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**Co-regulating with your child: exploring parent-infant
Interpersonal Neural Synchrony after an interactive
rupture**

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ABSTRACT

During the first thousand days, the infant's ability to iteratively co-regulate their neurophysiological states with the primary caregiver – better known as interpersonal neural synchrony (INS) – is believed to be a neuromarker for social interaction. Electroencephalograph (EEG) hyperscanning is a novel experimental paradigm that allows the simultaneous recording of neural activity between two interactive partners. Recent studies showed that behavioral synchrony in parent-infant dyads is established through repeated cycles of matched and mismatched states, which can be observed during the Face-to-Face Still-Face (FFSF) procedure. While the literature highlighted significant associations between INS and a series of interactive behaviors, no study to date has assessed changes in INS during experimentally induced interactive ruptures. The present dissertation aims at assessing how directed and non-directed INS between parents and infants changes before and after an interactive rupture. Additionally, the study assessed whether arbitrary decision-making during dual EEG data pre-processing impacted measures of INS. Parents and their nine-month-old infants had their brain activity measured via the EEG hyperscanning paradigm while they engaged in an adapted FFSF procedure. Interactive behaviors were microanalytically coded in Noldus. INS was computed using cross-frequency phase locking value (PLV; see Chapter 3), partial directed coherence (PDC; see Chapter 4), imaginary coherence (ICoh) and weighted phase lag index (wPLI; see Chapter 5). Our studies revealed the following: 1. The FFSF produced significant changes in PDC directionality and increases in wPLI from Play to Reunion; 2. Arbitrary decision-making during EEG data pre-processing has limited impact on INS metrics such as PLV; 3. Parent-infant INS is significantly correlated with dyadic behaviors, and 4. Significant associations between INS and dyadic behaviors were focused in fronto-posterior and centro-frontal clusters. The findings provide preliminary evidence regarding the association between INS and dyadic reparations, and reinforce the notion that biobehavioral synchrony and INS can mutually facilitate one another.

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PREFACE

The present dissertation is mainly composed of a collection of published, unpublished and submitted papers related to the 2-Brain Regulation to Achieve Improved Neuroprotection during Early Development (2-BRAINED) research project, i.e. the main project I have been collaborating on during my doctoral work. The 2-BRAINED project has been funded by the Italian Ministry of Health under the Ricerca Finalizzata 2021 program (project code: GR-2021 12375213) and it is part of the MNESYS partnership under the Spoke 1 (Neurodevelopment, Cognition and Social Interaction). The structure of the dissertation is provided below.

Chapter 1 describes the theoretical framework of the 2-BRAINED project and the aims that guided the studies presented in the subsequent chapters. Chapter 2 (adapted from Billeci et al. 2024, doi: 10.3389/fpsyg.2024.1516616) contains the details regarding the enrolment of the participants, the materials, the experimental procedure and the plan of analysis used in the studies described in the subsequent chapters. Chapters 3 (adapted from Pili et al. 2025, doi: 10.1016/j.jneumeth.2025.110400), 4 (adapted from Pili et al., *submitted to NeuroImage on October 9th, 2025*) and 5 (original production) contain the experimental studies conducted to tackle experimental aims 1, 2 and 3 respectively (see Chapter 1 for details). Finally, Chapter 6 contains a general discussion of the results described in the previous chapters and provides the limitations and future directions stemming from this study. The Appendix provides the original articles from which Chapter 2, 3 and 4 were adapted.

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CHAPTER 1 – INTRODUCTION AND OBJECTIVES

Chapter 1 provides the theoretical framework, the state of the art in the field of parent-infant biobehavioral and neural co-regulation during interactive exchanges, as well as the objectives, specific aims and structure of the present dissertation.

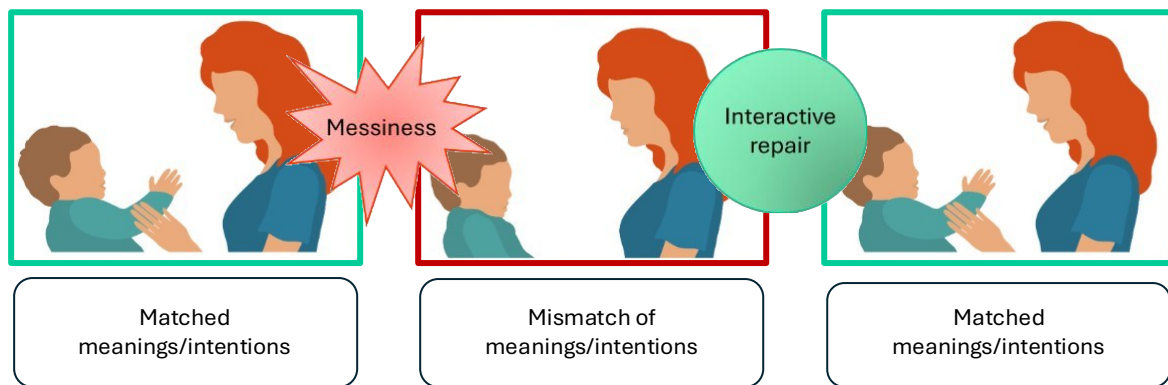
1. INTRODUCTION

1.1. *The first thousand days*

Growing bodies of research have highlighted how the “first thousand days” of child development, a catchphrase used to describe the period going from the conception to the second year postpartum ([Moore et al. 2017](#)), have a crucial impact on life-long health, well-being and learning capabilities of individuals ([Capitani et al. 2023](#)). During this timeframe, the human brain is particularly sensitive to the quality of the environment and living experiences, which in turn may shape the developing brain of infants through processes of neuroplasticity ([Montirosso and Provenzi 2015](#)).

In the first thousand days, the quality of environment and living experiences depends on the quality of the relationship with the primary caregiver ([O'Connor 2003; Swain et al. 2007](#)), a role which is taken up by the biological mothers of the infants in the majority of modern societies and cultures. Particularly, maternal sensitivity ([Salter Ainsworth et al. 2015](#)), the ability to appropriately respond to infants’ signals and needs, is of fundamental importance in fostering infants’ cognitive ([Malmberg et al. 2016](#)), socio-emotional ([Kivijärvi et al. 2001; Provenzi et al. 2015](#)) and regulatory capacities ([Conradt and Ablow 2010](#)), as well as peer relationships, school achievements and behavioral adaptation during later childhood and adolescence ([Endevelt-Shapira and Feldman 2023; Provenzi et al. 2018](#)). Despite the importance of this concept in understanding the mechanisms of infant attachment ([Behrens et al. 2016](#)), there is repeated evidence that mother-infant interactions are not of unidirectional nature, but rather a fully bidirectional exchange in which the infant is capable of being an active social partner ([Brazelton et al. 1975; Murray and Trevarthen 1986; Stern 2018; Tronick et al. 1978](#)). Starting from very early such as the third month of life, infants are capable of engaging in rhythmic face-to-face interactions with their primary caregiver, made not only of mutual gazes, co-vocalizations, and matching of affective expressions ([Feldman et al. 2011; Stern 2018; Tronick 2018](#)), but also of instances of behavioral and affective mismatches ([Feldman 2007; Provenzi et al. 2018](#)), an idea that is based on Gianino and Tronick’s Mutual Regulation Model (MRM; [Gianino and Tronick 2013](#); see Figure 1.1.).

Figure 1.1. The Mutual Regulation Model.



This iterative process of intermodal temporal coordination of verbal and non-verbal communicative and emotional behaviors between the infant and the parental figure is commonly referred to as “synchrony” (Leclère et al. 2014), and it has been repeatedly associated with positive long-term developmental outcomes such as emotion regulation and maturation of the social brain (including empathy) up to adulthood (Feldman 2007; Leclère et al. 2014; Turk et al. 2022). Contrarily, disruptions occurring during mother-infant interactions, such as maternal intrusiveness (Feldman et al. 2009), maternal postpartum depression or infant early life stress (Pratt et al. 2017; Levy et al. 2019; Feldman 2020) can have detrimental effects on infant socio-emotional development.

Following this framework, Feldman (2007) distinguished between three different prototypes of behavioral synchrony: non-directed (or concurrent), directed (or sequential), and organized. Synchrony is defined “non-directed” when the two members of the dyad are temporally matching their behavioral and affective states, whereas it is defined “directed”, or sequential, when the nature of the interaction entails a leader-follower dynamic. Organized synchrony, on the other hand, is reached when parents and infants are responsive to each other’s behavioral and affective states, and requires the infant to be able to engage in social interactions independently from the parental figure’s scaffolding strategies. All three types of synchrony can be observed in ecological parent-infant social interactions starting from the third month of age, although the “organized”, or “mutual”, type of

synchrony is more apparent from the infant's 9th month of age due to emergence of intersubjectivity ([Feldman 2007](#); [Stern 2018](#)).

According to previous research from Tronick and colleagues ([Tronick 2018](#); [Tronick and Cohn 1989](#)), parents and infants do not spend most of their playtime in temporally matched states, but rather in “repairing” miscoordinated behaviors and affective states. In light of this, it is believed that the central component of synchrony between parents and infants could be the constant attempts of the two members of the dyad to “repair” and “synchronize” their behavior ([Feldman 2007](#)), rather than the ability to stay constantly “in sync” with one another. For this reason, developmental neuroscientists and psychologists have started to study parent-infant synchrony by observing how the dyad repairs the interaction after experimentally induced interactive perturbations. In infants aged 0-9 months, one of the most effective experimental paradigms that allows to observe this dynamic interplay of mismatches and reparations during dyadic social interactions is the Face-to-Face Still-Face Paradigm (FFSF; [Tronick et al. 1978](#)), a procedure which presents an interactive perturbation during an ecological parent-infant interaction to purposefully withhold all the contingent responses from the parental figure that could promote synchronous behavior and affective states in the dyad.

1.2. The Face-to-Face Still-Face Paradigm: how the dyad “repairs” messiness

The FFSF paradigm was originally introduced by Tronick and colleagues ([1978](#)) to observe the active contribution of infants during social interactions ([Mesman et al. 2009](#)). Parents and infants are asked to engage in a three-step dyadic interaction consisting of the following episodes: a one-to-three minute baseline (called “Play” episode) in which the parent is instructed to play/interact with the infant “as they normally would”, a Still-Face episode (SF; 1-3 min) during which the parent is asked to stop responding to the infant and instead look at them with an emotionless (“still”) facial expression, and finally a resumption of the playful interaction (called “Reunion” episode). According to previous studies (see [Mesman et al. 2009](#) for a comprehensive overview), the “still-face effect” in infants is characterized by a decrease in gaze and in positive affect and a subsequent increase in negative affect from the baseline to the SF episode, with only a partial recovery during the reunion

episode ([Adamson and Frick 2009](#); [Tronick et al. 1978](#)). Additional changes in infant behavior from baseline to SF episode and reunion include increase in motor activity ([Jamieson 2004](#); [Lamb et al. 1987](#); [Sherilyn Adler and Tiffany 2024](#)), self-regulatory strategies such as tactile-, object-, and self-stimulation ([Moszkowski 2004](#); [Rosenblum et al. 2002](#); [Tronick 2005](#)), and increase in physiological indicators of stress such as heart rate (from baseline to SF episode only; see [Haley et al. 2006](#); [Ham and Tronick 2006](#); [Weinberg and Tronick 1996](#) and cortisol levels (see [Mesman et al. 2009](#); [Provenzi et al. 2016](#)). In some studies, these behavioral changes were found to be moderated by infant demographic characteristics such as age at assessment ([Mesman et al. 2009](#)) and gender ([Braungart-Rieker et al. 2001](#); [Mayes and Carter 1990](#); [Toda and Fogel 1993](#); [Weinberg et al. 1999](#)), infant temperament ([Conradt and Ablow 2010](#)), as well as by responses to maternal behavior ([Mastergeorge et al. 2014](#); [Mesman et al. 2013](#)). Maternal nurturing behaviors, such as affective touch, were also found to reduce the still-face effect ([Stack and Muir 1990](#); [Stack and Muir 1992](#)). Mesman and colleagues ([2009](#)) argue that the still-face effect could be ascribed not only to the lack of parental contingent responses, but most and foremost by the unexpected combination of the parent's infant-directed gaze and unresponsiveness. This hypothesis has been confirmed by previous studies that investigated the differential impact of facial, tactile and vocal cues on the still-face effect in infants, finding that the loss of facial and tactile cues were the factors that contributed the most to the still-face effect ([Gusella et al. 1988](#); [Legerstee and Markova 2007](#); [Stack and LePage 1996](#); [Stack and Muir 1990](#)). Despite the extensive evidence on the still-face effect in infants, stability of the aforementioned behavioral and physiological responses across different ages is still debated ([Mesman et al. 2009](#)).

In the last decade, there has been an increased interest in investigating the neurophysiological correlates of the still-face effect, and particularly the effects of the SF episode on maternal and infant frontal alpha asymmetry. Frontal alpha asymmetry (FAA) describes the neurophysiological underpinnings of emotion regulation processes, and it measures the difference between left and right hemisphere frontal activation in the *alpha* frequency band ([Killeen and Teti 2012](#); [Smith et al. 2017](#);

[Swider-Cios et al. 2024; Swingler et al. 2014](#)). Positive values indicate a “leftward” FAA and are associated with positive emotional states and approach motivation, whereas negative values correspond to a more “rightward” FAA and are related to negative emotional states and avoidance ([Coan and Allen 2004; Davidson 1993; Davidson 2004; Perone et al. 2020](#)). To date, FAA was employed as an outcome measure of the FFSF procedure in two exploratory studies conducted in infants aged 6-12 months engaged in an adapted version of the FFSF containing one additional SF episode and reunion ([Perone et al. 2020; Swider-Cios et al. 2024](#)). Findings from these two studies are, however, contrasting. Perone and colleagues ([2020](#)) have found a more rightward FAA during the FFSF, especially in dyads in which the mother was behaviorally coded as more responsive; Swider-Cios and colleagues ([2024](#)), on the other hand, have found a still-face effect only at the behavioral level, whereas no still-face effect was found in the neural data. Despite this, they have found a significant association between infant negative affect and leftward FAA during the two SF episodes, whereas mothers showcased more rightward FAA only during the first SF episode ([Swider-Cios et al. 2024](#)).

The FFSF is considered a viable experimental procedure not only to estimate the neurophysiological correlates of emotional processing, but also to study the neural underpinnings of social bonding between parents and infants. The most widely used and child-friendly experimental paradigm used to acquire simultaneously the neurophysiological data of parents and infants engaged in a social interaction is the hyperscanning paradigm ([Turk et al. 2022](#)), which has been increasingly employed in the last decade by developmental neuroscientists from different groups worldwide.

1.3. Hyperscanning: a privileged standpoint to observe dyadic co-regulation

The hyperscanning paradigm is defined as the simultaneous recording of brain activity in two or more individuals ([Czeszumski et al. 2020](#)). It can be performed with a variety of techniques such as functional magnetic resonance imaging (fMRI; [Montague et al. 2002](#)), magnetoencephalography (MEG; [Czeszumski et al. 2020; Hirata et al. 2014](#)), functional near-infrared spectroscopy (fNIRS; [Miller et al. 2019](#)) and, finally, electroencephalography (EEG; [Toppi et al. 2016](#)). Among these, the

one that has been designated as the most apt to capture the dynamic and fast-paced nature of parent-infant interactions is the EEG ([Turk et al. 2022](#)). Other than being a non-invasive technique for brain signal acquisition, EEG is vastly available in wireless, infant-friendly systems (e.g. mBrainTrain) that allow for brain activity recording in both experimental and naturalistic, ecological settings.

Following the biobehavioral synchrony model, which posits that alignment between the biological responses of parents and their infants may foster effective communication, emotion regulation and social bonding in infants ([Bi et al. 2023](#)), hyperscanning emerged as the preferential paradigm to study the neural underpinnings of parent-infant social interactions ([Provenzi et al. 2023](#)). Estimation of biobehavioral synchrony is allowed through computation of a construct that has been named Interpersonal Neural Synchrony (INS; [Koul et al. 2023](#)), defined as the temporal alignment of brain activity between two interactive partners. INS can be measured with a vast array of connectivity metrics, which have been divided into two macro-categories in line with the types of behavioral synchrony described by Feldman ([2007](#); see above). The first category of INS indexes is represented by non-directed, also known as “concurrent”, metrics, and measure the similarities between the parent and the infant neural activity throughout time. Like its behavioral counterpart, non-directed INS assumes that the neural synchrony between parents and infants is mutual and matched in nature. Among the non-directed INS metrics, a further distinction can be made between the phase-based and the amplitude-based connectivity metrics, which can be used to describe different aspects of parent-infant neural co-regulation ([Turk et al. 2022](#)). Phase-based metrics are inherently non-stationary and rely on the assumption that two signals with a consistent (non-directed) delay in phase reset can be considered “synchronized” ([Ayrolles et al. 2021](#)). As phase resets occur in a fine-grained timescale, in the order of milliseconds ([Zamm et al. 2024](#)), phase-based indices are recommended when the main focus of the research is to obtain high-precision temporal information about ongoing cognitive processing ([Ayrolles et al. 2021](#)). Examples of non-directed phase-based INS metrics include the Phase Locking Value (PLV; [Lachaux et al. 1999](#)), the Phase Lag Index (PLI; [Stam et al. 2007](#)), the weighted Phase Lag Index (wPLI; [Vinck et al. 2011](#)) and the Circular Correlation (CCorr; [Burgess](#)

[2013](#)). An overview of the specific assumptions and relative limitations of these indices is available on Table 1.1. Amplitude-based metrics, on the other hand, define INS as the coupling (correlation) of amplitude envelopes between two oscillation frequencies, which has notably been observed during cognitive functions ([Palva and Palva 2018](#)). Compared to phase resets, amplitude coupling is independent of neuronal spike-time relationships and changes at a slower rate, which makes it ideal to study the similarities in cognitive processing between the interactive partners on a larger timescale ([Palva and Palva 2018](#); [Turk et al. 2022](#); [Zamm et al. 2024](#)). Amplitude-based metrics are represented only by one index – the amplitude-amplitude coupling (AAC; [Palva and Palva 2018](#); see Table 1.1 for details). An additional sub-category of non-directed INS indices is constituted by coherence metrics, which account for both phase and amplitude ([Ayrolles et al. 2021](#); [Zamm et al. 2024](#)). An example of non-directed coherence metric is the Imaginary Coherence (ICoh; [Dikker et al. 2021](#); [Nolte et al. 2004](#)), which normalizes the imaginary component of the cross-spectrum by the signal amplitudes (see Table 1.1 for details). Regardless of whether they are based on phase, amplitude or both, all non-directed INS metrics have the advantage of supporting a “cross-frequency” approach to INS computation, which (albeit dramatically underrepresented in the current literature) is deemed particularly valuable when dealing with subjects at different developmental stages and with different frequency peaks such as infants and adults ([Marriott Haresign et al. 2022](#); [Noreika et al. 2020](#); [Turk et al. 2022](#)).

Together with non-directed INS, the second type of INS metrics are called the directed, or sequential, measures, and include all the connectivity measures that are able to capture “lead and follow” dynamics during a social interaction. Indeed, directed INS metrics are based on the assumption that the two interactive partners do not influence their neural activity in a bidirectional fashion; rather, there is a member of the dyad whose neural activity is “framing”, or “causing”, the neural activity of the other partner ([Feldman 2007](#)). This means that, contrary to non-directed INS, directed indices assume an asymmetric flow of information between the interactive partners, in which the connectivity from parent to infant is different from the one from infant to parent ([Zamm et al. 2024](#)). Directed INS

metrics include the Time-lagged Cross correlations ([Wass et al. 2018](#)), which examine how the changes in one time series precede (or follow) the changes in the other time series, as well as the Granger causality (GC; [Granger 1969](#)) and the Partial Directed Coherence (PDC; [Baccalá and Sameshima 2001](#)), which are computed from the multivariate autoregressive (MVAR) coefficients obtained from fitting the autoregressive models to the time series (GC) or to the raw amplitude data in the frequencies of interest (PDC; [Baccalá and Sameshima 2001](#); [Turk et al. 2022](#)). Except from the Time-lagged Cross Correlations, neither GC nor PDC support a cross-frequency approach. An overview of the theoretical assumptions and limitations of these directed indices is available on Table 1.1.

Table 1.1. Characteristics of the directed and non-directed INS indices. Adapted from [Choi and Kim 2018](#) and [Turk et al. 2022](#).

Metric	Category	Signal property	Supports cross-frequency?	Description	Assumptions	Limitations	References
Phase Locking Value (PLV)	Non-directed	Phase	Yes (available)	Measure of frequency-specific transients of phase locking independent of amplitude. Two signals $x(t)$ and $y(t)$ with instantaneous phases $\Phi_x(t)$ and $\Phi_y(t)$ are considered “synchronized” if their instantaneous phase difference is constant.	<ul style="list-style-type: none"> - Phase difference $\Delta\Phi$ is statistically independent of the amplitudes - No assumption of signal stationarity 	<ul style="list-style-type: none"> - Does not isolate self-interactions - Found to detect spurious hyper-connections - Might be susceptible to temporal jitters and poor signal to noise ratio 	Burgess 2013 Lachaux et al. 1999 Nolte et al. 2004 Santamaria et al. 2020
Granger Causality (GC)	Directed	Amplitude	No	Statistical measure that estimates whether the past values of time series $X(t)$ can predict the values of time series $Y(t)$ significantly better than the past values of $Y(t)$ can predict the values of $Y(t)$ itself.	<ul style="list-style-type: none"> - The cause must happen prior to its effect - The cause has information that could improve the prediction of the effect - “Cause” and “effect” time series are stationary (mean and covariance do not change over time) and stable (do not diverge to ∞) 	<ul style="list-style-type: none"> - Assumes stationarity and stability of the time series - Highly dependent on model order p for the vector autoregressive (VAR) modelling 	Granger 1969 Jia 2019
Partial Directed Coherence (PDC)	Directed	Amplitude	No	Frequency-domain equivalent of GC based on multivariate autoregressive (MVAR) modeling of multichannel signals. It quantifies relative strength of the influence of the signal x_i on the signal x_j at frequency f .	<ul style="list-style-type: none"> - The relationship between signal x_i and x_j can be explained by a linear model - Signal x_i and x_j are stationary - Signal x_i and x_j “synchronize” in a lagged fashion 	<ul style="list-style-type: none"> - Assumes linearity between the two signals - Highly dependent on model order p for the MVAR modelling - Less effective with zero-lagged data 	Baccalá and Sameshima (2001) Baccalá and Sameshima (2021) Burgess 2013 Choi and Kim 2018 Santamaria et al. 2020
Imaginary Coherence (ICoh)	Non-directed	Phase + Amplitude	Yes (not available)	Frequency-domain measure that uses the imaginary part of the coherency to capture synchrony between signals while specifically discarding zero-lag components.	<ul style="list-style-type: none"> - Two signals can be considered synchronized only when they show a time lag - The imaginary part of the coherency is insensitive to artifactual “self-interaction” caused by volume conduction - A lagged interaction with fixed time should produce a constant phase difference at values that are different than 0 and π 	<ul style="list-style-type: none"> - May be strongly influenced by the phase of the coherency: most effective in detecting synchronization with a phase lag corresponding to a quarter of cycle and less effective when signals are either in-phase or in phase opposition - Normalization by signal amplitudes may be sensitive to addition of uncorrelated noise sources 	Nolte et al. 2004 Jia 2019 Procissi et al. 2025, <i>in press</i> Stam et al. 2007 Vinck et al. 2011

Phase Lag Index (PLI)	Non-directed	Phase	Yes (not available)	Phase-based estimator of non-equiprobability of the leads and lags between two signals. Larger asymmetries between the two signals suggest larger connectivity.	<ul style="list-style-type: none"> - Two signals can be considered synchronized only when they show a time lag - Two signals can be considered synchronized when there is asymmetry of phase difference distribution 	<ul style="list-style-type: none"> - Discontinuous measure: small perturbations of the signal may turn phase lags into leads and vice versa - May be positively biased - Insensitive to true changes in phase-synchronization when the phase of the coherency lies very close to 0° or 180° 	Jia 2019 Stam et al. 2007 Vinck et al. 2011
Weighted Phase Lag Index (wPLI)	Non-directed	Phase	Yes (not available)	Phase-based estimator that extends the PLI by weighing the contribution of the observed phase leads and lags by the magnitude of the imaginary component of the cross-spectrum.	<ul style="list-style-type: none"> - Two signals can be considered synchronized only when they show a time lag - The imaginary component of the cross-spectrum is insensitive to artifactual “self-interaction” caused by volume conduction - Two signals can be considered synchronized when there is asymmetry of phase difference distribution 	<ul style="list-style-type: none"> - Insensitive to true changes in phase-synchronization when the phase of the coherency lies very close to 0° or 180° 	Jia 2019 Vinck et al. 2011
Circular Correlation (CCorr)	Non-directed	Phase	No	Alternative correlation metric for circular data measuring the circular covariance of differences between the observed phase and the expected (mean) phase.	<ul style="list-style-type: none"> - Real oscillators are imperfect, meaning that they will show small variations over time - Perfect oscillators have zero phase variance, as the frequency of oscillation will be constant - Knowing the phase of such an oscillator in its recent past allows to predict its phase in the near future - The phase variance of two related channels A and B will co-vary throughout time 	<ul style="list-style-type: none"> - Accuracy may be dependent on epoch length 	Burgess 2013 Zimmermann et al. 2024
Amplitude-amplitude coupling (AAC)	Non-directed	Amplitude	Yes (available)	Correlation of amplitude envelopes between two distinct oscillation frequencies.	<ul style="list-style-type: none"> - Two signals are synchronized when their envelopes change amplitude at the same time - Amplitude coupling is independent of neuronal spike-time relationships 	<ul style="list-style-type: none"> - Not dependent on the absolute amplitude of the envelopes - Cannot carry out integrative computational roles dependent on spike time correlations 	Koul et al. 2023 Palva and Palva 2018 Turk et al. 2022 Zamm et al. 2024
Time-lagged cross correlations	Directed	Amplitude	Yes (available)	Estimator of the correlation between two time series shifted relatively in time. It identifies whether changes in one time series preceded or followed changes in another time series.	<ul style="list-style-type: none"> - Two signals “synchronize” in a lagged fashion - Signal x_i and x_j are stationary 	<ul style="list-style-type: none"> - Assumes stationarity 	Shen 2015 Wass et al. 2018

The vast majority of the current knowledge on parent-infant EEG hyperscanning has been derived from studies that adopted non-directed INS metrics, particularly the PLV ([Atilla et al. 2023](#); [Leong et al. 2019](#); [Santamaria et al. 2020](#)), the wPLI ([Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#)) and more recently the CCorr ([Neel et al. 2025](#)). PLV and CCorr were predominantly measured in the *alpha* (6-9 Hz) frequency band, highlighting significant positive associations between phase-based parent-infant INS and some dyadic behaviors such as ostensive eye contact and maternal utterances ([Leong et al. 2019](#)), maternal visual and tactile stimulation ([Neel et al. 2025](#)), and maternal emotional feedback with positive valence ([Santamaria et al. 2020](#)). Positive associations with cognitive processing have been found as well, including learning likelihood ([Leong et al. 2019](#)) and observing the mother performing a task ([Atilla et al. 2023](#)). wPLI, on the other hand, has been exclusively measured in the *theta* (4-7 Hz) frequency band, and was found to be positively associated with maternal sensitivity ([Endevelt-Shapira and Feldman 2023](#)) as well as with increased infant social attention, positive arousal, and safety/approach behaviors ([Endevelt-Shapira et al. 2021](#)).

While the wPLI is considered a robust INS metric due to its reduced probability of detecting “false positive” connectivity in the case of noise sources with near-zero phase lag ([Endevelt-Shapira and Feldman 2023](#); [Vinck et al. 2011](#)), some concerns have arisen regarding the use of the PLV as an INS metric. In a previous study conducted on real EEG data collected on adults, Burgess ([2013](#)) highlighted the proneness of PLV to spurious hyper-connections, mainly in the *theta* frequency band (nearly 20% of all possible connections). While the *alpha* band was less affected (1.3% of the weak hyper-connections were spurious), caution in the interpretation of the findings is still recommended. While highly underrepresented in the current state of the literature, some authors have used directed metrics to explore parent-infant INS. The only two examples are the study of Santamaria and colleagues ([2020](#)), which computed the PDC in the *alpha* frequency band, and the study of Wass and colleagues ([2018](#)), in which the directed index of choice was the time-lagged cross correlations (with

theta as the frequency of interest). PDC in the *alpha* frequency band, particularly in the parent-to-infant direction (parent leading, infant following), was found to be significantly stronger when the parent provided a positive emotional feedback compared to when they provided a negative one ([Santamaria et al. 2020](#)). Time-lagged cross-correlation, on the other hand, highlighted a higher infant attention focus when the parents were more neurally responsive to them ([Wass et al. 2018](#)).

The presented evidence was predominantly collected during scaffolded dyadic interactions without any form of interactive perturbation. While this has allowed to reach a better understanding of parent-infant INS and what could be the potential behavioral cues to establish it, the present literature addresses solely the (few) instances of the social exchange in which parents and infants are actively matching their states. For what concerns the parent-infant EEG hyperscanning field, no study to date has addressed the inter-neural correlates of the mismatch and reparation process described in the MRM model and in Feldman's work, which is considered the core process through which synchrony itself is established ([Feldman 2007](#)). Understanding the neurophysiological correlates of the interactive reparation process could not only expand our knowledge about the importance of the caregiving figure on infant socioemotional, cognitive and neural development, but also constitute the basis for a better understanding (and potential improvement) the reparation skills in populations exposed to early stress (e.g., preterm infants and their parents).

2. OBJECTIVES

The present dissertation describes the evidence obtained from the “2-Brain Regulation for Improved Neuroprotection during Early Development” (2-BRAINED) study, a longitudinal randomized controlled trial (RCT) funded by the Italian Ministry of Health under the Ricerca Finalizzata 2021 program (research line: Giovani Ricercatori, project code: GR-2021 12375213) that aims at assessing the neuroprotective effect of an early Video-Feedback Intervention in very preterm dyads. Due to the very limited number of very preterm dyads enrolled for this study, this dissertation will focus

exclusively on the aims and results regarding the control group of full-term infants and their parents.

The dissertation will tackle the following experimental aims.

2.1. Aim 1: exploring the feasibility of an automated pre-processing method and its impact on INS.

Before computing any type of INS metric, neurophysiological data acquired via the hyperscanning paradigm must undergo pre-processing in order to minimize motion-related artifacts. Pre-processing is exceptionally important in this field of research due to the ecological nature of the experimental setting, which can increase the likelihood of artifactual portions of data, and due to having infants as experimental subjects, since they are unable to minimize movement if asked. Although Turk and colleagues ([2022](#)) provided practical guidelines to perform signal pre-processing on dual parent-infant EEG data, standardization studies on this topic are still lacking, posing risks on the comparability and replicability of the findings reported in existing parent-infant INS studies ([Pili et al. 2025](#)). Notably, a methodological study revealed that arbitrary methodological decisions taken during pre-processing of EEG data acquired through the hyperscanning paradigm have a significant impact on non-directed phase-based INS metrics such as the PLV and CCorr ([Zimmermann et al. 2024](#)), highlighting the need of standardization of dual EEG pre-processing methods. Following this evidence, as well as the dearth of attempts at standardizing the pre-processing steps of dyadic EEG data ([Kayhan et al. 2022](#)), the first aim of our study was to assess whether the degree of automatization of the pre-processing pipeline, i.e. the degree in which the subjective judgement of a human coder was required throughout the process, affected non-directed phase-based INS metrics such as the PLV. The results of this methodological analysis, conducted on a subgroup of 31 full-term dyads, will be tackled in Chapter 3 (adapted from [Pili et al. 2025](#), doi: 10.1016/j.jneumeth.2025.110400).

2.2. Aim 2: exploring dyadic “lead and follow” dynamics before and after an interactive perturbation and their relation to dyadic behavior.

The current literature on parent-infant EEG hyperscanning studies provides extensive evidence regarding the direct relationship between non-directed INS and dyadic behavior, seemingly delineating a framework in which higher behavioral matching, higher maternal sensitivity and

responsivity, and/or more positive interactive exchanges, are significantly associated with higher parent-infant INS. In the same way, higher levels of maternal intrusiveness and negative interactive exchanges are expected to be negatively associated with INS. While the existence of a positive association between non-directed INS and positive dyadic exchanges should be recognized and taken into account, researchers have argued that a biobehavioral synchrony model based on the assumption that “the higher the contingency, the better” would be too simplistic ([Mitsven et al. 2022](#); [Provenzi et al. 2023](#)). Considering that most social interactions are asymmetrical in nature and may imply different roles and turns in taking action ([Provenzi et al. 2023](#)), exploring different conceptualizations of biobehavioral synchrony, such as directed synchrony, could provide novel insights into the neural underpinnings of social interactions between infants and their parents.

Biobehavioral synchrony of the directed, “lagged” kind has already been observed in previous studies conducted on 3-4 month old infants and their parents ([Feldman et al. 1996](#); [Feldman et al. 1999](#)), featuring the infant as the “leading” member and the parent as the “following” member. As the infant grows and enters the stage of intersubjectivity, the lag between the behavior of the infant and that of the parent was found to decrease due to increased familiarity with the interactive partner’s rhythms, with a subsequent increase of biobehavioral synchrony of the organized, mutual kind. Despite this evidence, there are two factors that still remain unexplored. First, it is still unclear whether the neural equivalent of directed synchrony, i.e. directed/sequential INS, follows the same pattern described in Feldman’s works ([Feldman et al. 1996](#); [Feldman et al. 1999](#)). Lastly, it is still unknown whether an interactive perturbation, such as the FFSF procedure, could generate changes in the “lead and lag” structure of the parent-infant interaction at the neural level.

The second aim of this dissertation is thus to explore if and how the “lead and lag” structure of the dyadic interactions change after an interactive perturbation such as the FFSF procedure, and whether these changes are related to dyadic behaviors typically associated with a still-face effect (such as negative emotionality and gaze aversion) and/or parental soothing strategies (such as maternal

affective touch). The results of the study, conducted on a sample of 48 parent-infant dyads, are showcased in Chapter 4 (adapted from Pili et al. *under review*).

2.3. *Aim 3: exploring non-directed INS before and after an interactive perturbation and its relation to dyadic behavior.*

As previously mentioned, the “still-face effect” has been observed in multiple studies conducted in infants up to nine months (see [Mesman et al. 2009](#)) and is often characterized by a decrease in social gaze and an increase in negative affect from the baseline to the SF episode ([Adamson and Frick 2009](#); [Tronick et al. 1978](#)). Some maternal behaviors related to the global mother-infant communication system, such as affective touch, have been found to reduce the still-face effect ([Stack and Muir 1990](#); [Stack and Muir 1992](#)). The current research in parent-infant EEG hyperscanning literature has been focusing on exploring the association between non-directed INS and a series of dyadic behaviors such as extended eye contact ([Leong et al. 2019](#); [Neel et al. 2025](#)), maternal affective touch ([Neel et al. 2025](#)) and maternal emotional feedback valence ([Santamaria et al. 2020](#)); however, no study to date has focused on the impact of a social stressor, such as the FFSF procedure, on parent-infant INS.

The third and final aim of this dissertation is thus to explore changes in parent-infant non-directed INS before and after an interactive perturbation, as well as its association with dyadic behavior. Our focus will be primarily on infant behaviors that are related to the presence of a still-face effect, such as negative emotionality and gaze aversion, as well as on maternal sensitive behavior, particularly the instances of maternal affective vocalizations and affective touch, and on dyadic matched behaviors, such as mutual social gaze and mutual positive emotionality. Considering the repeated positive associations between non-directed INS and dyadic behaviors that are affected by the FFSF, such as social gaze ([Leong et al. 2019](#); [Neel et al. 2025](#)) and positive emotionality ([Santamaria et al. 2020](#)), we could reasonably expect that 1. the presence of a still-face effect in our sample will result in a significant reduction of mutual positive emotionality and social gaze and a consequent increase in infant negative emotionality and gaze aversion, and 2. an increase of negative emotionality and gaze aversion from baseline to SF and from baseline to reunion could result in significant changes in non-

directed INS across FFSF episodes. Lastly, we expect that non-directed INS metrics will be positively associated with behaviors with positive valence, such as maternal affective vocalizations and affective touch and infant positive emotionality and social gaze, and negatively associated with behaviors with negative valence, such as lack of maternal vocalizations and touch, as well as negative emotionality and gaze aversion. The results of the study, conducted on a subsample of 58 dyads, will be presented in Chapter 5.

CHAPTER 2 – METHODS AND STUDY PROTOCOL (adapted from Billeci et al. 2024)

Chapter 2 (adapted from [Billeci et al. 2024](#), doi: 10.3389/fpsyg.2024.1516616) provides an overview of the experimental setting, experimental procedure and plan of analysis of the 2-BRAINED project. The project has been funded by the Italian Ministry of Health under the Ricerca Finalizzata 2021 program (research line: Giovani Ricercatori, project code: GR-2021 12375213, Principal Investigator: Livio Provenzi). The recruitment strategy, inclusion and exclusion criteria for the participants, as well as the experimental procedures are valid for all the studies described in Chapter 3, Chapter 4 and Chapter 5. The original version of the article, published in “Frontiers in Psychology” (Volume 15, 2024 - 1516616), is available in the Appendix. The study protocol is also available on ClinicalTrials.gov (ID: NCT06659809).

1. METHODS AND PROCEDURES

The 2-BRAINED project is a randomized-controlled trial (RCT) with three arms, two of which include a group of very preterm dyads (VPT) and one is composed of full-term (FT) dyads. For the purposes of this dissertation, we will consider only the control group of FT dyads.

1.1. Population, enrollment, and arm allocation

FT infants were enrolled at birth by contacting their caregivers within the first 48 h after delivery. Infants were considered eligible if they met the following conditions: a gestational age of 37 weeks or more, were healthy, and showed no evident signs of neurodevelopmental risk or morbidities. Exclusion criteria included single-parent families, parental age under 18 years, lack of Italian language mastery, and the presence of documented parental psychiatric disorders.

