 2021). Conventionally, the presence of fontanelles,
membraneous gaps in the neonatal skull structure, have been considered to affect the localisation of EEG sources by creating an additional localisation error up to a couple of centimeters (Wallois et al., 2021), yet there is evidence that fontanelles, in fact, do not provide a special pathway for electrical spread, which is rather uniform across the skull due to the high skull conductivity in neonates (Odabaee et al., 2014). Functionally, the hallmark of premature EEG is its characteristic multifrequency spontaneous activity (Vanhatalo and Kaila, 2006; Wallois et al., 2021), which includes infra-slow components (< 0.5 Hz). Ideally, the use of direct current-coupled amplifiers would enable capturing the full frequency band of clinical interest (Vanhatalo et al., 2005). However, this is challenging in the clinical setting even with a suitable amplifier; to be reliable, low frequency recordings would require long epochs of excellent signal quality, which is challenging to obtain in the NICU. Due to the prevalence of artefacts in recordings of preterm infants in the NICU, signal processing is essential; for instance, the mains frequency as well as specific frequencies of NICU devices, may be suppressed by notch filtering, and electrocardiographic (ECG) artefacts may be removed by signal decomposition methods, such as independent component analysis (ICA).

2.3 Preterm infants: developmental adversities and effects of the NICU environment

Preterm birth constitutes a major risk for the infant’s survival and increases the prevalence of later developmental adversities (Chung et al., 2020; Johnson and Marlow, 2017; Organization, 2012; Saigal and Doyle, 2008). An infant born before 37 weeks GA is classified as preterm, with subcategories encompassing the extremely preterm (< 28 weeks GA), very preterm (28–32 weeks GA), moderate preterm (32–34 weeks GA), and late preterm (34–37 weeks GA). The survival rates of the highest-risk, extremely preterm infants have improved in recent reports (Bell et al., 2022; Costeloe et al., 2012; Helenius et al., 2019; Norman et al., 2019), yet differences between medical centers remain: survival probability is highest in specialized centers which offer active neonatal care services (Marlow et al., 2014). Annual rates of alive-born preterm infants also show an increasing trend (Costeloe et al., 2012; Helenius et al., 2019; Norman et al., 2019; Saigal and Doyle, 2008), highlighting the need to consider treatment practices to ensure long-term support for the consequences of prematurity.

The major sequelae related to premature birth include structural alterations inflicting cerebral palsy (CP), or, most often, altered functional maturation which is not accompanied by visible structural malformations, leading to developmental and sensory impairment. Outcome measure-
Literature review

ments conducted during the first two years have found a prevalence of 2–9% of CP in surviving preterm-born infants (Do et al., 2020; Johnson and Marlow, 2017), and, to a lesser extent, of sensory impairments (visual or hearing): 1% severe, and 5–6% moderate impairments (Johnson and Marlow, 2017). Studies of neurocognitive outcomes at 2–3 years show also decreased levels of cognitive, language and motor outcomes in preterms (Cheong et al., 2017; Do et al., 2020; Johnson and Marlow, 2017; Saigal and Doyle, 2008). These adversities seem to extend to later childhood: studies have identified an 11–12 point decrease in IQ in school-aged children (Johnson and Marlow, 2017) as well as increased rates of behavioural sequelae, such as attention deficit/hyperactivity disorder (ADHD), autism spectrum disorders (ASD), avoidant personality, and anti-social personality (Chung et al., 2020). Finally, anxiety, attention, and social difficulties are more prevalent in preterm-born children and adolescents (Chung et al., 2020; Johnson and Marlow, 2017). Recent literature has shown that the occurrences of many developmental conditions are decreasing, likely brought about by improvements in neonatal intensive care, and underscoring the importance of identifying individuals at risk and providing interventions at an early stage (Chung et al., 2020).

Structurally, preterm infants are susceptible to intraventricular hemorrhage (IVH) and white matter injuries (WMI), which range from the severe periventricular leukomalacia (PVL) to milder diffuse WMI (Back, 2017). Larger volumes of WMIs are significantly related to compromised neurodevelopment (Guo et al., 2017; Omidvarnia et al., 2015; Woodward et al., 2012). In addition, preterm infants show dysmaturation via altered volumes of both WM and GM (Back, 2017; Bouyssi-Kobar et al., 2016; Inder et al., 2005; Padilla et al., 2015; Srinivasan et al., 2007; Volpe, 2019), which carry out to childhood and adolescence (de Kieviet et al., 2012; Peterson et al., 2000), and correlate with neurodevelopmental outcomes (Keunen et al., 2016). Structural WM connectivity is also decreased in the preterm population (Batalle et al., 2017; Volpe, 2019). Notably, structural development is heterogeneous in the preterm population, with little overlap across individuals (Dimitrova et al., 2020). Functionally, while the development of local activity is considered to proceed similarly irrespective of the environment being in or ex utero (Shany et al., 2014), several studies support the idea that preterm cohorts exhibit altered functional networks (González et al., 2011; Omidvarnia et al., 2014; Tokariev et al., 2019a,b).

Preterm infants spend the time of the last trimester in the unnatural environment of the NICU. Concern of adverse effects caused by the sensory environment of the NICU, including, inter alia, monitoring devices, environmental noise, examinations, medication, respiratory support, and circadian light exposure (Beltrán et al., 2021; Vitale et al., 2021), persist (Lammertink et al., 2022; Smith et al., 2011). These considerations have evoked endeavours to control the environment by active noise cancella-
tion or cycled light (Beltrán et al., 2021; Vitale et al., 2021), to modulate the external sensory experience of the infant by music (Filippa et al., 2020; Haslbeck et al., 2020; Lordier et al., 2019), massage (Guzzetta et al., 2009), or skin-to-skin therapies (Moore et al., 2016), or to influence the endogenous stability of the infant by added support of parental care and parent-infant emotional connection (Mclean et al., 2021; Welch et al., 2015). Many of these studies have reported improvements on measures of local brain development and correlation with later outcomes; however, more evidence on the long-term effects of these interventions is needed (Beltrán et al., 2021).

2.4 Structural and functional brain development

Early brain development is characterized by spontaneous activity, which drives the formation cortical circuitry underlying the tightly associated structural and functional development (Molnár et al., 2020). At the early embryonic stage during the second trimester, neurons are not yet connected, and generate only sparse, asynchronous activity. In the consecutive developmental stage, neurons are linked primarily via electrical gap junctions, and synchronous bursts start to appear within local neuronal clusters and, mediated by axonal projections, between remote areas (Lagercrantz et al., 2010; Molnár et al., 2020). Thalamocortical connections are established, projecting first from the thalamus to the subplate, a prominent transient structure which underlies the immature cerebral cortex, functions as a "waiting zone", and exerts its own connections to the cortex (Kostović and Judaš, 2010; Lagercrantz et al., 2010; McConnell et al., 1989). The thalamus and subplate play a major role in integrating and mediating spontaneous and sensory-driven activity, which can originate from sensory organs or external stimuli, even when the sensory organs are not yet fully developed (Luhmann et al., 2016; Molnár et al., 2020; Wallois et al., 2021). When the thalamic afferents reach the cortex, even as early as 26 weeks CA, the endogenous thalamic and sensory peripheral activity start to reshape the cortical circuitry. The subsequent maturation of synapses increases synchronous activity between distant brain regions during the third trimester, as does the development of intraregional and interregional cortico-cortical connections develop first, while shorter intraregional connections are formed later at the end of gestation and during infancy (Pihko et al., 2013; Wallois et al., 2021). However, by term age, the major structural connections are established (Wallois et al., 2021). The third trimester is also characterized by prominent brain growth, cortical folding, and gyrrification (Wallois et al., 2021). Finally, external sensory stimuli have a progressively stronger impact on brain function,
and the synchrony of spontaneous activity decreases during a transition to "adult-like" low-amplitude desynchronized activity (Molnár et al., 2020).