1.2. Experimental procedure

When the infant reached 9 ± 1 months of age, parent-infant dyads were invited to our laboratory to perform the EEG-hyperscanning task. The task will feature the videotaping of mother–infant interaction according to a modified Face-to-Face Still-Face (FFSF) procedure ([Tronick et al. 1978](#)) and the simultaneous EEG data collection from both the interactive partners. The age range of 9 ± 1 months was chosen for our study due to it being the age in which infant develop intersubjectivity, defined as the ability to recognize one another’s impulses intuitively with or without cognitive and symbolic elaboration ([Bråten 1998](#)), and mutual regulation with the caregiving figure ([Feldman 2007](#)).

1.2.1. FFSF procedure

At 9 months, mothers and infants took part in a FFSF procedure in the laboratory (see *1.2.3. Experimental setting*). The FFSF included three episodes: During the Play (baseline) episode (2 min), mothers and infants interacted face-to-face avoiding the use of toys and pacifier; during the Still-Face episode (1 min), mothers were asked to interrupt any communication toward the infant, to maintain a

still, poker face, while maintaining eye-contact; and during the Reunion episode (2 min), unconstrained interaction was resumed. The procedure has been previously adopted to assess biobehavioral dimensions ([Provenzi et al. 2017](#); [Provenzi et al. 2019](#)) and physiological underpinnings ([Mantis et al. 2014](#); [Montirosso et al. 2010](#)) of socio-emotional stress regulation in VPT infants. The entire procedure was videotaped for the offline coding of specific maternal and infant interactive behaviors (see 1.2.4. *Demographic data and behavioral coding* for details).

1.2.2. *Neurophysiological procedures*

EEG data acquisition occurred at 500 Hz sampling frequency during the 9-month FFSF procedure employing the Smarting Pro (mBrainTrain, Belgrade, Serbia) system equipped with two 32-channel EEG caps featuring wireless Bluetooth connection between the amplifiers and the mBrainTrain Streamer software installed on two separate laptops. The laptops receiving data were linked to each other via a network cable to ensure synced data collection. The use of wireless EEG caps allowed greater flexibility and comfort for participating dyads.

Upon arrival, the infants were familiarized with the setting: a play mat and toys were available to aid in acclimatization to the environment. The researchers debriefed parents with a comprehensive explanation of the study's aims and procedures. Cap sizes were selected to fit participants' head circumference. The caps fitting process commenced with the caregiver to ensure greater infant comfort and familiarity with the equipment. The conductive gel was applied to optimize signal conductivity and minimize artifacts.

1.2.3. *Experimental setting*

In line with the guidelines on parent-infant EEG hyperscanning studies written by Turk and colleagues ([2022](#)), participants were tested in an infant-friendly room equipped with a playmat, age-appropriate toys, an infant highchair to allow the infant to sit at the same height as the parent during the FFSF procedure, and a sink to allow the participants to wash their heads at the end of the experiment. An illustration of our laboratory and pictures of our equipment is provided in Figure 2.1.

Figure 2.1. Experimental setting.



1.2.4. Demographic data and behavioral coding

Demographic (e.g., parental age, parental job, and parental educational level) and neonatal (e.g., gestational age, birth weight, and Apgar score) were obtained from medical charts. To code for dyadic behaviors, videotapes obtained from two cameras during the lab FFSF procedure were edited offline using Movavi Video Suite 2020 software and a single synced video showing both frontal views of the caregiver and the infant's face, hands, and torso was produced. Videos were micro-analytically coded for infants' and caregivers' target interactive behaviors according to an adaptation of the Parent–Infant Coding Scheme (PICS, Version 4.0; Brambilla et al. *unpublished manual*) as reported in Table 2.1. PICS codes will be computed as a percentage of time for each FFSF episode (Table 2.1.).

Table 2.1. Selection of codes from the Parent-Infant Coding Scheme (PICS, Version 4.0; Brambilla et al., *unpublished manual*). Adapted from [Billeci et al. 2024](#).

Variable	Levels	Description
Both interactive partners		
Emotional state	Negative	Clear display of negative emotionality (e.g., eyes, mouth, general movements of the face or the body, and other vocal or non-vocal signals) including fussing and crying.
	Neutral	No clear display of negative or positive emotionality.
	Positive	Clear display of positive emotionality (e.g., eyes, mouth, general movements of the face or the body, and other vocal or non-vocal signals) including smiles and laughs.
Gaze direction	Face-directed	Attention focus is on the interactive partner's face.
	Body-directed	Attention focus is on the interactive partner body (e.g., hands and torso).
	Object-directed/avoiding	The subject is actively avoiding eye contact as displayed by head and body movements/posture, or is looking at an inanimate object.
Approach/withdrawal	Withdrawal	Evident leaning backward and/or turning the head away to avoid interaction.
	Neutral	No evident backward or forward movements.
	Approach	Evident leaning forward and/or reaching forward to engage in interactive behaviors.
Parent-specific codes		
Vocal inputs	No voice	No vocal productions.
	Negative	Vocal comments that convey explicit critique or rejection of infants' behaviors or state.
	Pragmatic	Vocal comments that are finalized to modify or instruct the interactive partner's cognitive state, such as requests, attention-getting, and explanations.
	Affective	Vocal comments that convey playful and social engagement such as singing, laughing, and playing nursery rhymes. Includes expressions of appreciation or acceptance of infants' behaviors or state or are finalized to soothe infants' stress, as well as mind-related comments (e.g., "you think" and "you want") and mirroring of infants' communicative bids.
Tactile inputs	No touch	No tactile stimulations.

	Negative	Tactile stimulations that clearly appear intrusive and/or provoke or increase a negative emotionality state in the interactive partner.
	Pragmatic	Tactile stimulations that are finalized to modify or instruct the interactive partner postures or movements in the environment, such as holding, shadowing, and attention-getting.
	Playful	Tactile stimulations that convey playful and social engagement such as tickling, squeezing, and any other appropriate entertaining tactile stimulations that are fast-paced, dynamic, repetitive, and/or characterized by quick cinematic features. This category also includes tactile stimulations that are finalized to soothe or regulate the behavioral state of the interactive partners, such as stroking, kissing, massaging, and any other appropriate tactile stimulations with clear regulatory functions and conveying a sense of affective closeness.

1.3. Plan of EEG data elaboration

1.3.1. Pre-processing pipeline

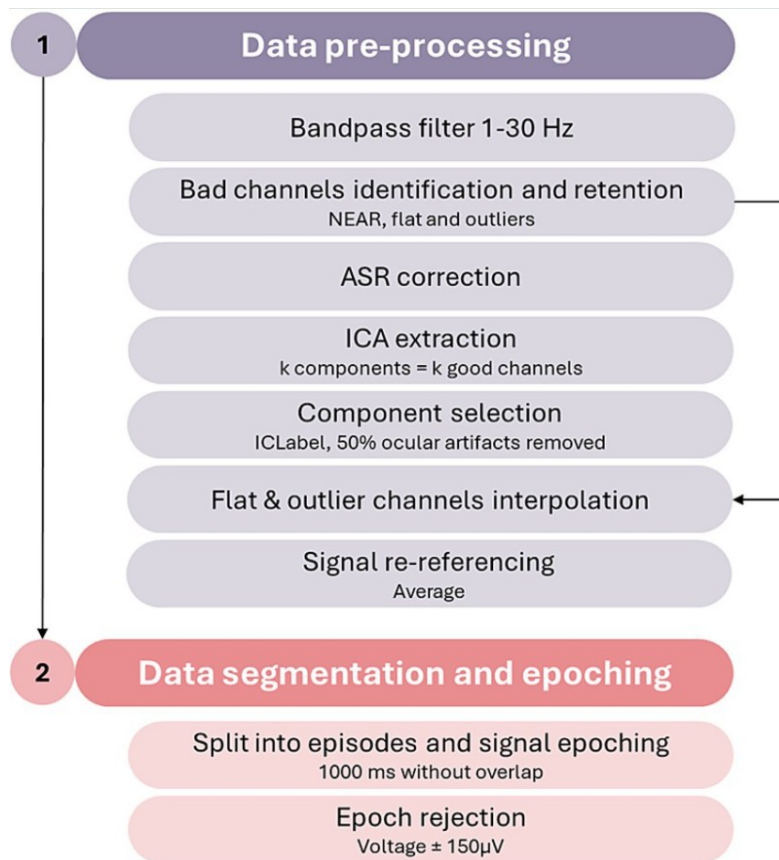
Dyadic EEG data was pre-processed with a fully automated pipeline built using the MATLAB-based (The MathWorks Inc., 2024) interacting toolbox EEGLAB ([Delorme and Makeig 2004](#)). A brief description of the main pre-processing steps is available in Figure 2.2.

The parent and infant signals were pre-processed separately with the same steps and parameters for both. First, data filtering was performed with the application of a Hamming windowed sinc FIR bandpass 1–30 Hz filter as the analyses (see below) were conducted on the lower (*theta* and *alpha*) frequency bands. Subsequently, flat and outlier channels were detected using the Neonatal EEG Artifact Removal (NEAR) plugin ([Kumaravel et al. 2022](#)) and retained (i.e., put in a separate temporary matrix) for later interpolation. Dyads in which at least one of the members displays a signal with more than 15% ($N > 5$) of flagged channels were excluded from further analyses. The NEAR plugin was preferred over other bad channel detection methods due to its validation on neural data of infants aged 8-10 months ([Kumaravel et al. 2022](#)). The EEG signal from all non-flagged channels underwent noise correction through the artifact subspace reconstruction (ASR), with burst criterion

(k) set at 10 in line with previous studies ([Chang et al. 2020](#); [Koul et al. 2023](#)); subsequently, analysis of the components of the signal was performed through the independent component analysis (ICA), producing as many components as the number of good channels. The algorithm of choice was Infomax. The resulting components were then classified through the ICLabel ([Pion-Tonachini et al. 2019](#)) plugin. Every component flagged as having a 50% or more probability of being an ocular artifact were rejected. At this stage, the matrix containing the flat and outlier channels were re-merged with the EEG matrix, and the bad channels were interpolated through spherical interpolation using the pre-processed signal. The signal was then re-referenced to the average signal of the channels and split into three different sets containing each phase of the experimental procedure (Play, Still-Face, and Reunion): Each of these sets was subsequently segmented into 1,000 ms epochs avoiding overlaps. Bad data segments containing residual artifacts in each of the three phases were identified. All segments in which at least one of the channels of interest used for INS metrics (see below) displayed a voltage exceeding $\pm 150 \mu\text{V}$ ([Debnath et al. 2020](#)) was marked as rejected. The rejected epochs in the infant's and parent's signals were merged to obtain the final pool of rejected epochs for the dyad. This ensured that all the rejected epochs for one interactive member of the dyad were similarly mirrored for the other partner. Dyadic data underwent further analyses if their signal contained at least 30 good epochs in both the Play and Reunion phases; if this criterion was not met, their signal underwent manual epoch rejection performed by an expert EEG coder. In case after the manual epoch rejection, the dyad resulted in having less than 30 good merged epochs in at least one of the FFSF episodes, the signal was excluded from further analyses.

The current pipeline has been used to pre-process dual EEG data in the studies presented in Chapter 4 and Chapter 5. For an overview of the pre-processing pipelines used in the study presented in Chapter 3, see the relative chapter (2.2. *Data pre-processing*).

Figure 2.2. EEG data pre-processing pipeline. NEAR = Neonatal EEG Artifact Removal; ASR = artifact subspace reconstruction; ICA = independent component analysis. Adapted from [Billeci et al. 2024](#).



1.3.2. Estimating indices of inter-brain co-regulation

Several inter-brain synchronization indices have been proposed so far to estimate the coupling between two brains ([Czeszumski et al. 2020](#)). Since there is still debate on the appropriateness of each inter-brain synchronization measure, we computed and compared several indices (see Table 2.2.).

Table 2.2. Indices of inter-brain co-regulation adopted in the 2-BRAINED study. Adapted from [Billeci et al. 2024](#).

Index	Description	Notes	Reference
Phase-Locking Value (PLV)	Frequency-specific transients of phase locking independent of amplitude. The value ranges from 0 to 1: values closer to 0 indicate random signals with unsynchronized phases; values closer to 1 indicate stronger coupling between the two signals.	While previous studies focused mainly on infant frequency bands, cross-frequency PLV indices will be obtained for the purposes of the 2-BRAINED study.	Lachaux et al. (1999) ; Canolty and Knight (2010)
Weighted Phase Lag Index (wPLI)	Estimates to what extent the phase leads and lags between signals from two sensors are non-equiprobable. It extends the Phase Lag Index by weighing the contribution of the observed phase lags and leads by the magnitude of the imaginary component of the cross-spectrum. Values range from 0 to 1, where 0 indicates random signals with unsynchronized phases and 1 indicates stronger coupling between the two signals.	/	(Vinck et al. 2011)
Imaginary Coherence (ICoh)	Computed through spectral density (power) of each participant and cross-spectral density between them to estimate the average phase difference and consistency of phase difference synchronization.	ICoh is expressed as a complex number: the real part represents how much the coherence is driven by instantaneous interactions; the imaginary part shows how much the coherence is based on lagged interactions.	(Nolte et al. 2004) (Dikker et al. 2021)
Partial Directed Coherence (PDC)	Frequency domain equivalent of Granger causality that measures the directed influences between a pair of signals in a multivariate dataset. Higher values (no defined range) indicate a higher probability for the connection between the two signals to be directed.	While previous studies focused mainly on infant frequency bands, we chose to compute PDC using “broadened” thresholds that allowed us to include the adult bands as well.	Baccalá and Sameshima (2001) Baccalá and Sameshima (2021)

The computation of synchronization measures occurred in pre-defined channels of interest located in the frontal and central areas of the scalp, considering both homologous and non-homologous channel combinations. As for the frequencies of interest, we considered *alpha* and *theta*. These frequencies have been found to be involved in parent–infant social tasks, with *theta* fluctuations linked to changes in shared attention during joint play of parent and infant ([Wass et al. 2018](#)), enhancement of *alpha*

and *theta* power linked to changes in directed gaze ([Leong et al. 2017](#)), and fluctuations in *alpha* band linked to changes in emotional states of mother and child ([Santamaria et al. 2020](#)).

1.4. Statistical power and sample size estimates

The sample size has been estimated according to the RCT design of the study, which included two groups of very preterm (VPT) infants alongside the control group of FT dyads. The parameters were thus set as follows: medium effect size, $f = 0.25$, $alpha = 0.05$, $beta = 0.20$, number of groups = 3 (VPT-intervention arm, VPT-care as usual arm, and FT). The procedure yielded a total sample size of 159 subjects, with 53 subjects per RCT arm. Nonetheless, considering the longitudinal nature of the study and the attrition rate related to EEG tasks with infants, an oversampling of $n = 80$ ($\sim +50\%$) subjects per RCT arm was planned to secure the minimum sample size for appropriately powered statistical analyses.

CHAPTER 3 – EXPLORING THE FEASIBILITY OF AN AUTOMATED PRE-PROCESSING METHOD AND ITS IMPACT ON INS (adapted from Pili et al. 2025)

This chapter addresses Aim 1 of the 2-BRAINED study, i.e. the feasibility of an automated pre-processing method and its impact on non-directed phase-based INS metrics such as the PLV. The introduction, methods, results and discussion sections, as well as the figures and tables, are adapted from the published version of this work, available at the following DOI: 10.1016/j.jneumeth.2025.110400. The original article ([Pili et al. 2025](#)), originally published in “Journal of Neuroscience Methods” (Volume 417, May 2025, 110400), is available in the Appendix.

1. INTRODUCTION

A fundamental step to estimate INS in EEG hyperscanning studies is signal pre-processing. The practical guidelines written by Turk and colleagues ([2022](#)) recommend applying a manual or semi-automated pre-processing routine which includes visual inspection of the raw data and removal of flat channels, data re-referencing, data filtering, interpolation or removal of spurious channels, manual artifacts rejection, Independent Component Analysis (ICA) or wavelet analysis, manual or automated artifacts rejection and segmentation of the signal into epochs. In addition, recently published pipelines, such as DEEP ([Kayhan et al. 2022](#)), MADE ([Debnath et al. 2020](#)) or the MATLAB-based HAPPE ([Gabard-Durnam et al. 2018](#)) provide standardized (DEEP) and/or fully automatized methods (HAPPE, MADE) to pre-process developmental EEG data. Despite this, a standardization of the pre-processing routine in this field seems to still be lacking. The majority of the authors of parent-infant EEG hyperscanning studies (see [Atilla et al. 2023](#); [Leong et al. 2017](#); [Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#); [Santamaria et al. 2020](#); [Wass et al. 2018](#)) seem to have opted for custom scripts for EEG data pre-processing, generating discrepancies among the literature in three main aspects: 1. the order in which the pre-processing steps are performed; 2. the parameters set for each step, and 3. the degree of automatization of the whole process. While it is known that different settings on some of the most commonly used pre-processing steps such as the type of filter ([Widmann et al. 2015](#); [Winkler et al. 2015](#)), ICA algorithm ([Dimigen 2020](#)), and other automatized algorithms for noise correction such as Artifact Subspace Reconstruction (ASR; [Chang et al. 2020](#)) may differently impact the properties of the EEG signal, the degree of automatization of the pipeline may pose an additional challenge to the reproducibility of the findings reported in developmental EEG hyperscanning research. Indeed, semi-automatized pre-processing methods were especially used in the first parent-infant EEG hyperscanning studies ([Leong et al. 2017](#); [Leong et al. 2019](#); [Santamaria et al. 2020](#)) and their usage was also recommended in dyadic EEG hyperscanning guidelines ([Turk et al. 2022](#)). However, discrepancies in artifact rejection stemming from individual sensitivity of the coder may produce variations in the pre-

processed signals that, in turn, could impact INS estimates to an unknown degree. Automatized pre-processing methods, on the other hand, may minimize the issue of individual differences in the pre-processing steps and sensitivity of the coders; however, while more recent studies pre-processed dyadic EEG data using an automatized approach ([Atilla et al. 2023](#); [Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#)), attempts at establishing standardized and automatized dyadic EEG data pre-processing routines are still rare in the literature ([Kayhan et al. 2022](#)). Moreover, artifact rejection based on visual inspection is still a diffused practice in dyadic EEG hyperscanning studies ([Kayhan et al. 2022](#)). For these reasons, it is crucial to understand whether different methodological decisions taken during the EEG signal pre-processing, particularly the degree of automatization of the pre-processing pipeline, have an impact on INS estimates.

In this first study, we aimed at comparing the performance of a fully automated pipeline for dual parent-infant EEG data with the one of a more traditional, “manual” dual EEG pre-processing method. As the names suggest, the two pipelines differed exclusively in their degree of automatization, whereas the order and thresholds of the specific steps were the same for both (see par. 2.2., *Data pre-processing*). Our interest was in two main outcomes: the main pre-processing outputs (e.g. number of interpolated channels, type and number of rejected independent components, rejected epochs) and INS estimates. PLV was used as the only proxy of INS due to its predominant use in parent-infant EEG hyperscanning studies ([Atilla et al. 2023](#); [Leong et al. 2017](#); [Leong et al. 2019](#); [Santamaria et al. 2020](#)) as well as for the purpose of testing the adequacy of this index for Aim 2 of the 2-BRAINED project.

By increasing the knowledge on the intricacies of data pre-processing and artifact correction in dyadic EEG data, we aim at encouraging developmental researchers to reach a common ground in regard to the modalities of EEG data pre-processing in this field and thus increase the comparability and reproducibility of the results that will be obtained in future studies.

2. METHODS

2.1. Participants

Dual EEG data was taken from a dataset of 31 mother-infant (8-10 months; 16 males and 15 females) dyads from the FT control group who had their brain activity recorded through the EEG hyperscanning paradigm ([Dumas 2011; Montague et al. 2002](#)) while they were engaged in a Face-to-Face Still-Face Procedure (FFSF; [Tronick et al. 1978](#)). For the details regarding the experimental procedure and EEG equipment, see Chapter 2 of the present dissertation. Participants for this study were recruited between June 2022 and April 2024.

2.2. Data pre-processing

EEG data was pre-processed on the MATLAB-based (MathWorks Inc. 2024, n.d.) interacting toolbox EEGLAB ver. 2024.0 ([Delorme and Makeig 2004](#)) using two different custom pipelines: the “automated” pipeline, which required no control from the coder, and the “manual” pipeline, in which the coder was directly involved in the processes of channel interpolation, independent components (ICs) rejection and visual inspection of the signal. A summarization of the main steps of the two pipelines is presented in Figure 3.1.

Both pipelines were built according to previous guidelines on dyadic EEG data pre-processing ([Debnath et al. 2020; Kayhan et al. 2022; Turk et al. 2022](#)), and differed from one another exclusively on their degree of automatization in the following steps: detection of bad channels, IC selection, epoch rejection. In both pipelines, the infants’ and the parents’ signals were pre-processed separately, the infant first and the parent last.

Data filtering. Both the automated and the manual pipeline applied the default EEGLAB Hamming windowed sinc FIR bandpass filter to the signals, with 1 Hz and 30 Hz as the lower and upper limits respectively. This was in line with the frequency bands of choice for the INS analyses (*theta* and *alpha*; see par. 2.3.).

Detection of bad channels. In both pipelines, flat and outlier channel detection was performed using the Neonatal EEG Artifact Removal (NEAR) plugin, which has been validated in EEG data acquired

both in adults and in infants aged 8-10 months ([Kumaravel et al. 2022](#)). In the automated pipeline, NEAR was the only method for detection of bad channels, whereas in the manual pipeline, the usage of NEAR was followed by a visual inspection of the signal by the coder, who checked the output of NEAR to either confirm it or to detect additional flat or outlier channels to interpolate.

Channel interpolation. In both pipelines, the channels that were previously detected as flat or outliers were interpolated using the spherical spline method. Interpolation of the channels with bad signal was performed on the whole recording rather than on the specific segments in which they resulted as flat or outliers.

Noise correction. Both pipelines proceeded with noise correction using the ASR algorithm ([Chang et al. 2020](#)). In our pipelines, ASR was set in correction mode in order to preserve the temporal synchronization of the two signals, and deployed a cutoff parameter (k) at the default value of $k = 20$. The cutoff parameter k is crucial in determining the sensitivity of the noise correction process: every segment of data that exceeds k is considered artifactual and thus corrected into EEG-like data ([Kumaravel et al. 2022](#)). Consequently, a low k (below 20 in the adult population) will result in a stricter noise correction, whereas a high k (over 30 in the adult population) will result in a looser noise correction.

Independent Component Analysis (ICA). Analysis of the components of the signals was performed through ICA in both pipelines using the default Infomax ICA algorithm (*runica*). In both the automated and the manual pipeline, *runica* produced as many components as the total number of channels ($N = 32$). Components were then categorized using the ICLabel plugin ver. 1.6 ([Pion-Tonachini et al. 2019](#)) and subsequently inspected for removal following different criteria for each pipeline. In the automated pipeline, the inspection was entirely automated, and ICs were rejected from the signal if they displayed a probability of 50% or higher of being an ocular artifact. In the manual pipeline, on the other hand, the inspection was performed by the coder after a preliminary assessment of the artifactual ICs performed with the ADJUST plugin ver. 1.1.1 ([Mognon et al. 2011](#)). The coder was required to 1. check the ICs flagged by ADJUST to confirm or reject the output; and 2. manually

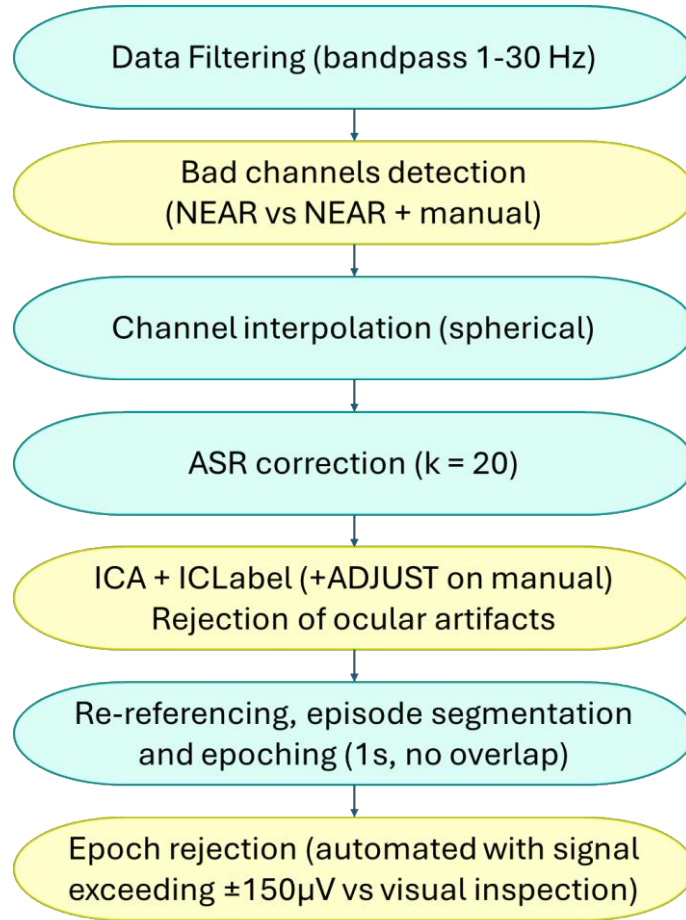
check the remaining ICs and reject those displaying a probability higher than 50% of containing ocular artifacts. Both ICLabel and ADJUST have been trained exclusively on adult EEG data; however, while ADJUST demonstrated slightly inferior performance in labeling infant EEG ICs compared to algorithms specifically designed for infant data, ICLabel's performance on infant EEG data was found to be non-inferior to that of infant-specific IC labeling plugins such as Adjusted ADJUST ([Leach et al. 2020](#)).

Signal re-referencing. In both pipelines, re-referencing was performed using the average signal of the channels.

Signal segmentation and epoching. In both pipelines, the signal was segmented into the three episodes of the experimental procedure: Play (120 s), Still-Face (60 s), Reunion (120 s). Each episode was subsequently epoched in 1 s epochs with no overlap coherently with previous EEG hyperscanning studies ([Ayrolles et al. 2021](#); [Bevilacqua et al. 2019](#); [Goldstein et al. 2018](#)).

Bad epochs rejection. After epoching, the Play and Reunion episodes were inspected for residual artifacts. At this stage, the signal acquired during the Still-Face was not included due to the larger percentage of artifacts that were expected to be found in this specific episode compared to the Play and Reunion. In the automated pipeline, the process was entirely automated: the script automatically flagged all the epochs in which at least one of the fourteen target channels for PLV computation (see par. 2.3.) displayed a voltage exceeding $\pm 150\mu\text{V}$ (parameter used in [Debnath et al. 2020](#)). In the manual pipeline, on the other hand, the process was performed by the coder, who would visually inspect the signal and flag all the epochs in which at least one of the fourteen target channels for PLV computation displayed either a voltage exceeding $\pm 150\mu\text{V}$ or residual artifacts. In both pipelines, all epochs flagged in one member of the dyad were automatically flagged in the other member to maintain the temporal synchronization of the signals.

Figure 3.1. Comparison between the two pre-processing pipelines. Steps in which the degree of automatization between the two pipelines was different are displayed in yellow. Adapted from [Pili et al. 2025](#).



2.3. INS indexes - PLV

PLV is a measure of frequency-specific transients of phase locking independent of amplitude ([Lachaux et al. 1999](#)) which has been widely used in EEG hyperscanning studies with developmental samples ([Atilla et al. 2023](#); [Leong et al. 2017](#); [Leong et al. 2019](#); [Santamaria et al. 2020](#)). After bandpass filtering the signals at the frequencies of interest and extracting their Hilbert phase, PLV is defined as

$$PLV_{mn} = \frac{1}{N} \left| \sum_{k=1}^{TN} e^{i(\Delta\varphi_k(f_n, f_m, t, k))} \right| \quad (1)$$

where N is the number of trials and $\Delta\varphi_k(f_n, f_m, t, k)$ is calculated as follows:

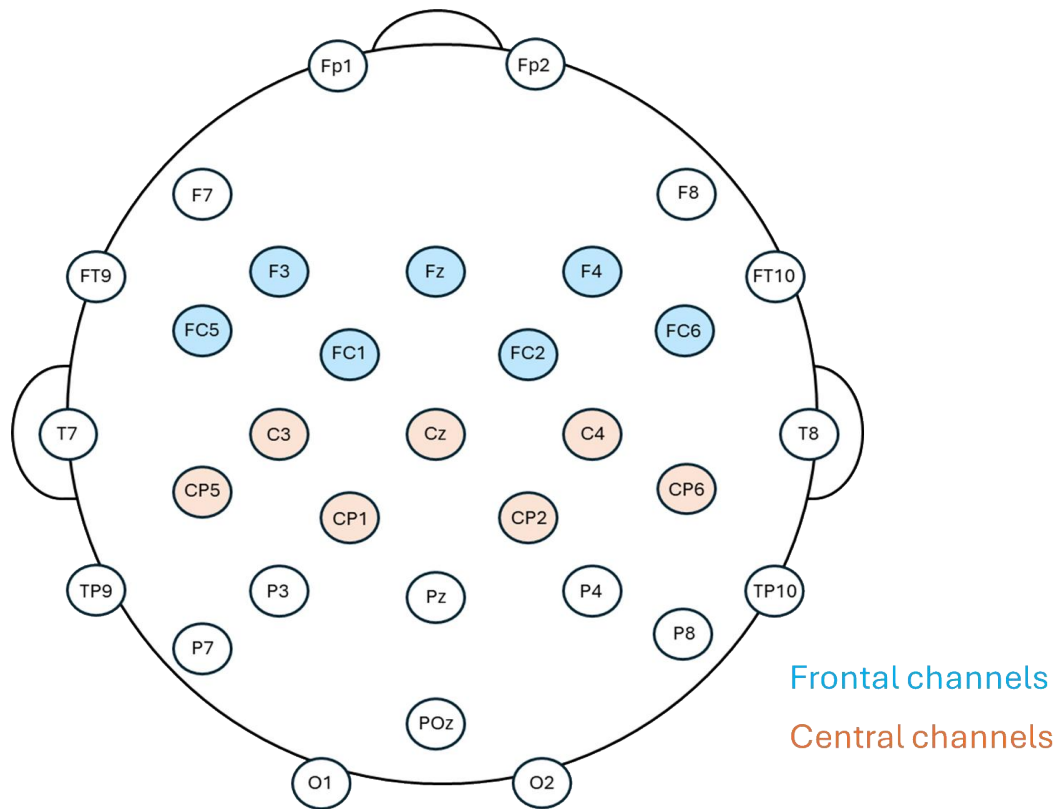
$$\Delta\varphi_k(f_n, f_m, t) = \left(\frac{n+m}{2m} \varphi(f_m, t, k) - \frac{m+n}{2n} \psi(f_n, t, k) \right) \quad (2)$$

where n and m are the center frequencies of the two signals and $\varphi(f_m, t)$ is the phase angle at channel φ , at time t , on trial k , and channel ψ .

PLV ranges from $[0, 1]$, where $PLV = 0$ indicates random signals with unsynchronized phases and $PLV = 1$ indicates stronger coupling between the two signals. In the present study, PLV was computed using MATLAB Fieldtrip ([Oostenveld et al. 2011](#)) custom script deploying functions from the semi-automated pipeline DEEP ([Kayhan et al. 2022](#)). The frequency bands of choice were *theta* and *alpha* ([Haartsen et al. 2020](#); [van der Velde et al. 2019](#)) due to their respective association with changes in directed gaze during joint and individual play ([Wass et al. 2018](#)) and in changes in emotional states during the interaction ([Perone et al. 2020](#); [Santamaria et al. 2020](#)). Considering the differences in frequency peaks between the infant and the adult brain ([Noreika et al. 2020](#)), we opted for a cross-frequency approach, computing *theta* and *alpha* PLVs using both the infant thresholds (3-5 Hz for *theta* and 6-9 Hz for *alpha*; [Marshall et al. 2002](#); [van der Velde et al. 2019](#)) and the adult thresholds (4-7 Hz for *theta* and 8-12 Hz for *alpha*; [Hill et al. 2020](#)).

Following a previous work ([Santamaria et al. 2020](#)), fourteen ($N = 14$) out of 32 total channels were considered for the computation, seven of which were frontal (F3, F4, Fz, FC1, FC2, FC5, FC6) and seven were central (C3, C4, Cz, CP1, CP2, CP5, CP6). As illustrated in Figure 3.2, the computation of the PLV was performed between homologous channels, resulting in a total of $N = 14$ different PLVs, one per channel. Single-channel PLV would be then grouped according to their region (frontal, central) and averaged in order to obtain a mean frontal and a mean central PLV per episode (Play, Reunion) and frequency band (*theta*, *alpha*).

Figure 3.2. Channels of interest for phase-based INS estimates. Adapted from [Pili et al. 2025](#).



2.4. Data quality assessment

To ensure that the quality of our data was adequate for the computation of the PLV, signal-to-noise ratio (SNR) was computed on the raw parent and infant signals before undergoing data pre-processing. SNR was calculated through a MATLAB EEGLAB custom script that, coherently with previous works ([Babiloni et al. 2004](#); [Donoghue et al. 2022](#); [Goldenholz et al. 2009](#)), used the spectral method to estimate the ratio between the power of the signal and the power of the noise for each channel. In our script, the signal was operationalized as the power displayed in the *theta* (3-5 Hz for the infants and 4-7 Hz for the parents) and the *alpha* (6-9 Hz for the infant and 8-12 Hz for the parents) frequency bands, whereas the noise was equal to the power of the white noise (≥ 100 Hz for both infants and parents, considering that frequency bands of neural activity are defined up to 95 Hz; see [Koul et al. 2023](#)). After computing the power of the signal and the power of the noise using the “bandpower” function, we calculated the ratio between them in each channel (flat and outlier channels were momentarily interpolated for this purpose) and transformed the results into decibels. SNR values

for each channel were then averaged in order to obtain a single global SNR value for each subject. Positive SNR values are regarded as indicative of a higher power in the “signal” and negative values indicate a higher power in the bands that are considered “noise”. No standardized cut-off for SNR values is present for studies including real EEG from parent-infant interactions; however, previous works deploying real and simulated data of adult EEG signals recommend a threshold up to 10 dB ([Babiloni et al. 2004](#)), so that oscillatory measures such as instantaneous phase and frequency ([Donoghue et al. 2022](#)) can be reliably estimated.

2.5. Statistical analysis

Data analysis was performed using Jamovi version 2.3.28 (The jamovi project 2024 n.d.). The graphs representing the mean SNR values of our signals were built with RStudio version 2023.12.1 (RStudio Team 2023). To explore the impact of the different methodological choices taken during the two different pre-processing methods on PLVs, the following plan of analysis was performed. Descriptive statistics and Student’s t tests for paired samples were applied to detect potential differences between the two pipelines on specific outputs of the data pre-processing, namely number of interpolated channels per signal, type of ICs, percentage of rejected ICs and epochs per episode. After computation of PLV on the signals pre-processed with the two different pipelines, Pearson’s correlations and repeated measures ANOVA accounting for Episode (Play, Reunion) and Pipeline (Automated, Manual) were computed for each Region (Frontal, Central) and frequency band (*theta*, *alpha*) to compare the impact of the different pre-processing methods on this index.

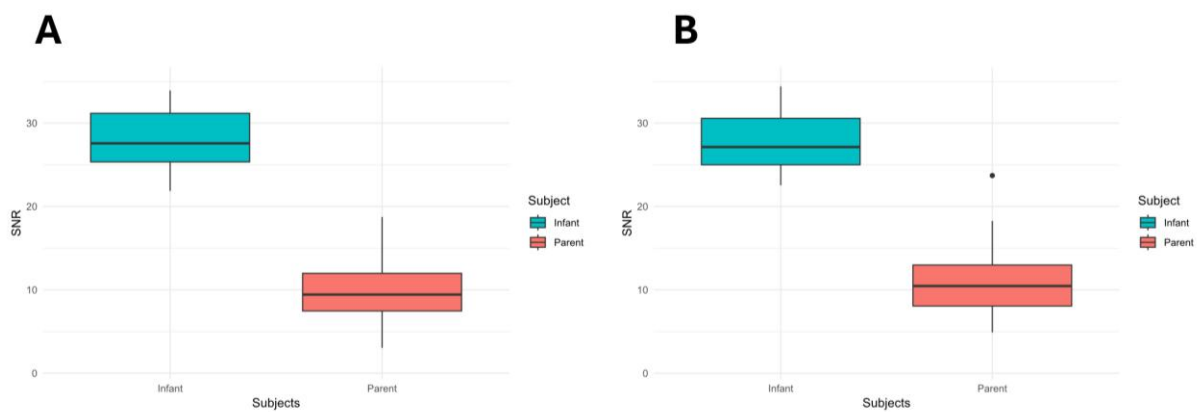
3. RESULTS

3.1. SNR estimation

Global SNR values for the parents’ and infants’ signals during Play (A) and Reunion (B) episodes are showcased in Figure 3.3. All subjects revealed positive SNR values in both episodes, indicating a good quality of the data used for PLV computation. However, SNRs in the infants’ signals were found to be significantly higher compared to the ones computed in the parents’ signals in both episodes ($F(1,30) = 579.47$; $p < 0.001$). Indeed, SNRs computed in the infants’ signals ranged [21.86

33.9] in the Play episode (mean global value: 28.06 ± 3.40 dB) and [22.54 34.4] in the Reunion (mean global value: 27.64 ± 3.40 dB); the parents' signals, on the other hand, had significantly lower SNRs, with values ranging [3.05 18.7] in the Play episode (mean global value: 9.74 ± 3.84 dB) and values ranging [4.90 23.7] in the Reunion episode (mean global value: 11.10 ± 4.01 dB). Despite these differences, all the infants' and the majority of the parents' signals showcased a SNR that exceeded the threshold of 10 dB, meaning that our dataset was adequate for a reliable computation of the PLV in our frequency bands of interest.

Figure 3.3. Mean SNR values of the raw infants' and parents' signals during the Play (A) and Reunion (B) episodes. Adapted from [Pili et al. 2025](#).



3.2. Data pre-processing outputs

A summary of the descriptive statistics, along with the results of the statistical analyses, is available on Table 3.1.

Table 3.1. Descriptive statistics and paired samples Student's t tests comparing the performance of the two pipelines (automated to manual). Significant comparisons are reported in bold. Adapted from [Pili et al. 2025](#).

Parameter	Mean ± SD (range) - Automated	Mean ± SD (range) - Manual	Student's t	p	C.I.
N interpolated channels (Parent)	2.44 ± 2.02 (0-8)	2.27 ± 2.02 (0-8)	1.64	0.110	-0.04; 0.39
N target interpolated channels (Parent)	0.15 ± 0.36 (0-1)	0.15 ± 0.36 (0-1)	NA	NA	NA
N interpolated channels (Infant)	1.65 ± 1.65 (0-6)	1.88 ± 1.84 (0-6)	-1.49	0.147	-0.56; 0.09
N target interpolated channels (Infant)	0.18 ± 0.58 (0-3)	0.35 ± 0.81 (0-3)	-1.79	0.083	-0.38; 0.02
Perc. removed components (Parent)	7.74 ± 6.27% (0-33.33%)	21.98 ± 12.67% (3.13-46.88%)	-6.48	<0.001	-18.7; -9.75
Perc. removed components (Infant)	4.25 ± 2.37% (0-12.5%)	14.92 ± 11.52% (0-28.13%)	-5.24	<0.001	-14.8; -6.51
Merged rejected epochs (Play)	5.10 ± 11.27 (0-55)	20.71 ± 19.07 (1-74)	-6.27	<0.001	-20.7; -10.53
Merged rejected epochs (Reunion)	6.45 ± 12.23 (0-54)	22.55 ± 21.65 (2-85)	-5.04	<0.001	-22.6; -9.57

3.2.1. Interpolated channels

As shown in Table 3.1, we have compared the performance of the two pipelines during channel interpolation based on two parameters: overall number of interpolated channels (out of $N = 32$) and number of interpolated “target” channels for PLV computation (out of $N = 14$). In both these parameters and in both the parents’ and the infants’ signals, the performance of the two pipelines did not significantly differ from one another, as demonstrated by the non-significant p-values yielded by the paired samples Student’s t test (see Table 3.1). The number of overall interpolated channels ranged [0 8] (0-25%) for the parents’ signals and [0 6] (0-18.75%) for the infants’ signals, whereas the number of interpolated target channels ranged [0 1] for parents (0-3.13%) for parents and [0 3] (0-16.67%) for infants’ signals.

3.2.2. Type of ICs

Figures 3.4 illustrates the ICs detected by ICLabel during each pre-processing method in the infants’ (A) and the parents’ (B) signals. Results of Student’s t tests comparing the results of the automated and manual pre-processing pipelines are showcased in Table 3.2.

Figure 3.4. Comparison of ICLabel results between the two pipelines for the infants’ (A) and parents’ (B) signals. Adapted from [Pili et al. 2025](#).

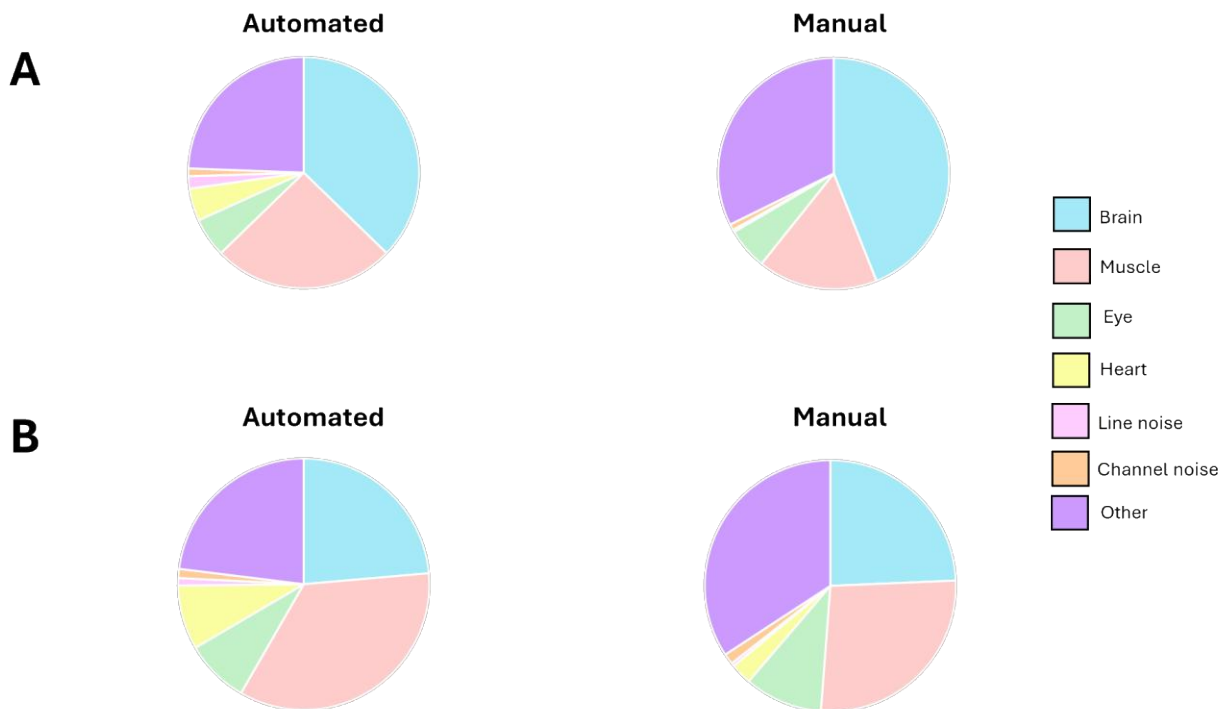


Table 3.2. Paired samples Student’s t tests comparing the type of ICs resulting from ICLabel for each pipeline (automated to manual). Significant comparisons are reported in bold. Adapted from [Pili et al. 2025](#).

Component	Infant		Parent	
	Student’s t	p-value (C.I.)	Student’s t	p-value (C.I.)
Brain	-1.89	0.067 (-13.56; 0.49)	-0.35	0.731 (-4.97; 3.53)
Muscle	2.59	0.014 (1.88; 15.66)	1.99	0.056 (-0.22; 15.91)
Eye	-0.39	0.697 (-1.81; 1.22)	-2.58	0.015 (-3.26; -0.38)
Heart	2.10	0.044 (0.12; 8.53)	2.89	0.007 (1.63; 9.43)
Line noise	2.06	0.048 (0.015; 3.21)	1.45	0.158 (-0.22; 1.29)
Channel noise	0.63	0.536 (-0.59; 1.13)	-0.34	0.735 (-1.44; 1.03)
Other	-2.07	0.048 (-15.41; -0.09)	-2.96	0.006 (-18.88; -3.45)

Figures 3.4. shows how the distributions of the ICs in the infants’ and the parents’ signals are slightly different from one another. While the infants’ signals showcased a majority of “Brain” ICs both after automated (37.25%) and manual (43.78%) pre-processing, followed by “Muscle” (25.49% for automated and 16.72% for manual) and “Other” (24.41% for automated and 32.16% for manual), “Eye” (5.50% for automated and 5.79% for manual), and then “Heart” (4.55% for automated and 0.22% for manual), “Channel noise” (1.08% for automated and 0.82% for manual) and “Line noise” (1.72% for automated and 0.11% for manual), the parents’ signals displayed a majority of either “Muscle” (34.73% for automated and 26.88% for manual) and “Other” (23.04% for automated and 34.20% for manual) ICs, followed by “Brain” (23.61% for automated and 24.33% for manual), “Eye” (8.24% for automated and 10.05% for manual), and then “Heart” (8.26% for automated and 2.73% for manual), “Channel noise” (1.18% for automated and 1.38% for manual) and “Line noise” (0.95% for automated and 0.41% for manual).

In the infants’ signals, ICLabel ran in the automated pipeline found significantly more “Muscle” ($t(30) = 2.59$; $p = 0.014$), “Heart” ($t(30) = 2.10$; $p = 0.044$) and “Line Noise” ($t(30) = 2.06$; $p = 0.048$) ICs, as well as significantly less “Other” ICs ($t(30) = -2.07$; $p = 0.048$), compared to the manual

pipeline. In the parents' signals, on the other hand, ICLabel ran in the automated pipeline found significantly more "Heart" ($t(30) = 2.89$; $p = 0.007$) ICs and significantly less "Eye" ($t(30) = -2.58$; $p = 0.015$) and "Other" ($t(30) = -2.96$; $p = 0.006$) ICs compared to the manual pipeline.

3.2.3. Rejected ICs

We also explored potential differences in performance of the two pipelines in terms of percentage of removed ICs. Paired samples Student's *t* test highlighted significant differences in the percentage of ICs rejected by the two pipelines both in the parents' ($t(30) = -6.48$; $p < 0.001$) and the infants' signals ($t(30) = -5.24$; $p < 0.001$), with the manual pipeline removing 2.84 times more ICs in the parents' signals and 3.51 times more ICs in the infants' signals compared to the automated one (see Table 3.1).

3.2.4. Rejected epochs

Another parameter of interest when preliminarily comparing the performance of the two pipelines is the number of rejected (merged) epochs in the Play and Reunion episodes, considering that this step was performed by EEGLAB in the automated pipeline and by a human coder in the manual pipeline using comparable exclusion criteria for bad epochs.

All recordings contained at least 30 (25%) good epochs in both Play and Reunion episodes, ensuring sufficient data to compute PLVs. Significant differences emerged in the number of epochs rejected by the two pipelines both in the Play ($t(30) = -6.27$; $p < 0.001$) and Reunion ($t(30) = -5.04$; $p < 0.001$) episodes: more specifically, the manual pipeline tended to reject 16 more epochs both in the Play and Reunion episodes (see Table 3.1).

3.3. PLV

3.3.1. Theta band

Central tendency measures for *theta* PLVs computed in the Play and Reunion episodes, as well as the correlations matrices comparing the PLVs obtained after automated and manual pre-processing, are available respectively on Table 3.3 and 3.4. Figure 3.5 plots the trends of the two pipelines across episodes in the frontal (A) and central (B) channels.

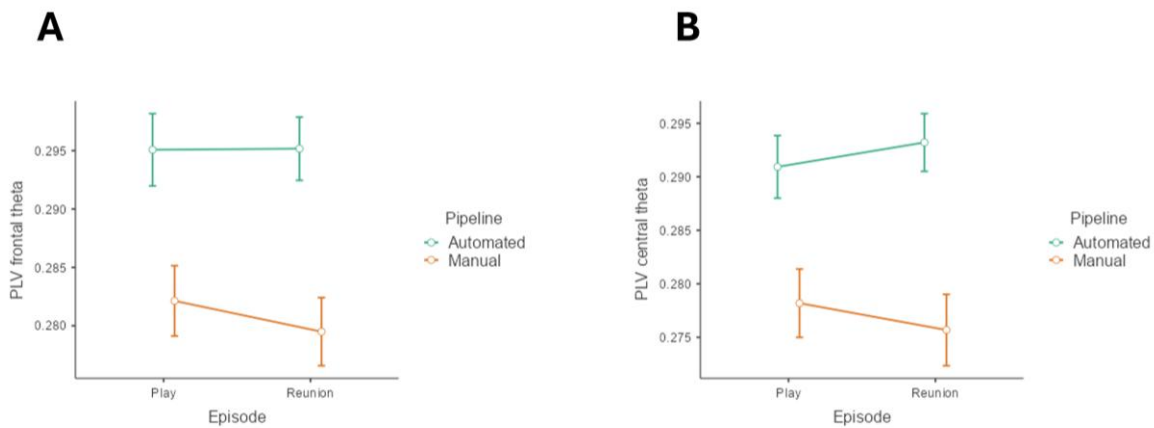
Table 3.3. Descriptive statistics comparing the PLVs calculated on the *theta* and *alpha* frequency bands after automated and manual pre-processing across episodes and regions. Adapted from [Pili et al. 2025](#).

Variable	Mean ± SD (range) - Automated	Mean ± SD (range) - Manual
PLV <i>theta</i> – Play - Frontal	0.294 ± 0.003 (0.254-0.329)	0.282 ± 0.017 (0.263-0.334)
PLV <i>theta</i> - Play - Central	0.289 ± 0.003 (0.260-0.324)	0.278 ± 0.017 (0.250-0.341)
PLV <i>theta</i> – Reunion - Frontal	0.295 ± 0.003 (0.270-0.328)	0.279 ± 0.016 (0.259-0.329)
PLV <i>theta</i> – Reunion - Central	0.293 ± 0.003 (0.270-0.322)	0.276 ± 0.019 (0.247-0.329)
PLV <i>alpha</i> - Play - Frontal	0.206 ± 0.002 (0.188-0.235)	0.206 ± 0.011 (0.184-0.239)
PLV <i>alpha</i> - Play - Central	0.210 ± 0.002 (0.191-0.232)	0.211 ± 0.009 (0.190-0.229)
PLV <i>alpha</i> – Reunion - Frontal	0.207 ± 0.002 (0.190-0.238)	0.208 ± 0.012 (0.189-0.239)
PLV <i>alpha</i> – Reunion - Central	0.212 ± 0.002 (0.190-0.244)	0.211 ± 0.013 (0.185-0.241)

Table 3.4. Correlation matrix (Pearson’s *r*) comparing the PLVs calculated on the *theta* frequency band after automated and manual pre-processing across episodes and regions. Note: **p* ≤ 0.05; ***p* ≤ 0.01; ****p* ≤ 0.001. Correlations between PLVs computed on homologous episodes and regions across pipelines are written in bold. Adapted from [Pili et al. 2025](#).

	Manual PLV <i>theta</i> – Play - Frontal	Manual PLV <i>theta</i> – Play – Central	Manual PLV <i>theta</i> – Reunion - Frontal	Manual PLV <i>theta</i> – Reunion - Central
Automated PLV <i>theta</i> – Play - Frontal	r = 0.746 *** p < 0.001	r = 0.678*** p < 0.001	r = 0.606*** p < 0.001	r = 0.571*** p < 0.001
Automated PLV <i>theta</i> – Play – Central	r = 0.597*** p < 0.001	r = 0.730*** p < 0.001	r = 0.488** p = 0.005	r = 0.576*** p < 0.001
Automated PLV <i>theta</i> – Reunion - Frontal	r = 0.528** p = 0.002	r = 0.561** p = 0.001	r = 0.575*** p < 0.001	r = 0.592*** p < 0.001
Automated PLV <i>theta</i> – Reunion - Central	r = 0.513** p = 0.003	r = 0.583*** p < 0.001	r = 0.568*** p < 0.001	r = 0.750*** p < 0.001

Figure 3.5. Trends of the PLVs calculated across episodes (Play, Reunion) in the frontal (A) and central (B) channels in the *theta* frequency band. Adapted from [Pili et al. 2025](#).



PLVs computed after automated and manual pre-processing yielded comparable values: PLVs after automated pre-processing ranged [0.254 0.329], whereas PLVs after manual pre-processing ranged [0.247 0.334]. Pearson’s correlations across homologous episodes and regions were all significant, positive and strong (see Table 3.4). Repeated measures ANOVA revealed a significant effect of Pipeline, but not of Episode, both in the frontal ($F(1,30) = 42.17$; $p < 0.001$) and central ($F(1,30) = 56.84$; $p < 0.001$) channels. In addition, PLV computed on central channels exhibited a significant Episode*Pipeline effect ($F(1,30) = 5.86$; $p = 0.022$). The findings indicate that, although the PLVs computed on the *theta* frequency band were strongly and significantly correlated with one another across the two pipelines, the automated pipeline tended to produce significantly higher *theta* PLVs in the frontal and central channels compared to the manual pipeline.

3.3.2. Alpha band

Results on the analyses - central tendency measures, Pearson’s correlations - conducted on PLVs calculated on the *alpha* frequency band are available respectively on Table 3.3 and 3.5. Figure 3.6 plots the trends of the two pipelines across episodes in the frontal (A) and central (B) channels.

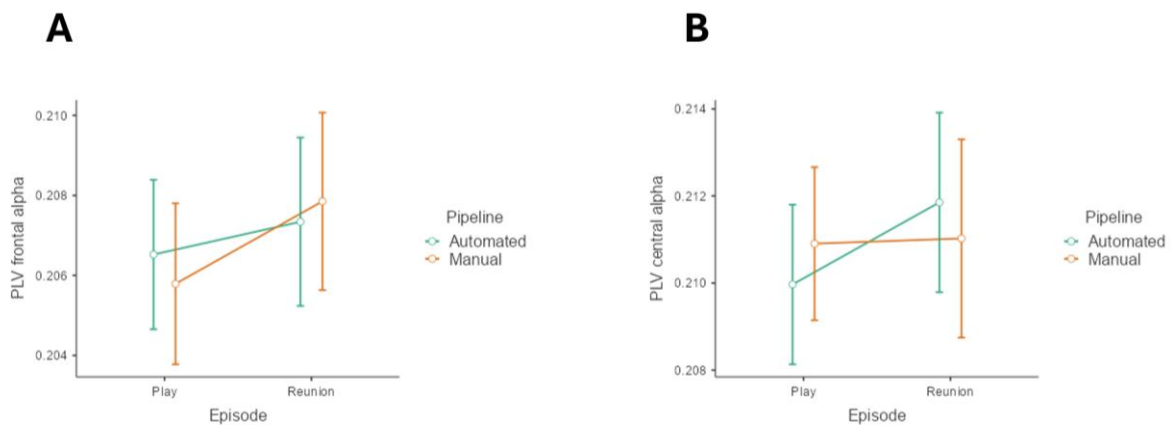
Table 3.5. Correlation matrix (Pearson’s r) comparing the PLVs calculated on the *alpha* frequency band after automated and manual pre-processing across episodes and regions. Note: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001. Correlations between PLVs computed on homologous episodes and regions across pipelines are written in bold. Adapted from [Pili et al. 2025](#).

	Manual PLV <i>alpha</i> – Play - Frontal	Manual PLV <i>alpha</i> – Play – Central	Manual PLV <i>alpha</i> – Reunion - Frontal	Manual PLV <i>alpha</i> – Reunion - Central
Automated PLV <i>alpha</i> – Play - Frontal	r = 0.879*** p < 0.001	r = 0.693*** p < 0.001	r = 0.613*** p < 0.001	r = 0.701*** p < 0.001
Automated PLV <i>alpha</i> – Play – Central	r = 0.650*** p < 0.001	r = 0.824*** p < 0.001	r = 0.523** p = 0.003	r = 0.733*** p < 0.001
Automated PLV <i>alpha</i> – Reunion - Frontal	r = 0.597*** p < 0.001	r = 0.569*** p < 0.001	r = 0.928*** p < 0.001	r = 0.709*** p < 0.001
Automated PLV <i>alpha</i> – Reunion - Central	r = 0.670*** p < 0.001	r = 0.644*** p < 0.001	r = 0.690*** p < 0.001	r = 0.900*** p < 0.001

Table 3.6. Results of the repeated measures ANOVA accounting for Episode (Play, Reunion) and Pipeline (Automated, Manual) conducted on the *alpha* PLVs for each region of interest (Frontal, Central). Adapted from [Pili et al. 2025](#).

Variable		df	F	p-value
PLV <i>alpha</i> – Frontal	Episode	1,30	0.026	0.874
	Pipeline	1,30	0.715	0.404
	Episode*Pipeline	1,30	1.148	0.292
PLV <i>alpha</i> - Central	Episode	1,30	0.505	0.483
	Pipeline	1,30	0.005	0.942
	Episode*Pipeline	1,30	1.550	0.223

Figure 3.6. Trends of the PLVs calculated across episodes (Play, Reunion) in the frontal (A) and central (B) channels in the *alpha* frequency band. Adapted from [Pili et al. 2025](#).



PLVs computed after automated and manual pre-processing yielded comparable values, with PLVs ranging [0.184 0.241] after automated pre-processing and PLVs ranging [0.185 0.247] after manual pre-processing. Pearson’s correlations across homologous episodes and regions were all significant, positive and strong (see Table 3.5). Repeated measures ANOVA revealed no significant effects of Episode, Pipeline, or Episode*Pipeline interaction both in the frontal and central channels (see Table 3.7).

4. DISCUSSION

To investigate the impact of different methodological decisions during dyadic EEG data pre-processing, we have pre-processed the signal of 31 mother-FT infant dyads with two pipelines – the “automated” and the “manual” pipelines, featuring the same pre-processing steps, but different degrees of automatization. After computing SNR of the raw signals and after pre-processing them with each of the two pipelines, we have computed cross-frequency PLVs in the *theta* (3-5 and 6-9 Hz) and *alpha* (6-9 and 8-12 Hz) frequency bands and compared the values resulting after each pre-processing method. The two pipelines showcase significant differences both in the outputs they generated (particularly type of ICs and rejected ICs and epochs) and in the way they affected PLVs. The automated pipeline tended to preserve the original signals by rejecting less ICs and epochs, and produced significantly higher PLVs in the *theta* frequency band compared to the manual pipeline. Despite this, the automated pipeline tended to contain more ICs labeled as various sources of noise

such as "Heart" and "Line Noise", whereas the manual pipeline contained more ICs labeled as ocular artifacts or "Other". No significant differences were found between the two pipelines in terms of interpolated channels, ICs labeled as brain activity, and PLVs computed in the *alpha* frequency band. Moreover, PLVs produced by the two pipelines were highly correlated in both frequency bands of interest. These results suggest that phase-based INS estimates such as the PLV seem to be affected, although only partially, by the choice of using a manual or automated pre-processing pipeline.

To the authors' knowledge, this is the first study comparing different methodological decisions during dyadic EEG data pre-processing using real dyadic EEG data including infants. I will break down each result and implication of the findings, to then proceed with analyzing the potential implications and limitations of the present study.

4.1. Differences in pre-processing outputs

Our analyses revealed that the automated pipeline tended to label significantly more ICs labeled as "Muscle" (infants' signals only), "Heart" (infant and parent signals) and "Line Noise" (infants' signals only) compared to the manual pre-processing; conversely, it tended to label significantly less ICs labeled as "Eye" (parents' signals only) and "Other" (infant and parent signals) compared to the manual pipeline. In addition, the automated pipeline rejected significantly less ICs and epochs per episode compared to the manual one. No significant differences across pipelines emerged when looking at the number of interpolated channels and the percentage of ICs labeled as "Brain" activity. The origin of the differences in the types of ICs produced by the two pre-processing methods is not clear, especially considering that the two pre-processing pipelines have no additional differences apart from their levels of automatization. One possible explanation for this result is that the slight discrepancies between the two pipelines at the bad channel detection and interpolation level, although not statistically significant, could have still determined significant changes in ICA decomposition. Notably, differences in the sets of interpolated channels were more common in the infants' signals ($N = 8$) compared to the parents' signals ($N = 3$), which is consistent with the differences in the IC ratios across pipelines being more frequent in the formers compared to the latter. In the parents'

signals with discrepancies in the set of channels interpolated by the two pipelines, the automated pipeline tended to systematically interpolate more channels than the manual one, which could have in turn reduced the global detection rate of some artifactual ICs such as ocular artifacts and those labelled as “Other”. The opposite trend was found, however, in the infants’ signals with discrepant channel interpolation across pipelines, since the majority of the signals (N = 5) had more interpolated channels in the manual pipeline compared to the automated one (although the remaining three displayed the opposite trend). This could explain, for instance, the tendency of the automated pipeline to detect significantly more artifactual ICs (e.g. muscular, cardiac and “line noise” artifacts) compared to the manual pipeline; despite this, further investigation is needed to assess the impact of channel interpolation on ICA decomposition. This applies especially to the parents’ data, since ICLabel as well as ADJUST were validated on an adult population ([Leach et al. 2020](#); [Mognon et al. 2011](#); [Pion-Tonachini et al. 2019](#)), whereas discrepancies in the IC classification in infant data are not unlikely due to the structural and functional differences between the infant and the adult brain ([Noreika et al. 2020](#)). Although in fact ICLabel was found to have a comparable performance to IC-classification plugins built specifically for infant EEG data such as adjusted- ADJUST ([Leach et al. 2020](#)), it is possible for it to underperform when dealing with EEG data recorded on different subjects such as infants ([Leach et al. 2020](#); [Pion-Tonachini et al. 2019](#)).

Notably, in the parents’ EEG recordings, the automated pipeline was found to label significantly less ICs as ocular artifacts compared to the manual pipeline, whereas this was not true for the infants’ EEG data (see par. 3.2.2.). This difference could provide a partial explanation to the tendency of the manual pipeline to reject more ICs in the parents’ signals compared to the automated one; however, the majority of the discrepancies in the ICs rejection process between the two pipelines could be easily ascribed to the level of automatization, analogously to the differences in the number of rejected epochs per episode. In addition, the manual pipeline implemented the use of the ADJUST plugin to support the coder in ICs rejection, which is trained not only to reject eye blinks and vertical and horizontal eye movements, but also to detect generic discontinuities in the signals (e.g. impedance

fluctuations or electronic devices interferences; [Mognon et al. 2011](#)). It is plausible to think that these characteristics of the ADJUST plugin may have constituted an additional reason behind the tendency of the manual pipeline to reject significantly more ICs than the automated pre-processing.

Overall, these findings suggest that the degree of automatization of a dual EEG pre-processing pipeline has a significant impact on the type of extracted ICs and, consequently, on its capacity to detect and reject a various number of artifacts such as motion-related artifacts, eye movements and general discontinuities of the EEG signals. An automated pre-processing method has been found to be more conservative of the original signal properties and thus reject less ICs and epochs compared to a human coder, who can be more prone to reject ICs and epochs that display general discontinuities or motion-related artifacts. While these findings provide insights that could aid researchers in choosing between a manual or automated pre-processing method, it does not provide evidence on the ideal thresholds and parameters that should be used when processing EEG data recorded on parent-infant dyads. Considering that this is the first study comparing the performance of two different dual EEG data pre-processing methods, however, further evidence is needed to support these findings and their respective interpretations.

4.2. Pipeline-dependent changes in PLVs

Together with their differences in terms of rejected ICs and epochs, the automated and the manual pipelines seemed to affect cross-frequency PLVs in different fashions. Repeated measures ANOVA revealed that *theta* PLVs computed in the frontal (Figure 3.5A) and central (Figure 3.5B) channels after automated pre-processing were significantly higher than those computed after the manual pre-processing. However, no significant differences were displayed by the *alpha* PLVs (both in frontal and central regions) between the two pipelines; moreover, Pearson's correlations among *theta* and *alpha* PLVs calculated after automated and manual pre-processing in homologous episodes (Play, Reunion) and regions (Frontal, Central) were all positive, strong and significant.

PLV is one of the most widely used measures of INS in EEG hyperscanning research due to its aptness at capturing the dynamic flow of information between two social agents during interactions; despite

this, the literature provides evidence on the proneness of this index to spurious hyperconnections (i.e. between non-matched participants; [Burgess 2013](#)) and on its tendency to inflate with shorter epoching ([Zimmermann et al. 2024](#)). While our results cannot provide a direct support to these claims, it is clear that the choice of an automated or manual pre-processing pipeline has, to some degree, affected our INS estimates, particularly those that were computed on the infant and adult *theta* frequency band. We propose two potential explanations as to why only the *theta* PLVs displayed a significant effect of the Pipeline factor.

Framework 1 - contamination from ocular artifacts. In our study, the automated and the manual pipelines were made to reject ICs that showcased a high probability (50% or more) of being an ocular artifact. According to our analyses, the manual pipeline tended to label significantly more ICs as ocular artifacts in the parents' signals and, in general, rejected significantly more ICs in both infants and parents compared to the automated one. Notably, previous works assessing ocular activity in the adult brain have consistently detected these types of artifacts in the lower frequencies, including adult delta (0.5-4 Hz) and *theta* (4-7 Hz; [Gasser et al. 1985](#); [Kanoga et al. 2016](#)), but not *alpha*. It is thus possible that, contrary to the *alpha* PLVs, the PLVs computed in the *theta* frequency band were inflated by some leftover ocular artifacts in the parents' signals that remained undetected by ICLabel during automated, but not manual, pre-processing.

Framework 2 - different signal conservation rates. According to our analyses, the manual pipeline tended to reject significantly more ICs and epochs in both members of the dyad (infant and parent) compared to the automated one. While this could have implied that the manual pipeline had a lower probability than the automated one to contain residual noise – e.g. ocular artifacts – that could have inflated PLVs in the *theta* band, it could have also inadvertently removed portions of the signal that displayed *theta* oscillations, thus leading to lower PLVs in this frequency band compared to those computed after automated pre-processing. Notably, while ICA is a reliable method to separate brain sources from stereotypical artifacts (e.g. eye movement, muscle artifacts), mixed ICs containing both brain and artifactual data are not uncommon ([Winkler et al. 2015](#)), including ICs with mixed brain

and ocular sources. Consequently, it is possible that the manual pipeline, being stricter in terms of ICs and epoch rejection, could have rejected more portions of the signal in which vertical and horizontal eye movements were mixed with *theta* oscillations stemming from brain activity, something that may not have happened with *alpha* oscillations due to the location of ocular artifacts at lower frequencies.

Comprehensively, our findings seem to suggest that choosing an automated or manual pre-processing method, although equal in terms of order of the specific steps and parameters chosen to minimize artifacts, has a significant impact on phase-based INS measures such as PLV. However, as we have no access to the "ground truth" PLVs of our datasets, we have no means to assess whether one pipeline was more fitting than the other for dealing with dyadic EEG data. Moreover, as there is limited evidence regarding the impact of the degree of automatization of EEG data pre-processing pipelines on INS estimates, EEG technicians and other experts in the field are encouraged to take a critical approach to the proposed interpretations and to test the reproducibility of our findings through further methodological studies. It is also recommended that future studies make comparisons not only between pipelines with different degrees of automatization, but also between pipelines with different pre-processing steps and thresholds in order to determine how different pipelines impact the signal quality and, subsequently, INS estimates in dyadic EEG data.

4.3. Towards the use of automated pre-processing pipelines for dyadic EEG data processing

Overall, the significant differences revealed by our analyses in the *theta* PLVs obtained after automated and manual pre-processing suggest the need for further research and systematization of EEG data pre-processing collected in parent-infant dyads. A potential path towards this endeavor could be the employment of fully automatized dyadic EEG pre-processing methods accounting for the structural and functional differences between the adult and the infant brain. Employing an automated pipeline for EEG data pre-processing would have several advantages such as increased rapidity of analysis and enhanced reproducibility of the findings. Rapidity of analysis is a significant perk when dealing with dual EEG data, especially when dealing with large datasets: manually pre-

processing one dyad, especially with pipelines containing highly time-consuming steps such as ICA decomposition and epoch rejection, may take up to one hour per dyad, whereas our automated pre-processing pipeline, for instance, pre-processes at least two more dyads in the same time frame. This would allow EEG hyperscanning researchers not only to obtain the results of their data collections in a reasonable time frame, but also to make significant advances on the field of EEG data pre-processing methodology by exploring the impact of different pre-processing parameters on the signals. Regarding reproducibility, it is clear that a fully automated pre-processing pipeline, with parameters and thresholds set in advance, is less prone to subjective decision making compared to a manual pipeline requiring an expert coder for IC and epoch rejection. Employing a highly reproducible pipeline may be an invaluable asset in hyperscanning research, especially when considering the dearth of reproducibility studies addressing data analysis techniques in this field of research ([Kayhan et al. 2022](#)). However, we recognize that this study represents nothing more than a small contribution towards this endeavor. Notably, we have no means to reliably assess whether our automated pipeline improved SNR in a significantly more adequate way compared to our manual pre-processing; in addition, we do not know whether our findings regarding automated and manual pre-processing pipelines can be replicated with INS indexes based on different signal properties such as power amplitude (e.g. amplitude envelope coupling). Phase and amplitude are indeed known for being inter-independent properties of the EEG signal, and although there are methodological works challenging this claim ([Zimmermann et al. 2024](#)), it remains important to assess whether phase and amplitude-based INS indexes are affected in similar or different ways by the type of pre-processing method and by different steps and thresholds. We therefore highlight the need for further evidence regarding the feasibility of fully automated pre-processing in developmental EEG hyperscanning studies with the use of control measures such as SNR and power analysis in the frequency bands of interest in the field such as *theta* and *alpha*, as well as the investigation of the impact of different pre-processing methods on INS indexes based on other signal properties such as amplitude.

5. LIMITATIONS

Despite the presented findings and implications, the present study has notable limitations, including the exclusive use of the PLV as INS index, the absence of quality assessment on the signal modified by the two pipelines such as SNR on the selected frequency bands, lack of control measures of our INS synchrony of choice (PLV), and the absence of comparison of our automated pipeline with other automated and open-source pipelines (e.g. DEEP, HAPPE, MADEnnn).

5.1. Usage of one INS index as outcome measure

As previously mentioned in the paper, the only INS index used as a metric to understand the impact of different pre-processing methods on dyadic EEG data was the PLV, calculated as co-variance of phase between the activity recorded on the parent and the infant on homologous channels. This constitutes a significant limitation to our findings, especially considering that an increasing number of hyperscanning studies is employing other and more robust measures of INS such as the Circular Correlation (CCorr; [Neel et al. 2025](#)), the Partial Directed Coherence (PDC; [Leong et al. 2017](#); [Santamaria et al. 2020](#)) and the Amplitude-Amplitude Coupling using the envelope (AAC; [Koul et al. 2023](#); [Palva and Palva 2018](#)). While the CCorr is a phase-based INS estimate like the PLV and measures the circular co-variance between the observed and the expected phase of the two signals ([Burgess 2013](#)), PDC and AAC are based on different signal properties and theoretical assumptions. While all these indexes are optimized for minimizing spurious correlations, we cannot exclude that the use of different methodological choices may impact these indexes differently as seen in the paper of Zimmermann and colleagues ([2024](#)). Future studies comparing different dual EEG data pre-processing methods should therefore use at least two INS indexes as outcome measures, possibly based on different signal properties (phase, amplitude) and/or different theoretical assumptions (directional vs non-directional synchronization).

5.2. No quality assessment of the pre-processed signal (SNR)

A second limitation to our findings is the fact that, while we have performed a quality check of the raw signals through computation of SNR, we did not assess potential differences on the degree in

which SNR was improved after automated and after manual pre-processing. While we can assume that both pipelines have increased SNR from its baseline levels through filtering and artifacts rejection, the literature does not provide any standardized procedure on how to reliably estimate SNR in dyadic EEG data after signal pre-processing due to a lack of consensus of what portions of data should be defined as “noise”. Considering how SNR has been found to positively impact phase-based INS measures such as PLV and CCorr in Zimmermann and colleagues’ methodological work ([Zimmermann et al. 2024](#)), as well as how failure to properly conduct some of the earliest pre-processing steps such as data filtering, artifact rejection and re-referencing may lead to lower SNR due to the introduction of new artifactual data into the signal ([Chiarion et al. 2023](#)), we posit that computation of SNR should be implemented as one of the control measures of signal quality before and after pre-processing, together with other procedures such as dyads shuffling aimed at ensuring that the synchrony values obtained after data processing reflect interaction-driven neural synchronization rather than, for instance, sensorimotor communication ([Pezzulo et al. 2013](#); [Pezzulo et al. 2019](#)). Advantages of SNR computation after data pre-processing include a better understanding of how different methodological decisions could affect signal quality in both adult and infant EEG, which in turn would promote an informed standardization of dyadic EEG data pre-processing routines.

5.3. No control measures on the INS index of choice

While the present study was not aimed at validating the PLV as the gold standard for mother-infant INS studies, it is important to point out that we did not implement control measures such as dyads shuffling to reliably assess the nature of the INS patterns reported in this work. It has been indeed argued that, while it has been extensively used to estimate INS during parent-infant interactions ([Atilla et al. 2023](#); [Atzaba-Poria et al. 2017](#); [Endevelt-Shapira et al. 2021](#); [Krzeczkowski et al. 2022](#); [Leong et al. 2017](#); [Leong et al. 2019](#) ; [Perone et al. 2020](#); [Santamaria et al. 2020](#); [Wass et al. 2018](#)), PLV could rather reflect the similarities in frequency of oscillation between the two subjects or, simply put, the fact that the two members of the dyad are engaged in the same task during neural data

acquisition ([Burgess 2013](#)). Notably, the present work includes SNR as a control measure for the adequacy of our dataset for PLV computation; despite this, usage of control measures such as dyads shuffling in both methodological works and dyadic EEG hyperscanning studies is highly recommended.

5.4. No comparison with other validated pipelines (DEEP, HAPPE, MADE)

It is also important to note that the performance of our automated and manual pipelines was not compared to the one of other open-source pre-processing methods such as DEEP ([Kayhan et al. 2022](#)), MADE ([Debnath et al. 2020](#)) or HAPPE ([Debnath et al. 2020](#); [Gabard-Durnam et al. 2018](#)). By comparing the performance of multiple pre-processing pipelines, it would have been possible to assess more precisely which steps of the pre-processing make the most significant difference in INS estimates such as PLV or other indexes based on other signal properties.

6. CONCLUSIONS

Despite these limitations, the present study delivers preliminary evidence on the importance of the choice of the pre-processing method when analyzing dyadic EEG data, and highlights once more the need for standardization of the EEG data pre-processing methods in developmental hyperscanning research in terms of degree of automatization of noise correction, as well as order of the steps and their specific thresholds. To achieve this, it is recommended that EEG experts and developmental neuroscientists conduct further methodological studies on dyadic EEG data pre-processing methods, something that could potentially lead establishment of best practices and the creation of validated and reproducible automated or semi-automated pre-processing tools. Furthermore, we highlight the importance of assessing the impact of different methodological decisions on INS estimates based on other signal properties (e.g. amplitude) or theoretical assumptions (e.g. directed INS).

CHAPTER 4 – EXPLORING DYADIC “LEAD AND FOLLOW” DYNAMICS BEFORE AND AFTER AN INTERACTIVE PERTURBATION AND ITS RELATION TO DYADIC BEHAVIOR (adapted from Pili et al., *under review*)

This chapter addresses Aim 2 of the 2-BRAINED study, i.e. assessing whether parent-infant INS follows a directed pattern and how said pattern changes in response to an interactive perturbation. The introduction, methods, results and discussion sections, as well as the figures and tables, are adapted from the original draft of this work (Pili et al. *under review*, available in the Appendix), which has been submitted to NeuroImage on October 9th, 2025 and is currently undergoing peer review.