The changes in the activity and coupling of the early networks brought about by the complex interplay between the thalamus, subplate, and cortical columns are reflected in the EEG recordings of preterm human infants. The hallmark of early premature EEG is the alternating burst-like activity with periods of relative quiescence (Vanhatalo and Kaila, 2006), which later develops to abundantly continuous activity seen in full-term infants (Fig. 2.1). This development may be essentially described by two distinct trajectories: the development of intermittent bursts, here collated under the term spontaneous activity transients (SATs), and the development of continuous background oscillations (Vanhatalo and Kaila, 2006).

SATs are multi-frequency events of transient bursting, which encompass multiple high frequencies (theta, alpha–beta) nested in an infra-slow intrinsic frequency (0.1–0.5 Hz), and have striking similarity between species (Vanhatalo and Kaila, 2006; Wallois et al., 2021). There is abundant terminology on these multiband events in the literature, which, however, has likely risen from variability of measurement setups and low sensitivity of suboptimal electrode numbers (Lagercrantz et al., 2010). SATs are fully visible only in direct current mode recordings, which are able to record the infra-slow component, eliminated with the conventional 0.5 Hz high-pass filtering in the typical alternating current mode recordings (hence producing the conventional appearance of SATs, often termed "delta brushes"). SATs are found to arise by 24 weeks CA, emerging first focally in sensory cortices, and spreading later over other regions. Their peak occurrence is at around 32–35 weeks CA (Martini et al., 2021). The synchrony of SAT events is initially very poor but increases with maturation. Namely, rough coincidence between SAT events in homologous sensory cortices arises at around 30 weeks CA, and more consistent synchrony develops from 35 weeks CA, concomitantly with the formation of callosal fibres and onset of long-distance connectivity (Vanhatalo and Kaila, 2006). The frequency of occurrence and amplitude of SATs decline with maturation in tight association to the functional role of the GABA neurotransmitter. Namely, GABA first functions as an excitatory neurotransmitter between pyramidal neurons, facilitating spontaneous activity, but eventually turns into the main inhibitory neurotransmitter, thus resulting in the disappearance of SAT events shortly after term-equivalent age (TEA, typically around 40 weeks CA in human infants). In contrast to the developmental decrease of SAT events, there is a gradual increase in the "adult-like" continuous background EEG, operating on high frequency bands (alpha, beta, gamma) (Vanhatalo and Kaila, 2006). This ongoing activity eventually overcomes the immature bursts. However, it requires functioning large-scale thalamo-cortical and cortico-cortical circuits with high temporal precision and thus
Distinct vigilance states during sleep, active (AS) and quiet sleep (QS), emerge gradually from around 28 weeks CA (André et al., 2010; Dereymaeker et al., 2017; Wallois et al., 2021). The most salient difference is that discontinuous bursts are present in AS only until 32 weeks CA, while in QS they remain visible until term age (see Fig. 2.1) (Wallois et al., 2021). AS is characterized by continuous activity, rapid eye movement (REM), myoclonic twitches, and intermittent muscle activity seen in frontal and/or temporal sensors (André et al., 2010). On the contrary, QS is identified by

Figure 2.1. Development of neonatal EEG. 1-minute epochs are presented from longitudinal EEG recordings of a single infant at 33, 36, 39, and 40 weeks CA in active sleep (AS, left) and quiet sleep (QS, right). The traces represent activity from the electrodes C3 (left/top) and C4 (right/bottom) in average montage. Below the EEG traces are time-frequency plots (continuous wavelet transform) for the C3 electrode activity in the same time window. Note the transition from the intermittent burst-quiescence pattern to increasing continuous activity, salient in QS. In AS, discontinuity is present only until around 32 weeks CA.
discontinuous background pattern, absence of REMs, low muscle tone, and regular respiration (André et al., 2010). While the developmental role of the neonatal sleep states is still largely unknown, it has been suggested that the different components of sleep impact neurodevelopment via sensory feedback and regulation of molecular signaling and release of plasticity-promoting neuromodulators (Blumberg et al., 2022). For instance, AS has been considered crucial for activity-dependent brain development by providing sensory experience via self-generated myoclonic twitches promoting neuronal oscillations and synchronization of functional networks (Del Rio-Bermudez and Blumberg, 2018). Conversely, most abnormalities are more discernible in QS, which has been conventionally considered the more sensitive sleep state. Finally, the transition between sleep states has been shown to affect the organization of functional networks and carry prognostic information (Tokariev et al., 2019a).

2.5 Conceptualisation of functional connectivity

Dynamic communication of functionally specialized brain areas is considered a key requirement for cognitive processes of the human brain (Bressler and Menon, 2010; Uhlhaas et al., 2010) and is mediated by the temporal coordination of neuronal oscillations (Bastos and Schoffelen, 2016; Engel et al., 2013; Fries, 2005). The strength of coordination (functional connections or, in terms of graph theory, edges) between neuronal populations (nodes) is estimated by FC. Notably, FC, a measure of statistical interactions between physiological brain signals, differs from structural connectivity (SC), which refers to the anatomical connections between cortical sites, revealed by post mortem histological studies or quantifying the preferential direction of water in the brain volume (diffusion-weighted MRI) (Bressler and Menon, 2010; Dubois et al., 2015). FC is also generally ignorant to the direction of communication and is thus differentiated from effective connectivity, which specifically refers to measures of direct statistical causation between signals (Bastos and Schoffelen, 2016).

The term 'functional connectivity' is most often associated exclusively with studies investigating the synchrony of blood-oxygen-level-dependent (BOLD) signals measured by fMRI or functional near-infrared spectroscopy (fNIRS). However, computing FC from direct neurophysiological signals, obtained by M/EEG, has two primary advantages in comparison to techniques dependent on neurovascular coupling. First, EEG and MEG enable a high temporal resolution, paramount to studying temporally accurate correlations between neuronal oscillations and unachievable with techniques that measure the BOLD signal. Second, measuring brain activity directly instead of using BOLD as a proxy to it is essential in studies on neonates, whose vascular network is still immature, showing age-dependent neu-
rovascular coupling delays up to 18 s (Allievi et al., 2016) and variable hemodynamic responses to neuronal activity (Nourhashemi et al., 2020). Notably, there is increasing evidence on the absence of correlation between EEG and BOLD signals in neonatal studies both in animals (Kozberg et al., 2016) and in humans (Omidvarnia et al., 2014). This Thesis is set apart from fMRI-based connectome studies, and the following sections will focus on measures of FC achievable with M/EEG.

Synchronization between neuronal oscillations occurs in distinguishable modes, referred to as intrinsic coupling modes (ICMs) (Engel et al., 2013), which are thought to reflect different mechanisms on distinct temporal scales (Palva and Palva, 2012). First, band-limited phase synchrony (PS) between neuronal oscillations is a mode of accurate, subsecond neuronal synchronization and is referred to as, inter alia, phase-phase correlations (PPCs), phase-ICMs, or phase-based connectivity. Functionally, PPCs are considered to play an important role in regulating neuronal communication by providing time windows of accurate alignment in neuronal peak excitability, thus facilitating communication between neuronal populations with mutually coordinated phase difference, and suppressing oscillations arriving at cancelling phase (Engel et al., 2013; Fries, 2005; Palva and Palva, 2012).

Second, a complementary mode of interareal synchrony, amplitude-amplitude correlation (AAC; also referred to as, inter alia, envelope-ICMs or power-based connectivity) is found between the amplitude envelopes of oscillations in longer time scales of multiple seconds. AACs measure gross cortical connectivity between neuronal populations. While their mechanistic role is somewhat less clear, they are considered to involve in the temporal regulation of the neuronal populations engaged in a functional network by gating oscillatory activity (Engel et al., 2013).