1. INTRODUCTION

Although underrepresented in the current literature, directed INS metrics, such as Partial Directed Coherence (PDC; [Baccalá and Sameshima 2021](#)) and Time-lagged cross-correlations ([Wass et al. 2018](#)) are believed to better reflect the time-lagged nature of dyadic interactions ([Turk et al. 2022](#)). Directed INS is based on the assumption that the neurobehavioral activity of one member of the dyad is “framing”, or “causing” the neurobehavioral patterns showcased by the other member of the dyad, a pattern that has been observed multiple times in a strictly behavioral level during ecological observational studies ([Cohn and Elmore 1988](#); [Cohn and Tronick 1987](#); [Feldstein et al. 1993](#); [Kaye and Fogel 1980](#)). At the neural level, Santamaria and colleagues ([2020](#)) have observed that, when mothers displayed positive emotionality towards a toy in front of their ten-month-old infants, mother-to-infant directed INS measured via PDC in the *alpha* band (6-9 Hz) was significantly stronger compared to the one measured during displays of negative emotionality. Similarly, Wass and colleagues ([2018](#)) have explored the relationship between parents’ neural responsivity and infant attention focus during joint versus solo play, highlighting how, during joint play, infants were more attentive when their parents were more neurally responsive to them.

While there is an increasing number of EEG hyperscanning studies exploring non-directed INS during structured and unstructured dyadic exchanges (see for instance [Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#); [Leong et al. 2019](#); [Neel et al. 2025](#)), changes in parent-infant directed INS during social interactions remain prevalently unexplored. Additionally, the current literature still lacks studies exploring the impact of interactive ruptures, a common occurrence during real-life interactive exchanges, on parent-infant INS. INS metrics that are able to capture changes in the “leader and follower” dynamics during social interactions, such as PDC, could provide invaluable insights into how parents and infants re-negotiate their roles as social partners after an interactive mismatch. Considering all this, our objective was to explore changes in the neural “leader and follower dynamics” (directed INS) before and after the FFSF, and whether said changes are related

to the degree in which infants showcased behaviors that constitute the “still-face effect”, such as negative emotionality and gaze aversion, and to maternal sensitive behaviors such as affective touch. In line with previous research on parent-infant directed behavioral synchrony ([Feldman 2007](#); [Feldman et al. 1996](#); [Feldman et al. 1999](#)) and on the still-face effect (see [Mesman et al. 2009](#) for an overview), we aim at testing the following experimental hypotheses. Our first hypothesis (H1) is that, during the baseline (play) episode of the FFSF procedure, directed INS between parents and their nine-month-old infants will follow a bidirectional pattern, meaning that we expect no significant differences between the infant-led INS (I->P, infant leading and parent following) and the parent-led INS values (P->I, parent leading and infant following). This hypothesis is supported by previous behavioral studies on directed parent-infant behavioral synchrony, which showcased a prevalence of mutual synchrony among parents and infants aged 9 months due to infants reaching the phase of intersubjectivity ([Feldman 2007](#); [Feldman et al. 1996](#); [Feldman et al. 1999](#)). After the interactive perturbation (reunion episode), we hypothesize (H2) that the dyads whose infants showcase high percentages of negative affect or gaze aversion during the Still-Face or whose parents showcased high percentages of affective touch during Reunion will display a shift in the direction of INS, going from bidirectional to parent-led (P->I). This hypothesis is based on the assumption that parents whose infants showcase a marked still-face effect, characterized by numerous instances of negative emotionality and/or gaze aversion, will actively try to bring their infant back to a state of positive engagement, thus leading the dyadic interaction both on a behavioral and neural level.

2. METHODS

2.1. Participants and procedure

Our sample consisted of 74 infants (age range 8-11 months) and their primary caregivers (their mothers) enrolled as control subjects (FT group) for the 2-BRAINED project (see Chapter 2, “*1.1. Population, enrolment and arm allocation*”, for details on the recruitment strategy). The experimental

procedure was the same described in Chapter 2 (“1.2. Experimental procedure”). Participants for this study were recruited between June 2022 and February 2025.

2.2. Synchrony analysis

Dual EEG data collected on parents and infants was pre-processed according to the pipeline described in Chapter 2 (“1.3. Plan of EEG data elaboration”). Eligibility criteria for synchrony analyses are the same defined in Chapter 2.

Since our aim was to investigate whether parent-infant INS followed a directed pattern, we chose to compute INS using the Partial Directed Coherence (PDC; [Baccalá and Sameshima 2021](#)). PDC is the frequency domain equivalent of Granger causality ([Granger 1969](#)) and measures the directed influences between a pair of signals in a multivariate dataset ([Baccalá and Sameshima 2001](#); [Choi and Kim 2018](#); [Santamaria et al. 2020](#)). Assuming that the simultaneously recorded m channel signals $x(t) = [x_1(t), \dots, x_m(t)]^T$ can be described by a multivariate vector autoregressive (MVAR) model as follows:

$$x(t) = \sum_{n=1}^p A_n x(t-n) + e(t) \quad (1)$$

where p is the model order, A_n is the matrix of model coefficients at time lag n , and $e(t) = [e_1(t), \dots, e_m(t)]^T$ is a multivariate Gaussian white noise with mean equal to zero and covariance matrix Σ . Transforming said MVAR model by Fourier Transform (FT), we obtain:

$$\bar{A}(f) = I - A(f) = [\bar{a}_1(f) \bar{a}_2(f) \dots \bar{a}_m] \quad (2)$$

where $A(f)$ is the FT of the model coefficients and $\bar{a}_{i,j}(f)$ is the i, j th element of $\bar{A}(f)$. From the transformed model coefficients, the PDC can be computed as

$$PDC_{i \rightarrow j}(f) = \frac{\bar{a}_{i,j}(f)}{\sqrt{\bar{a}_j^H(f) \bar{a}_j(f)}} \quad (3)$$

where H indicates the transpose and complex conjugate operator. PDC is thus a normalized measure able to quantify the relative strength of the influence of the signal x_i on the signal x_j at frequency f ([Choi and Kim 2018](#); [Santamaria et al. 2020](#)). While it does not have a predetermined range of values, higher PDC values indicate a higher probability for the connection between signal x_i and signal x_j to be directed ([Choi and Kim 2018](#)). When both the feedback and feedforward connections between signal x_i and signal x_j are strong, it is likely that they are influencing each other in a bidirectional fashion ([Pereda et al. 2005](#)).

In the present study, PDC was computed through a custom script that calls functions from the MATLAB toolbox Fieldtrip ([Oostenveld et al. 2011](#)). To minimize the effect of volume conduction as well as the amount of data contained in the MVAR modeling, inter-neural PDC was computed across a subset of six channels, two of which frontal (F3, F4), two central (C3, C4) and the remaining two posterior (P3, P4). This selection was aimed at enhancing comparability with previous EEG hyperscanning studies measuring PDC between parents and infants, in which directed INS was computed on a subset of frontal, central and posterior channels ([Leong et al. 2017](#); [Santamaria et al. 2020](#)). Moreover, considering that the current EEG hyperscanning literature reports mixed findings regarding the involvement of specific brain regions in the establishment of parent-infant INS ([Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#)), we chose to adopt a more explorative approach, and thus measured PDC across all possible combinations across our channels of interest. For what concerns the frequencies of interest, we chose to compute PDC in the *theta* and *alpha* frequency bands due to their association with cognitive ([Leong et al. 2017](#); [Wass et al. 2018](#)) and socio-emotional processing ([Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#); [Santamaria et al. 2020](#)). To account for the differences in the adult and infant neural rhythms ([Noreika et al. 2020](#)) while following the original mathematical formula of the PDC that does not allow the use of a “true” cross-frequency approach to INS ([Turk et al. 2022](#)), we chose to consider as *theta* frequency the neural activity comprised between 3 and 7 Hz, and the *alpha* frequency all the neural

activity comprised between 6 and 12 Hz. These broadened *theta* and *alpha* thresholds allowed us to include both the adult rhythms (4-7 Hz for *theta* and 8-12 Hz for *alpha*) and the infant rhythms (3-5 Hz for *theta* and 6-9 Hz for *alpha*).

Another important parameter to choose when computing PDC is the model order p . In line with previous literature ([Jia 2019](#); [Santamaria et al. 2020](#)), our custom script selects the model order by an iterative process that computes the lowest best fitting value according to the Akaike Information Criterion (AIC; [Akaike 1979](#)) and the Bayesian Information Criterion (BIC; [Schwarz 1978](#)). In our dataset, the optimal model order for 44 out of 48 dyads was consistently $p = 5$ both during Play and during Reunion, a value consistent with a previous work that computed PDC in a sample of mother-infant dyads ([Santamaria et al. 2020](#)). Regarding the remaining four dyads, two ($N = 2$) of them consistently reported a model order different than 5 in both FFSF episodes ($p = 4$ for one dyad and $p = 6$ for the other), whereas the remaining two ($N = 2$) dyads displayed a different model order across FFSF episodes, with $p = 5$ in the Play episode and $p = 4$ or 7 in the Reunion.

2.3. Behavioral coding

Videos of the FFSF procedure were microanalytically coded for interactive behaviors according to an adaptation of the Parent-Infant Coding Scheme (PICS ver. 4.0; Brambilla et al. *unpublished manual*). Behavioral coding was performed via the Noldus The Observer XT software ([The Observer XT](#)) by two undergraduate students and two research assistants trained in the usage of our coding scheme. Behaviors of interest were coded for each member of the dyad and for each episode and measured as the percentage of time spent showcasing said behavior. For the purposes of this study, we considered only the following behaviors: percentage of infant negative emotionality during the SF episode, percentage of infant gaze aversion during the SF episode, and percentage of maternal affective touch during the Reunion episode. A definition of the behaviors of interest is provided in Chapter 2 (*Table 2.1*).

2.4. *Statistical analysis*

Analyses were performed on RStudio 2023.12.1 (Posit team 2024). Due to the Shapiro-Wilk test revealing a non-normal distribution of the variables of interest, non-parametric tests were deemed as more suitable for statistical precision. The following plan of analysis was followed.

Preliminary analyses – behavioral data. To explore potential behavioral changes across the FFSF episodes, a non-parametric repeated measures ANOVA (Friedman test) was run on the three behavioral variables of interest (see above) to test the effect of the Episode (Play, SF, Reunion) factor. Subsequently, in order to explore potential differences in directed INS (as measured by PDC) according to the percentage of time spent displaying the behaviors of interest, dyads were median-split according to their scores in the following variables: infant gaze aversion during the SF episode, infant negative emotionality during the SF episode, and maternal affective touch during the Reunion episode. For each of these variables, dyads who displayed scores higher than the median were included in the “high” group, whereas dyads who displayed scores lower than the median were included in the “low” group.

Main analyses. For each frequency band and channel combination, an Aligned Rank Transform Analysis of Variance (ART ANOVA; [Wobbrock et al. 2011](#)) was run on the PDC values with the following factors: Episode (Play vs Reunion), Direction (I→P vs P→I) and Behavior (high vs low; one model per behavioral variable). Alongside the effect of the single factors, interaction effects were also explored, including two-way effects (Episode*Direction, Episode*Behavior and Direction*Behavior) as well as the three-way interaction between Episode, Direction and Behavior. ART ANOVA is a statistical test for factorial non-parametric analysis that relies on a pre-processing step that first “aligns” the data for each effect before assigning ranks, averaged when ties occur ([Wobbrock et al. 2011](#)). For the purposes of our study, ART ANOVA was deemed as the most appropriate statistical test for non-parametric samples due to its capacity to handle repeated measures and multivariate study designs ([Wobbrock et al. 2011](#)). In RStudio, ART ANOVA was run using the

package ARTool ([Kay et al. 2025](#); [Wobbrock et al. 2011](#)). Results were controlled for multiple comparisons via False Discovery Rate (FDR; Benjamini-Hochberg method), with $\alpha = 0.05$.

Post-hoc analyses. We were also interested in exploring whether the impact of the Episode and the Direction factors (as single factors) was different according to the percentage of time spent by the dyads displaying one of the behaviors of interest. Thus, for each frequency band, channel combination and behavior of interest, we ran Wilcoxon tests for paired samples on each subsample (separately for low and high dyads) in order to compare the PDC values according to the Episode (Play vs Reunion) and then to the Direction (I→P vs P→I). Results were controlled for multiple comparisons via False Discovery Rate (FDR; Benjamini-Hochberg method), with $\alpha = 0.05$. After identifying the number of connections displaying a dominant direction, we ran a two proportions Fisher exact test (`fisher.test` function in RStudio) in order to explore potential episode-related changes in direction dominance across episodes (Play vs Reunion). The two-proportions Fisher exact test was performed for each frequency band (*alpha*, *theta*), PDC direction (I→P, P→I) and behavioral subgroup (low and high infant gaze aversion, low and high infant negative emotionality, low and high maternal affective touch).

Sensitivity analyses. The statistical tests performed to assess the changes in parent-infant PDC in function of the FFSF episode and direction of connectivity were previously ran on the whole sample in order to explore potential between episode and between direction changes in PDC regardless of the behavioral group. In addition, we checked the percentage of overlap between the low and high subgroups of the three behaviors of interest to better assess the extent of the interaction between the three behaviors of interest, as well as its impact on parent-infant PDC fluctuations.

2.5. *Data reduction*

Among the 74 dyads, a total of 26 dyads were excluded from the final sample for the following reasons: failure to complete the experimental procedure (N = 12), failure to acquire the neural data

on the infant (N = 10), technical issues with the EEG equipment (N = 1) and inadequate EEG signal for synchrony analyses (N = 3; among which N = 2 for 6 or more interpolated channels of interest and N = 1 for less than 30 good epochs per episode). Thus, the final sample consisted of 48 parent-infant dyads, meaning that the sample size for each behavioral “high” and “low” subgroup was N = 24.

3. RESULTS

Our final sample included 48 parent-infant dyads (mean infant age at assessment = 9 months and 15 days, range 8-11 months; 28 males and 20 females), all pertaining to the full-term control arm of the study (mean gestational age 39.43 weeks). The participating parents were, in all dyads, the biological mother of the infant (mean parent age at assessment = 34.65 ± 3.74 years). Table 4.1 provides the central tendency measures of the global PDC values.

Table 4.1. Grand central tendency measures of the PDC values for each frequency band, FFSF episode and direction of connectivity. Adapted from Pili et al. (under review).

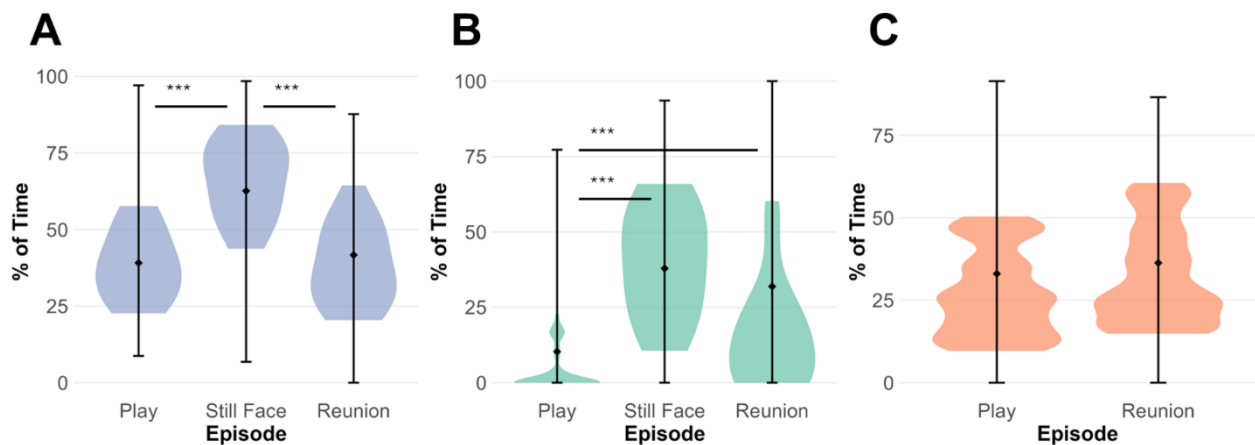
Frequency, Episode	I→P PDC		P→I PDC	
	Mean ± SD	Range	Mean ± SD	Range
<i>Alpha, Play</i>	0.048 ± 0.047	0.001-0.42	0.029 ± 0.031	0.001-0.31
<i>Alpha, Reunion</i>	0.044 ± 0.038	0.0004-0.29	0.035 ± 0.039	0.001-0.39
<i>Theta, Play</i>	0.029 ± 0.033	0.001-0.34	0.029 ± 0.031	0.001-0.32
<i>Theta, Reunion</i>	0.026 ± 0.023	0.0003-0.23	0.036 ± 0.042	0.001-0.39

3.1. Behavioral Still-face effect

The Friedman test revealed a significant effect of the Episode factor for infant gaze aversion ($\chi^2 = 32.2$; $df = 2$; $p < 0.001$) and infant negative emotionality ($\chi^2 = 32.1$; $df = 2$; $p < 0.001$), but not for maternal affective touch (see Figure 4.1). Post-hoc analyses highlighted a significantly higher percentage of infant gaze aversion during SF compared to both Play (Durbin-Conover statistic = 6.19; $p < 0.001$) and Reunion (Durbin-Conover statistic = 5.70; $p < 0.001$), as well as a significantly lower

percentage of infant negative emotionality during Play compared to both SF (Durbin-Conover statistic = 6.56; $p < 0.001$) and Reunion (Durbin-Conover statistic = 5.05; $p < 0.001$). The findings, overall, indicate the presence of a still-face effect in our sample. Supplementary Materials (Ch.5.S1, “Behavioral data”, see Appendix) report additional information regarding the behavioral characteristics of our sample, including the distribution of the dyads in the low and high behavioral subgroups.

Figure 4.1. Central tendency measures of infant gaze aversion (A), infant negative emotionality (B) and maternal affective touch (C) across the different FFSF episodes. Brackets indicate the episodes which significantly differed in terms of percentage of time spent in the specific coded behavior (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). Adapted from Pili et al. (under review).



3.2. PDC changes in function of episode, direction and behavior

The full results of the ART ANOVA for each frequency band and channel combination are reported in Supplementary Materials (Ch.2.S2, “Full statistical report”, Table S2.1. See Appendix) for both *alpha* and *theta* band. Whenever connections would display significant effects for more than one factor, priority was given to the factor (or interaction factor) with the highest F statistic and lowest p-value (called dominant factor).

3.2.1. Alpha band (6-12 Hz)

The majority of the channel combinations (19/36) had direction as the dominant significant factor ($F \geq 1.973$; $p < 0.05$), followed by episode*gaze aversion (dominant in 4/36 channel combinations; $F \geq 4.971$; $p < 0.05$), episode*direction (dominant in 3/36 channel combinations; $F \geq 4.952$; $p < 0.05$) and episode*negative emotionality (dominant in 2/36 channel combinations; $F \geq 5.174$; $p < 0.05$). PDC in 2/36 channel combinations showed a dominant direct effect of negative emotionality ($F \geq 5.740$; $p < 0.05$), and 1/36 channel combinations showed a dominance of the three-way effect between episode, direction and negative emotionality ($F = 13.28$; $p \leq 0.001$). 5/36 channel combinations showcased no significant effect of any single or interaction factors on PDC. However, after controlling for multiple comparisons, only the single effect of direction maintained statistical significance for 18/36 connections ($F \geq 6.392$; $p_{\text{adj}} < 0.05$), whereas 1/36 connections maintained a significant interaction effect of episode and gaze aversion ($F = 14.471$; $p_{\text{adj}} = 0.045$). The remaining 17 channel combinations showcased no effect of any of the single and interaction effects after controlling for multiple comparisons.

3.2.2. Theta band (3-7 Hz)

The majority of the channel combinations (19/36) had direction as the dominant significant factor ($F \geq 0.687$; $p < 0.05$), followed by episode*direction (dominant in 3/36 channel combinations; $F \geq 5.826$; $p < 0.05$) and episode*negative emotionality (dominant in 2/36 channel combinations; $F \geq 6.023$; $p < 0.05$). Significant two-way interaction effects between episode and affective touch (dominant in 1/36 channel combinations; $F = 5.83$; $p = 0.02$) and episode and gaze aversion (dominant in 1/36 channel combinations; $F = 8.74$; $p = 0.005$) have been found, as well as a three-way interaction between episode, direction and all the three behavioral variables (dominant in 1/36 channel combinations for interaction with affective touch, 1/36 for interaction with gaze aversion and 1/36 for interaction with negative emotionality; $F \geq 4.279$; $p < 0.05$). One channel combination displayed a dominance of the single effect of episode ($F = 4.30$; $p = 0.044$), whereas the remaining 6/36 channel

combinations showcased no significant effect of any single or interaction factor on PDC. After controlling for multiple comparisons, only the single effect of direction maintained statistical significance for 17/36 channel combinations ($F \geq 6.762$; $p_{\text{adj}} < 0.05$), including 1/36 connections that maintained a significant interaction effect of episode and gaze aversion factors ($F = 14.939$; $p_{\text{adj}} = 0.045$). The remaining 18 channel combinations showcased no effect of any of the single and interaction effects after controlling for multiple comparisons.

3.3. *Inter-episode differences (Play vs Reunion) in PDC in low vs high behavioral subgroups*

3.3.1. *Alpha band (6-12 Hz)*

Among the two available directions ($I \rightarrow P$ and $P \rightarrow I$), $P \rightarrow I$ is the PDC direction that displays a greater amount of significant episode*behavior interactions. $I \rightarrow P$ PDC, on the other hand, displays only limited episode*behavior interactions. Inter-episode changes in PDC values in our sample, however, do not survive control for multiple comparisons via FDR. Statistics are reported in Supplementary Materials (Ch.4.S2, “*Full statistical report*”, Table S2.2. See Appendix).

3.3.2. *Theta band (3-7 Hz)*

Similarly to the *alpha* band, the PDC direction that displays a greater amount of significant episode*behavior interactions is the $P \rightarrow I$. Despite this, between-episodes differences in $I \rightarrow P$ and $P \rightarrow I$ PDC did not survive FDR correction. Statistics are reported in Supplementary Materials (Ch4.S2, “*Full statistical report*”, Table S2.3. See Appendix).

3.4. *Intra-episode differences ($I \rightarrow P$ vs $P \rightarrow I$ connectivity) in PDC in low vs high behavioral subgroups*

3.4.1. *Alpha band (6-12 Hz)*

Both during Play and Reunion episodes, PDC seemed to be significantly stronger in the $I \rightarrow P$ direction compared to the $P \rightarrow I$ direction. However, the ratio of connections that display stronger $I \rightarrow P/P \rightarrow I$

PDC has been shown to change according to specific maternal and infant behaviors during the SF episode and Reunion. Statistics are shown in Supplementary Materials (Ch4.S2, “*Full statistical report*”, Table S2.4. See Appendix). Results are summarized in Figure 4.2. The following paragraphs report exclusively the intra-episode differences that survived post-hoc correction via FDR (adjusted p-value instead of raw p-value; $\alpha = 0.05$).

Whole sample. Paired Wilcoxon tests conducted on the whole sample revealed a clear pattern of I->P directionality in our sample during both Play and Reunion. Specifically, 22/36 connections during Play ($W \geq 818$; mean difference ≥ 0.009 ; $p_{\text{adj}} < 0.05$) and 13/36 during Reunion ($W \geq 814$; mean difference ≥ 0.015 ; $p_{\text{adj}} < 0.05$) displayed a significantly higher PDC in the I->P compared to the P->I direction. The majority of the I->P-dominated connections were of the following types: Infant-Frontal to Parent-Central (F->C; $N = 4/22$ during Play and $N = 4/13$ during Reunion), Infant-Frontal to Parent-Posterior (F->P; $N = 4/22$ during Play and $N = 4/13$ during Reunion), Infant-Central to Parent-Central (C->C; $N = 4/22$ during Play and $N = 2/13$ during Reunion) and Infant-Posterior to Parent-Central (P->C; $N = 4/22$ during Play and $N = 2/13$ during Reunion) (see Figure 3A and Table S2.4). Conversely, only 0/36 during Play and 2/36 connections during Reunion ($W \leq 329$; mean difference ≤ -0.016 ; $p_{\text{adj}} < 0.025$) displayed the opposite pattern, i.e. significantly higher PDC in the P->I direction. Notably, at the regional level, both P->I-dominated connections found during Reunion involved a frontal channel of the parent and a posterior channel of the infant (F->P). 2 proportion z-test revealed no significant differences between proportions of I->P and P->I-dominated connections from Play to Reunion. A graphical representation of the results is available in Figure 4.2A.

Gaze aversion. Dyads with low infant gaze aversion and those with high gaze aversion showcased similar directionality patterns across episodes. Results are summarized in Figure 4.2B. In the subgroup of low infant gaze aversion dyads, 13/36 connections during the Play episode ($W \geq 241$; mean difference ≥ 0.016 ; $p_{\text{adj}} < 0.05$) and 6/36 in the Reunion ($W \geq 240$; mean difference ≥ 0.029 ; $p_{\text{adj}} < 0.05$) displayed a significantly higher PDC in the I->P direction compared to P->I; conversely, no connection displayed significantly stronger PDC in the P->I direction compared to I->P in both

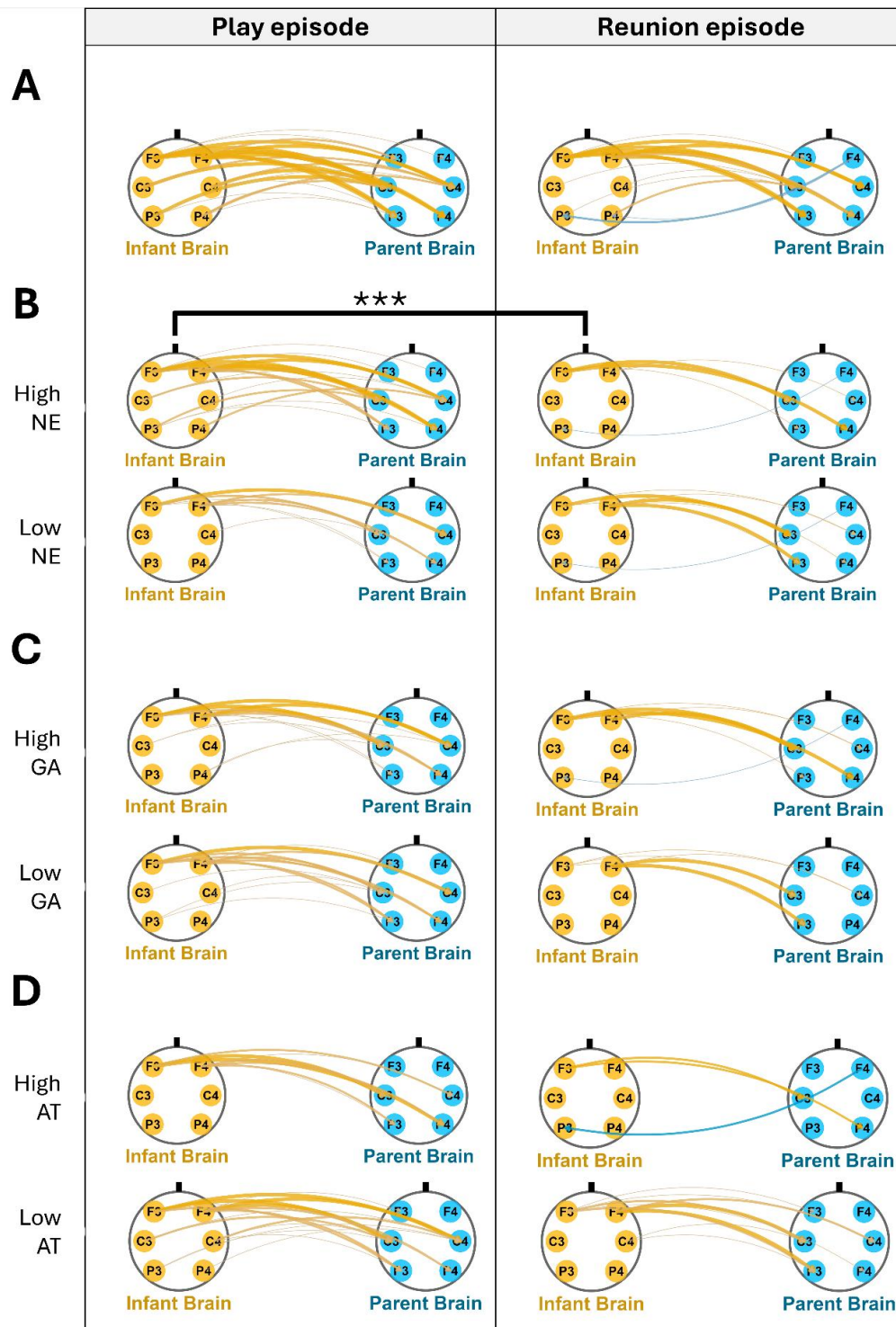
FFSF episodes. A similar pattern was observed in the subgroup of high infant gaze aversion dyads, where 12/36 channel combinations during Play ($W \geq 241$; mean difference ≥ 0.020 ; $p_{\text{adj}} < 0.05$) and 6/36 during Reunion ($W \geq 240$; mean difference ≥ 0.022 ; $p_{\text{adj}} < 0.05$) displayed a significantly higher I->P PDC compared to the P->I counterpart. As illustrated in Figure 4.2B and Table S2.4, the majority of the I->P-dominated connections were either of the F->C type (Infant-Frontal to Parent-Central; $N = 4$ during Play and $N = 4$ during Reunion) or of the F->P type (Infant-Frontal to Parent-Posterior; $N = 4$ during Play and $N = 2$ during Reunion) in both low and high gaze aversion subgroups. Only one channel combination (1/36; F4 of the parent to P3 of the infant) displayed a significantly higher PDC in the P->I direction compared to I->P in the Reunion, but not in the Play episode ($W = 50$; mean difference = -0.025 ; $p_{\text{adj}} = 0.022$). The inter-episode changes in I->P PDC detected in both subgroups were not statistically significant.

Negative emotionality. The PDC directionality patterns across Play and Reunion differed according to the percentage of infant negative emotionality displayed during the SF episode. More specifically, in the low subgroup, 9/36 connections during the Play episode ($W \geq 249$; mean difference ≥ 0.033 ; $p_{\text{adj}} < 0.025$) and 7/36 during the Reunion ($W \geq 244$; mean difference ≥ 0.023 ; $p_{\text{adj}} < 0.05$) displayed significantly stronger I->P PDC compared to P->I, whereas no connections during Play and 1/36 during Reunion ($W = 61$; mean difference = -0.022 ; $p_{\text{adj}} = 0.044$) were significantly stronger in the P->I direction. At the regional level, the majority of the I->P-dominated connections were of the Infant-Frontal to Parent-Central type ($N = 4/9$ during Play and $N = 4/7$ during Reunion) and Infant-Frontal to Parent-Posterior type ($N = 4/9$ during Play and $N = 3/7$ during Reunion), whereas the P->I-dominated connection found in Reunion was between the channel F4 of the parent and the channel P3 of the infant. Conversely, in the high negative emotionality subsample, 17/36 connections during the Play ($W \geq 240$; mean difference ≥ 0.015 ; $p_{\text{adj}} < 0.05$) were found to showcase a significantly stronger I->P PDC, with a reduction to 5/36 ($W \geq 241$; mean difference ≥ 0.030 ; $p_{\text{adj}} < 0.05$) during the Reunion episode. This change was observed to be statistically significant ($p = 0.004$; 95% CI: 1.58-21.95), whereas the same change was not significant in the subgroup of low negative

emotionality dyads. As illustrated in Figure 4.2C and in Table S2.4 of the Supplementary Materials, the majority of the I->P-dominated connections were of the F->C (N = 4/17 during Play and N = 3/5 during Reunion) and F->P type (N = 3/17 during Play and N = 2/5 during Reunion). Connections with significantly stronger PDC in the P->I direction, on the other hand, went from 0/36 during Play to 1/36 (F4 of the parent and P3 of the infant) during Reunion (W = 62; mean difference = -0.021; $p_{\text{adj}} = 0.048$), and the change in proportions was not significant.

Affective touch. Similarly to the results reported on infant gaze aversion, splitting the sample in low and high affective touch dyads did not highlight behavior-dependent differences in PDC directionality. Results are summarized in Figure 4.2D. In the subgroup of low affective touch dyads, 17/36 connections in Play (W ≥ 238 ; mean difference ≥ 0.019 ; $p_{\text{adj}} < 0.05$) and 10/36 in Reunion (W ≥ 239 ; mean difference ≥ 0.025 ; $p_{\text{adj}} < 0.05$) displayed a significantly stronger PDC in the I->P direction compared to P->I. The majority of the I->P-dominated connections were of the F->C (N = 4/17 during Play and N = 4/10 during Reunion), F->P (N = 4/17 during Play and N = 3/10 during Reunion) and C->C type (N = 4/17 during Play and N = 1/10 during Reunion). As for the subgroup of dyads with high percentages of parental affective touch, the I->P direction dominance was less pronounced. 8/36 during Play (W ≥ 245 ; mean difference ≥ 0.021 ; $p_{\text{adj}} < 0.05$) and 2/36 during Reunion (W ≥ 252 ; mean difference ≥ 0.027 ; $p_{\text{adj}} < 0.025$) displayed a significantly higher PDC in the I->P direction, whereas no connections during Play and 1/36 connections during Reunion (W = 40; mean difference = -0.031; $p_{\text{adj}} = 0.01$) were found to have a higher PDC in the P->I direction. Similarly to the low affective touch subgroup, the majority of the I->P-dominated connections were of the F->C (N = 3/8 during Play and N = 1/2 during Reunion) and F->P type (N = 3/8 during Play and N = 1/2 during Reunion), whereas the P->I-dominated connection found in Reunion involved the F4 channel of the parent and the P3 channel of the infant. In both low and high affective touch subsamples, changes in proportion of I->P and P->I-dominated connections did not reach statistical significance.

Figure 4.2. Brain maps representing the ratio of I->P-dominant and P->I-dominant PDC in the alpha (6-12 Hz) frequency band. The analyses were performed on the whole sample (A) as well as for the median split samples according to the level of infant negative emotionality (NE) during the SF (B), infant gaze aversion (GA) during the SF (C) and maternal affective touch (AT) during the Reunion (D). The arrows indicate the connections having a I->P dominance (gold) or a P->I dominance (blue) at a significance level of adjusted p-value ≤ 0.05 . Thickness of the connections represents the significance level (low thickness: $\text{padj} \leq 0.05$; medium thickness: $\text{padj} \leq 0.01$; high thickness: $\text{padj} \leq 0.001$). Square brackets indicate significant inter-episode changes in number of directed connections according to the two proportions Fisher exact test (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). Adapted from Pili et al. (under review).



3.4.2. Theta band (3-7 Hz)

In the *theta* frequency band, the impact of infant and maternal behavior during the FFSF on PDC directionality was more pronounced compared to the *alpha* band, with more evident and significant differences in the high percentage subgroups compared to the low percentage ones. Results are described in Supplementary Materials (Ch4.S2, “Full statistical report”, Table S2.5. See Appendix). A graphic representation of the findings is available in Figure 4.3. The following paragraphs report exclusively the intra-episode differences that survived post-hoc correction via FDR (adjusted p-value instead of raw p-value; $\alpha = 0.05$).

Whole sample. Analyses conducted on the whole sample revealed different patterns of direction dominance in *theta* PDC across Play and Reunion. Results are illustrated in Figure 4.3A. A total of 7/36 connections during Play ($W \geq 841$; mean difference ≥ 0.015 ; $p_{\text{adj}} < 0.025$) and 2/36 during Reunion ($W \geq 851$; mean difference = 0.012; $p_{\text{adj}} < 0.025$) displayed a significantly higher PDC in the I->P direction compared to P->I. Said connections were exclusively of the F->C ($N = 4/7$ during Play and $N = 1/2$ during Reunion) and F->P type ($N = 3/7$ during Play and $N = 1/2$ during Reunion). Moreover, 7/36 connections during the Play episode ($W \leq 336$; mean difference ≤ -0.011 ; $p_{\text{adj}} < 0.025$) and 9/36 during Reunion ($W \leq 364$; mean difference ≤ -0.018 ; $p_{\text{adj}} < 0.05$) showcased significantly stronger PDC in the P->I direction. Similarly to the I->P-dominated connections, P->I-dominated connections were almost exclusively of the F->C ($N = 3/7$ during Play and $N = 4/9$ during Reunion) and F->P type ($N = 4/7$ during Play and $N = 4/9$ during Reunion). No significant changes in proportion of I->P and P->I-dominated connections from Play to Reunion were observed.

Gaze aversion. PDC directionality across Play and Reunion episodes followed similar patterns for low and high infant gaze aversion dyads; however, dyads pertaining to the high infant gaze aversion subgroup displayed slightly stronger shifts in directionality across episodes. Results are illustrated in Figure 4.3B. In the subgroup of low infant gaze aversion dyads, 5/36 connections during Play ($W \geq 243$; mean difference ≥ 0.021 ; $p_{\text{adj}} < 0.05$) were significantly stronger in the I->P direction compared

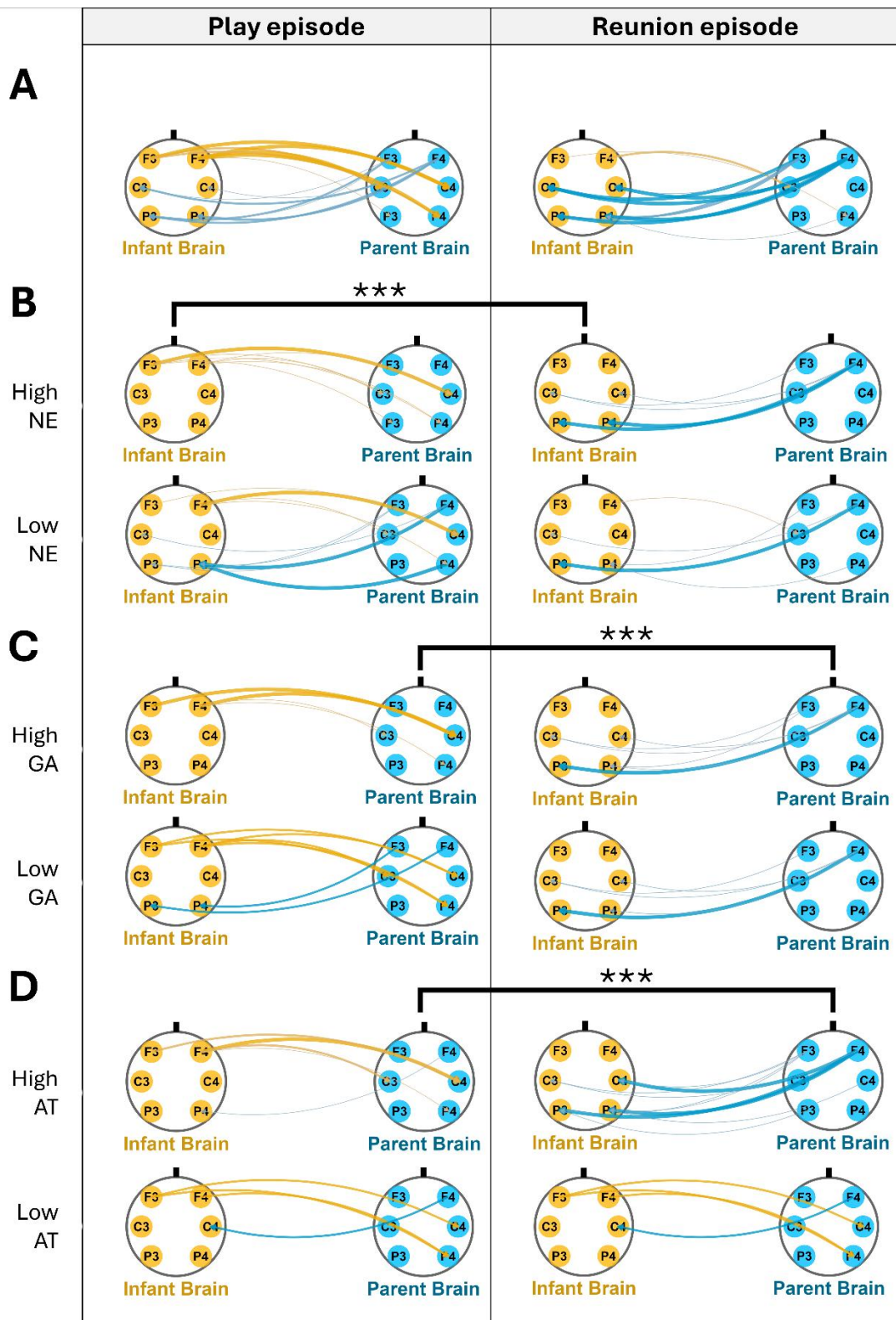
to P->I, a number that was reduced to 0/36 during Reunion; conversely, connections with significantly stronger PDC in the P->I direction went from 2/36 during Play ($W \leq 62$; mean difference ≤ -0.017 ; $p_{\text{adj}} < 0.05$) to 5/36 during Reunion ($W \leq 59$; mean difference ≤ -0.016 ; $p_{\text{adj}} < 0.05$). These changes in I->P and P->I connection ratios did not reach statistical significance. Regarding the subgroup of dyads with high percentage of infant gaze aversion, 4/36 connections displayed a significantly stronger PDC in the I->P direction in the Play episode ($W \geq 247$; mean difference ≥ 0.016 ; $p_{\text{adj}} < 0.05$), a number that decreased to 0/36 during Reunion. The increase was not significant. Connections displaying a significantly stronger P->I, on the other hand, went from 0/36 during Play to 6/36 during Reunion ($W \leq 60$; mean difference ≤ -0.026 ; $p_{\text{adj}} < 0.05$); this increase was found to be statistically significant ($p = 0.025$; 95% CI: 0-0.78). Both in low and high gaze aversion subgroups, the I->P-dominated as well as the P->I-dominated connections were either of the F->C or the F->P type (see Table S2.5 and Figure 4.3B).

Negative emotionality. Different PDC directionality patterns across Play and Reunion were shown by the subgroup of low infant negative emotionality and high infant negative emotionality dyads. Results are illustrated in Figure 4.3C. For the group of low negative emotionality, 6/36 connections during Play ($W \leq 60$; mean difference ≤ -0.017 ; $p_{\text{adj}} < 0.05$) and 4/36 during Reunion ($W \leq 61$; mean difference ≤ -0.017 ; $p_{\text{adj}} < 0.05$) were found to display a significantly stronger PDC in the P->I direction, whereas 4/36 connections during Play ($W \geq 239$; mean difference ≥ 0.015 ; $p_{\text{adj}} < 0.05$) and 1/36 during Reunion ($W = 246$; mean difference = 0.018; $p_{\text{adj}} = 0.029$) showcased an opposite pattern (I->P stronger than P->I PDC). These changes across the two FFSF episodes were not statistically significant. In the subgroup of high negative emotionality dyads, 7/36 connections ($W \geq 244$; mean difference ≥ 0.013 ; $p_{\text{adj}} < 0.05$) were found to display a significantly stronger PDC in the I->P direction during Play, a number that was significantly reduced to 0/36 during Reunion ($p = 0.011$; 95% CI: 1.61-Inf). Conversely, 0/36 connections during Play and 5/36 during Reunion ($W \leq 56$; mean difference ≤ -0.028 ; $p_{\text{adj}} < 0.05$) displayed a significantly stronger PDC in the P->I direction. Said inter-episode ratio change was, however, non-significant. Both in low and high negative emotionality

subgroups, the I->P-dominated as well as the P->I-dominated connections were almost exclusively of the F->C or the F->P type (see Table S2.5 and Figure 4.3C).

Affective touch. The subgroup of high maternal affective touch dyads displayed the largest changes in PDC directionality across episodes compared to the low affective touch subgroup. Results are illustrated in Figure 4.3D. In the low parental affective touch dyads, 4/36 connections during Play ($W \geq 246$; mean difference ≥ 0.027 ; $p_{\text{adj}} < 0.05$) and 4/36 during Reunion ($W \geq 241$; mean difference ≥ 0.018 ; $p_{\text{adj}} < 0.05$) displayed a significantly stronger PDC in the I->P direction, whereas 1/36 connections during Play ($W = 55$; mean difference = -0.018 ; $p_{\text{adj}} = 0.032$) and 3/36 during Reunion ($W \leq 60$; mean difference ≤ -0.018 $p_{\text{adj}} < 0.05$) showcased the opposite pattern. Inter-episode changes in I->P and P->I PDC-dominated connections were not statistically significant. As for the high parental affective touch dyads, 3/36 connections during Play ($W \geq 248$; mean difference ≥ 0.013 ; $p_{\text{adj}} \leq 0.025$) and 0/36 during Reunion showcased a significantly higher I->P PDC. Conversely, 1/36 connections during Play ($W = 62$; mean difference = -0.014 ; $p_{\text{adj}} = 0.048$) and 10/36 during the Reunion episode ($W \leq 61$; mean difference ≤ -0.021 ; $p_{\text{adj}} < 0.05$) displayed a higher P->I PDC, with the increase from Play to Reunion being statistically significant ($p = 0.006$; 95% CI: 0.002-0.60). Both in low and high affective touch subgroups, the I->P-dominated as well as the P->I-dominated connections were almost exclusively of the F->C or the F->P type (see Table S2.5 and Figure 4.3D).

Figure 4.3. Brain maps representing the ratio of I->P-dominant and P->I-dominant PDC in the theta (3-7 Hz) frequency band. The analyses were performed on the whole sample (A) as well as for the median split samples according to the level of infant negative emotionality (NE) during the SF (B), infant gaze aversion (GA) during the SF (C) and maternal affective touch (AT) during the Reunion (D). The arrows indicate the connections having a I->P dominance (gold) or a P->I dominance (blue) at a significance level of adjusted p-value ≤ 0.05 . Thickness of the connections represents the significance level (low thickness: $\text{padj} \leq 0.05$; medium thickness: $\text{padj} \leq 0.01$; high thickness: $\text{padj} \leq 0.001$). Square brackets indicate significant inter-episode changes in number of directed connections according to the two proportions Fisher exact test (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). Adapted from Pili et al. (under review).



4. DISCUSSION

We have collected dual EEG data from 48 parent-infant dyads during an adapted FFSF procedure and computed I->P and P->I PDC in order to explore if and how dyadic lead and follow dynamics changed after an interactive perturbation. We were also interested in understanding to what extent individual differences in how the dyads responded to the FFSF procedure associated with I->P and P->I PDC across Play and Reunion episodes. Our findings indicate that, in our sample, a still-face effect occurred at the behavioral level, with a significant increase in infant negative emotionality during SF and Reunion compared to Play and a significant increase in infant gaze aversion during the SF compared to Play and Reunion. At the neural level, direction of connectivity (I->P vs P->I) was the factor that best explained the changes in parent-infant PDC, suggesting that neurobehavioral synchrony may have been established in a directed fashion. Post-hoc analyses conducted in the subgroups of dyads with low versus high percentages of infant gaze aversion, infant negative emotionality and maternal affective touch complemented the results obtained from the ART ANOVA, highlighting differences in the interplay between PDC direction and dyadic behavior among the two frequencies of interest. More specifically, changes in *alpha* PDC directionality were exclusively driven by the degree of infant negative emotionality during the SF episode, with high percentages (>36.78% of total time, corresponding to 22s or more) being associated with a significant decrease in the proportion of I->P-dominated connectivity from Play to Reunion. Changes in *theta* PDC directionality, on the other hand, were related to all the behavioral variables of interest; specifically, high percentages of infant negative emotionality during the SF (>36.78% of total time) were associated with a significant decrease in the proportion of I->P-dominated connections from Play to Reunion, whereas high percentages of infant gaze aversion during the SF (>66.01% of total time, corresponding to 39s or more) and maternal affective touch during the Reunion (>28.96% of total time, corresponding to 34s or more) were related to a significant increase of the proportion of P->I-dominated connections from Play to Reunion. In light of this, our experimental hypotheses (H1 and H2, see *Introduction*) were partially supported for *theta* PDC and refuted for *alpha* PDC.

4.1. The dyadic “lead and follow” dynamic in *alpha* versus *theta* frequencies

Contrary to our expectations, parent-infant PDC in the *alpha* band seemed to follow a clear directed pattern during the Play episode, featuring the infant as the leader and the parent as the follower. In this frequency band and episode, dyads from our sample displayed a remarkably higher number of I->P-directed connections compared to the P->I counterparts, which were almost non-existent. These results are in contrast with previous observations of behavioral synchrony in parents and their nine-month-old infants, where synchrony was prevalently of the mutual (bidirectional) type due to the emergence of intersubjectivity ([Feldman 2007](#); [Feldman et al. 1996](#); [Feldman et al. 1999](#)). Interestingly, our *alpha* PDC results are more consistent with the findings on parent-infant dyads with younger infants (3-8 months), where the synchrony structure was found to be of the lagged/directed type and featuring the infant as the “leader” ([Feldman et al. 1996](#); [Feldman et al. 1999](#)). Our findings on *alpha* PDC are also in contrast with Santamaria and colleagues’ findings of a significantly stronger PDC in the P->I condition compared to I->P ([Santamaria et al. 2020](#)), although this difference might be ascribed to the characteristics of the study design. Conversely, trends of parent-infant *theta* PDC during the FFSF procedure seemed to be more in line with our experimental hypotheses, as well as with the current literature on parent-infant directed biobehavioral synchrony. Both in the full sample and in the median-split subsamples, parent-infant *theta* PDC during the Play episode seemed to follow a prevalently bidirectional pattern (or otherwise a weakly infant-led pattern in the subgroup of high infant negative emotionality and gaze aversion), which then changed into a directed, parent-led type of connectivity during the Reunion episode. This change in PDC directionality, however, was evident only in the whole sample of 48 dyads, as well as in the subsamples of high affective touch dyads and high gaze aversion dyads, whereas no significant changes in *theta* PDC directionality were found in the low behavioral subsamples.

One tentative explanation to the different results showcased by *alpha* and *theta* PDC could lie in the different functional roles of these frequency bands during dyadic interaction, and how these roles relate to the potential underlying mechanisms of the still-face effect. It is generally accepted that the

distressing nature of the FFSF procedure can be ascribed to the violation of social expectations, and particularly the violation of the expectations regarding maternal availability ([DiCorcia et al. 2016](#); [Legerstee and Markova 2007](#); [Mesman et al. 2009](#)). Notably, INS in the *theta* frequency band has been previously associated with attentional and cognitive components of the dyadic interaction, including changes in shared attention during joint play ([Wass et al. 2018](#)) and changes in directed gaze ([Leong et al. 2017](#); [Turk et al. 2022](#)), and *theta* activity in the infant brain has been associated with attention to social stimulation ([Orekhova et al. 2006](#)). While parent-infant INS in the *alpha* frequency band has also been related to changes in directed gaze in a previous study ([Leong et al. 2017](#)), this band has been often associated with changes in emotional states ([Atzaba-Poria et al. 2017](#); [Krzeczkowski et al. 2022](#); [Perone et al. 2020](#); [Swider-Cios et al. 2024](#); [Turk et al. 2022](#)) as well as valence of maternal emotional feedback ([Santamaria et al. 2020](#)). It is thus possible that, due to the more cognitive nature of the FFSF procedure, the *theta* band may have better captured the potentially disruptive effect of the interactive perturbation on I->P PDC, whereas PDC in the *alpha* band could have been more sensitive to other behavioral characteristics of the dyads such as emotional valence. It is important to note, however, that this interpretation may be challenged by the partial overlap between the *theta* and *alpha* ranges adopted in the present study, particularly in the 6 Hz and 7 Hz data points. Replication studies assessing directed INS on non-overlapping frequency ranges, such as the infant *theta* (3-5 Hz) and *alpha* rhythms (6-9 Hz), could help clarify the potential functional differences between *theta* and *alpha* brain-to-brain directed coupling.

4.2. *The neural underpinnings of the still-face effect*

The dyads from our sample showcased a clear still-face effect at the behavioral level, with a significant increase in infant negative emotionality from Play to SF which carried over during the Reunion episode. Conversely, infant gaze aversion increased from Play to SF and returned to baseline levels during Reunion, whereas no significant differences in percentages of maternal affective touch were found across episodes. The findings are consistent with Mesman and colleagues' meta-analytical work, which reported a significant increase in negative emotionality from Play to SF and Reunion

and no significant changes in other dyadic behaviors such as social gaze from Play to Reunion in infants aged 0-9 months ([Mesman et al. 2009](#)).

At the neural level, changes in PDC did not seem to be driven by the FFSF procedure per se, but rather by the interplay between dyadic behavior and PDC directionality (I->P-dominant or P->I-dominant) across the FFSF episodes. While episode-dependent changes in I->P and P->I PDC (*alpha*, *theta*) did not reach statistical significance after correction for multiple comparisons, more robust changes in *alpha* and *theta* PDC were detected when assessing the direction (I->P versus P->I) changes from Play to Reunion in the subsample of dyads displaying high percentages of the behaviors of interest, particularly of infant negative emotionality during the SF (*theta*, *alpha*), infant gaze aversion during the SF and maternal affective touch during Reunion (*theta* only). Our findings suggest that neural co-regulation between parents and their nine-month-old infants might follow a directed fashion, and particularly shift from bidirectional to parent-led, only in dyads whose infants are highly reactive (e.g., frequent fussing, crying, negative emotional expressions and frequent gaze aversion) to interactive ruptures such as the FFSF. As for the dyads whose infants reacted to the SF with lower-than-the-median gaze aversion and negative emotionality, it is possible that their neural co-regulation patterns are better explained by non-directed connectivity metrics, as seen in previous parent-infant EEG hyperscanning works (see for instance [Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#); [Leong et al. 2019](#); [Neel et al. 2025](#)). There are two possible tentative interpretations of these findings, which are not mutually exclusive. Compared to parents of less reactive infants, parents of infants with high-intensity displays of distress during the SF might have felt an increased need to soothe their baby and have him/her re-engaged in a positive exchange during the Reunion episode. This might have resulted in the parent being more proactive in the social exchange, leading to a shift to P->I *theta* PDC (in the subgroup of high infant gaze aversion), or to a reduction of the I->P connections in the high infant negative emotionality subgroup (both *theta* and *alpha* frequency), from Play to Reunion. Simultaneously, infants showcasing high negative emotionality and/or high gaze aversion during the SF might have felt a higher need of co-regulating with their parents

compared to less reactive infants, making them more receptive to the parent's proposals and thus decreasing the strength of their leading role in the interactive exchange compared to the Play episode. Nonetheless, future parent-infant EEG hyperscanning studies measuring both non-directed and directed INS before and after interactive ruptures are needed in order to support these claims, as the current literature revealed that biobehavioral synchrony between parents and their nine-month-old infants is more commonly established in a non-directed, mutual fashion (see [Feldman 2007](#) for an overview). Comparing both non-directed and directed measures would thus provide further insights into the neurobehavioral correlates of dyadic co-regulation processes, and particularly on how parents and infants repair interactive mismatches.

A similar tentative interpretation could be made for the findings on the impact of maternal affective touch on *theta* PDC directionality. Together with its role in the reduction of the still-face effect (see [Stack and Muir 1990](#); [Stack and Muir 1992](#)), maternal affective touch was previously reported to direct the infants' attention to the mother's hands ([Stack and Muir 1990](#)), to be temporally organized with infants' affect and attention ([Jean et al. 2014](#)), and also to associate with higher parent-infant Circular correlation (a non-directed INS metric measuring phase synchronization between two signals, see [Burgess 2013](#) for details) when accompanied by direct maternal eye contact ([Neel et al. 2025](#)). Our results suggest that maternal affective touch might be a cue through which INS of the directed, parent-led kind is established. This could be especially relevant in the Reunion compared to the Play episode, since the distress experienced during the SF episode could make infants more receptive to the parental co-regulating strategies, thus changing the INS directionality compared to the baseline levels. It is worth noting that, in our sample, maternal affective touch did not seem to be reactive to the infants' behavior during the SF episode. Notably, we did not find significant differences in the percentage of time dedicated to affective touch from Play to Reunion, and the majority of the mothers displaying high affective touch during the Reunion episode (17/24) also displayed high levels of affective touch during the Play episode. It is possible that, for the parents from our sample, affective touch was just a way for them to positively engage with their infant

regardless of his/her emotional state, and that the association between this behavior and P→I PDC changes in the *theta* band could indeed be ascribed to an increased infant receptiveness to parental co-regulating attempts during the Reunion episode compared to Play.

While maternal affective touch has been associated with the construct of maternal sensitivity ([Botero et al. 2020](#); [Endevelt-Shapira and Feldman 2023](#); [Feldman et al. 2010](#)), we have not assessed whether parents showing higher percentages of affective touch scored also high in maternal sensitivity scales. Interestingly, levels of maternal sensitivity during dyadic interactions showcased a positive association with parent-infant non-directed INS as measured by the wPLI ([Endevelt-Shapira and Feldman 2023](#)), meaning that there could be a potential link between *theta* INS, maternal sensitivity and maternal affective touch. Further studies on parent-infant biobehavioral synchrony are needed to assess the interplay between these three variables.

4.3. The potential involvement of frontal areas in directed INS

It is worth noting that the vast majority of the connections showcasing a significant I→P or P→I directed pattern started from a frontal channel (F3, F4) and arrived at either a central (C3, C4) or posterior (P3, P4) channel. This is true for both *theta* and *alpha* frequency bands, for both Play and Reunion episodes, and for both whole and median-split samples (see Figures 5 and 6). While it is known that EEG presents a low spatial resolution, as the current generated by the brain sources is blurred by resisting layers such as the cerebrospinal fluid, the skull and the scalp ([Burle et al. 2015](#); [Makeig et al. 1995](#); [Srinivasan et al. 1996](#); [Srinivasan 1999](#)), it is still relevant to discuss how our findings compare with the existing literature about the neural correlates of parent-infant INS, and particularly with the body of evidence collected from functional near infrared spectroscopy (fNIRS) and magnetoencephalography (MEG) hyperscanning. Previous studies conducted on parent-infant and parent-child dyads have indeed highlighted the involvement of frontal regions, such as the bilateral lateral and median prefrontal cortex (lPFC and mPFC) ([Nguyen et al. 2020](#); [Nguyen et al. 2021](#)) and the inferior frontal gyrus (IFG) ([Lin et al. 2023](#)), in establishing INS during interactive and

cooperative tasks. The PFC has been found to be implied in all the processes related to the engagement in a mutual interaction, including the detection of communicative signals directed towards the self, mentalization and reward ([Nguyen et al. 2021](#); [Redcay and Schilbach 2019](#)), as well as higher order cognitive functions such as attentional regulation, working memory, regulation of the emotional experiences and planning of self-initiated social behaviors ([Azhari et al. 2022](#)). Similarly, the IFG is part of the “mirror neuron” network and has been associated with the detection of communicative signals related to social interaction, and particularly to the verbal components of social communication ([Lin et al. 2023](#); [Redcay and Schilbach 2019](#)). In their study, Endevelt-Shapira and Feldman ([Endevelt-Shapira and Feldman 2023](#)) argued that parent-infant INS could involve the activation of the frontotemporal network, which is implied in processes of social cognition and empathy ([Atzil et al. 2012](#); [Frith and Frith 2001](#)). Notably, several studies have reported a joint involvement of frontal and temporal areas, the latter including the temporo-parietal junction ([Minagawa et al. 2023](#)) and the right angular gyrus ([Lin et al. 2023](#)). While we were not able to assess the involvement of the frontotemporal network due to the exclusion of the temporal channels from the analysis (which was motivated by their higher presence of myogenic speech confounds in these peripheral channels; see [Brooker and Donald 1980](#)), the consistent role of the frontal channels as the “sending hub” of directed parent-infant INS may be indicative of the infants’ and parents’ intention of initiating and maintaining social behavior. As for the involvement of central and posterior channels as the “receiving hubs”, the result could be in line with a previous EEG hyperscanning study which highlighted a significant connection between the maternal right central area (channel C4) and the infant right occipito-temporal area (channel P8) ([Endevelt-Shapira et al. 2021](#)). In the adult brain, activity in the centroparietal regions has been proposed as a neuromarker for social coordination during spontaneous inter-neural coupling ([Dumas et al. 2010](#); [Tognoli et al. 2007](#)), whereas in the infant brain, there is evidence of the inferior parietal cortex, and particularly the inferior parietal lobule, being involved in the processing of the similarities and differences between the self and the other, as well as to imitation and the recognition of being imitated ([Meltzoff and Decety 2004](#)). Again,

we have no means to determine which were the brain sources underlying the activity recorded from the central and posterior channels of choice; however, the involvement of these channels as the “receiving hubs” both in parents and infants may underlie their intention of being receptive of each other’s behaviors and communicative signals. Evidence from further studies on parent-infant directed INS is needed in order to support the involvement of a fronto-parieto-occipital network during dyadic lead and follow social dynamics.

5. LIMITATIONS AND IMPLICATIONS

The present study contains several limitations, which restrict the generalizability and interpretability of the obtained results.

The first limitation relates to the enlarged and partially overlapped thresholds used to select the *theta* and *alpha* frequency bands in our study. While our intention was to adopt the assumption that parents and infants have different peaks for the same frequency bands ([Noreika et al. 2020](#); [Turk et al. 2022](#)) while strictly following Baccalà and Sameshima’s original formulation of the PDC ([Baccalà and Sameshima 2021](#)), it should also be considered that our thresholds partially overlap with frequencies other than *theta* and *alpha* in the adult and infant brain respectively. More specifically, the 3-7 Hz interval for the *theta* band partially overlaps with the adult *delta* waves (1-3 Hz; [Koul et al. 2023](#)) and the infant *alpha* (6-9 Hz; [Xie et al. 2018](#)). In the same fashion, the 6-12 Hz interval for the *alpha* band partially overlaps with the adult *theta* (4-8 Hz; [Koul et al. 2023](#)) and the infant *beta* frequency (9-14 Hz; [Xie et al. 2018](#)). While a previous work has encouraged the use of a cross-frequency approach when dealing with parent-infant INS ([Turk et al. 2022](#)), it is important to note that the majority of the studies in this field ([Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#); [Leong et al. 2017](#); [Leong et al. 2019](#); [Neel et al. 2025](#); [Santamaria et al. 2020](#); [Wass et al. 2018](#)) still work under the assumption that parents and infants showcase the same frequency peaks during social interaction, which are conventionally set at 4-7 Hz for *theta* ([Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#)) and at 6-9 Hz for *alpha* ([Neel et al. 2025](#); [Santamaria et al. 2020](#)). Conduction of

further studies utilizing the “conventional” *theta* and *alpha* thresholds are encouraged in order to assess the robustness of the results presented in the current work.

Another important limitation lies in the lack of a significance threshold for our PDC values. While we tried to compensate for this limitation by assessing the presence of significant differences between the I-->P and P-->I PDC and correcting for multiple comparisons, we did not use any data-driven or pre-selected threshold to determine whether our INS estimates were strong enough to describe a truly causal relationship between the parents’ and the infants’ signals. Notably, Santamaria and colleagues ([Santamaria et al. 2020](#)) have employed proportional thresholding to eliminate spurious PDC connections in their study, and while this method is expected to lead to more stable networks metrics ([Garrison et al. 2015](#)), other researchers have risen concerns regarding the robustness of this methodology due to significant changes in global connectivity measures in function to the chosen threshold ([Adamovich et al. 2022](#); [Tsolisou 2023](#); [Zakharov et al. 2021](#)). Future research on parent-infant PDC ought to address this limitation and propose robust thresholds through which directed INS can confidently describe a “causal relationship” between the signal of the leading partner and the one of the following partner.

Lastly, our coding system, including the use of the median split to divide our sample in “high” and “low” display of the behaviors of interest, might constitute another limitation of our study. While previous studies employing non-directed INS metrics have reported significant associations between parent-infant INS and specific dyadic behaviors such as mutual gaze ([Leong et al. 2017](#); [Leong et al. 2019](#)) and positive emotional valence ([Santamaria et al. 2020](#)), we cannot exclude the interplay between different sensory stimuli (visual, tactile, auditory) in shaping the mutual neural coordination between parents and their infants. Interestingly, a recent study from Neel and colleagues ([Neel et al. 2025](#)) described a significant increase in INS measured through the CCorr in the *alpha* band when maternal tactile stimulation was added to visual stimulation in 3-month-old infants, indicating that parent-infant INS could also be fostered by multimodal sensory input rather than only by single

behavioral cues. Moreover, our categorization of infant and parental behavior into “high” and “low” rates was highly dependent on the individual characteristics of our dyads, with median percentages of negative emotionality during SF and maternal affective touch during Reunion not even reaching fifty percent. This meant that, for instance, some of the “high negative emotionality” infants displayed behaviors that were coded as negative emotionality for less than thirty seconds during the SF episode, and the same applies to mothers who were in the “high affective touch” group (affectionately touching their infant for less than a minute during the Reunion episode). While it is more unlikely that an infant displaying truly high negative emotionality during the SF episode can complete the FFSF and provide adequate EEG data, we encourage researchers in the developmental neuroscience field who are interested in investigating changes in parent-infant INS in function to behavior to adopt more robust methods to define subsamples based on the behavior duration, such as rating scales. Moreover, we encourage to investigate the impact of multimodal sensory stimulation in fostering parent-infant directed INS, as well as the impact of macroscopic dyadic behaviors, such as maternal sensitivity and maternal intrusiveness. Additionally, it is important to note that our coding system did not include parent-infant leading behaviors, which could have complemented the findings concerning the directed neurophysiological measures and provided more robustness to our experimental hypotheses. Previous studies from Feldman and colleagues ([Feldman et al. 1996](#); [Feldman et al. 1999](#)) have utilized the monadic phase coding system to code for parent-infant leading behaviors (infant age 3-9 months) during a free play interaction, which is based on a definition of synchrony as a behavioral match in the direction of change during specific timeframes. Other than these studies, the literature concerning parent-infant leading behaviors during unconstrained social exchanges remained scarce, as there is little consensus on how to define directed synchrony at a behavioral level. Future EEG hyperscanning studies focusing on directed INS should compare the results of the neurophysiological lead and follow dynamics with robust behavioral measures of leading behaviors.

Even with the aforementioned limitations, the present study has several implications, including being the first study to assess parent-infant directed INS in an ecological setting including an interactive

perturbation. It provides novel insights into the “lead-and-follow” mechanisms of parent-infant interaction, and how parents and infants negotiate their roles after an interactive perturbation. Moreover, it highlights the complementary role of alpha and theta INS during parent-infant interaction, and how these two frequencies are related to different dyadic behaviors. If consistently replicated in future studies, these findings could open the possibility of utilizing PDC as a neuromarker for parent infant co-regulation in typical and at-risk conditions.

**CHAPTER 5 – EXPLORING NON-DIRECTED INS BEFORE AND AFTER AN
INTERACTIVE PERTURBATION AND ITS RELATION TO DYADIC BEHAVIOR**

(original article)

This chapter addresses Aim 3 of the present dissertation, i.e. the exploration of changes in non-directed parent-infant INS before and after an interactive perturbation and its potential associations with infant, maternal and dyadic matched behaviors. To date, the results of this study have not been published or submitted to any journal.

1. INTRODUCTION

In the last decade, there has been an increasing interest in studying the neurophysiological correlates of parent-infant social interactions, with a particular focus on which dyadic behaviors could act as cues for establishing brain-to-brain co-regulation. A growing body of research has explored parent-infant INS in both controlled ([Atilla et al. 2023](#); [Bánki et al. 2024](#); [Endevelt-Shapira et al. 2021](#); [Leong et al. 2017](#); [Leong et al. 2019](#); [Neel et al. 2025](#); [Santamaria et al. 2020](#)) and naturalistic ([Endevelt-Shapira and Feldman 2023](#); [Phillips et al. 2023](#); [Wass et al. 2018](#)) contexts, highlighting its positive associations with a vast array of dyadic behaviors such as eye contact and maternal utterances ([Leong et al. 2019](#)), maternal visual and tactile stimulation ([Neel et al. 2025](#)), maternal emotional feedback with positive valence ([Santamaria et al. 2020](#)), maternal sensitivity ([Endevelt-Shapira and Feldman 2023](#)) and infant social attention, positive arousal, and safety/approach behaviors ([Endevelt-Shapira et al. 2021](#)). The parent-infant EEG hyperscanning literature shows a marked preference for non-directed, phase-based metrics for the INS computation due to their fine-grained temporal resolution and aptness at measuring fluctuations of cognitive processing on a finer timescale ([Ayrolles et al. 2021](#); [Turk et al. 2022](#)). Among these, the most used INS metric in parent-infant EEG hyperscanning studies is the PLV ([Atilla et al. 2023](#); [Leong et al. 2019](#); [Santamaria et al. 2020](#)), with only a limited number of studies adopting alternative metrics such as the wPLI ([Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#)) and the CCorr ([Neel et al. 2025](#)). While PLV is one of the few INS metrics that is solely dependent on the phase reset rather than on other signal properties such as signal amplitude and coherence ([Nolte et al. 2004](#)), this metric introduces an important limitation: it does not mitigate the spurious phase synchrony resulting from common sources (volume conduction) ([Choi and Kim 2018](#)). Moreover, in his methodological work, Burgess ([2013](#)) argued that simply measuring the consistency of the phase difference between two signals may not be sufficient to determine true phase synchronization, and our previous methodological study ([Pili et al. 2025](#), see Chapter 3) showed that PLV computed in the theta band, even in its cross-frequency variant, is sensitive to arbitrary methodological choices and level of pre-processing

automatization. These limitations are relevant in the parent-infant EEG hyperscanning literature, as they could pose issues regarding the generalizability, robustness and reproducibility of the results. In this regard, other available phase synchronization metrics, such as wPLI, CCorr, and even ICoh, although severely underrepresented in the current parent-infant EEG hyperscanning literature, might be more suitable metrics to measure non-directed INS during parent-infant interaction due to their increased statistical power and their being less sensitive to volume-conducted sources compared to PLV ([Burgess 2013](#); [Nolte et al. 2004](#); [Vinck et al. 2011](#)).

The vast majority of the parent-infant EEG hyperscanning studies have focused their attention on potential behavioral cues through which parents and infants can establish INS; however, little to no studies have explored how parent-infant INS changes after an experimentally induced interactive perturbation such as the FFSF procedure. Exploring changes in non-directed parent-infant INS could not only provide further evidence on the relationship between dyadic behavior and inter-neural co-regulation, but also help us understand the impact of stress and (social) expectancy violation on INS.

In light of this, the present study aims are twofold. The first aim is to explore changes in parent-infant INS before and after an interactive perturbation, whereas the second aim is to assess the association between parent-infant INS and a series of parental and infant behaviors. For this second aim, our focus will be on the infant behaviors that have been previously associated with the presence of a still-face effect, such as negative emotionality and gaze aversion, parental behaviors that have been previously associated with the constructs of maternal sensitivity and responsivity, such as affective vocalizations and affective touch, as well as dyadic matched behaviors such as mutual positive emotionality and mutual social gaze.

2. METHODS

2.1. Participants and procedure

Our sample consisted of 103 infants (age range 8-11 months) and their primary caregivers (their mothers) enrolled as control subjects (FT group) for the 2-BRAINED project (see Chapter 2, “1.1. Population, enrolment and arm allocation”, for details on the recruitment strategy). The experimental procedure was the same described in Chapter 2 (“1.2. Experimental procedure”). Participants for this study were recruited between June 2022 and August 2025.

2.2. Synchrony analysis

Dual EEG data collected on parents and infants was pre-processed according to the pipeline described in Chapter 2 (“1.3. Plan of EEG data elaboration”). Eligibility criteria for synchrony analyses are the same defined in Chapter 2.

In line with the guidelines on parent-infant EEG hyperscanning studies ([Turk et al. 2022](#)), non-directed INS between our dyads was measured using two different non-directed connectivity metrics: Imaginary Coherence (ICoh) and weighted Phase Lag Index (wPLI). As described in the Introduction, this choice was motivated both by the higher robustness of these metrics compared to the previously used PLV ([Nolte et al. 2004](#); [Vinck et al. 2011](#)) as well as by the results showcased in our previous methodological work ([Pili et al. 2025](#), see Chapter 3).

ICoh is a coherency measure that was created by Nolte and colleagues ([Nolte et al. 2004](#)) to overcome some of the limitations of the coherence and phase synchronization measures, particularly the sensitivity to “self-interactions” (volume conduction problem). To achieve this, ICoh utilizes the imaginary component of the coherency, which describes lagged interactions and is insensitive to artifactual “self-interactions” caused by volume conduction.

In the present study, ICoh was computed with a custom script built on MATLAB R2024a originally used in a previous work from our group (see Procissi et al. 2025, *in press*). After performing the time-

frequency decomposition of the signal through the use of Morlet wavelet convolutions in the frequency bands of interest (see below), the script computed the cross-spectral density $S_{x,y}(f)$ of the parent and infant signals. The cross-spectral density is a measure of correlation between the two signals based on their power spectra, and is defined as

$$S_{xy}(f) = \frac{1}{T} \sum_{t=1}^T X(t, f) Y^*(t, f) \quad (1)$$

where $X(t, f)$ is the wavelet spectrum of $x(t)$, T is the number of epochs, and $Y^*(t, f)$ is the complex conjugate of $Y(t, f)$. By considering the auto-spectral densities:

$$S_{xx}(f) = \frac{1}{T} \sum_{t=1}^T |X(t, f)|^2 \quad (2)$$

$$S_{yy}(f) = \frac{1}{T} \sum_{t=1}^T |Y(t, f)|^2 \quad (3)$$

the complex coherency was computed as

$$C_{xy}(f) = \frac{S_{xy}(f)}{\sqrt{S_{xx}(f)S_{yy}(f)}} \quad (4)$$

ICoh is a complex number, in which the real part quantifies the instantaneous interactions and the imaginary part $Im(ICoh)$ describes lagged interactions. ICoh was computed as the absolute imaginary part of the complex coherency $C_{xy}(f)$:

$$ICoh(f) = \left| Im \left(C_{xy}(f) \right) \right| \quad (5)$$

While it cannot effectively be spuriously increased by volume conduction of independent sources ([Vinck et al. 2011](#)), the ICoh has been reported to be strongly influenced by the phase of the coherency, making it more effective when the lag between the two signals corresponds to a quarter

of cycle ($\pi/4$) and less effective when the two signals are either in phase or in phase opposition ([Jia 2019](#); [Stam et al. 2007](#)). For this reason, we decided to operationalize parent-infant INS using a second phase synchronization metric that was specifically created to overcome some of the limitations of the ICoh – the wPLI.

wPLI is a phase synchronization index that extends the original Phase Lag Index (PLI; [Stam et al. 2007](#)) by weighing the contribution of the observed phase lags and leads by the magnitude of the imaginary component of the cross-spectrum ([Vinck et al. 2011](#)). In our study, the wPLI was computed through a custom script that utilized functions from the MATLAB (R2024a)-based Fieldtrip toolbox ([Oostenveld et al. 2011](#)). After running the Hilbert transform on the bandpass-filtered data at the frequencies of interest (see below), inter-neural wPLI was computed with a function that applied the following formula:

$$\Phi \equiv \frac{|E\{\Im\{X\}\}|}{E\{|\Im\{X\}|\}} = \frac{|E\{|\Im\{X\}| \text{sgn}(\Im\{X\})\}|}{E\{|\Im\{X\}|\}} \quad (6)$$

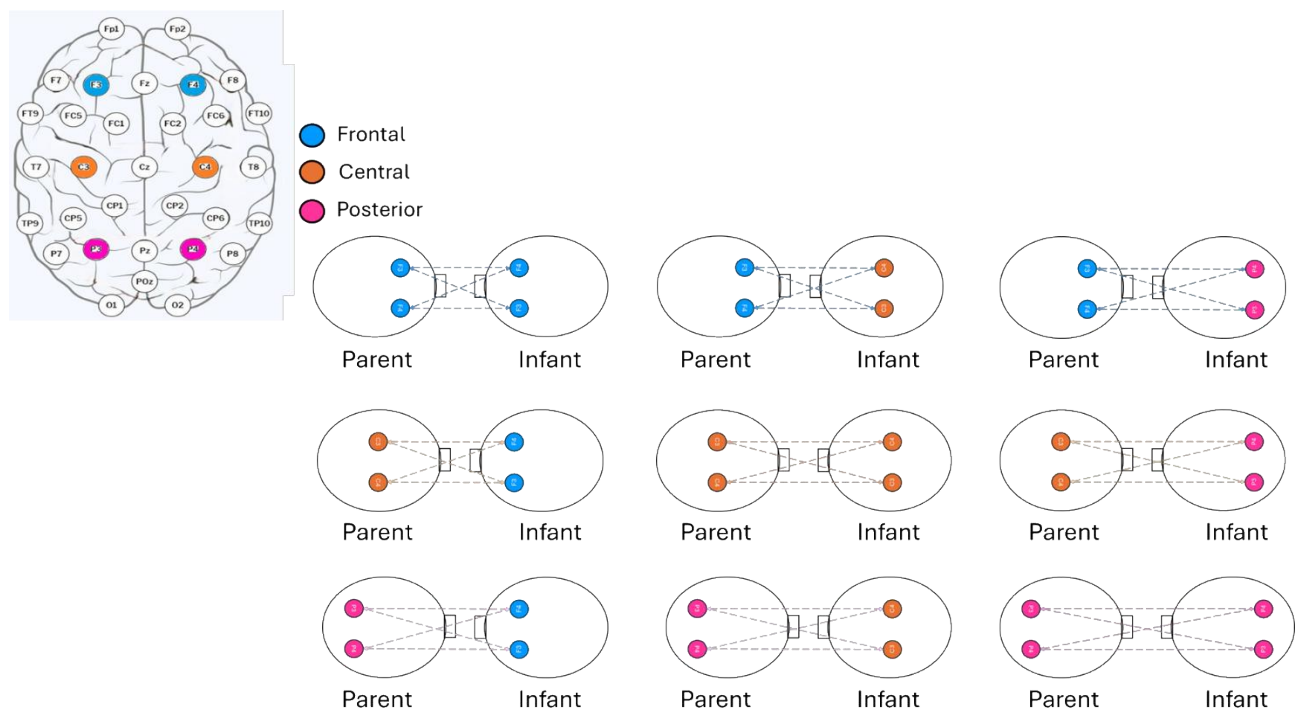
The wPLI assumes a value between 0 and 1, with higher wPLIs indicating higher coupling between neural oscillations ([Jia 2019](#)).