Finally, cross-frequency coupling encompassing \( n:m \) PPCs and phase-amplitude coupling (PAC) are deemed to coordinate spectrally distributed interactions (Palva and Palva, 2018, 2012) of the brain. \( n:m \) PPC, also termed cross-frequency phase synchrony (CFS), is simply the general case of PPC, where the frequencies of the two oscillations are not identical. PAC reflects modulation of the amplitude envelope of a high-frequency oscillation by the phase of a low-frequency oscillation. An important example of this modulation in preterm neonates are the SAT events, where high-frequency bursts are embedded into a low frequency carrier wave (Vanhatalo and Kaila, 2006). The low-frequency component would likely stem from activity generated in the subplate, which would activate bursts of cortical activity in overlying layers, reflected in the high-frequency component.
2.6 Mathematical methods for estimating functional connectivity

The mathematical methods for estimating FC are presented schematically in Fig. 2.2. For estimating FC between neurophysiological time series, the real-valued measurement data must first be transformed to complex form in order to enable separation of the phase- and amplitude-related components of the signal. By means of the Hilbert transform, we can thus obtain the analytic signal \( s_a(t) \) from a real-valued signal \( s(t) \):

\[
s_a(t) = s(t) + i \hat{s}(t),
\]

where \( \hat{s}(t) \) is the Hilbert transform of \( s(t) \), \( t \) is time, and \( i \) is the imaginary unit. The spectral representation of this signal is:

\[
s_a(t) = A e^{i \phi},
\]

where \( A \) reflects the amplitude and \( \phi \) the instantaneous phase of the signal.

The synchrony of two time series \( x \) and \( y \) can be mathematically represented in the complex plane with the cross-spectrum \( S_{x,y}(\omega) \), which corresponds to the multiplication of the analytic complex signal \( x \) by the complex conjugate of \( y \):

\[
S_{x,y}(\omega) = (s_x(t) + i \hat{s}_x(t)) \ast (s_y(t) - i \hat{s}_y(t))
= A_xA_y e^{i(\phi_x - \phi_y)}.
\]

This results in a vector, at a prefiltered frequency \( \omega \), whose magnitude reflects the product of the signals’ amplitudes \( A_xA_y \) and angle to the real axis their phase difference \( \phi_x - \phi_y = \Delta \phi \). \( \omega \) is omitted from the following equations for readability.

The traditional method of estimating of PS is coherence, which is the frequency domain equivalent of the cross-correlation function and is obtained from the cross-spectrum as:

\[
Coh_{x,y} = \frac{|S_{x,y}|}{\sqrt{S_{x,x} \ast S_{y,y}}},
\]

where \( S_{x,x} \) and \( S_{y,y} \) are the power estimates of signals \( x \) and \( y \). Alternatively, coherence can be computed as magnitude squared coherence (MSC) by taking the square of Eq. 2.4. Often the imaginary part of coherence (IMC) is used in order to reduce smearing by volume conduction (Nolte et al., 2004). However, the limitation of coherence is that it does not reflect
Figure 2.2. Analyzing interactions of brain signals. a) Extracting signal attributes. Band-pass filtered signals are presented from two brain regions (green and orange), with the signal attributes separated: amplitude time series $A_x$ and $A_y$ (top) are obtained from the real part of the complex signals $s_x$ and $s_y$, respectively, and phase time series $\phi_x$ and $\phi_y$ (bottom) from their argument. b) Phase-phase correlations (PPC) quantify the consistency of phase differences $\Delta \phi_{xy}$. The cross-spectrum of the outlined time window is shown on the unit circle, where the grey lines represent instantaneous phase differences at different time points, and the dark line represents the mean phase difference. c) Amplitude-amplitude correlation (AAC) measures the correlation between amplitude envelopes, obtained as the absolute values of the complex signals $s_x$ and $s_y$. d) Phase-amplitude coupling (PAC) estimates cross-frequency phase synchrony between a low-frequency band-pass filtered signal and the envelope of a high-frequency signal, filtered to the same frequency range. The signals can originate from the same brain region (local PAC, l-PAC) or from different brain regions (distant PAC, d-PAC).

uniquely phase relationships and may thus be affected by changes in power (Lachaux et al., 1999).

Measures of strict PS aim to characterize the clustering or consistency of the phase differences across time. The first is reflected by the phase-locking value (PLV) (Lachaux et al., 2000, 1999), which is defined as:
\[ PLV_{x,y} = \frac{1}{N} \sum_{t=1}^{N} e^{i\Delta\phi(t)}, \]  

(2.5)

where \( N \) is the number of time points \( t \). However, PLV is sensitive to artificial correlations generated by volume conduction. This has motivated an alternative approach, the phase-lag index (PLI) (Stam et al., 2007), which reflects the consistency of non-zero phase differences across time:

\[ PLI_{x,y} = |E\{\text{sgn}[\Delta\phi(t)]\}| 
\]

\[ = |E\{\text{sgn}[\text{Im}(S_{x,y})]\}|, \]  

(2.6)

where \( E\{.\} \) is the expected value operator and \( \text{sgn}[.] \) the signum function. This measure is based on the idea that artificial correlations caused by volume conduction are instantaneous and lead to phase lags of exactly 0 or \( \pi \) (Stam et al., 2007). Ignoring these artificial interactions comes at the cost of missing true zero-phase interactions. An extension of the PLI is the weighted PLI (wPLI) (Vinck et al., 2011), which weights phase lags that are further from the real axis to reduce discontinuities:

\[ wPLI_{x,y} = \frac{|E\{|\text{Im}(S_{x,y})| \ast \text{sgn}[\text{Im}(S_{x,y})]\}|}{|E\{|\text{Im}(S_{x,y})|\}|}, \]

\[ = \frac{|E\{|\text{Im}(S_{x,y})|\}|}{E\{|\text{Im}(S_{x,y})|\}|}. \]  

(2.7)

Finally, inflation due to sample size may be corrected by a debiased version of the wPLI, debiased wPLI (dwPLI) (Vinck et al., 2011) computed as:

\[ dwPLI_{x,y} = \frac{\sum |\text{Im}(S_{x,y})|^2 - \sum (\text{Im}(S_{x,y}))^2}{(\sum |\text{Im}(S_{x,y})|)^2 - \sum (\text{Im}(S_{x,y}))^2). \]  

(2.8)

Cross-frequency coupling modes are computed with similar PS metrics; CFS is reduced from \( n:m \) to \( 1:1 \) frequency ratio by multiplying the lower frequency component with a constant, after which the measures above may be used (Siebenhühner et al., 2016). PACs are estimated by computing PLV between a low frequency signal and the amplitude envelope of a high frequency signal filtered to the low frequency range (Siebenhühner et al., 2016; Vanhatalo et al., 2004).

For estimating AAC, the most simple metric is the correlation coefficient (CC) between the amplitude envelope time series (Schoffelen and Gross, 2009). Again, however, the effect of volume conduction produces artificial correlations, which may be addressed by orthogonalizing the amplitude.
Literature review

envelopes (Brookes et al., 2012; Hipp et al., 2012). Here, a univariate prediction of the effect of complex signal $x$ on complex signal $y$ is estimated and subtracted from signal $y$ to generate an orthogonalized signal $y_R$:

$$y_R = y - x \beta = y - x \ast (x^+ y),$$  \hspace{1cm} (2.9)

where $x^+$ is the pseudo-inverse of $x$. The orthogonalized correlation coefficient (oCC) may then be computed as the mean of the orthogonalizations in both directions:

$$oCC_{x,y} = \frac{corr[|x|,|y_R|] + corr[|x_R|,|y|]}{2},$$  \hspace{1cm} (2.10)

where $corr[.]$ is the correlation function and $x_R$ and $y_R$ are the orthogonalizations of signals $x$ and $y$ in regard to the other.

A central challenge of FC analysis is, as discussed above, the effect of volume conduction (Bastos and Schoffelen, 2016; Palva and Palva, 2012). Volume conduction itself is essential for the ability to measure neurophysiological signals from the scalp; however, it limits the separability of true interactions from artificial correlations due to linear mixing of nearby sources. While artificial correlations may be reduced by source reconstruction and using measures insensitive to zero-phase lags, such as PLI or IMC, or measures which reject the common component of a signal pair, such as oCC, the vicinity of true interactions gives rise to spurious correlations (or ghost interactions) to which no analysis measures are immune (Palva et al., 2018). Additionally, in bivariate FC analysis, the common influence of unobserved sources cannot be determined and will bias the results positively, precluding causal interpretations (Bastos and Schoffelen, 2016). Finally, directionality cannot be determined by the phase differences, due to the fact that phase is circular modulo $360^\circ$, rendering phase leads and lags inseparable (Bastos and Schoffelen, 2016).

2.7 Current state of neonatal functional connectivity studies

Since its advent by González et al. (2011), functional connectivity studies characterizing PS in EEG recordings of newborn infants have evoked substantial expectations in uncovering crucial phenomena of early brain activity and development in a powerful, quantitative manner (Vanhatalo and Palva, 2011). However, after more than a decade of these studies, we are still challenged in deriving overarching conclusions due the variety of methods and study designs employed for this goal. So what have we really learned from FC studies carried out on neonates? Specifically, what
do we know about the effect of prematurity on functional networks, the frequency- and sleep state-related changes in these networks, and, finally, the normal development of different coupling modes?

Studies identifying uniquely phase-related metrics as discussed in Section 2.6 are to this date scarce, yet provide the most specific information on PS, the most accurate mode of connectivity (Palva and Palva, 2012; Womelsdorf et al., 2007). In their pioneering work, González et al. (2011) found clear effects of developmental stage, frequency band, spatial areas, and sleep state on sensor-level PLI; most significantly, preterm-born infants showed higher PLI between homologous frontal and central areas in QS delta band as compared to healthy fullterm infants. Tokariev et al. (2019b) had similar findings measured at TEA: preterm infants had higher dwPLI compared to fullterm controls in the low frequency bands (0.7–1 Hz), driven by frontal and central regions. Intriguingly, they also showed that the networks were more dispersed in the preterm population, and that connectivity strength correlated with neurological outcomes associated with cognitive development in the 1.4–4 Hz frequency band. Yet most studies have focused on AAC relationships, and the literature on PPC-related changes in premature infants is highly insufficient.

The more often studied amplitude correlations provide a complementary mode of overall gross communication between neuronal populations on a longer temporal scale, and their organization is recently shown to be highly context-dependent. For instance, Omidvarnia et al. (2014) showed that the strength of EEG-derived oCC networks of neonates is tightly linked with the characteristic bursting–quiescence interplay of neonatal EEG: during low modes (relative quiescence) there are weak spatial correlations between sensor-level EEG signals, but during high modes (related to bursts) correlations increase across almost all electrode pairs. This effect was more widespread in preterm infants compared to fullterm controls, and notably, the effect was not present in fMRI-derived signals. Furthermore, Tokariev et al. (2019a) found clear reorganization of oCC networks between sleep states and, intriguingly, that this reorganization was attenuated in preterm infants and correlated with later visual performance at 2 years of age. Finally, preterm infants with structural brain lesions (IVH) show significantly decreased connectivity strength, expressing sparser networks with correlation to later neurodevelopment (Omidvarnia et al., 2015).

Parallel to the literature of PPC and AAC works on premature infants exists a wider set of studies using coherence-derived measures. These studies have found sleep state-, region-, and frequency-dependent networks of both increased and decreased coherence in preterm infants (de la Cruz et al., 2007; Duffy et al., 2003; Grieve et al., 2008), and associations of lower levels of imaginary coherence with favorable language outcomes (Shellhaas et al., 2022). However, coherence does not represent uniquely phase- or amplitude-related attributes of the EEG time series but a mixture of them,
rendering its interpretation challenging and susceptible to changes in the signals’ power covariance (Lachaux et al., 1999).

Sleep state-related changes of different FC modes are shown in multiple studies. For instance, Tokariev et al. (2016b) found that QS showed stronger PLV in the lowest (< 1 Hz) and highest (4–22.6 Hz) frequencies, while AS was stronger in the mid-range frequencies (1–4 Hz). In a later study, Tokariev et al. (2019b) repeated this finding by identifying stronger dwPLI in QS at low (0.7–1 Hz) and high (16–22.6 Hz) frequencies, and stronger AS connectivity from 2.8–4 Hz. In terms of AAC, Tokariev et al. (2019b, 2016b) found that the frequency content of CC/oCC networks also differs between sleep states, with higher AS connectivity at low frequencies (< 2 Hz) and prominent QS connectivity at higher frequencies (> 2 Hz) at TEA. PAC was found to be higher in QS than AS and decrease during the first postnatal weeks in both sleep states at frequencies over 10 Hz (Tokariev et al., 2016b).

Studies characterizing the normal development of FC coupling modes during the time window from the third trimester to a couple of weeks post-delivery limit to very few. The development of PLI between 28–45 weeks CA was investigated by van de Pol et al. (2018), with results of a consistent group-level decrease in global PLI across the frequency spectrum. Amplitude correlations showed robust decrease with maturation across the spectrum in QS during the first weeks after delivery (Tokariev et al., 2016b). In coherence studies, Meijer et al. (2014) found that the peak of MSC decreases with GA globally in the delta and theta band, and in visual cortices in the beta band; yet it increases with CA in frontopolar sensors in the theta band. Clearly, there is a critical gap in literature on the normal development of these functional coupling modes within the neonatal period.
3. Aims

The overall aim of this Thesis was to study multiple aspects on the development of EEG-based functional connectivity in preterm neonates and its association with clinical outcomes. The specific aims were to:

• Investigate prematurity-related effects on phase-based functional connectivity, and study the link between functional connectivity networks and later neurocognitive outcomes (Study I).

• Examine the longitudinal development of distinct modes of functional cortical coupling in neonates during early maturation (Study III).

• Assess the direct network effects of a multimodal environmental enrichment intervention, the bedside facilitation of the mother-infant emotional connection, on preterm infants during the NICU stay (Study II).
4. Methods

4.1 Data acquisition

EEG recordings of preterm and term-born neonates during daytime sleep were obtained as part of previously published cohorts (Leikos et al., 2020; Nevalainen et al., 2015; Omidvarnia et al., 2014; Rahkonen et al., 2013; Tokariev et al., 2019a,b, 2016b; Welch et al., 2012, 2014). In Study I, recordings were obtained from early preterm (EP, \(N = 46\)) infants (GA: \(26.4 \pm 1.2\) weeks, mean \(\pm\) standard deviation, SD) and term-born healthy controls (HC, \(N = 67\), GA: \(40.4 \pm 1.1\) weeks) at TEA (41.4 \(\pm\) 1.4 weeks CA). Sample descriptions are presented in Table 4.1. The study was approved by the Ethics Committee of the Helsinki University Central Hospital. Written informed consent was obtained from the parent or guardian of each subject. Recordings were performed with NicOne (Natus, USA) or Cognitrace (ANT-Neuro, Germany) EEG systems using a 19-channel EEG cap (Waveguard, ANT-Neuro) with vertex reference and sampling frequency 256 Hz or 500 Hz (resampled to 250 Hz).

Table 4.1. Background information on data used in Study I. Age and weight data are shown as mean \(\pm\) standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Early preterms (EP)</th>
<th>Healthy controls (HC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>46</td>
<td>67</td>
</tr>
<tr>
<td>GA (weeks)</td>
<td>26.2 (\pm) 1.2</td>
<td>40.4 (\pm) 1.1</td>
</tr>
<tr>
<td>CA at EEG (weeks)</td>
<td>40.8 (\pm) 1.5</td>
<td>41.8 (\pm) 1.1</td>
</tr>
<tr>
<td>Female (%)</td>
<td>30</td>
<td>32</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>880 (\pm) 200</td>
<td>3710 (\pm) 450</td>
</tr>
</tbody>
</table>

In Studies II and III, high-density EEG recordings were obtained from infants in the NICU receiving either standard care (SC) or an additional family nurture intervention (FNI). FNI is a NICU-based EE intervention,
whose aim is to facilitate the development of the early parent-infant emotional connection via engagement of mothers and their preterm infants in intimate sensory interactions, such as emotional talking paired with gentle touch, holding, exchange of scent cloths, and visual (eye-to-eye) contact (Welch et al., 2012). Sample descriptions are presented in Table 4.2. The study was approved by CUMC Institutional Review Board and written informed consent was obtained from a parent of each subject. The recordings were obtained longitudinally (1–4 recordings/infant from 33–45 weeks CA) with a 128-channel system (Electrical Geodesics Inc., USA) using vertex reference and 1000 Hz sampling frequency. Four electrodes were discarded from analysis due to location on the face. In Study II, analysis was performed on a subset of recordings measured at TEA while in Study III, the full longitudinal recordings were included. Study pipelines are summarized in Fig 4.1.