The main difference between ICoh and wPLI lies in the way they normalize the data. While the wPLI is based solely on the imaginary component of the cross-spectrum, the ICoh normalizes the imaginary component by the signal amplitudes ([Vinck et al. 2011](#)). This means that the addition of uncorrelated noise sources in the signals could potentially decrease the magnitude of the ICoh due to the increase in signal power ([Stam et al. 2007](#)).

Both the ICoh and the wPLI were computed for each FFSF episode (Play, Reunion) in the baby *theta* (3-5 Hz) and baby *alpha* (6-9 Hz) frequency bands, coherently with the existing literature (see [Endeveldt-Shapira et al. 2021](#); [Endeveldt-Shapira and Feldman 2023](#); Procissi et al. 2025 *in press*). The INS metrics were computed only in a subset of channels of interest, two of which frontal (F3, F4), two central (C3, C4) and two posterior (P3, P4). Notably, these channels have yielded significant INS values in previous adult-infant EEG hyperscanning studies ([Leong et al. 2017](#); [Santamaria et al.](#)

2020). ICoh and wPLI were computed both across homologous channel combinations as well as across different channels, for a total of $6^2 = 36$ channel combinations. In order to reduce the number of dependent variables for the correction for multiple comparisons (see 2.4. *Plan of analysis*), the ICoh and wPLI values for each frequency band (*theta*, *alpha*) and FFSF episode (Play, Reunion) were clustered together according to the region of the channel couples and averaged to obtain a single “regional” value per dyad. A cluster for all possible region combinations was created, for a total of $3^2 = 9$ region clusters: Frontal-Frontal (FF), Frontal-Central (FC), Frontal-Posterior (FP), Central-Frontal (CF), Central-Central (CC), Central-Posterior (CP), Posterior-Frontal (PF), Posterior-Central (PC), and Posterior-Posterior (PP). An illustration of all the channels and channel combinations of interest is available in Figure 5.1.

Figure 5.1. Channels of interest and channel combinations for which wPLI and ICoh were computed.



2.3. Behavioral coding

Videos of the FFSF procedure were microanalytically coded for interactive behaviors according to an adaptation of the Parent-Infant Coding Scheme (PICS ver. 4.0; Brambilla et al. 2023, *unpublished manual*). Behavioral coding was performed via the Noldus The Observer XT software ([The Observer](#)

[XT](#)) by two undergraduate students and two research assistants trained in the usage of our coding scheme. Behaviors of interest were coded for each member of the dyad and for each episode and measured as the percentage of time spent showcasing said behavior. For the purposes of this study, we considered both infant and maternal behaviors, as well as matching behavior. For the infant behaviors, our choice went to the behaviors which have been repeatedly associated with a still-face effect, namely the percentage of infant negative emotionality (NE_I) and gaze aversion (GA_I) per episode (Play, SF, Reunion). As for parental behaviors, we selected all the behaviors that have been previously related to the so-called global mother-infant communication system ([Botero et al. 2020; Endevelt-Shapira and Feldman 2023; Feldman et al. 2010](#)), particularly maternal affective touch (AT_M) and maternal affective vocalizations (AV_M) per episode (Play, Reunion). In the maternal measures, we also considered the percentage of time in which the mothers did not vocalize (no voice, NoV_M) to or touch (no touch, NoT_M) the infant in order as control measures. Lastly, for what concerns the matched behaviors, we considered the percentage of time per episode (Play, Reunion) in which the parents and infants simultaneously showcased positive emotionality (PE_{match}) and social gaze (SG_{match}). A definition of the behaviors of interest is provided in Chapter 2 (*Table 2.1*).

2.4. *Plan of analysis*

Data analysis was performed on RStudio 2023.12.1 (Posit team 2024) and Jamovi 2.3.28 (The jamovi project 2024). Due to the violation of the assumption of normality ($p \leq 0.05$ to the Shapiro-Wilk test) of our variables of interest, we winsorized our data (between 0 and 5 dyads per INS measure) by replacing the outlier values with the values located at two standard deviations from the mean. Winsorization was performed in RStudio using the Winsorize function available on the DescTools package. Subsequently, we performed the following statistical analyses. Preliminary analyses included a non-parametric repeated measures analysis of variance (ANOVA, Friedman test) in Jamovi aiming at assessing between-episode (Play, SF, Reunion) changes in the percentage of time spent by the dyads in showcasing the behaviors of interest (see 2.3. *Behavioral coding*). As for the

main analysis, we were interested in exploring 1. the correlations between the non-directed INS metrics of choice (ICoh, wPLI) and the maternal, infant and matched behaviors of interest, and 2. potential changes in *theta* and *alpha* ICoh and wPLI across episodes (Play, Reunion). For point 1, we ran Pearson's correlations in Jamovi between *theta* and *alpha* ICoh and the maternal, infant and matched behaviors of interest for each FFSF episode (Play, Reunion). The same analysis was performed for *theta* and *alpha* wPLI and the maternal and infant behaviors of interest during Play and Reunion. As for point 2, we performed repeated measures ANOVA in Jamovi for each non-directed INS metric (ICoh, wPLI) and frequency band (*theta*, *alpha*) with Episode (two levels, Play and Reunion) and Region (nine levels: FF, FC, FP, CF, CC, CP, PF, PC and PP) as the main factors. Results were corrected post-hoc in RStudio for multiple comparisons via False Discovery Rate (FDR), Benjamini-Hochberg method, using the `p_adjust` function ("BH" method, $\alpha = 0.05$). Lastly, we performed Pearson's correlations between ICoh and wPLI for each Episode (Play, Reunion) and frequency band (*theta*, *alpha*) in order to assess whether the two metrics were significantly correlated.

2.5. Data reduction

Out of the 103 dyads, $N = 45$ were excluded from the analysis for the following reasons: inability to conclude the FFSF procedure ($N = 15$), unavailable infant recording ($N = 10$), technical issues ($N = 9$), less than 30 good epochs in at least one FFSF episode ($N = 9$), and more than 5 interpolated channels of interest ($N = 2$). Thus, our final sample comprised $N = 58$ parent-infant dyads. Infants' mean age was 9 months and 16 days \pm 22 days (36 males and 22 females), whereas parents' mean age was 35.57 ± 4.09 years. All the participating parents were the biological mothers of the infants.

3. RESULTS

3.1. Maternal, infant and matched behaviors across the FFSF

Descriptive statistics (means, standard deviations and range of values) for the infant, maternal and dyadic behavioral data are reported in Table 5.1.

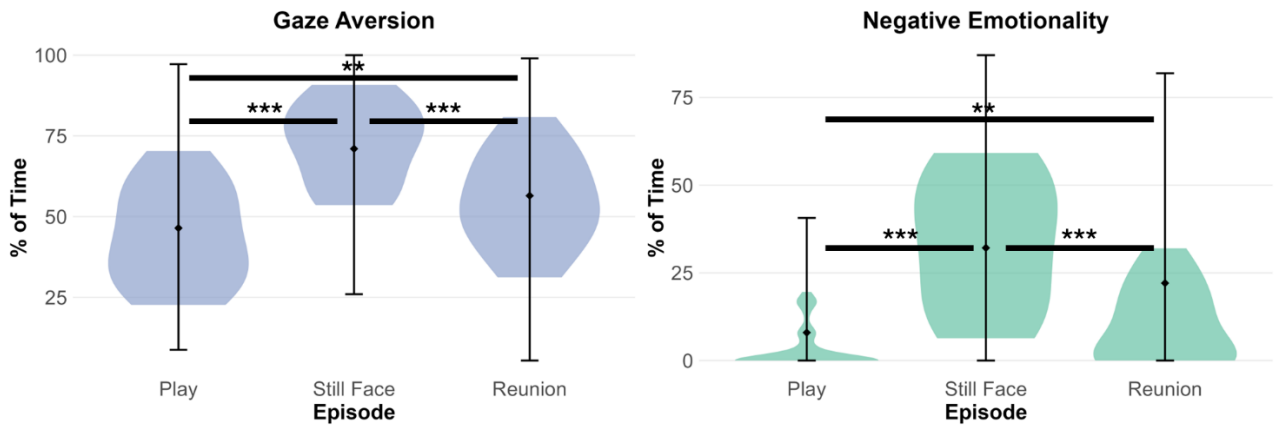
Table 5.1. Descriptive statistics of the infant, maternal and dyadic behaviors of interest. Infant and maternal behaviors are expressed in percentage of time [0 100] in which the subjects expressed the behaviors per FFSF episode. Dyadic behaviors are expressed in proportion [0 1] of time in which parents and infants expressed the behaviors of interest simultaneously per FFSF episode.

Behavior	Play (mean±SD, range)	SF (mean±SD, range)	Reunion (mean±SD, range)
Infant behavior			
Negative emotionality	7.97 ± 13 [0 40.6]	32.12 ± 27.5 [0 87.1]	22.07 ± 26.2 [0 81.9]
Gaze aversion	46.45 ± 25.3 [8.75 97.2]	71.01 ± 22 [25-97 100]	56.47 ± 25.5 [5.44 99]
Maternal behavior			
Affective vocalizations	58.8 ± 25.8 [6.65 96.7]	NA	57.1 ± 23.3 [9.37 98.8]
Affective touch	30.2 ± 25.5 [0 82]	NA	31.1 ± 25.1 [0 81.5]
No vocalizations	20.5 ± 19.5 [0.21 61.1]	NA	21.6 ± 17.5 [0 62]
No touch	64.3 ± 26.6 [10.94 100]	NA	60 ± 27.3 [4.94 100]
Matched behavior			
Positive emotionality	0.273 ± 0.214 [0 0.709]	NA	0.192 ± 0.203 [0 0.644]
Social gaze	0.372 ± 0.206 [0 0.789]	NA	0.292 ± 0.194 [0.008 0.685]

3.1.1. Infant behaviors

The repeated measures ANOVA revealed a significant effect of the Episode factor for both NE_I ($\chi^2(2) = 45.3, p < 0.001$) and GA_I ($\chi^2(2) = 41.7, p < 0.001$). More specifically, percentage of NE_I was found to be significantly higher during the SF episode compared to both Play (Durbin-Conover = 8.47, $p < 0.001$) and Reunion episodes (Durbin-Conover = 3.26, $p = 0.001$). In addition, percentage of NE_I was significantly higher during Reunion compared to Play (Durbin-Conover = 5.21, $p < 0.001$). An analogous pattern was found for the percentage of GA_I , which was found to be significantly higher during SF compared to Play (Durbin-Conover = 7.94, $p < 0.001$) and Reunion (Durbin-Conover = 4.83, $p < 0.001$) and significantly higher during Reunion compared to Play (Durbin-Conover = 3.11, $p = 0.002$). A graphic representation of the results is provided in Figure 5.2.

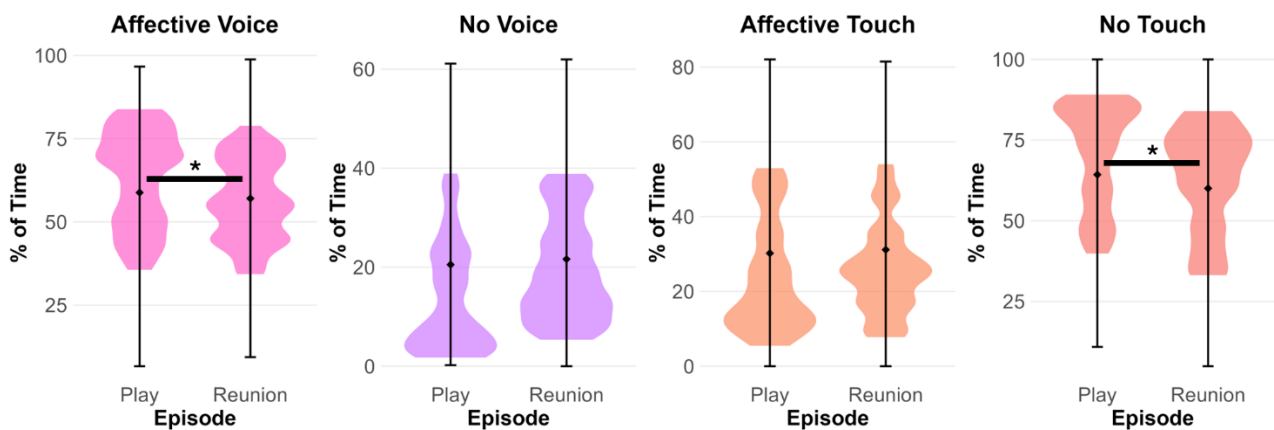
Figure 5.2. Central tendency measures of the infant behaviors of interest across the FFSF episodes. The diamond and the violin represent the mean and one standard deviation respectively, whereas the whiskers indicate the range of values reported by our sample. The brackets indicate the between-episodes differences in terms of percentage of time spent in the specific coded behavior that reached statistical significance (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).



3.1.2. Maternal behaviors

The analyses revealed a significant effect of the Episode factor for the variables AV_M ($\chi^2(1) = 3.95$, $p = 0.047$) and NoT_M ($\chi^2(1) = 4.41$, $p = 0.036$), with percentages of AV_M and NoT_M being significantly higher during the Play Episode compared to Reunion. No significant effect of the Episode factor was found for the AT_M ($\chi^2(1) = 0.296$, $p = 0.586$) and NoV_M ($\chi^2(1) = 2.12$, $p = 0.145$) variables. Results are illustrated in Figure 5.3.

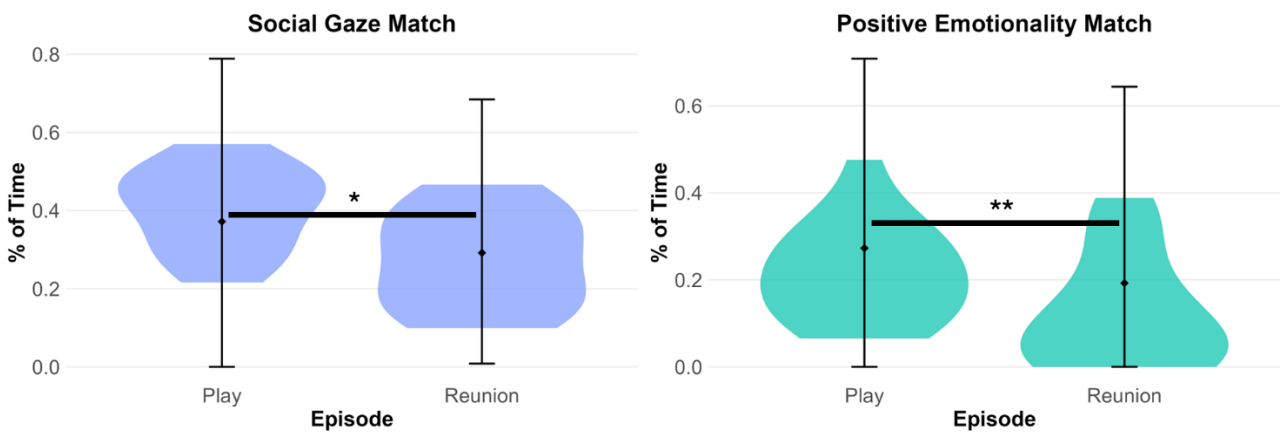
Figure 5.3. Central tendency measures of the maternal behaviors of interest across the FFSF episodes. The diamond and the violin represent the mean and one standard deviation respectively, whereas the whiskers indicate the range of values reported by our sample. The brackets indicate the between-episodes differences in terms of percentage of time spent in the specific coded behavior that reached statistical significance (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).



3.1.3. Matched behaviors

Repeated measures ANOVA revealed a significant effect of Episode for both PE_{match} ($\chi^2(1) = 10.7$, $p = 0.001$) and SG_{match} ($\chi^2(1) = 3.95$, $p = 0.047$), with percentages of the two matching behaviors being significantly higher during Play compared to Reunion. Descriptive statistics are graphically represented in Figure 5.4.

Figure 5.4. Central tendency measures of the dyadic behaviors of interest across the FFSF episodes. The diamond and the violin represent the mean and one standard deviation respectively, whereas the whiskers indicate the range of values reported by our sample. The brackets indicate the between-episodes differences in terms of percentage of time spent in the specific coded behavior that reached statistical significance (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).



3.2. Imaginary Coherence (ICoh)

3.2.1. Alpha band (6-9 Hz)

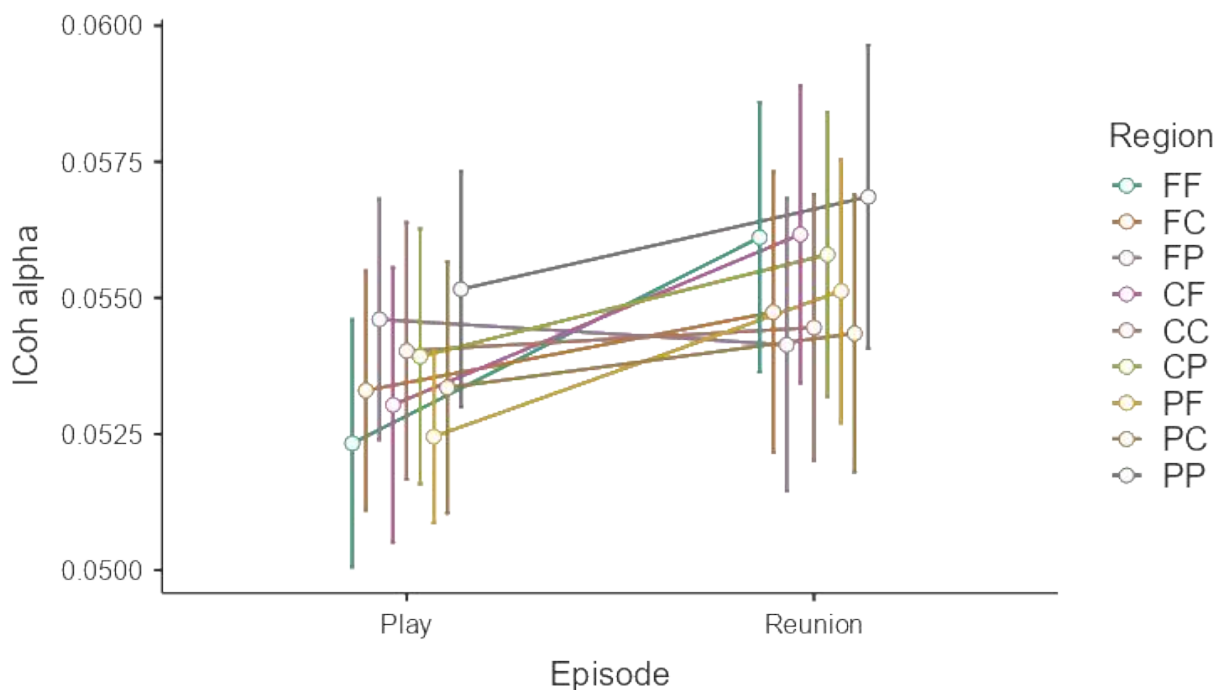
Table 5.2 provides the descriptive statistics (means, standard deviations, ranges) of the *alpha* ICoh values measured in each FFSF episode (Play, Reunion) and connectivity clusters. Results of the correlational analysis and the ANOVA are provided below.

Table 5.2. Descriptive statistics (means, standard deviations, ranges) of the ICoh computed in the *alpha* (6-9 Hz) frequency band. Statistics are provided for each FFSF episode (Play, Reunion) and for each connectivity cluster.

Region	Play (mean \pm SD, range)	Reunion (mean \pm SD, range)
Parent Frontal-Infant Frontal (FF)	0.052 \pm 0.009 [0.038 0.070]	0.056 \pm 0.009 [0.038 0.076]
Parent Frontal-Infant Central (FC)	0.053 \pm 0.008 [0.037 0.073]	0.054 \pm 0.010 [0.036 0.076]
Parent Frontal-Infant Posterior (FP)	0.055 \pm 0.008 [0.037 0.072]	0.054 \pm 0.010 [0.038 0.079]
Parent Central-Infant Frontal (CF)	0.053 \pm 0.010 [0.038 0.073]	0.056 \pm 0.010 [0.039 0.081]
Parent Central-Infant Central (CC)	0.054 \pm 0.009 [0.036 0.073]	0.055 \pm 0.009 [0.042 0.076]
Parent Central-Infant Posterior (CP)	0.054 \pm 0.009 [0.036 0.073]	0.056 \pm 0.010 [0.040 0.078]
Parent Posterior-Infant Frontal (PF)	0.053 \pm 0.006 [0.039 0.067]	0.055 \pm 0.009 [0.036 0.075]
Parent Posterior-Infant Central (PC)	0.053 \pm 0.009 [0.036 0.072]	0.054 \pm 0.010 [0.039 0.075]
Parent Posterior-Infant Posterior (PP)	0.055 \pm 0.008 [0.041 0.073]	0.057 \pm 0.011 [0.036 0.079]

Inter-episode changes in INS. The ANOVA revealed no significant (omnibus) effect of the Episode and Region factors for what concerns ICoh measured in the *alpha* band. Results are graphically represented in Figure 5.5.

Figure 5.5. Inter-episode changes in ICoh measured in the *alpha* (6-9 Hz) frequency band for each connectivity cluster of interest.



Correlations with infant, maternal and dyadic behavior. The ICoh in the *alpha* frequency band showcased no significant correlations with any of the infant, maternal and matched behaviors of interest during the Play and Reunion episodes.

3.2.2. *Theta band (3-5 Hz)*

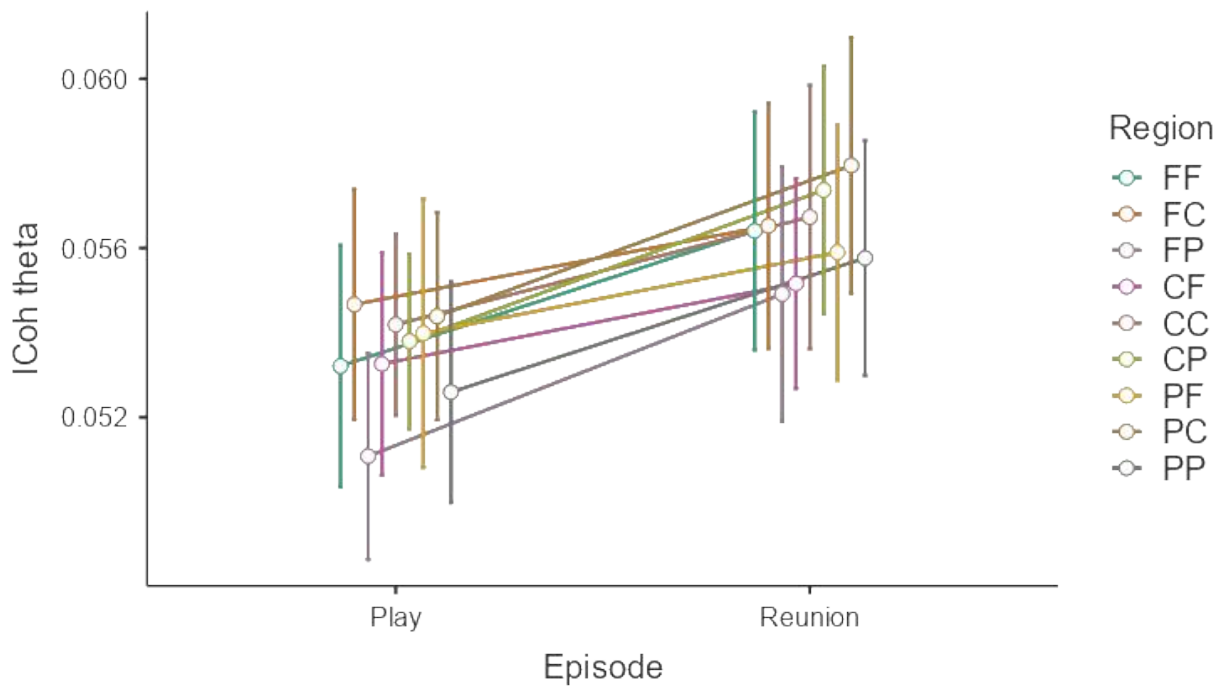
Descriptive statistics of the *theta* ICoh for each FFSF episode and connectivity cluster are available in Table 5.3.

Table 5.3. Descriptive statistics (means, standard deviations, ranges) of the ICoh computed in the *theta* (3-5 Hz) frequency band. Statistics are provided for each FFSF episode (Play, Reunion) and for each connectivity cluster.

Region	Play (mean ± SD, range)	Reunion (mean ± SD, range)
Parent Frontal-Infant Frontal (FF)	0.053 ± 0.011 [0.031 0.076]	0.056 ± 0.011 [0.036 0.080]
Parent Frontal-Infant Central (FC)	0.055 ± 0.010 [0.037 0.076]	0.057 ± 0.011 [0.036 0.082]
Parent Frontal-Infant Posterior (FP)	0.051 ± 0.009 [0.033 0.071]	0.055 ± 0.011 [0.033 0.083]
Parent Central-Infant Frontal (CF)	0.053 ± 0.010 [0.037 0.075]	0.055 ± 0.009 [0.034 0.077]
Parent Central-Infant Central (CC)	0.054 ± 0.008 [0.036 0.072]	0.057 ± 0.012 [0.033 0.085]
Parent Central-Infant Posterior (CP)	0.054 ± 0.008 [0.036 0.070]	0.057 ± 0.011 [0.037 0.084]
Parent Posterior-Infant Frontal (PF)	0.054 ± 0.012 [0.030 0.078]	0.056 ± 0.011 [0.034 0.083]
Parent Posterior-Infant Central (PC)	0.054 ± 0.009 [0.033 0.076]	0.058 ± 0.012 [0.040 0.080]
Parent Posterior-Infant Posterior (PP)	0.053 ± 0.010 [0.034 0.074]	0.056 ± 0.011 [0.037 0.078]

Inter-episode changes in INS. A significant omnibus effect of the Episode ($F(1,57) = 6.71, p = 0.012$) factor was found when considering ICoh in the *theta* frequency band, indicating significantly higher *theta* ICoh values in the Reunion compared to the Play episode (see Figure 5.6). Post-hoc analyses revealed that *theta* ICoh computed in the Parent-Frontal to Infant-Posterior (FP) connectivity cluster significantly increased in Reunion compared to Play ($t(57) = -2.185; p = 0.033$); however, this effect did not survive FDR correction.

Figure 5.6. Inter-episode changes in ICoh measured in the theta (3-5 Hz) frequency band for each connectivity cluster of interest.



Correlations with infant, maternal and dyadic behavior. ICoh in the *theta* band showed significant correlations with all the three categories of behaviors of interest (infant, maternal, matched). Regarding infant behaviors, ICoh in the *theta* band correlated significantly and negatively with the percentage of GA_I during the Play episode. The correlation was found specifically in the ICoh measured in the Parent-Frontal to Infant-Posterior (FP) connectivity cluster ($r(56) = -0.402, p = 0.002$) as well as in the Parent-Posterior to Infant-Posterior (PP) connectivity cluster ($r(56) = -0.301, p = 0.022$). No significant correlations between *theta* ICoh and infant behaviors were found during the Reunion episode.

Significant correlations between *theta* ICoh and maternal and dyadic behaviors were found during both the FFSF episodes of interest. For what concerns the maternal behaviors, a significant positive correlation was found between *theta* ICoh measured in the Parent-Frontal to Infant-Posterior (FP) connectivity cluster and the percentage of AV_M during the Play episode ($r(56) = 0.280, p = 0.035$). The positive correlation with AV_M was replicated in the Reunion episode, but with the ICoh measured in the Parent-Central to Infant-Frontal (CF) connectivity cluster ($r(56) = 0.317, p = 0.016$).

Additionally, a significant negative correlation was found between the percentage of AT_M and *theta* ICoh measured in the Parent-Posterior to Infant-Posterior connectivity cluster (PP) during the Play episode only ($r(56) = -0.282, p = 0.032$). As for the dyadic behaviors, significant positive correlations were found between *theta* ICoh measured in the Parent-Frontal to Infant-Posterior (FP) connectivity cluster and percentage of PE_{match} during Play ($r(56) = 0.262, p = 0.047$), as well as between *theta* ICoh measured in the Parent-Posterior to Infant-Central (PC) connectivity cluster and percentage of SG_{match} during Play ($r(56) = 0.275, p = 0.036$). Percentage of SG_{match} correlated positively and significantly with *theta* ICoh during the Reunion episode as well, but only with the ICoh measured in the Parent-Central to Infant-Frontal (CF) connectivity cluster ($r(56) = 0.267, p = 0.043$).

3.3. Weighted Phase Lag Index (wPLI)

3.3.1. Alpha band (6-9 Hz)

Table 5.4. provides the descriptive statistics of the wPLI values computed on the *alpha* frequency band in each FFSF episode and connectivity cluster. Results of the correlational analysis and the repeated measures ANOVA are provided below.

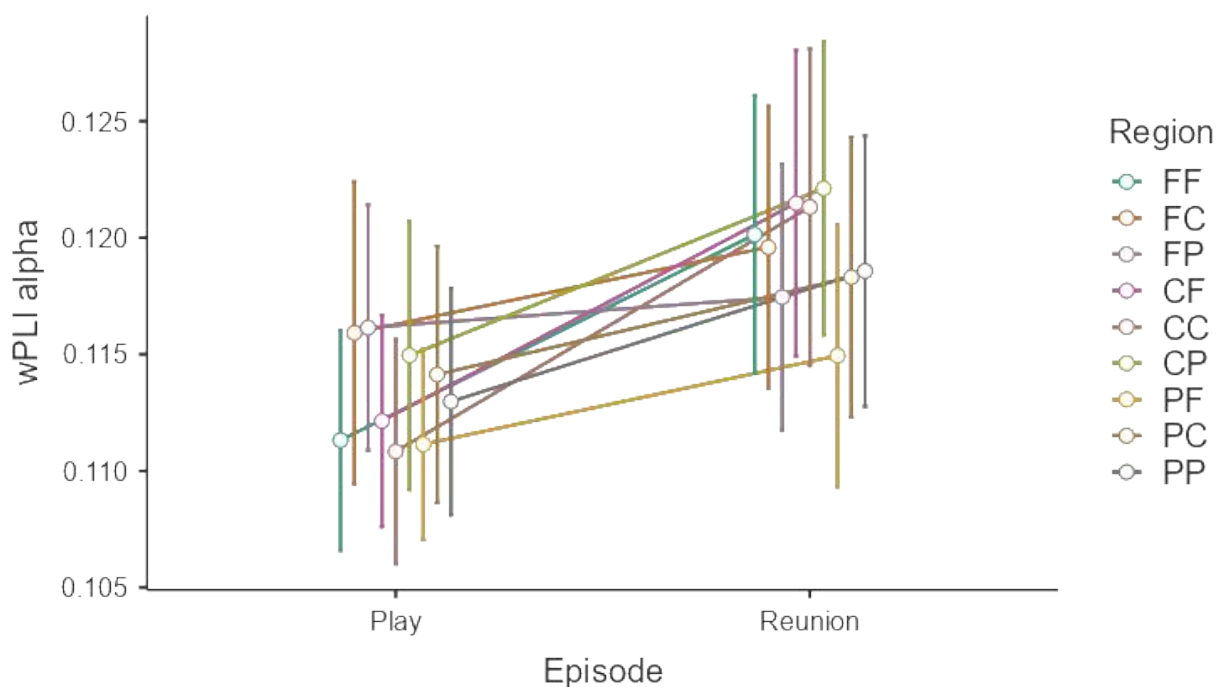
Table 5.4. Descriptive statistics (means, standard deviations, ranges) of the wPLI computed in the *alpha* (6-9 Hz) frequency band. Statistics are provided for each FFSF episode (Play, Reunion) and for each connectivity cluster.

Region	Play (mean \pm SD, range)	Reunion (mean \pm SD, range)
Parent Frontal-Infant Frontal (FF)	0.111 \pm 0.018 [0.081 0.151]	0.120 \pm 0.023 [0.081 0.168]
Parent Frontal-Infant Central (FC)	0.116 \pm 0.025 [0.061 0.174]	0.120 \pm 0.023 [0.082 0.170]
Parent Frontal-Infant Posterior (FP)	0.116 \pm 0.020 [0.076 0.158]	0.117 \pm 0.022 [0.085 0.171]
Parent Central-Infant Frontal (CF)	0.112 \pm 0.017 [0.077 0.147]	0.121 \pm 0.025 [0.081 0.177]
Parent Central-Infant Central (CC)	0.111 \pm 0.018 [0.080 0.153]	0.121 \pm 0.025 [0.081 0.179]
Parent Central-Infant Posterior (CP)	0.115 \pm 0.022 [0.075 0.162]	0.122 \pm 0.024 [0.079 0.178]
Parent Posterior-Infant Frontal (PF)	0.111 \pm 0.016 [0.082 0.144]	0.115 \pm 0.021 [0.072 0.160]
Parent Posterior-Infant Central (PC)	0.114 \pm 0.021 [0.074 0.159]	0.118 \pm 0.023 [0.079 0.167]
Parent Posterior-Infant Posterior (PP)	0.113 \pm 0.019 [0.077 0.150]	0.119 \pm 0.022 [0.083 0.164]

Inter-episode changes in INS. wPLI computed in the *alpha* frequency band showcased a significant omnibus effect of the Episode factor ($F(1,57) = 6.80; p = 0.012$). Post-hoc analyses and correction

for multiple comparisons revealed that these changes occurred mainly in three connectivity clusters: Parent-Frontal to Infant-Frontal (FF) ($t(57) = -2.59$; $p_{\text{adj}} = 0.045$), Parent-Central to Infant-Frontal (CF) ($t(57) = -2.50$; $p_{\text{adj}} = 0.045$) and Parent-Central to Infant-Central (CC) ($t(57) = -2.90$; $p = 0.045$), with wPLI measured in the *alpha* frequency band displaying significantly higher values during Reunion compared to Play. Figure 5.7. illustrates the *alpha* wPLI trends across the two FFSF episodes of interest and connectivity clusters.

Figure 5.7. Inter-episode changes in wPLI measured in the alpha (6-9 Hz) frequency band for each connectivity cluster of interest.



Correlations with infant, maternal and dyadic behavior. Alpha wPLI showcased a significant correlation only with one infant variable – percentage of GA_1 during the Play episode. The correlation was negative, weak and was limited to the *alpha* wPLI measured in the Parent-Frontal to Infant-Frontal (FF) connectivity cluster ($r(56) = -0.306$; $p = 0.019$). No significant correlations were found between *alpha* wPLI and maternal and matched behaviors, and no significant correlations with any of the dyadic behaviors emerged during the Reunion episode.

3.3.2. Theta band (3-5 Hz)

Descriptive statistics of the wPLI measured in the *theta* frequency band are provided in Table 5.5.

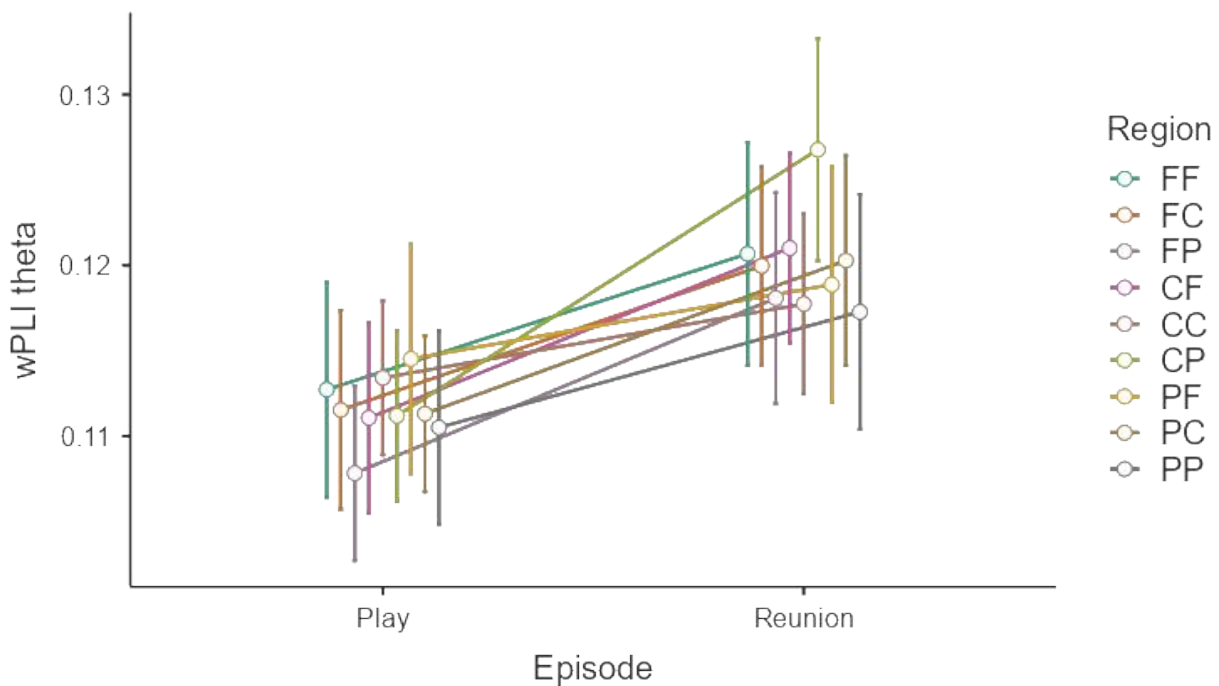
Statistics are available for each FFSF episode and connectivity cluster.