Table 4.2. Background information on data used in Studies II–III. Age and weight data are shown as mean ± standard deviation.

<table>
<thead>
<tr>
<th></th>
<th>Family Nurture Intervention (FNI)</th>
<th>Standard care (SC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>75</td>
<td>62</td>
</tr>
<tr>
<td>GA (weeks)</td>
<td>31.1 ± 2.2</td>
<td>31.1 ± 2.3</td>
</tr>
<tr>
<td>Female (%)</td>
<td>48</td>
<td>54</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>1420 ± 360</td>
<td>1520 ± 440</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Number of subjects in subset for Study II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>59</td>
<td>50</td>
</tr>
<tr>
<td>GA in subset (weeks)</td>
<td>31.0 ± 2.4</td>
<td>30.9 ± 2.5</td>
</tr>
<tr>
<td>CA at EEG in subset (weeks)</td>
<td>39.9 ± 1.6</td>
<td>40.1 ± 1.7</td>
</tr>
</tbody>
</table>

For Studies I and II, follow-up outcome scores were obtained from neurodevelopmental examinations. In Study I, newborn neurological assessment scores were measured at TEA using the Hammersmith Neonatal Neurological Examination (HNNE) (Dubowitz et al., 1999). Neurodevelopmental outcomes at two years of age were obtained with the Bayley Scales of Infant and Toddler Development – Edition III (BSID–III) (Bayley, 2006) and the Griffiths Mental Developmental Scales (GMDS) (Huntley, 1996). In Study II, BSID-III scores were measured at 18 months of age.
Methods

Study I

Study II

Study III

Subjects

Parcels

PPC

EP

HC

Parcels

FNI

SC

33 weeks

45 weeks

Data acquisition

Source reconstruction

Connectivity analysis

Group difference

Development

Figure 4.1. Methodological pipeline of Studies I–III. EEG recordings were obtained from cohorts of preterm infants and healthy term-born controls. Epochs of active (AS) and quiet sleep (QS) were extracted for analyses. The data was filtered into 21–24 narrow frequency bands and reconstructed in 8014 source signals, which were collated into 58 cortical parcel signals covering four cortical regions: frontal, central, temporal, and occipital. In all studies, FC was estimated by pairwise measures of PPC in all frequency bands and for each sleep state. In addition, in Study III, AACs, PACs (local and distant), and power signals were computed for comparison of different neuronal coupling modes. In Study I, prematurity-related network effects were studied by computing group difference in PPC networks between early preterm (EP) and healthy control (HC) infants. In addition, edge-wise correlations between PPC strength and neurodevelopmental outcomes were investigated. In Study II, the effect of a bedside intervention, FNI, was investigated by computing group difference in PPC networks between infants receiving either FNI or standard care (SC). These networks were correlated with later neurodevelopment and compared to the same HC cohort as in Study I. In Study III, longitudinal measurements between 33 and 45 weeks CA were analyzed by computing mixed-effects models for different neuronal coupling modes, PPC, AAC, and PAC, as well as signal power. Modified with permission from Studies I–III.
4.2 Signal preprocessing

In all studies, segments of EEG with no major artefacts were selected and classified into 3–5 minute epochs of active (AS) and quiet sleep (QS) visually by an EEG expert using NicoletOne Reader software (Natus, USA). The subsequent quantitative analyses were performed using Matlab R2020a (MathWorks, Natick, USA).

In Study I, EEG signals were prefiltered from 0.15–45 Hz, and only subjects with sufficient data quality in all channels were included. In Studies II and III, data was prefiltered from 0.4–40 Hz and data quality was inspected on channel level with channels of insufficient quality excluded from analysis. ECG artefacts were removed by a semiautomated approach using ICA implemented with FastICA (Hyvarinen, 1999). In this approach, the ECG-related component of the data is identified and subtracted from each channel. ICA was similarly used in Study III for detecting and removing any remaining artefacts related to electric devices.

In all studies, data was resampled to 100 Hz sampling rate and converted to average montage. Notch filtering of power line interference was also applied for individual recordings in Study III.

The data was filtered into narrow frequency bands spanning over the physiologically relevant range of interest. In Study I, the range was defined as 21 frequency bands from 0.4–22 Hz, but in Studies II and III, it was increased to 24 bands from 0.4–38 Hz. All filtering was implemented in forward and backward directions to account for phase delays introduced by the infinite impulse response filter. The central frequencies ($F_c$) were determined as $1.2 \times F_c$ with $F_c = 0.5$ Hz as the first central frequency. Cutoff frequencies were defined as $0.85 \times F_c$ and $1.15 \times F_c$, while the bandstop frequencies were implemented as $0.5 \times F_c$ and $1.5 \times F_c$. This produced frequency bands with semi-equal bandwidth and 50 % overlap on a logarithmic scale in the frequency domain. In Study III, separate filtering was performed for PAC analysis: parcel-level signals were filtered into a broad-band low-frequency ($0.4–1.4$ Hz) component, and multiple narrow-band high-frequency components ($F_c = 3.1, 5.3, 7.7, 11, 16, 23, 33$ Hz).

Source reconstruction was applied to preprocessed data in all studies to convert sensor-level signals to source level. The head model was previously developed from the magnetic resonance imaging (MRI) data of a healthy full-term infant (Odabaee et al., 2014; Tokariev et al., 2019b) using the Brainstorm (Tadel et al., 2011) and the openMEEG (Gramfort et al., 2010) software packages. The MRI data was segmented into scalp, skull, and intacranial volume, with conductivities set as 0.43 S/m, 0.2 S/m, and 1.79 S/m, respectively (Odabaee et al., 2014). The inverse operator was computed with dSPM (Dale et al., 2000). The source space was built from $N = 8014$ dipoles oriented normal to the cortex, and the parcel space was
generated from previous simulations by K-means clustering the source dipoles into \( N = 58 \) parcels (Tokariev et al., 2019b). Parcel signals were obtained by taking the weighted mean of the source signals within each parcel.

### 4.3 Connectivity analysis

In all studies, PPC, the temporally most accurate mode of FC, was estimated by pairwise computations of dwPLI (Vinck et al., 2011) between the narrow-band filtered parcel signals. In **Study I**, the resulting connectivity matrices were corrected for the suboptimal 19-electrode recording setup with a previously developed Fidelity Operator, which rejects connections that are identified as unreliable (Tokariev et al., 2019b). Briefly, the Fidelity Operator was generated with simulations where each parcel pair was consecutively set to full PS and the resulting dwPLI was computed. For each parcel pair, this was repeated 100 times, the mean dwPLI was computed over these iterations, and its 99th percentile was compared to a surrogate dwPLI value generated from simulations with no PS. The connections with mean dwPLI under the surrogate threshold were rejected from further analysis. The reliability of FC analysis is highly dependent on the number of recording electrodes (Tokariev et al., 2016a); thus, this procedure was not necessary in **Studies II and III**, which utilized recordings performed with 124 electrodes.

In addition to PPC, multiple metrics of FC and local cortical activity were computed in **Study III** to enable identification of different modes of neuronal coupling. AAC was computed using oCC (Brookes et al., 2012), where Pearson correlation is computed between signals orthogonalized with respect to each other. Orthogonalization was performed in both directions and the mean oCC was used for further analysis. PAC was estimated by computing the PLV between a broad low-frequency component and the amplitude envelope of seven high-frequency components filtered to the same low-frequency range (Siebenhühner et al., 2016; Vanhatalo et al., 2004). The upper and lower triangles of the directional connectivity matrix were averaged to obtain gross non-directional estimates of distant PAC (d-PAC), and the diagonal of the matrix corresponded to local PAC (l-PAC). Finally, power was computed as the squared amplitude envelope signal, obtained with the Hilbert transform. The visualization of brain networks was performed using BrainNet Viewer (Xia et al., 2013).
4.4 Statistical analysis

The following sections summarize the methods used in Studies I–III, which are described in detail in the respective publications.

In Study I, prematurity-related difference networks were characterized by computing group difference in PPC strength on the level of individual edges between EP and HC infants (Wilcoxon rank-sum test, \( \alpha = 0.01 \)), scanning 21 narrow frequency bands and both AS and QS sleep states. Furthermore, to assess the clinical meaning of PPC networks, edge-level values were correlated (Spearman correlation, \( \alpha = 0.05 \)) to immediate newborn neurological assessment scores (HNNE) and later 2-year follow-up neurocognitive outcomes (BSID–III and GMDS). Multiple comparisons were corrected by the adaptive false discovery rate (FDR) (Storey, 2003).

In Study II, we studied whether the PPC networks of preterm infants are affected by the FNI bedside intervention by comparing a cohort of premature infants receiving standard care (SC) to a cohort of preterm infants receiving an additional treatment of FNI (Wilcoxon rank-sum test, \( \alpha = 0.05 \)). Multiple comparisons were adjusted for using adaptive FDR. The found group-difference networks were correlated with follow-up neurodevelopmental outcomes at 18 months of age (BSID—III, Spearman correlation, \( \alpha = 0.05 \)). Similar group difference and correlation analyses were performed with measures of local maturation of functional brain age (FBA, for details, see Study II). Finally, we compared the connectivity levels of both cohorts to those of an additional cohort of HCs (Wilcoxon rank-sum test, \( \alpha = 0.05 \)). Correlation tests were FDR-corrected using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995).

In Study III, we analyzed the development of five metrics of local activity or large-scale connectivity, PPC, AAC, d-PAC, l-PAC, and power, as a function of CA from longitudinal recordings using linear mixed-effects models (LMMs), which account for the non-independence of repeated measures. First-order (linear) and second-order (quadratic) models were applied, and the model yielding the lower Bayesian information criterion (BIC) value was selected. LMMs were first computed on the level of individual edges, and then collated to global, local, or regional measures. FDR-correction was computed with the adaptive FDR and Benjamini-Hochberg methods.
5. Results

5.1 Preterm birth changes phase-based connectivity networks (I)

In this study, we investigated the impact of prematurity on PPC networks by comparing edge-level connectivity values of early preterm (EP) infants to fullterm healthy controls (HCs). The proportion of edges ($K$) was computed to describe the extent of group difference networks and plotted on a 3-dimensional brain surface to visualize their spatial distributions.

Comparison of PPC networks in EP and HC infants showed widespread frequency- and sleep state-dependent differences (Fig. 5.1). The most salient change in AS was found in theta frequencies (4–9 Hz with $K$ up to 10 % of all possible edges), where EP infants showed a vast broadband network of increased PPC strength, while an opposite peak, showing decreased connectivity in EP infants, was seen in the higher frequencies (peak at 16 Hz, $K = 8 \%$). Lower peaks ($K = 2–4 \%$) were found in delta frequencies, with subnetworks of both increased and decreased connectivity in EP infants (peaks at around 1 Hz and 3 Hz). In contrast, the QS results showed the most prominent networks in delta frequencies, where group difference in both directions was found in separate, wide-spread networks ($K = 8–10 \%$). As in AS, high frequencies present networks ($K = 8 \%$) of decreased connectivity strength in the EP group, with a similar basal organization of connections linking frontal areas to occipital and temporal lobes.

5.2 Phase-based connectivity networks correlate with immediate and long-term outcomes in preterm infants (I)

The clinical significance of edgewise PPC strength was also evaluated in Study I by correlating each edge to multiple scores reflecting immediate newborn neurology (HNNE scores) measured at TEA or neurodevelopmen-
Results

Figure 5.1. Effects of prematurity on PPC networks. **a)** The proportion of edges \((K)\) with significantly increased (red) or decreased (blue) PPC strength in a cohort of early preterm (EP) infants compared to term-born healthy controls (HCs, two-tailed Wilcoxon rank-sum test, \(\alpha = 0.01\)) as a function of frequency in AS (left) and QS (right). The grey shaded area depicts the level of false positive discoveries. **b)** 3-dimensional visualizations of the spatial organization of the FDR-corrected peak networks from (a). The color coding of edges is identical to (a). Node colours represent cortical regions (frontal: yellow, central: magenta, temporal: green, occipital: black). Reproduced with permission from Study I.

tal performance in later childhood (BSID–III and GMDS) measured at two years of age. A summary of the correlations is presented in Fig. 5.2.

We found broad networks whose strength correlated significantly with immediate newborn neurological assessment scores. The most prominent correlation was found in both sleep states at delta frequencies (AS: 1.2–3.7 Hz; QS: 1.2–3.1 Hz), where a vast network \((K \text{ up to } 14\%)\) correlated positively with cognitive outcomes. Most intriguingly, there were no positively correlating networks in the HC cohort (for details, see Study I). Smaller networks \((K < 10\%)\) of negative correlation to cognitive outcomes were found in the lowest frequencies \(<0.7\text{ Hz}\) in AS. Motor scores were associated with restricted networks at delta frequencies in both sleep states (AS: positive correlation at 2.6–3.1 Hz; QS: positive correlation at 3.7 Hz, negative correlation at 1.5–1.8 Hz).

Neurodevelopmental scores in later childhood showed significant correlations with spatially constrained networks at narrow frequency bands. The lowest delta frequencies \(<0.7\text{ Hz}\) depicted positive correlation with visual scores in AS and negative correlation with cognitive and language scores across sleep states. Mid-delta frequencies (1.2–1.5 Hz) showed positive correlation to motor outcomes in both sleep states, while high-delta (2.1–2.6 Hz) indicated negative relationship to visual scores in AS. The highest frequencies \((>6\text{ Hz})\) from high-theta to low-beta showed negative correlations to visual, cognitive, and language scores uniquely in AS.

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5.3 Preterm brain networks are improved by supporting the parent-infant emotional connection (II)

The focus of this study was to investigate the direct brain network effects of FNI, a bedside intervention aiming to improve the parent-infant emotional connection during the NICU stay by engaging mothers and their preterm infants in multiple emotional interactions. To achieve this aim, we compared PPC networks of FNI and SC cohorts, and assessed the clinical impact of the findings.

The group difference analysis (Fig. 5.3) revealed extensive networks with reduced PPC strength in the FNI group across a broad range of frequencies (5–16 Hz, K up to 12 %). The effect was salient in both sleep states and peaked at around alpha frequency (11 Hz). These alpha networks had, however, different topologies across sleep states: the network in AS showed denser connections in the right hemisphere spanning between central and frontal cortices, while the network in QS had additional connections to occipital areas and connected inter-hemispheric central and temporal regions (for further details, see Study II). Secondary peaks were found at theta (5.3 Hz) and delta (0.5, 1.8, and 3.1 Hz) frequencies, of which only the 1.8 Hz network showed increased connectivity in the FNI cohort.
Intriguingly, no differences in local neuronal maturation, assessed by FBA, were found between cohorts.