Table 5.5. Descriptive statistics (means, standard deviations, ranges) of the wPLI computed in the *theta* (3-5 Hz) frequency band. Statistics are provided for each FFSF episode (Play, Reunion) and for each connectivity cluster.

Region	Play (mean \pm SD, range)	Reunion (mean \pm SD, range)
Parent Frontal-Infant Frontal (FF)	0.113 \pm 0.024 [0.070 0.161]	0.121 \pm 0.025 [0.070 0.174]
Parent Frontal-Infant Central (FC)	0.112 \pm 0.022 [0.066 0.157]	0.120 \pm 0.022 [0.078 0.167]
Parent Frontal-Infant Posterior (FP)	0.108 \pm 0.020 [0.065 0.151]	0.118 \pm 0.024 [0.078 0.172]
Parent Central-Infant Frontal (CF)	0.111 \pm 0.021 [0.068 0.156]	0.121 \pm 0.021 [0.085 0.166]
Parent Central-Infant Central (CC)	0.113 \pm 0.017 [0.082 0.149]	0.118 \pm 0.020 [0.074 0.163]
Parent Central-Infant Posterior (CP)	0.111 \pm 0.019 [0.080 0.151]	0.127 \pm 0.025 [0.078 0.180]
Parent Posterior-Infant Frontal (PF)	0.115 \pm 0.026 [0.066 0.167]	0.119 \pm 0.026 [0.072 0.173]
Parent Posterior-Infant Central (PC)	0.111 \pm 0.017 [0.075 0.147]	0.120 \pm 0.023 [0.076 0.171]
Parent Posterior-Infant Posterior (PP)	0.111 \pm 0.022 [0.067 0.158]	0.117 \pm 0.026 [0.074 0.173]

Inter-episode changes in INS. Repeated measured ANOVA revealed a significant omnibus effect of the Episode factor for what concerns the *theta* wPLI ($F(1,57) = 12.86$, $p < 0.001$). Post-hoc analyses and FDR correction for multiple comparisons indicate a significant increase of *theta* wPLI during the Reunion compared to the Play episode in five out of nine connectivity clusters: Parent-Frontal to Infant-Central (FC) ($t(57) = -2.48$; $p_{\text{adj}} = 0.029$), Parent-Frontal to Infant-Posterior (FP) ($t(57) = -2.87$; $p = 0.027$), Parent-Central to Infant-Frontal (CF) ($t(57) = -2.50$; $p = 0.029$), Parent-Central to Infant-Posterior (CP) ($t(57) = -3.79$; $p = 0.008$) and Parent-Posterior to Infant-Central (PC) ($t(57) = -2.59$; $p = 0.029$). Results are graphically represented in Figure 5.8.

Figure 5.8. Inter-episode changes in wPLI measured in the theta (3-5 Hz) frequency band for each connectivity cluster of interest.



Correlations with infant, maternal and dyadic behavior. Analogously to the *theta* ICoh, wPLI computed in the *theta* frequency band correlated significantly with infant behaviors only in the Play episode and with matched behaviors during both FFSF episodes. Significant correlations with maternal behaviors, on the other hand, were reported only during the Reunion episode.

Regarding infant behaviors, *theta* wPLI was found to be negatively correlated with GA_I during the Play episode. More specifically, percentage of GA_I was significantly and negatively correlated with *theta* wPLI measured in the Parent-Frontal to Infant-Posterior (FP) ($r(56) = -0.349$, $p = 0.007$) and in the Parent-Posterior to Infant-Posterior (PP) connectivity clusters ($r(56) = -0.267$; $p = 0.043$). No significant correlations were found between *theta* wPLI and infant behaviors during the Reunion episode.

For what concerns maternal behaviors, *theta* wPLI measured in the Parent-Central to Infant-Frontal (FC) and in the Parent-Posterior to Infant-Frontal (PF) connectivity clusters was found to positively correlate with percentage of AV_M during the Reunion episode (CF: $r(56) = 0.366$; $p = 0.005$; PF: $r(56) = 0.277$; $p = 0.037$). Conversely, a significant negative association was found between percentage of

NoV_M during the Reunion episode and *theta* wPLI measured in the Parent-Frontal to Infant-Frontal (FF) connectivity cluster ($r(56) = -0.331$; $p = 0.012$). No significant correlations between maternal behaviors and *theta* wPLI emerged during the Play episode.

As for the matched behaviors, *theta* wPLI was found to significantly and positively correlate with percentage of SG_{match} during Play and with percentage of PE_{match} during both Play and Reunion episodes. More specifically, the positive correlation with SG_{match} during the Play episode was limited to the wPLI measured in the Parent-Frontal to Infant-Posterior (FP) connectivity cluster ($r(56) = 0.331$, $p = 0.011$); the positive correlations with PE_{match}, on the other hand, involved different connectivity clusters across FFSF episodes. During Play, the positive correlation was found in the Parent-Frontal to Infant-Posterior (FP) connectivity cluster ($r(56) = 0.290$; $p = 0.027$), whereas during Reunion, it emerged in two different connectivity clusters: Parent-Frontal to Infant-Central (FC) ($r(56) = 0.278$; $p = 0.034$) and Parent-Posterior to Infant-Frontal (PF) ($r(56) = 0.299$; $p = 0.022$).

3.4. Correlations between ICoh and wPLI

Table 5.6. provides the Pearson's *r* and the respective *p*-values between the ICoh and wPLI values in homologous frequency bands, episodes and connectivity clusters. The correlations between ICoh and wPLI measured in the *alpha* and *theta* frequency bands were significant, strong and positive for all connectivity clusters and FFSF episodes.

Table 5.6. Pearson's correlations between *alpha* (6-9 Hz) and *theta* (3-5 Hz) ICoh and wPLI across homologous episodes (Play, Reunion) and connectivity clusters. Asterisks (*) indicate significant correlations according to this legend: *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$.

Connectivity cluster	Indices	<i>Alpha</i>	<i>Theta</i>
Play Episode			
Parent Frontal-Infant Frontal (FF)	Pearson's <i>r</i>	0.634***	0.738***
	df	56	56
	p-value	<0.001	<0.001
Parent Frontal-Infant Central (FC)	Pearson's <i>r</i>	0.752***	0.714***
	df	56	56
	p-value	<0.001	<0.001

Parent Frontal-Infant Posterior (FP)	Pearson's r df p-value	0.705*** 56 <0.001	0.612*** 56 <0.001
Parent Central-Infant Frontal (CF)	Pearson's r df p-value	0.645*** 56 <0.001	0.452*** 56 <0.001
Parent Central-Infant Central (CC)	Pearson's r df p-value	0.724*** 56 <0.001	0.453*** 56 <0.001
Parent Central-Infant Posterior (CP)	Pearson's r df p-value	0.787*** 56 <0.001	0.472*** 56 <0.001
Parent Posterior-Infant Frontal (PF)	Pearson's r df p-value	0.718*** 56 <0.001	0.771*** 56 <0.001
Parent Posterior-Infant Central (PC)	Pearson's r df p-value	0.689*** 56 <0.001	0.665*** 56 <0.001
Parent Posterior-Infant Posterior (PP)	Pearson's r df p-value	0.763*** 56 <0.001	0.739*** 56 <0.001
Reunion Episode			
Parent Frontal-Infant Frontal (FF)	Pearson's r df p-value	0.737*** 56 <0.001	0.625*** 56 <0.001
Parent Frontal-Infant Central (FC)	Pearson's r df p-value	0.784*** 56 <0.001	0.725*** 56 <0.001
Parent Frontal-Infant Posterior (FP)	Pearson's r df p-value	0.736*** 56 <0.001	0.723*** 56 <0.001
Parent Central-Infant Frontal (CF)	Pearson's r df p-value	0.806*** 56 <0.001	0.765*** 56 <0.001
Parent Central-Infant Central (CC)	Pearson's r df p-value	0.812*** 56 <0.001	0.795*** 56 <0.001
Parent Central-Infant Posterior (CP)	Pearson's r df p-value	0.822*** 56 <0.001	0.727*** 56 <0.001

Parent Posterior-Infant Frontal (PF)	Pearson's r df p-value	0.734*** 56 <0.001	0.800*** 56 <0.001
Parent Posterior-Infant Central (PC)	Pearson's r df p-value	0.836*** 56 <0.001	0.693*** 56 <0.001
Parent Posterior-Infant Posterior (PP)	Pearson's r df p-value	0.813*** 56 <0.001	0.743*** 56 <0.001

4. DISCUSSION

The present study aimed at exploring how non-directed INS between parents and their nine-month-old infants changed after an experimentally induced interactive perturbation, as well as whether changes in parent-infant INS displayed correlations with infant, maternal and matched behaviors during a naturalistic social interaction. We have thus collected neural data via the EEG hyperscanning paradigm from 58 parent-infant dyads while they were engaged in an adapted version of the FFSF procedure; subsequently, we have estimated INS in the *alpha* and *theta* frequency bands using two non-directed metrics, ICoh and wPLI, and microanalytically coded infant, parental and matched behaviors, particularly infant behaviors that are strictly related to the still-face effect, maternal nurturing behavior, as well as dyadic, temporally matched behaviors. At the behavioral level, the analyses indicate that the infants from our sample showcased a clear still-face effect, with percentages of GA_I and NE_I being significantly higher during the SF episode compared to both Play and Reunion and significantly higher during Reunion compared to Play. Additionally, maternal behaviors such as AV_M and NoT_M , as well as all matched behaviors of interest, particularly SG_{match} and PE_{match} , were found to be showcased significantly more frequently during the Play episode compared to Reunion. At the neural level, wPLI computed in the *theta* (3-5 Hz) and *alpha* (6-9 Hz), frequency bands was found to be significantly higher during the Reunion episode compared to Play; the same effect was not significant for ICoh computed in the *theta* band after post-hoc correction for multiple comparisons. Finally, ICoh and wPLI measured in the *theta* frequency band (3-5 Hz) were found to

be significantly correlated with infant, maternal and matched behaviors, particularly with percentage of GA_I during Play, percentage of AV_M during Reunion and percentage of SG_{match} and PE_{match} during Play. To the best of our knowledge, this is the first parent-infant EEG hyperscanning study that assesses INS using both the ICoh and the wPLI, and one of the first studies that explored changes in non-directed INS after an interactive perturbation.

4.1. Inter-episode differences in ICoh and wPLI: INS and the reparation process

Our analyses revealed a significant effect of the Episode (Play vs Reunion) factor on wPLI computed in both *theta* and *alpha* bands as well as on ICoh computed in the *theta* frequency band. More specifically, non-directed INS as measured by these two metrics was found to yield significantly higher values during Reunion compared to Play in the following connectivity clusters: FF, CF and CC for *alpha* wPLI, FC, FP, CF, CP and PC for *theta* wPLI and FP for *theta* ICoh. With the exception of the effect of Episode emerging from *theta* ICoh measured in the FP connectivity cluster, all these effects survived post-hoc correction for multiple comparisons (FDR, Benjamini-Hochberg method).

The increase of *theta* and *alpha* wPLI from Play to Reunion was unexpected, especially considering the existing literature on parent-infant INS and changes in parent-infant biobehavioral synchrony throughout the FFSF procedure. Previous studies on parent-infant EEG hyperscanning repeatedly reported positive associations between INS, particularly phase-based non-directed INS metrics such as PLV and wPLI, and dyadic behaviors with positive valence such as cooperative play ([Wass et al. 2018](#)), positive emotionality ([Santamaria et al. 2020](#)), social gaze ([Leong et al. 2017](#)), maternal joint visual and tactile stimulation ([Neel et al. 2025](#)) and maternal sensitivity ([Endevelt-Shapira and Feldman, 2023](#)); conversely, lower INS was found during individual play ([Wass et al. 2018](#)), back-to-back and stranger-infant interaction ([Endevelt-Shapira et al. 2021](#)), and in relation to maternal intrusive behavior ([Endevelt-Shapira and Feldman 2023](#)). Considering that our sample showcased a significant increase from Play to Reunion of negative valence behaviors, such as infant gaze aversion (GA_I) and infant negative emotionality (NE_I), and a subsequent decrease of dyadic, matched

behaviors commonly associated with parent-infant INS such as mutual social gaze (SG_{match}) and mutual positive emotionality (PE_{match}), the opposite pattern was to be expected, i.e. a significant decrease in ICoh and wPLI from Play to Reunion. Another body of evidence that makes our finding unexpected is constituted by the studies assessing changes in biobehavioral synchrony during the FFSF procedure. In their study, Coppola and colleagues ([Coppola et al. 2016](#)) have observed a significant decrease of instances of playful, well-regulated (behavioral) exchanges from Play to Reunion in parent-infant dyads with infants aged 3-6 months, suggesting that the maternal attempts to regulate the infant's affective state during the Reunion episode may not be sufficient to allow the infant to recover from the interactive perturbation. Coherently to this hypothesis, a study from Conradt and Ablow ([Conradt and Ablow 2010](#)) highlighted a significant increase of infant heart rate (HR) and a significant decrease of infant respiratory sinus arrhythmia (RSA, an indicator of parasympathetic activation) from Play to SF episode, which did not return to baseline levels during Reunion. While the infants from our sample also displayed a clear still-face effect, with a significant increase of NE_1 and GA_1 from Play to SF that (partially) carried over to the Reunion episode, infants' levels of social stress did not seem to affect parent-infant non-directed INS (as measured by wPLI) in the same way: on the contrary, the partial behavioral carryover effect of the FFSF procedure seemed to have been accompanied by a significant increase in wPLI from Play to Reunion in multiple connectivity clusters. We argue that these findings may be interpreted by referring to the concept of dyadic reparation introduced by Gianino and Tronick in their Mutual Regulation Model (MRM; [Gianino and Tronick 2013](#)). Dyadic reparation is defined as the ability of parents and infants to repair affective and behavioral mismatched states in order to recover a behaviorally and affective "matched" state ([Gianino and Tronick 2013](#); [Müller et al. 2015](#)), which allows the infant to acquire better stress and emotion regulation strategies ([Provenzi et al. 2018](#)). Under this theoretical framework, it is possible that the increase in wPLI from Play to Reunion reflected the attempts by our dyads to repair from the dyadic mismatch caused by the SF episode. More specifically, the interactive rupture caused by the SF episode and the subsequent carryover of the still-face effect might have brought the dyads

from our sample to display a neural “compensatory mechanism” in order to recover the positive exchange that occurred during the Play episode, which subsequently led to an increase in wPLI. Said “compensatory mechanism” could have been associated with variables that were not accounted for in the present study but were previously found to contribute to infant recovery from SF to Reunion, such as infant hetero-regulation strategies applied by the parent ([Provenzi et al. 2015](#)), parental sensitivity ([Conradt and Ablow 2010](#); [Coppola et al. 2016](#)) or even infant social engagement and temperament ([Swider-Cios et al. 2024](#)). Alternatively, it is possible that the interactive rupture caused by the SF episode might have increased the infants’ receptiveness to the parental proposals and their need of hetero-regulation compared to the levels displayed during Play, resulting in a significant increase in wPLI. Naturally, further studies exploring changes in parent-infant INS in response to interactive ruptures are of paramount importance in order to achieve a better understanding of the relationship between neural dyadic co-regulation and dyadic reparation processes. Indeed, at the current state of the literature, it is not possible to advance precise hypotheses about the functional and developmental role of this increase in parent-infant non-directed INS, and particularly on whether these patterns of dyadic neural co-regulation are widespread in the general population of infants born full-term with typical risk of neurodevelopmental conditions. Similar studies involving clinical populations, such as infants born preterm, visually impaired infants or infants with elevated likelihood of neurodevelopmental conditions ([Provenzi et al. 2023](#)) could provide invaluable insights into the functional role of parent-infant INS in typical and atypical development.

It is worth noting that significant changes in non-directed parent-infant INS across Play and Reunion emerged only in wPLI, whereas limited, and ultimately non-significant, changes were found when considering ICoh measured in the *theta* frequency band. Considering that ICoh and wPLI were significantly, strongly and positively correlated across homologous connectivity clusters and FFSF episodes, we hypothesize that this discrepancy might be ascribed to the computational differences displayed by the two INS metrics. While both wPLI and ICoh are regarded as robust connectivity measures which effectively overcome the issue of volume conduction, previous works reported that

ICoh is strongly influenced by the phase of the coherency, which makes it more apt at capturing leads and lags between the two signals which correspond to a quarter of cycle ($\pi/4$) and less effective when the two signals are either in phase or in phase opposition ([Jia, 2019; Stam et al. 2007](#)). Additionally, ICoh normalizes the imaginary component of the coherency by the signal amplitudes, which means that the addition of uncorrelated noise sources in the signal could potentially decrease the magnitude of the ICoh due to the increase in signal power ([Stam et al. 2007](#)). wPLI, on the other hand, is exclusively based on the imaginary component of the cross-spectrum, with no normalization process ([Vinck et al. 2011](#)). In the present study, it is possible that the leads and lags between the parents and infants did not correspond to a quarter of cycle, or that the signal amplitudes in our frequency bands of choice did not undergo changes across the FFSF procedure. Future methodological and experimental studies comparing the two metrics during experimentally induced interactive perturbations are needed in order to support this interpretation. The comparison of wPLI and ICoh with non-directed INS metrics based on amplitude coupling ([Palva and Palva 2018](#)) could help clarify how phase and amplitude are affected by interactive ruptures.

4.2. *The complementarity of ICoh and wPLI*

For what concerns the relationship between non-directed INS and behavior during the FFSF, ICoh measured in the *theta* frequency band and wPLI measured on both *theta* and *alpha* bands showcased significant, albeit weak correlations with all types of behaviors of interest (infant, maternal, matched). In both INS metrics and frequency bands, the correlations were more likely to be significant during Play episode; nonetheless, significant correlations between parent-infant INS and behavior have been found during the Reunion episode as well.

Regarding INS in the *theta* frequency band, ICoh and wPLI showcased similar correlational patterns with infant, maternal and matched behaviors. More specifically, both metrics were found to be negatively correlated with percentage of infant gaze aversion (GA_I) during Play and to be positively correlated with the following maternal and matched variables: percentage of mutual positive

emotionality (PE_{match}) and mutual social gaze (SG_{match}) during Play and percentage of maternal affective vocalizations (AV_M) during Reunion. Notably, said correlations with the dyadic behaviors of interest were found in the same connectivity clusters across metrics: FP and PP for the negative correlation with GA_I , CF for the positive correlation with AV_M and FP for the positive correlation with PE_{match} . Correlations with SG_{match} , on the other hand, were displayed in different connectivity metrics across INS metrics: PC for the *theta* ICoh and FP for *theta* wPLI. In addition to these, *theta* ICoh was found to be positively associated with percentage of AV_M during Play (FP cluster), percentage of SG_{match} during Reunion (CF cluster), and to be negatively associated with percentage of maternal affective touch (AT_M) during Play (PP cluster); wPLI, on the other hand, was found to be positively correlated with percentage of PE_{match} during Reunion (FC and PF clusters) and to be negatively correlated with percentage of instances of no maternal vocalizations (NoV_M) during Reunion (FF cluster). Our findings are in line with previous research which linked non-directed INS in *theta* to a series of dyadic behaviors that included changes in shared attention during joint play ([Wass et al. 2018](#)) and changes in directed gaze ([Leong et al. 2017](#)), emotional processing ([Endevelt-Shapira et al. 2021](#)) and maternal sensitivity as compared to maternal intrusiveness ([Endevelt-Shapira and Feldman 2023](#)). Significant correlations with maternal affective vocalizations were expected as well, given the role of maternal contingent vocalizations in fostering parent-infant physiological synchrony in previous works ([Feldman et al. 2011](#); [McFarland et al. 2020](#)). The results reinforce the role of the *theta* frequency in the establishment of parent-infant non-directed INS, with attentional (GA_I , SG_{match}) and emotional/affective behaviors (PE_{match} , AV_M) acting as cues that enable the process of dyadic neural co-regulation. The negative correlation between *theta* ICoh and percentage of AT_M during Play, on the other hand, was an unexpected finding, as maternal affective touch has been previously associated with maternal sensitivity ([Botero et al. 2020](#); [Endevelt-Shapira and Feldman 2023](#); [Feldman et al. 2010](#)) and it has been found to foster mutual engagement during parent-infant interactions ([Nguyen et al. 2021](#)). Considering that maternal sensitivity has been found to be positively associated with *theta* wPLI in a previous study ([Endevelt-Shapira and Feldman 2023](#)) and

that maternal affective touch was previously found to be temporally coordinated with infants' affect and attention ([Jean et al. 2014](#); [Stack and Muir, 1990](#)), we would have expected both ICoh and wPLI to showcase a positive, rather than negative, correlation with AT_M . A possible explanation to our findings is that affective touch alone may not be sufficient to drive non-directed INS during parent-infant interaction. This hypothesis is supported by an EEG hyperscanning study by Neel and colleagues ([2025](#)), who reported a significant positive association between non-directed INS (as measured by the Circular correlation) and maternal touch only when the latter was accompanied by directed gaze. Alternatively, it is possible that this effect was mediated or moderated by additional variables that were not accounted for in our analyses, such as infant temperament and sensory profile. Notably, infant temperament is hypothesized to play a role in the recovery from SF to Reunion (see [Mesman et al. 2009](#)), whereas the infant's sensory profile may have impacted the way they reacted to maternal tactile stimulation. Further studies assessing the mediating/moderating role of these variables, as well as the impact of multisensory stimulation (e.g., visual and tactile, auditory and tactile), on the relationship between maternal affective touch and parent-infant INS are needed in order to shed light into the neural mechanisms driving neural dyadic co-regulation between parents and their infants. Considering that the negative correlation with AT_M was not consistent across INS metrics and frequency bands, it is also possible that the correlation was mainly driven by random fluctuations rather than by an underlying neurobehavioral process.

For what concerns ICoh and wPLI computed in the *alpha* frequency band, correlations with infant behaviors were limited, whereas no significant correlations were found with maternal and matched behaviors. Particularly, *alpha* wPLI measured in the FF connectivity cluster was found to be significantly and negatively correlated with percentage of GA_1 during Play; however, this finding was not replicated in *alpha* ICoh, which showcased no significant correlations with any of the behaviors of interest. Our findings are in contrast with the existing literature on parent-infant EEG hyperscanning studies, which reports significant associations between neural activity in the *alpha* (6-9 Hz) frequency band and changes in emotional states ([Atzaba-Poria et al. 2017](#); [Krzeczkowski et al.](#)

[2022; Perone et al. 2020; Swider-Cios et al. 2024](#)), valence of the emotional feedback during structured interactions ([Santamaria et al. 2020](#)), maternal joint visual and tactile stimulation ([Neel et al. 2025](#)) and positive social exchanges in which the infant observed the parent performing a task ([Atilla et al. 2023](#)). This evidence, unitedly with the significant increase in *alpha* wPLI displayed during Reunion compared to Play, suggests that the fluctuations in *alpha* INS reported in our sample were not driven by changes in the interactive behaviors of interest across the FFSF episodes, but rather by other dyadic behaviors that were not accounted for in the present study. Notably, previous studies that reported significant associations between parent-infant INS in the *alpha* band and changes in emotional states during parent-infant structured and naturalistic social interactions were mainly found either in studies computing parent-infant Frontal Alpha Asymmetry (FAA; [Atzaba-Poria et al. 2017; Krzeczowski et al. 2022; Perone et al. 2020; Swider-Cios et al. 2024](#)) or other non-directed INS such as PLV ([Atilla et al. 2023; Santamaria et al. 2020](#)) or CCorr ([Neel et al. 2025](#)); *alpha* ICoh and wPLI, on the other hand, were never utilized in the literature prior to the present study to measure parent-infant INS. All these metrics, particularly PLV and CCorr, compute phase synchronization using different computational approaches compared to ICoh and wPLI, which could have determined the discrepancies between the results of the present study and the ones reported in the existing literature. Future experimental and methodological studies comparing how different non-directed INS metrics are related to dyadic behaviors could provide further insights into the comparability of the results yielded by different INS metrics in the current body of literature.

It is worth noting that the majority of the significant associations between non-directed INS and the dyadic behaviors of interest were more common during the Play episode compared to Reunion, even while *alpha* and *theta* wPLI during this FFSF episode was found to be significantly lower compared to that measured during the Reunion episode. This finding suggests that parent-infant non-directed INS during Play and Reunion could be driven by different underlying processes. During unconstrained and naturalistic playful interactions such as the Play episode of the FFSF procedure, non-directed INS might be prevalently driven by infant, maternal and mutual positive behaviors,

which function as cues for the dyad to co-regulate their neural activity. Conversely, during the Reunion episode, in which both the infant and the parent have just experienced a social stressor and in which positive dyadic behaviors tend to be shown less frequently compared to Play, behavioral matches and affective infant and maternal behaviors may not be sufficient to drive INS; rather, a more complex interplay of individual, cognitive and socio-emotional factors is needed in order to re-establish the previous levels of INS. Future studies examining both microanalytical as well as macro-analytical behaviors and individual characteristics of infants and parents could shed light into the neural and social mechanisms underlying parent-infant INS before and after interactive ruptures.

4.3. The role of fronto-posterior connectivity in parent-infant interaction

While the use of the EEG technique does not allow precise source localization due to its low spatial resolution (unless methods such as the spline Laplacian are used; see [Nunez and Pilgreen 1991](#); [Srinivasan 1999](#)), it could be relevant to note that, in the present study, several of the infant, maternal and matched behaviors showed significant correlations with ICoh and wPLI measured in specific subsets of connectivity clusters. During the Play episode, all the significant correlations between *theta* wPLI and dyadic behaviors, as well as 60% (three out of five) of the significant correlations between *theta* ICoh and dyadic behaviors, were found to involve the Parent-Frontal to Infant-Posterior (FP) connectivity cluster, which makes it the connectivity cluster that displays the highest number of significant correlations between non-directed INS and dyadic behaviors in the present study. During the Reunion episode, however, the connectivity cluster that was found to be the most frequently involved in the significant correlations between parent-infant INS and dyadic behaviors was the Parent-Central to Infant-Frontal (CF) connectivity cluster; more specifically, it was implied in one out of three significant correlations between *theta* wPLI and dyadic behavior as well as in all (N = 2) the significant correlations between *theta* ICoh and dyadic behaviors. Additionally, the CF connectivity cluster was found to display a significant increase in both *theta* and *alpha* wPLI from Play to Reunion, making it the connectivity cluster that was most affected by the interactive rupture.

The involvement of the fronto-posterior connectivity pattern during unconstrained free play interactions between parents and infants is not surprising, as it is well documented in several previous studies employing neuroimaging methods with higher spatial resolution (compared to EEG) such as functional near infrared spectroscopy (fNIRS) and magnetoencephalography (MEG). Additionally, this finding is in line with the results of our previous study on directed INS (Pili et al. *under review*; see Chapter 4), in which the connections displaying a dominance of the “parent leader, infant follower” type (P->I) were prevalently those involving the frontal channels of the parent (F3, F4) and the posterior channels of the infant (P3, P4). The role of frontal regions in the establishment of INS during parent-infant and parent-child social interactions is well documented in the literature, with studies finding a preferential involvement of the bilateral median and bilateral lateral prefrontal cortex (mPFC and IPFC; [Nguyen et al. 2020](#); [Nguyen et al. 2021](#)) and the inferior frontal gyrus (IFG; [Lin et al. 2023](#)). The prefrontal cortex (PFC) has been related to a series of social and higher order cognitive processing, including but not limited to the detection of communicative signals directed towards the self, mentalization and reward ([Nguyen et al. 2021](#); [Redcay and Schilbach 2019](#)), as well as attentional regulation, working memory, regulation of emotional experiences and planning of self-initiated social behaviors ([Azhari et al. 2022](#)). Similarly, the IFG has been found to be involved with the detection of communicative signals related to social interaction, and particularly to the verbal components of social communication ([Lin et al. 2023](#); [Redcay and Schilbach, 2019](#)). Notably, parent-infant INS in the frontal regions was found to be associated with the duration of affectionate touch ([Nguyen et al. 2021](#)) and frequency of vocal turn-taking ([Nguyen et al. 2023](#)) as well, especially when considering slow rhythms up to 0.3 Hz and the *theta* frequency band ([Hoehl et al. 2025](#)). As for the involvement of the infants’ posterior channels, a previous EEG hyperscanning study by Endevelt-Shapira and colleagues ([2021](#)) reported a significantly strong parent-infant INS (as measured by the wPLI in the *theta* band) involving the mothers’ right central area (channel C4) and the infant right occipito-temporal area (channel P8). Additionally, several temporo-parietal areas of the infant brain have been found to be related to the processing of social cues, including the inferior parietal lobule

(IPL; [Meltzoff and Decety 2004](#)), the right angular gyrus ([Lin et al. 2023](#)) the superior temporal sulcus (STS) and the temporo-parietal junction (TPJ; [Hoehl et al. 2025](#); [Minagawa et al. 2023](#)). The IPL was found to be involved in the processing of imitative behavior and the distinction between the self and the other ([Meltzoff and Decety 2004](#)). The TPJ and STS, on the other hand, are considered key areas in the social brain network, and are involved in integrating multimodal cues, social attention and social perception ([Hoehl et al. 2025](#)). The TPJ in particular is believed to be involved in the attribution of mental states to others ([Minagawa et al. 2023](#)) and it has been repeatedly found to be implied in the establishment of parent-infant INS ([Hoehl et al. 2025](#); [Nguyen et al. 2020](#)), whereas the STS processes the action prediction of others ([Minagawa et al. 2023](#)). While the low spatial resolution of EEG does not allow a precise estimation of the correspondence between each channel and the underlying brain structures, our findings regarding the correlations between dyadic behavior during the Play episode and non-directed INS measured in the Parent-Frontal to Infant-Posterior (FP) connectivity cluster might be considered in line with the hypothesis that parent-infant INS during structured and unstructured positive interactive exchanges involves the so-called frontotemporal network, which is implied in social cognition and empathy ([Endevelt-Shapira and Feldman 2023](#)). The fronto-temporal network includes frontal brain structures such as the mPFC and the IFG and temporo-parietal areas such as the STS, the IPL and the superior temporal gyrus (STG) ([Atzil et al. 2012](#); [Frith and Frith 2001](#)). Coherently with this framework, it is possible that, especially during the initial baseline (Play) interaction, the parent's main focus is to initiate social behavior and find the most appropriate way to respond to the infant's social and behavioral cues; conversely, the infant might prevalently recruit temporo-parietal areas in order to process the multimodal stimuli coming from the parent, as well as to enhance social attention.

For what concerns the increase of *theta* and *alpha* wPLI observed in parental central areas and infant frontal brain areas during the Reunion episode, as well as its association with dyadic behaviors, references to the existing literature are sparse. While the involvement of infant frontal regions during social interactions and its associations with dyadic behaviors have been extensively documented in

the fNIRS hyperscanning literature (see above), only three previous EEG hyperscanning studies ([Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#); [Leong et al. 2017](#)) reported the involvement of parental central areas (C3 and C4 channels) during parent-infant and stranger-infant social interactions, particularly during instances of mutual social gaze ([Leong et al. 2017](#)). Additionally, previous studies proposed that activity of the centroparietal regions in the adult brain could function as a neuromarker for social coordination during spontaneous inter-neural coupling ([Dumas et al. 2010](#); [Tognoli et al. 2007](#)). Unitedly with the role of frontal connectivity in the detection and planning of cognitive, social and regulatory mechanisms (see [Azhari et al. 2022](#); [Lin et al. 2023](#); [Nguyen et al. 2021](#); [Redcay and Schilbach 2019](#)), it is possible that the observed increase in centro-frontal wPLI observed in our sample from Play to Reunion, as well as its significant associations with dyadic behaviors during the Reunion episode, reflected the dyads' efforts at recovering the previous positive interactive exchange after the SF episode. At the resumption of the unconstrained free play interaction during the Reunion episode, the parents from our sample might have increased activity in their central brain areas in order to achieve the previous levels of social coordination with their infants; conversely, infants might have increased activity in the frontal areas for self-regulation purposes as well as in order to enhance their receptiveness of the parents' new communicative signals. Interestingly, *theta* ICoh and wPLI measured in the CF connectivity cluster during the Reunion episode were mainly correlated with maternal affective vocalizations (AV_M), which was one of the few maternal behaviors that were found to significantly decrease across the FFSF episodes. It is possible that, during the Reunion episode, maternal affective vocal stimulations become a more relevant stimulus for the infant to trigger self- and hetero-regulation mechanisms due to the previous interactive rupture, whereas during the Play episode, infants might have relied more on other types of socio-emotional cues such as positive emotional valence and social gaze. This hypothesis would explain the prevalent involvement of the CF connectivity cluster during this episode and its associations with AV_M , as parent-infant INS in the frontal regions was also related to the frequency of vocal turn-taking ([Nguyen et al. 2023](#)). Naturally, further parent-infant hyperscanning studies

assessing the impact of interactive ruptures on INS using different neuroimaging techniques could provide invaluable insights into the role of the fronto-temporal and centro-frontal network in naturalistic social interactions.

5. STUDY LIMITATIONS AND IMPLICATIONS

While the present study provides novel evidence regarding how non-directed INS is affected by interactive perturbations, it showcases a series of limitations that slightly hinder the generalizability of these findings. The first limitation concerns the thresholds that were chosen to compute INS in the *theta* and *alpha* frequency bands, which reflect exclusively the infants' neural rhythms ([Noreika et al. 2020](#); [van der Velde et al. 2019](#)). While the majority of the parent-infant EEG hyperscanning studies adopted this approach, some researchers have argued that computing INS using a cross-frequency approach (i.e. by including both the infant rhythms and the adult rhythms as the frequencies of interest, see *Chapter 1 – Introduction*) might be more apt at capturing how parents and infants co-regulate their neural activity during social interactions ([Noreika et al. 2020](#); [Turk et al. 2022](#)). Future parent-infant EEG hyperscanning studies should include estimations of (non-directed) INS that utilize both a same-frequency and a cross-frequency approach. This would allow not only to assess the comparability of the INS estimates obtained with these two different approaches, but also provide a defined framework for developmental neuroscientists to choose the most appropriate frequency ranges and INS computation approach according to their research questions.

Another important limitation of our study is a lack of sensitivity analyses for our INS metrics of choice. While including multiple INS metrics in the analysis could reduce the likelihood of false positives, especially when it comes to correlational analyses between INS and dyadic behavior ([Turk et al. 2022](#)), control measures such as bootstrapping and comparison of INS between real couples with INS computed on randomly generated couples (e.g., by pairing infants with random parents or vice versa) could ensure that the obtained INS estimates can be ascribed to true synchronization derived by the affiliative bond between parents and infants and not by random fluctuations or

contextual factors (such as the dyad responding to the same stimuli and setting) ([Hamilton, 2021](#)). Running either one of these control measures in parent-infant EEG hyperscanning studies, or alternatively including a stranger-infant interaction in the experimental task, could enhance the reliability of the results obtained from these types of studies.

The third limitation concerns our microanalytical behavioral coding scheme, which allowed us to code exclusively for either isolated or homologous matched behaviors. Notably, parent-infant non-directed INS has been previously found to be associated with maternal multisensory stimulation, such as the combination of social gaze and affective touch ([Neel et al. 2025](#)) and broader behavioral constructs such as maternal sensitivity ([Endevelt-Shapira and Feldman 2023](#)). Maternal sensitivity in particular could have been a critical construct to include in the analyses, since it has been found to significantly contribute to the infant recovery from SF to Reunion in previous behavioral studies ([Conradt and Ablow 2010](#); [Coppola et al. 2016](#)). In addition, other individual and interactive factors, such as infant self- and hetero-regulation strategies ([Provenzi et al. 2015](#)) and infant temperament and sensory profile ([Mesman et al. 2009](#)), could have acted as moderators or mediators in the direct relationship between parent-infant non-directed INS, dyadic behaviors and the presentation of the interactive rupture. Future parent-infant EEG hyperscanning studies employing the FFSF paradigm to study the neural underpinnings of dyadic reparation should include both microanalytical and macroscopic measures of parental and infant behavior, as well as parental and infant regulatory and individual factors, in order to provide a broader perspective on the dyadic behaviors that foster neural co-regulation between parents and their infants.

Regardless of these limitations, the present study has several implications for the current literature on parent-infant EEG hyperscanning. Other than being one of the first studies assessing non-directed INS between parents and infants during the FFSF procedure, it provides novel evidence on the impact of interactive ruptures on wPLI and ICoh, which could prompt researchers to investigate the neurophysiological correlates of dyadic reparation in conditions of typical and atypical development.

Additionally, it further supports the relationship between parent-infant INS measured in the *theta* frequency band and a series of dyadic attentional and affective behaviors, particularly social gaze, mutual positive emotionality and maternal affective vocalizations. Lastly, this study supports previous EEG hyperscanning works that hypothesized the involvement of frontal and temporo-parietal brain areas in the establishment of parent-infant INS and interactive behaviors, and provides preliminary evidence on the role of centro-frontal connectivity in the recovery of parent-infant non-directed INS after an interactive perturbation.

CHAPTER 6 – GENERAL DISCUSSION, LIMITATIONS AND FUTURE DIRECTIONS

This chapter contains a discussion of the results illustrated in Chapter 3, 4 and 5 of the present dissertation, as well as a description of the future directions of the field of parent-infant EEG hyperscanning.