The most prominent group difference networks at alpha frequency (11 Hz) were investigated further to evaluate their clinical significance (Fig. 5.4). First, we correlated the strength of these networks with follow-up scores of neurodevelopmental outcomes (BSID–III) measured at 18 months of age. We found that lower PPC strength in these networks was associated with favourable cognitive and language outcomes (AS: cognitive, \(P = 0.006\) and \(\rho = -0.39\); QS: cognitive: \(P = 0.03\) and \(\rho = -0.31\); language: \(P = 0.02\) and \(\rho = -0.42\)). Finally, we compared both cohorts to an additional group of full-term HC infants. Strikingly, we found that the cohort receiving FNI treatment did not differ from HC infants (AS: \(P = 0.5\) and \(r = 0.08\); QS: \(P = 0.08\) and \(r = 0.19\)), while the SC cohort showed significantly increased levels of PPC strength (AS: \(P < 0.001\) and \(r = 0.66\); QS: \(P < 0.001\) and \(r = 0.73\)).
Results

**Figure 5.4.** Clinical significance of the FNI-related network effects. **A)** Correlation of mean PPC strength in the alpha networks (left: AS, right: QS) to cognitive and language outcome scores (BSID–III) assessed at 18 months of age (two-tailed Spearman correlation). Asterisks indicate correlations which passed correction for multiple comparisons (Benjamini-Hochberg). **B)** Comparison of preterm cohorts (FNI and SC) to full-term healthy controls (HC). Raincloud plots show the distributions of mean PPC strength in the alpha network within cohorts in AS (left) and QS (right). Medians are depicted with a horizontal line and the interquartile range with darker shading. Group difference is shown between HC–FNI and HC–SC (two-tailed Wilcoxon rank sum test, effect size (r): rank-biserial correlation). All presented HC–SC comparisons passed multiple comparisons correction (Benjamini-Hochberg). *n.s.* stands for non-significant. Reproduced with permission from Study II.

5.4 Development of functional connectivity during the neonatal period is characterized by distinct trajectories of cortical coupling modes (III)

In this study, we aimed to decipher how functional networks develop through the neonatal period. We studied this question by analysing the development of five complementary mechanisms of neuronal communication and activity, PPC, AAC, d-PAC, l-PAC, and power, in a large longitudinal cohort of preterm infants with high-density scalp EEG-recordings. A collation of selected results is presented in Fig. 5.5.

PPC development is characterized by subnetworks of both increasing and decreasing connectivity strength. The most extensive change is a broadband decrease ($P < 0.05$) of large-scale networks involving all regions of the brain. Peaks occur in both sleep states at mid-delta (1.8 Hz), high-
Results

Figure 5.5. Development of PPC, AAC, and l-PAC networks. a) $K$ plots depicting the proportion of connections with significant increase (red) or decrease (blue) in PPC strength as a function of CA (first order LMM, $P < 0.05$) in AS (left) and QS (right). The grey shaded box depicts the FDR level. Below are the spatial organizations of FDR-corrected networks at peak frequencies. Node colouring denotes cortical region (frontal, F: yellow; central, C: magenta; temporal, T: green; occipital, O: black). Mean adjusted $R^2$ values of the significant networks are presented below each brain plot. b) Scatter plots showing the trajectories of global AAC as a function of CA (first- or second-order LMM based on BIC value) in the same peak frequencies as in (a) in AS (top) and QS (bottom). Corrected $P$-values (Benjamini-Hochberg) and adjusted $R^2$ values of the interactions are presented above each plot. c) Regional l-PAC interaction as a function of CA (first- or second-order LMM based on BIC value) in four cortical regions (F, C, T, O) is presented for five high frequency bands in AS (top) and QS (bottom). The fitted functions are presented as dark lines with shaded areas depicting the confidence intervals of fixed effects. Vertical ticks on the x-axis denote the age with peak connectivity in regions with second-order interaction. Asterisks indicate tests which passed multiple comparisons correction (Benjamini-Hochberg, corrected $P < 0.05$). Minimum and maximum adjusted $R^2$ values of the interactions are presented above each plot.
Results

delta (3.7 Hz) and beta (33 Hz) frequencies. On the contrary, there are spatially constrained networks of increasing PPC strength at low-delta (< 1 Hz), mid-delta (2.1 Hz), and alpha (11 Hz) frequencies. The increasing mid-delta network is more salient in QS, involving connections within frontal regions with projections to central and occipital areas, while the alpha network was present only in AS, with connections from frontal to central, temporal, and occipital regions (for details, see Study III).

AAC networks demonstrate a robust developmental decrease \((P < 0.001)\), which is present on the global level, involving nearly all connections across the spectrum. Only frequencies < 1 Hz in AS show increasing AAC strength. The decrease is linear in all except the alpha frequencies (8–13 Hz), which show a quadratic relationship with age that approaches a plateau near TEA. The decrease of AAC led to wonder, whether concomitant changes in power could explain the effect. However, results from a similar developmental analysis with power show that power increases \((P < 0.05)\) with age across the spectrum (for details, see Study III), and that AAC and power values showed only few significant correlations in the lowest frequencies (< 1 Hz) in AS and either no correlations or negative correlations in QS. Analysis of d-PAC showed a similar global decline with CA as well as correlations between d-PAC and AAC values, albeit with considerable individual variance, being compatible with the idea that AAC and d-PAC depict different underlying mechanisms (for details see Study III).

Finally, networks of l-PAC reveal region- and sleep state-specific trajectories of development. Analysis in AS show a linear decline of l-PAC levels in all regions and frequencies. On the contrary, in QS, there are prominent quadratic developmental trajectories especially in occipital, central, and temporal regions. Here, l-PAC levels show an increase until a region-specific peak (36–39 weeks CA), followed by a prominent decrease. Finally, frontal regions show either a slight increase (3.7–7.7 Hz; \(P < 0.05\)) or no significant change (> 7.7 Hz; \(P > 0.05\)) as a function of age.
6. Discussion

Preterm birth disrupts a critical period of cortical development and is associated with lifelong neurocognitive sequelae; yet, the development of the underlying functional cortical networks, and the effect of prematurity on them, are poorly understood. This Thesis studied early brain development in preterm infants using FC analysis methods adapted to the physiological and technical requirements unique to neonatal brain function. In Study I, we identified prematurity-related differences in large-scale PPC networks and found associations of PPC strength to newborn and long-term neurocognitive performance. In Study II, we used FC analysis as a method to assess the impact of the FNI bedside intervention on preterm brain networks, finding clinically significant effects, which even rendered the networks of the intervention cohort comparable to full-term healthy controls. Finally, in Study III, we showed that the different cortical coupling modes have distinct developmental trajectories through the neonatal period, providing developmental models, or functional growth charts, for future scientific work.

This work showed that preterm birth inflicts changes in frequency-specific PPC networks, with distinct spatial distributions and dependency on sleep state, which is consistent with the recent evidence that brain function is dependent on multiple concomitantly existing PPC networks (Siebenhühner et al., 2016; Vidaurre et al., 2018) and that the different sleep states incorporate distinct spatial network organizations (Tokariev et al., 2019a,b, 2016b). Our findings showed similar increased levels of PPC in preterm infants at delta frequencies as reported earlier (González et al., 2011; Tokariev et al., 2019b), and extend this literature by identifying spatially constrained networks in this frequency band and in higher bands (theta, alpha, beta). This is well compatible with the idea that the current optimized methods for neonatal FC analysis are able to disclose more subtle differences than have previously been achievable. Our study also showed significant clinical relevance of preterm PPC networks as associations to outcome measures of neurological performance at TEA and long-term neurocognitive performance at two years of age, most promi-
nently in delta frequencies, but present also in the lowest and highest frequency bands as reported earlier (Tokariev et al., 2019b). Intriguingly, correlations to immediate neurological performance were absent in the HC cohort, which showed only few small networks with negative correlation. This somewhat unexpected result poses exciting avenues for future research. For instance, could this finding be mechanistically explained by the well-established link of neural activity networks to diffuse WMIs prevalent in preterm infants (Back, 2017; Guo et al., 2017; Volpe, 2019), which would amplify the association between network function and immediate neurological performance in the preterm infants? Alternatively, would the effects reflect a genuine difference that remains to later childhood or a delayed maturation of brain function in the preterm cohort, which would catch up at later age?

Early functional brain development is guided by rich endogenous activity and in utero external stimuli (Luhmann et al., 2022; Molnár et al., 2020; Wallois et al., 2021), which are undoubtedly altered when a preterm infant is exposed to the unnatural ex utero environment of the NICU (Lagercrantz et al., 2010). Attempts to improve preterm neurodevelopmental care have evoked multiple strategies designed to modify the NICU environment via various EE approaches (Beltrán et al., 2021; Filippa et al., 2020; Guzzetta et al., 2009; Haslbeck et al., 2020; Lordier et al., 2019; Mclean et al., 2021; Moore et al., 2016; Vitale et al., 2021; Welch et al., 2012); yet, their evidence-based development has lacked investigation of their effects on the therapeutic target, functional brain networks. We showed that FNI, a multimodal EE intervention employing bedside facilitation of the mother–infant emotional connection, has large-scale effects on the functional networks, but not on local neuronal activity, of preterm infants. Further, these effects correlate with neurodevelopmental outcomes in later childhood. Most strikingly, the FNI-related reduced PPC levels rendered the cohort receiving FNI comparable to healthy full-term infants, which was in sharp contrast to the control cohort receiving standard clinical care. The findings together suggest that supporting the parent-infant contact, the most fundamental contact of the infant’s life, has widespread and long-term effects on the infant’s neurodevelopment, disputing the need for external sensory enrichment practices in the NICU. Future work would necessarily require replication of these findings in similar cohorts. Mechanistically, it is challenging to explicitly harness the direct effects of FNI, yet the current findings would be compatible with the explanation that FNI is mediated indirectly by the physiological stabilization of the infant, leading to restoration of WM abnormalities, which have been shown to considerably affect neuronal synchrony by altered conduction velocities (de Faria et al., 2021; Noori et al., 2020).

Despite well-mapped developmental trajectories of the physical organization of brain networks (Vanhatalo and Kaila, 2006; Wallois et al., 2021), the
emergence of functional networks within the structural connectome have been poorly understood, despite their established relevance in early brain development (Molnár et al., 2019, 2020) and association to higher cognitive functions in the adult brain (Palva and Palva, 2018, 2011). Preterm infants provide an attractive population for studying this question. Our work shows that specific neuronal coupling modes follow distinct trajectories, highlighting the need to consider them separately. Previous studies conducted at different time intervals have shown decreases in PPC, AAC, and PAC networks (Tokariev et al., 2016b; van de Pol et al., 2018), which are all found within the growth charts provided in our work. However, our findings extend this literature by presenting extensive frequency-, sleep state- and region-specific development of different coupling modes. The temporally accurate PPC networks show a widespread global decrease, concomitantly with an increase in the spatially constrained frontally connected networks. On the contrary, the development of AAC networks, which operate on a temporally longer scale, manifests as a robust global decrease throughout the frequency spectrum. Finally, multifrequency l-PAC networks exhibit linear decrease in AS but spatially selective biphasic development in QS at central, temporal, and occipital areas, peaking at a regionally specific time just before TEA. The results fit well into the existing framework which characterizes neonatal EEG development as a transition from intermittent burst-like activity to mature continuous oscillations. The overall decreasing global AAC is consistent with the established decrease in synchronization of spontaneous activity patterns in response to changes in the developing cortical circuitry (Molnár et al., 2020). The region-specific biphasic development of l-PAC shows detailed multifrequency interactions within cortical regions, possibly originating from interplay of the subplate and cortex, which are considered to relate to the activity-dependent organization of sensory and motor areas that develop at different rates (Blumberg et al., 2022; Colonnese et al., 2017; Molnár et al., 2020). Finally, increasing PPC in frontal areas would most saliently be explained by the increase in early continuous activity in frontal regions, which are associated with the emergence of higher cortical functions (Kolk and Rakic, 2022).

The findings of this Thesis together highlight the complexity of early FC networks, which have been shown to be strongly context-dependent in several recent works (González et al., 2011; Omidvarnia et al., 2014; Tokariev et al., 2019a,b, 2016b). Intriguingly, the results of this Thesis suggest that decreased connectivity would often relate to more mature networks. For instance, Study II showed that decreased FNI-related networks in alpha frequencies relate to favourable neurodevelopmental outcomes. Study III showed a general direction of decreasing connectivity as a function of CA, albeit with several coupling mode-specific networks of increasing connectivity. Study I showed multiple networks of both
increased and decreased connectivity in preterm infants, yet associations with outcomes in the HC cohort were uniquely negative, pointing perhaps to the same general decrease of connectivity levels. This decrease is in line with the general decline of SAT events, which manifest as immature EEG in preterm infants. Future work should address whether the distinction between preterm infants to their fullterm counterparts arises from permanently altered networks inflicted by brain lesions, or whether the underlying effect is a transient dysmaturity. Answering the latter alternative would inform us on the generalizability of the growth templates presented in Study III on normal in utero development, which cannot be directly measured by EEG. Additionally, the current studies focus on quantifying FC within narrow frequency bands (with the exception of PAC). However, investigating the cross-talk between frequencies could potentially build a more comprehensive view of functional coordination in the neonatal brain and should be pursued in the future.

Limitations of the present Thesis include technical and patient population related considerations. First, the bivariate (pairwise) connectivity analysis is evidently a simplified view of the multivariate brain network activity; however, bivariate measures were used as they provide readily interpretable results, with established relevance to underlying neuronal mechanisms (Cohen, 2014; Engel et al., 2013; Wallois et al., 2021). Second, as no methods are completely immune to “ghost” interactions (Palva et al., 2018), some spatial blurring is unavoidable despite the stringent efforts to reduce it by our source-level analyses. Finally, Study III used the brain activity of prematurely born infants as a proxy to normal in utero brain development. While studies have indicated no qualitative effects of preterm birth on the overall course of EEG development (André et al., 2010; Shany et al., 2014), it cannot be ruled out that the initial prematurity of the infants might have affected the network measures at the later time points. Additionally, preterm infants are a heterogeneous population (Dimitrova et al., 2020; Tokariev et al., 2019b), which was accounted for by the LMM analysis. Nonetheless, preterm infants provide arguably the most direct means of studying the development of brain activity, compared to the options of in utero MEG or fMRI recordings.

Analysis of FC provides a method for systems-level analysis of temporally coordinated inter-areal brain communication. In the context of neonates, the arguably most direct method to analyze it stems from recordings of direct brain activity, in this age group practically EEG. FC measures have been shown to be clinically relevant and correlate with later neurocognitive outcomes, both in this Thesis and elsewhere (Omidvarnia et al., 2015; Stevenson et al., 2020; Tokariev et al., 2019a,b; van de Pol et al., 2018). However, it is evident that there are significant individual differences, especially in the heterogeneous preterm population (Dimitrova et al., 2020; Tokariev et al., 2019b), as well as large disparity in both recording and
analysis methods, currently precluding collection of reference values for individual clinical evaluations. Rather, FC analysis would currently be most beneficial for evaluating the efficacy of clinical interventions on neurodevelopmental care, as presented in Study II, and for providing insight to multiple developmental mechanisms in basic research (Study III), or between clinical populations (Study I). Currently, great attention must be paid to unifying methods in EEG-based FC studies for improving comparability and reproducibility of overarching conclusions.
This Thesis studied the early functional brain networks of preterm infants with EEG-based FC analysis, addressing changes in functional networks related to prematurity, the possibility to improve these networks by a bedside intervention, and the development of different cortical coupling modes through the neonatal period. The results together show that the functional organization of the neonatal brain is affected by preterm birth, yet these changes may be overcome by supporting the development of the natural parent-infant emotional contact. Finally, the growth charts presented in the Thesis describe extensively the development of different cortical coupling modes, underscoring the need for careful methodological considerations in both the performance and interpretation of FC analysis. Future endeavours in the field must include unification of methodological implementations, in order to improve reproducibility of findings and to identify the clinical use cases of FC analysis. As suggested in this Thesis, these use cases would most likely lie in the evaluation of the efficacy of clinical interventions and in studies of basic research or between different clinical populations.
References


References


References


Electroencephalographic functional connectivity analysis in preterm infants

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