1. GENERAL DISCUSSION

In the present dissertation, I presented three experimental studies involving respectively 31 (Chapter 3), 48 (Chapter 4) and 58 (Chapter 5) parent-infant dyads, with infants aged between 8 and 11 months and born full-term (≥ 37 gestational weeks) with no atypical neurological or genetic conditions, enrolled between June 2022 and August 2025. Parent-infant dyads participated in an EEG hyperscanning session while they were engaged in an adapted version of the FFSF procedure, comprising the following episodes: two minutes of free play (Play episode), one minute of maternal still-face (SF episode) and two minutes of resumption of the free play interaction (Reunion episode). The study presented in Chapter 3 was performed to tackle Aim 1 of the dissertation and examined the impact of *manual* and *automated* pre-processing pipelines on parent-infant Phase Locking Value (PLV) computed in *theta* and *alpha* frequency bands. Dual EEG data was pre-processed in MATLAB EEGLAB using two versions of the same pipeline – the *automated* and the *manual* – and cross-frequency PLVs stemming from *manual* and *automated* pre-processing were calculated and compared through repeated measures analysis of variance (ANOVA). Conversely, the studies presented in Chapter 4 and Chapter 5 tackled respectively Aim 2 and Aim 3 of the present dissertation and examined the impact of an interactive perturbation on parent-infant directed (Chapter 4) and non-directed (Chapter 5) INS metrics, as well as their relationship with dyadic behaviors displayed by the dyad during the FFSF procedure. In the study presented in Chapter 4, after dual EEG data pre-processing via our custom automated pipeline, Partial Directed Coherence (PDC) in the broad *theta* (3-7 Hz) and broad *alpha* (6-12 Hz) frequency bands were computed in both “infant leader, parent follower” (I->P) and “parent leader, infant follower” (P->I) directions; moreover, percentage of time spent showcasing specific dyadic behaviors per FFSF episode, particularly infant negative emotionality and infant gaze aversion during the SF as well as maternal affective touch during the Reunion, were extracted and used to perform a median split on the sample according to each of the three behaviors of interest. Dyads displaying percentages of time above the median for the behaviors of interest were included in the “high” subgroup, whereas dyads displaying percentages of time equal

or below the median for a specific behavior were included in the “low” group. Non-parametric repeated measures ANOVA were run in order to assess inter-episode (Play vs Reunion) and intra-episode (I->P vs P->I) changes in PDC in each behavioral subgroup. As for the study presented in Chapter 5, non-directed INS was measured using a phase synchronization metric, the weighted Phase Lag Index (wPLI) and a coherence metric, the Imaginary Coherence (ICoh). These indexes were computed in the baby *theta* (3-5 Hz) and baby *alpha* (6-9 Hz) frequency bands. Behaviors of interest included infant behaviors, such as percentage of negative emotionality (NE_I) and gaze aversion (GA_I), maternal behaviors, including affective vocalizations (AV_M), affective touch (AT_M), lack of vocalizations (NoV_M) and lack of touch (NoT_M), and dyadic matched behaviors, particularly instances of matched positive emotionality (PE_{match}) and matched social gaze (SG_{match}). Behaviors were measured during both Play and Reunion. After data winsorization, repeated measures ANOVA and Pearson’s correlations were run in order to assess inter-episode changes in non-directed INS (as measured by ICoh and wPLI) and potential associations with the dyadic behaviors of interest. A summarization of the main findings, their coherency across studies and their potential interpretation is provided in the following sub-paragraphs.

1.1. The behaviors of infants and parents during the FFSF procedure

From the behavioral point of view, both studies described in Chapter 4 and 5 revealed a clear still-face effect in the infants from our sample: the percentage of time in which infants displayed negative emotionality and gaze aversion during the SF episode was significantly higher compared to Play; additionally, Chapter 5 revealed a significant decrease of parent-infant matched behaviors, particularly mutual positive emotionality and mutual social gaze, from Play to Reunion. Nonetheless, the studies in Chapter 4 and Chapter 5 yielded different results for what concerns the carryover effect of the FFSF procedure. In the study described in Chapter 4, infants seemed to have experienced a carryover of the still-face effect from SF to Reunion only for the variable “negative emotionality”; percentage of gaze aversion, on the other hand, decreased significantly from SF to Reunion to baseline

(Play) levels, indicating a full recovery in regard to this specific behavior. The sample examined in Chapter 5, on the other hand, seemed to have experienced no carryover effect of negative emotionality and no full recovery from gaze aversion. While indeed the percentage of these behaviors decreased from SF to Reunion, displays of negative emotionality and gaze aversion during the Reunion episode were found to be significantly more frequent compared to those reported during the Play episode. Globally, these findings are in line with the current literature on the behavioral still-face effect in infants aged 3-9 months (see [Mesman et al. 2009](#)), which describe a significant decrease in social gaze and positive emotionality and a significant increase in negative emotionality from Play to SF, with a partial recovery from SF to Reunion and from Play to Reunion. The trends reported in Chapter 4 for what concerns infant behaviors across the FFSF procedure are those that are most in line with the existing literature on the FFSF procedure: in previous studies, infants aged 3-9 months were shown to experience a carryover of negative emotionality from SF to Reunion, whereas gaze, especially in older infants, was shown to undergo a complete recovery to baseline levels from SF to Reunion ([Mesman et al. 2009](#)). The partial recovery of infant negative emotionality and partial carryover effect of infant gaze aversion described in Chapter 5 are thus an unexpected finding, especially considering that the two samples enrolled for these studies are partially overlapped (N = 38 dyads in common). A potential interpretation for this discrepancy is that infant temperament, especially of the infants involved in only one of the considered studies, might have played a role in the infants' response to the FFSF procedure. Notably, infant temperament has been already hypothesized to function as a moderator of infants' affect recovery after interactive perturbations: more specifically, highly reactive infants are believed to be low on soothability, and thus might showcase a decreased chance of recovery from SF to Reunion compared to infants with easier temperaments ([Mesman et al. 2009](#)). Future studies assessing infants' behavioral response to the FFSF procedure should include a measurement of infant temperament as a covariate in order to better assess the factors impacting infants' affect recovery.

As for parental behaviors, both studies described in Chapter 4 and Chapter 5 reported no significant changes in percentage of maternal affective touch across Play and Reunion episodes. However, the study presented in Chapter 5 revealed a significant decrease in maternal affective vocalizations, as well as of instances of no maternal touch (of any kind, including intrusive and pragmatic touch; *see Chapter 2 for the full behavioral coding*), from Play to Reunion. No significant changes from Play to Reunion emerged in regard to percentage of time in which the parent did not provide vocal stimulation (of any kind, including negative and pragmatic ones; *see Chapter 2 for the full behavioral coding*) to the infant. Altogether, these findings suggest that parents who were enrolled for the third study (Chapter 5) reacted to the SF episode by increasing the tactile inputs and by decreasing the frequency of affective vocalizations from Play to Reunion. The FFSF procedure is regarded as a stressful procedure not only for the infants, but also for the parents, who are asked to maintain an emotionless expression even in face of infant distress. Previous studies revealed that over fifty percent of the participating mothers experienced discomfort or anxiety during the SF episode ([Mayes et al. 1991](#); [Tronick et al. 1978](#)), and that mothers tend to show signs of a physiological mobilization response (particularly a decrease in vagal tone) during moments of peak infant distress ([Oppenheimer et al. 2013](#)). In light of this evidence, it would have been expected to observe an increase of all soothing behaviors, particularly affective touch and affective vocalizations, during the Reunion episode, especially in dyads whose infants scored high in negative emotionality during the SF episode. However, our findings suggest that this was not the case, and this is supported not only by the significant decrease in maternal affective vocalizations from Play to Reunion, but also by the findings reported in our second study (Chapter 4). Particularly, it was observed that, among the dyads whose parents displayed high (as in higher than the median) affective touch during the Reunion episode, only half of them were paired with an infant displaying high negative emotionality and/or high gaze aversion during the SF episode (*see Appendix*), suggesting that affective touch was not a maternal reaction to infant distress. In light of this, we hypothesize that the parents from our sample did not react to the SF episode by displaying more strictly nurturing behaviors, but rather by increasing tactile

stimulation altogether. For what concerns tactile stimulation, our behavioral coding scheme (*see Chapter 2*) distinguished between instances of no tactile stimulation and instances in which the parent touched the infant. Instances of touch could then be categorized as intrusive, pragmatic or affective according to the intentionality of the parent. This means that the observed decrease of instances of no maternal tactile stimulation from Play to Reunion implies that there was a significant increase, during the Reunion, of the opposite behavior – tactile stimulation. It is possible that parents from our sample increased tactile stimulation in order to signal to the infant their availability to resume social interaction; alternatively, it is possible that parents used tactile stimulation as a form of distraction from the infants' distress and as a cue to shift their attention to the assigned task. Considering that affective vocalizations were found to be less frequent during Reunion compared to Play, but no changes were reported in regard to instances of no maternal vocal stimulation, it is possible that tactile stimulation and other dyadic behaviors were accompanied by other forms of vocal stimulation, such as pragmatic vocalizations (i.e. instructions, explanations, attention-getting). If this was the case, our hypothesis of parents wanting to re-engage the infant on a cognitive-attentional level would receive further support. Notably, we did not assess changes in this specific type of vocalizations across the FFSF procedure and their co-occurrence with other types of maternal inputs, which means that we cannot clearly define the nature of the observed changes in maternal behaviors in our sample. Further studies focusing on maternal behavior during the FFSF procedure could provide further insight into maternal reactions to having been unresponsive to infants.

1.2. How INS is affected by interactive ruptures: a mutual, dyadic recovery process

Overall, the studies described in Chapters 3, 4 and 5 suggest that an interactive perturbation, such as the one triggered by the FFSF procedure, leads to significant changes in both non-directed (cross-frequency PLV, ICoh and wPLI) and directed (PDC) INS from Play to Reunion, although the robustness of said changes is highly dependent on the INS metric of choice. For what concerns our non-directed INS metrics of interest (*see Chapter 3 and 5*), only infant *theta* (3-5 Hz) and *alpha* (6-9

Hz) wPLI were found to showcase a significant and robust increase during the Reunion episode in multiple connectivity clusters compared to Play; ICoh measured in infant *theta* and *alpha*, as well as cross-frequency PLV measured in the *theta* (3-5 Hz and 4-7 Hz) and *alpha* (6-9 Hz and 8-12 Hz) frequency bands, on the other hand, showed little to no changes across the FFSF procedure. Specifically, *theta* ICoh showcased a significant increase during Reunion compared to Play in the Parent-Frontal to Infant-Posterior (FP) connectivity cluster which did not survive post-hoc correction for multiple comparisons, whereas ICoh measured in the *alpha* band, as well as cross-frequency PLV measured in *theta* and *alpha* on homologous frontal and central channels, displayed no significant changes from Play to Reunion. As for directed INS, our second study (*see Chapter 4*) revealed that changes in PDC measured in the broad *theta* (3-7 Hz) and broad *alpha* (6-12 Hz) frequency bands were highly dependent on the dyadic behaviors displayed by infants and parents throughout the FFSF procedure, particularly during the SF episode and Reunion. While no robust and significant changes in “infant leader, parent follower” (I->P) and “parent leader, infant follower” (P->I) PDC were found across Play and Reunion episodes, our analyses revealed that dyads displaying frequent infant negative emotionality and gaze aversion during the SF episode, as well as dyads displaying frequent maternal affective touch during the Reunion, undergo a shift in PDC directionality across Play and Reunion, indicating a re-negotiation of the roles in the social interaction after the interactive perturbation. More specifically, the subgroup of dyads with high infant negative emotionality during the SF showcased a significant reduction, from Play to Reunion, of connections with a significantly higher (FDR-corrected) I->P PDC measured in both *theta* and *alpha* frequency bands, which was not replicated in the subgroup of dyads with low infant negative emotionality during the SF. A similar finding was reported for the subgroup of dyads with high (but not low) infant gaze aversion during the SF and high maternal affective touch during the Reunion, in which a significant increase of connections with a significantly higher (FDR-corrected) P->I PDC measured in the *theta* frequency band was found between Play and Reunion. Overall, these findings suggest that interactive perturbations, such as the one triggered by the FFSF procedure, can exert an impact not only on the

ability of parents and infants to co-regulate their behaviors and physiological activity, but also on the way they co-regulate their neural activity. This adds to the existing literature that theorizes the existence of a link between behavioral synchrony and INS ([Alonso et al. 2024](#); [Bi et al. 2023](#); [Levy et al. 2021](#); [Turk et al. 2022](#)), and supports the body of research that regards biobehavioral synchrony, including INS, as a mechanism that might be established by repeated and predictable cycles of matched and mismatched affective states ([Feldman, 2007](#); [Provenzi et al. 2018](#); [Provenzi et al. 2023](#)). Under this theoretical framework, it is possible that specific types of non-directed and directed INS metrics are more sensitive than others to fluctuations in parent-infant INS in response to interactive matches and mismatches, and that non-directed and directed INS metrics, overall, are capturing different aspects of the neural co-regulation between parents and infants during structured and unstructured social interactions. Notably, our four INS metrics of choice (cross-frequency PLV, PDC, ICoh and wPLI) display different computational characteristics, which may have had a role in the partial lack of comparability between the results (*see Chapter 1, Table 1.1 for details*). PDC, for instance, is a directed INS metric that is regarded as the frequency domain equivalent of the Granger causality ([Choi and Kim, 2018](#)) and is computed by fitting autoregressive models to the ‘raw’ power/amplitude data in a specific frequency domain ([Baccalá and Sameshima 2001](#); [Turk et al. 2022](#)). This computational approach allows to infer causal relationships between two brain signals, a factor that is normally overlooked by any of the other available non-directed and directed INS metrics; however, its assumptions of brain signal linearity (i.e. the relationship between the two brain signals can be explained by linear modelling) and stationarity (the mean and variances of the signals do not tend to change over time), which some researchers have considered to be too strict for naturally occurring EEG signals ([Baccalá and Sameshima 2021](#); [Cassani and Falk 2019](#); [Jia 2019](#)), could constitute a potential limitation to its capability to capture changes in parent-infant INS compared to inherently non-stationary metrics (unless preemptive measures to improve signal stationarity are implemented, see [Jia 2019](#)). In the same fashion, cross-frequency PLV, ICoh and wPLI are each based on different computational assumptions. PLV, for instance, is a measure of the mean phase coherence

between two brain signals, which is not weighted by the amplitude of the signals and accounts for every type of lag in its computation, including zero-phase lags ([Lachaux 1999](#); [Nolte et al. 2004](#)). While it is one of the most broadly used metrics in the current parent-infant EEG hyperscanning literature ([Pili et al. 2025](#)), its computational properties have been found to increase its vulnerability to “self-interactions” caused by volume conduction ([Nolte et al. 2004](#)) and to make this index prone to the detection of spurious hyper-connections ([Burgess 2013](#)). These limitations, for instance, are not valid for ICoh and wPLI, since they are both based on the imaginary component of the cross-spectrum and thus automatically exclude zero-lagged data from the computation ([Nolte et al. 2004](#); [Vinck et al. 2011](#)). While strictly related at a computational level, even ICoh and wPLI showcase some differences in the way they define and quantify INS between two interactive partners. ICoh, for instance, is a coherence metric that weighs the imaginary component of the coherency by the signal amplitudes ([Nolte et al. 2004](#); [Vinck et al. 2011](#)). This computational characteristic has been found to create a vulnerability to the addition of uncorrelated noise sources, which would result in an increase of the signal amplitudes and the subsequent inflation of the normalization term ([Vinck et al. 2011](#)). wPLI, on the other hand, is a phase synchronization metric based solely on the imaginary component of the cross-spectrum, which reduces its probability of detecting “false positive” connectivity in the case of noise sources with near-zero phase lag ([Endevelt-Shapira and Feldman, 2023](#); [Vinck et al. 2011](#)). Its robust computational properties, paired with its finer-grained account of neural content and timing due to it being a phase-based estimator (*see Chapter 1, paragraph 1.3*), could have allowed the wPLI, among the non-directed metrics of choice, to be more sensitive to non-directed changes in dyadic neural co-regulation from Play to Reunion in the presented studies compared to both cross-frequency PLV and ICoh. A different tentative interpretation, on the other hand, could clarify the discrepancies between the results yielded by wPLI and our directed INS metric of choice (PDC): more specifically, it is possible that these indices simply reflected different aspects of inter-neural co-regulation between parents and infants after the interactive rupture. In longitudinal developmental studies focusing on behavioral synchrony, synchrony of the directed, sequential kind

was prevalently observed in dyads with three- to six-month-old infants; conversely, when the infant reached nine months of age and acquired more independence from the mother's moment-by-moment support, behavioral synchrony was found to be more frequently of the mutual, reciprocal kind ([Feldman, 2007](#)). In light of this, our findings might suggest that, after the presentation of an interactive mismatch, nine-month-old infants and their parents might attempt at "repairing" the previous neurally co-regulated state in a bi-directional (non-directed) modality rather than in a directed/sequential fashion, unless the infant has frequent displays of negative affect and gaze aversion or unless the parent provides frequent tactile stimulation. This view would support the notion that nine-month-old infants are able to actively contribute to interactive exchanges with the parental figure in a true "give-and-take" mutuality ([Feldman, 2007](#); [Stern, 2018](#)) and might provide preliminary evidence of the existence of a neurophysiological correlate of the dyadic reparation process. However, while the significant increase in P->I PDC observed in our second study (Chapter 4) can be easily attributed to individual differences in parents' and infants' interactive behaviors, as said changes were significant only in the subgroup of dyads with high percentages of the dyadic behaviors of interest, the interactive mechanisms that impacted on the significant increase in *theta* and *alpha* wPLI from Play to Reunion in our third study (Chapter 5) remain unclear, especially at the current state of the EEG hyperscanning literature. Interactive and individual factors that were not accounted for in the present dissertation, such as parental sensitivity ([Endevelt-Shapira and Feldman 2023](#)), infant temperament ([Mesman et al. 2009](#)), infant self- and hetero-regulation mechanisms ([Provenzi et al. 2015](#); [Reindl et al. 2018](#)) as well as infant sensory profile, could have played a role in the neural co-regulation recovery after the interactive rupture. Considering the promising results reported in this dissertation, we encourage the conduction of future research exploring the relationship between parent-infant INS and the dyadic reparation process with experimental paradigms such as the FFSF procedure, as well as the role of behavioral, interactive and individual factors in promoting processes of behavioral and neurophysiological interactive repairs. If consistently replicated across studies, these findings could provide invaluable insights into the relationship between behavioral and

(interpersonal) neural synchrony, as well as on the putative neurophysiological underpinnings of interactive repairs. In addition, considering the methodological plurality in the quantification of parent-infant INS and its potential implications in the comparability of the results in this dissertation as well as in the broader EEG hyperscanning literature, we encourage the conduction of methodological studies exploring how connectivity metrics based on different theoretical assumptions and signal properties capture INS changes during parent-infant interaction.

It is worth noting that, in all three studies described in this dissertation, parent-infant INS was estimated on the whole duration of the Play and Reunion episodes, meaning that we were unable to obtain a finer-grained estimation on how non-directed and directed INS evolves throughout the FFSF procedure. Estimating INS separately for the first and second minute of the Play and Reunion episodes, for instance, could have allowed for a more precise estimation of the moment in which parents and infants were able to effectively recover from the SF episode at the neurophysiological level. This could have been done by assessing the existence of significant differences in parent-infant INS between the first minute and the second minute of Reunion, or between parent-infant INS estimated during Play and during the first and second minute of Reunion. It is in fact unlikely that the full recovery of non-directed INS observed in our studies occurred right at the start of the Reunion episode, but rather that it emerged at later timepoints. Moreover, we did not compute INS during the SF episode, which could have allowed us to assess the direct effect of the interactive perturbation. Future studies employing the EEG hyperscanning paradigm during a FFSF procedure should perform finer-grained analyses of parent-infant non-directed and directed INS, possibly including two different metrics, in order to obtain a more precise estimation of the INS trends throughout different moments of the FFSF episodes.

1.3. INS and associations with dyadic behaviors: the role of theta and alpha activity

In line with the existing literature, the studies described in Chapter 4 and 5 suggest the existence of a link between parent-infant INS and dyadic behavior. In the study exploring changes in leader and

follower dynamics across the FFSF procedure (see Chapter 4), significant changes in proportion of “directed” neural connections (as measured by PDC) were significant only in the subgroups of dyads displaying higher-than-the-median percentages of the behaviors of interest. More specifically, we found a significant decrease, from Play to Reunion, of connections with a “dominant” I->P PDC measured in the *alpha* (6-12 Hz) and *theta* (3-7 Hz) bands only in the subgroup of dyads with high infant negative emotionality during the SF episode; similarly, the analyses highlighted a significant increase, from Play to Reunion, of connections with a “dominant” P->I PDC measured in the *theta* band, but only in the subgroup of dyads with high infant gaze aversion during the SF and in the subgroup of dyads with high maternal affective touch during the Reunion. In our third study assessing changes in non-directed INS across the FFSF procedure (Chapter 5), *theta* ICoh and wPLI were found to be significantly associated to multiple individual (infant, maternal) and matched/mutual behaviors, especially during the Play episode. Particularly, *theta* ICoh and wPLI were both found to be negatively correlated with the percentage of infant gaze aversion (GA_I) showcased during Play, positively correlated with percentage of mutual social gaze (SG_{match}) and positive emotionality (PE_{match}) showcased during Play, and positively correlated with percentage of maternal affective vocalizations (AV_M) during the Reunion episode. Moreover, *alpha* wPLI was found to be significantly and negatively correlated with percentage of GA_I during Play. These findings further support the notion that parent-infant INS co-occurs with non-verbal and verbal dyadic behaviors, spanning from gaze direction, emotional states, as well as vocal and tactile stimulation ([Alonso et al. 2024](#); [Bi et al. 2023](#)), and support the hypotheses that regard INS as a neuromarker for social interaction ([Bi et al. 2023](#)) and that see behavioral synchrony and INS as two complementary mechanisms that can mutually influence and facilitate one another ([Alonso et al. 2024](#); [Levy et al. 2021](#)). Additionally, the results further support the role of *theta* and *alpha* connectivity in sustaining parent-infant dyadic co-regulation during structured and unconstrained interactive exchanges ([Turk et al. 2022](#)). In our studies, INS measured in the *theta* frequency band was the one that yielded the highest number of significant associations with dyadic behaviors, with all the non-directed (ICoh,

wPLI) and directed (PDC) metrics of choice showcasing significant associations with multiple dyadic behaviors, including infant gaze aversion and mutual social gaze, infant negative emotionality and mutual positive emotionality, as well as maternal affective touch and affective vocalizations. Considering the body of evidence linking activity in the *theta* frequency band with the display of a series of attentional and affective social cues (see Chapter 5 for an overview), it is plausible that neurobehavioral co-regulation between parents and infants during unconstrained social interactions could be facilitated prevalently through mechanisms of neural coupling in this specific frequency band. This hypothesis is also in line with the notion that the FFSF procedure may involve both socio-cognitive and affective processing, as the changes in emotional states and social gaze observed throughout Play, SF and Reunion are believed to originate from the violation of the infants' social expectancy of maternal directed gaze being followed by contingent stimulation ([Mesman et al. 2009](#)). Activity in the *alpha* frequency band during social interactions, on the other hand, has been prevalently associated with changes in emotional states ([Atzaba-Poria et al. 2017](#); [Krzeczkowski et al. 2022](#); [Perone et al. 2020](#); [Swider-Cios et al. 2024](#)), whereas evidence on its role in attention to social cues has been sparse ([Leong et al. 2017](#)). Our results regarding *alpha* non-directed and directed INS are only partially in line with this evidence, as decrease in I->P PDC from Play to Reunion was found to be exclusively linked to higher-than-the-median displays of infant negative emotionality during the SF episode. *Alpha* ICoh and wPLI, on the other hand, showed no significant correlations with behaviors related to emotional states such as infant negative emotionality and mutual positive emotionality. However, *alpha* wPLI was indeed negatively associated with percentage of infant gaze aversion during Play, which could be seen as supporting Leong and colleagues' finding ([Leong et al. 2017](#)) regarding a possible involvement of the *alpha* frequency band in the elaboration of visual/attentional cues. It is worth noting that, together with our findings on the relationship between INS in the *alpha* frequency band and interactive behaviors expressed during the FFSF, some of our findings regarding the relationship between INS measured in *theta* and dyadic behaviors could also be seen as contrasting. Particularly, proportion of connections with significantly higher I->P *theta*

PDC was found to significantly decrease from Play to Reunion in response to higher-than the median displays of infant negative emotionality during the SF episode, whereas *theta* ICoh and wPLI did not seem to co-vary with this specific interactive behavior. In the same fashion, proportion of connections with significantly higher P->I *theta* PDC was found to significantly increase from Play to Reunion in response to higher-than the median displays of maternal affective touch during the Reunion episode, whereas ICoh measured in the *theta* frequency band was found to display a significant negative correlation with the same variable measured during Play. We argue that these slight discrepancies could be merely attributed to the differences in the plan of analysis of our second and third study, as well as to potential individual differences in the two samples: displays of infant negative emotionality measured during the SF, for instance, were found to significantly more frequent than those displayed during the Play and Reunion episodes, which could have determined the significant associations with PDC, but not ICoh and wPLI (which were compared with displays of infant negative emotionality during the Play and Reunion episodes). The contrasting results obtained from the associations between *theta* INS and maternal affective touch are, on the other hand, more surprising, as the two samples from Chapter 4 and Chapter 5 did not seem to display different trends in this specific behavioral display. We cannot exclude that infant and maternal individual factors could have moderated the relationship between the two variables.

1.4. Localizing parent-infant INS: the putative role of fronto-posterior and centro-frontal connectivity

While EEG is a neuroimaging technique known for its low spatial resolution ([Burle et al. 2015](#); [Makeig et al. 1995](#); [Srinivasan et al. 1996](#); [Srinivasan 1999](#)), it could be relevant to explore similarities and discrepancies in connectivity patterns which emerged as significant across the studies described in the present dissertation. In our study on parent-infant directed INS (Chapter 4), the majority of the connections with a significantly higher I->P and P->I PDC measured in *theta* (3-7 Hz) and *alpha* (6-12 Hz) were either fronto-posterior (frontal channel of the leader, posterior channel of

the follower) or fronto-central (frontal channel of the leader, central channel of the follower) regardless of FFSF episode and percentage of time spent displaying the behaviors of interest. Similarly, in our study on parent-infant non-directed INS (Chapter 5), the majority of the significant correlations between *theta* ICoh and wPLI during the Play episode involved the fronto-posterior (frontal channel of the parent, posterior channel of the infant) cluster, whereas the majority of the significant correlations between *theta* ICoh and wPLI during the Reunion episode, as well as the increase in *alpha* and *theta* wPLI observed from Play to Reunion, involved connectivity of the centro-frontal type (central channel of the parent, frontal channel of the infant). As previously discussed (see Chapter 5), the consistent involvement of fronto-posterior connectivity, particularly of frontal activity of the parent and of posterior activity of the infant, could support the hypothesis of the implication of the fronto-temporal network during dyadic interactive exchanges, which involves brain areas such as the medial prefrontal cortex (mPFC), the superior temporal sulcus (STS), the superior temporal gyrus (STG), the inferior parietal lobule (IPL), the inferior frontal gyrus (IFG) and the temporoparietal junction (TPJ) ([Endevelt-Shapira and Feldman 2023](#); [Minagawa et al. 2023](#)). The fronto-temporal network (also known as fronto-temporoparietal network) was found to underpin key functions related to social cognition and empathy ([Atzil et al. 2012](#); [Endevelt-Shapira and Feldman 2023](#)), including mentalization ([Frith and Frith, 2001](#)) and theory of mind ([Grosse Wiesmann et al. 2017](#)). It is important that future studies in the field of developmental neuroscience explore the links between parent-infant INS and social cognition at later stages of development, as the involvement of the fronto-temporoparietal network in both processes could help at ascertaining whether INS could configure as an early neuromarker of social cognition and provide novel instruments to distinguish between patterns of typical and atypical development at early developmental stages. Notably, the current knowledge of early markers of social development is still lacking ([Schüler et al. 2024](#)) and a previous study observed atypical connectivity patterns in fronto-temporoparietal areas in children aged 7-13 years with autism spectrum disorder ([Urbain et al. 2016](#)). For what concerns the significant increase in centro-frontal non-directed INS from Play to Reunion, future parent-infant hyperscanning

studies should ascertain the role of centro-frontal connectivity in infant self- and hetero-regulatory patterns. Notably, our study on directed parent-infant INS (Chapter 4) has found this connectivity pattern to be frequently infant-led during Play, meaning that the relationship between parental central and infant frontal neural activity and behavioral regulatory processes during interactive exchanges could be more complex.

2. STUDY LIMITATIONS

Naturally, the experimental procedure, as well as the results described in the present dissertation, contain limitations that narrow the generalizability of the findings. Said limitations include the discrepancies in the choice of the thresholds to define *theta* and *alpha* frequency across studies, no application of control measures to minimize the impact of shared environmental inputs on parent-infant INS and no inclusion of infant and parental individual, regulatory and interactive characteristics in the analyses.

2.1. Different thresholds for *theta* and *alpha* frequencies

The first limitation of the present dissertation concerns the different thresholds for *theta* and *alpha* frequencies used in the first, second and third study, which might reduce the comparability of the findings. Particularly, PLV in Chapter 3 was computed using a cross-frequency approach, meaning that phase synchronization between the two signals was computed using the specific dominant frequencies of the parents (4-7 Hz for *theta* and 8-12 Hz for *alpha*) and infants (3-5 Hz for *theta* and 6-9 Hz for *alpha*). PDC, ICoh and wPLI, on the other hand, were computed using a “same frequency” approach, meaning that the parents’ and infants’ signals were filtered using the same thresholds. However, while with PDC both infant and parent thresholds were included (3-7 Hz for *theta* and 6-12 Hz for *alpha*), the same was not performed for ICoh and wPLI, in which the thresholds of choice were conventionally adapted to the infant brain rhythms (3-5 Hz for *theta* and 6-9 Hz for *alpha*). These discrepancies in the choice of the *theta* and *alpha* thresholds are motivated prevalently by computational reasons, as well as comparability with the existing literature. At the current state of

parent-infant inter-neural connectivity analyses, the only metric for which an open-source code supporting cross-frequency analysis was made available is the PLV ([Kayhan et al. 2022](#)). While wPLI and ICoh could also potentially support a cross-frequency approach, no studies to date have attempted as utilizing a cross-frequency version of these metrics in order to estimate parent-infant INS. Considering that ICoh in its “same frequency” version was never used in previous parent-infant EEG hyperscanning studies in the first place, and considering that wPLI as well was employed relatively recently in the EEG hyperscanning literature ([Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#)), we decided to opt for the usage of a “same frequency” approach on the study presented in Chapter 5. As for PDC, a robust cross-frequency version of this index would be challenging to obtain due to its directed, causal nature, as using different dominant frequencies for parents and infants would imply the assumption that directed influences between brain signals could also happen across different frequency ranges. Since, to date, there is no evidence of such a functioning of causal relationship between brain signals in the current EEG hyperscanning literature, we decided to compute this index by including both adult and infant thresholds, so that we could account for the differences in adult and infant dominant frequencies without assuming a cross-frequency-like directed influence between the brain signals. Although the comparability of directed and non-directed INS metrics is not well known in the current EEG hyperscanning literature due to the dearth of studies utilizing both types of indices, we recognize that the different thresholds used to compute *theta* and *alpha* non-directed and directed INS in our studies could have limited the comparability of the findings. While the usage of a cross-frequency approach in computing metrics of parent-infant INS is encouraged by some authors due to the fact that it allows to overcome the issue of measuring synchronization across different dominant frequencies ([Noreika et al. 2020](#); [Turk et al. 2022](#)), we cannot exclude that, while interacting with their infants, parents try to align their brain rhythms to those of their interactive partner. Future methodological and experimental studies computing INS using both a “same frequency” and cross-frequency approaches could provide

invaluable insights on the comparability of these two approaches and on how parents and infants co-regulate their brain activity despite the functional differences in the adult and infant brains.

2.2. No minimization of the impact of shared environmental inputs

Another relevant limitation of the present dissertation concerns the lack of application of control measures to minimize the impact of shared environmental inputs on the parent-infant INS metrics of interest. This interpretational problem of EEG hyperscanning data is a current object of debate among developmental neuroscientists, as previous neuroimaging studies highlighted that common cognitive processing of a shared stimulus can contribute to enhanced coherence between brain activity of two participants (see [Hamilton, 2021](#)). Notably, the studies described in the present dissertation adopted robust control measures in order to minimize “false positive” connectivity between parents and infants; however, these measures may not be sufficient to ensure that the obtained INS values are stemming exclusively from the interactive exchange. The study described in Chapter 3, for instance, included a quality assessment of the raw parent and infant raw signals performed via the computation of signal-to-noise ratio (SNR). Results highlighted mean SNR levels equal or higher than 10 dB, indicating that the quality of the data was likely to be adequate for minimization of spurious hyperconnections. The studies described in Chapter 4 and Chapter 5, on the other hand, relied on robust INS metrics (PDC, ICoh and wPLI) and statistical testing in order to minimize the occurrence of false positive results, particularly FDR correction (Benjamini-Hochberg method) of statistics stemming from parametric and non-parametric repeated measures ANOVA. Additionally, Chapter 5 included the utilization of two non-directed and robust INS metrics instead of one, which is one of the recommended procedures to minimize false positives in the statistical analyses (see [Turk et al. 2022](#)). While these measures should address the issues of false positive interactions between the parent and infant signals and dyadic behaviors, as well as reducing the likelihood of INS to be driven by motion-related artifacts, they might not be sufficient to ensure that the detected INS patterns are exclusively the result of the interactive exchange. Future parent-infant EEG hyperscanning studies should

accompany robust statistical analyses of INS with additional control measures that minimize the impact of shared environmental inputs, which include but are not limited to comparing INS values from real pairs with those computed on randomly generated couples or comparing INS measured on parent-infant dyads with INS measured during stranger-infant interaction (see [Turk et al. 2022](#) for an overview).

2.3.No inclusion of parental and infant individual, regulatory and interactive variables

Another limitation that narrows the interpretability of our findings concerns the lack of inclusion of infant and parental measures of individual, regulatory and interactive behaviors. Said variables include, but are not limited to, infant temperament and sensory profile, parental sensitivity and responsiveness, quality of the affiliative bond, and infants' self-regulatory and parental hetero-regulatory strategies applied during the FFSF. Notably, these factors were found in previous research to impact the way in which infants are able to recover from the FFSF procedure ([Mesman et al. 2009](#); [Provenzi et al. 2015](#)); additionally, maternal sensitivity, as well as infants' individual factors, were found to be associated with parent-infant INS ([Alonso et al. 2024](#); [Endevelt-Shapira and Feldman 2023](#)). While the lack of inclusion of these variables in our studies was mainly motivated by our willingness to avoid data overfitting, this choice hindered the interpretability of our findings, as the microanalytically coded behaviors shown throughout the FFSF procedure did not seem sufficient to explain the obtained results. In light of this, it is recommended that future studies explore the moderating role of individual, regulatory and interactive factors in the establishment of INS before and after experimentally induced interactive ruptures.

3. FUTURE DIRECTIONS AND CONCLUSIONS

Despite their limitations, the studies described in the present dissertation have several methodological and empirical implications for research in developmental neuroscience as well as for clinical practice. Primarily, the observed results reinforce the notion that parent-infant INS is associated with a series of non-verbal and verbal dyadic behaviors, and support the view that INS and behavioral synchrony

might mutually influence and facilitate one another. Moreover, our studies provide preliminary evidence of a possible neurophysiological correlate of the dyadic reparation process, and are in line with the view that biobehavioral synchrony could be established through repeated and predictable cycles of interactive matches and mismatches. At the methodological level, our studies suggest the feasibility of automated dual EEG data pre-processing when computing parent-infant INS, as this method enhances reproducibility while not altering INS trends across repeated measures. From here on, developmental neuroscientists could proceed in several ways. From a methodological point of view, our study on automated and manual pre-processing methods could prompt researchers to run further studies on the impact of arbitrary decision-making during dual EEG data pre-processing on different INS metrics, as well as to validate custom pre-processing tools by comparing their performance with open-source scripts such as DEEP ([Kayhan et al. 2022](#)) or HyPyP ([Ayrolles et al. 2021](#)). While some methodological studies are already present in the EEG hyperscanning literature (see [Zimmermann et al. 2024](#)), the full implications of arbitrary decision-making during dual EEG data pre-processing on the comparability of the available parent-infant EEG hyperscanning studies are still unclear. From an empirical and clinical point of view, a future direction suggested by our results is to enhance knowledge about the link between parent-infant non-directed and directed INS and the process of dyadic reparation. This could be performed by running studies assessing parent-infant INS before and after interactive ruptures (such as the FFSF procedure), and assessing how potential increases or decreases of INS from Play to Reunion are associated with infant and parental individual, regulatory and interactive factors. This could be relevant both on an empirical and clinical level, as the process of dyadic reparation is considered a key element of dyadic functioning with a direct impact on behavioral and emotional health and well-being ([Müller et al. 2015](#); [Provenzi et al. 2018](#); [Tronick 2007](#)), as well as for the development of infant regulation, resilience and other domains ([DiCorcia and Tronick 2011](#); [Provenzi et al. 2018](#)). Being able to trace the neurophysiological underpinnings of dyadic reparation would open the possibility to detect early neuromarkers of infant regulatory capacities, which could be implemented for research purposes as well as in family-centered

care (particularly in families who underwent early parenting-related stress; see [Provenzi et al. 2023](#)). Lastly, the studies described in the present dissertation could prompt developmental neuroscientists to investigate whether INS could be an early neuromarker of later socio-cognitive development. Similarly to biobehavioral synchrony, INS is believed to facilitate the emotional connection between parents and infants and, in turn, foster the development of the child's cognitive abilities, behavioral competences ([Bi et al. 2023](#)) and attachment ([Atzaba-Poria et al. 2017](#); [Djalovski et al. 2021](#); [Feldman, 2017](#); [Markova et al. 2019](#)). However, empirical evidence at the current state of the literature remains scarce, as no longitudinal study has examined the relationship between parent-infant INS and later development of social cognition. Understanding the link between parent-infant INS and later socio-cognitive development would be invaluable for developmental neuroscience as well as for clinical practice, as early neuromarkers of socio-cognitive functioning could constitute a novel instrument for the early detection of patterns of atypical development. For this reason, it is fundamental that populations who underwent early stress (e.g. preterm infants, families with high financial stress or with parental mental health conditions) and/or display elevated likelihood of neurodevelopmental conditions (e.g. siblings of children with autism spectrum disorder) are included in future hyperscanning studies, possibly using a longitudinal study design, in order to reach a better understanding of early predictors of (sub)optimal socio-cognitive development and of the neuroprotective role of optimal caregiving environments.

REFERENCES

- Adamovich, T., Zakharov, I., Tabueva, A., and Malykh, S. (2022). The thresholding problem and variability in the EEG graph network parameters. *Scientific Reports*, *12*(1), 18659. doi: 10.1038/s41598-022-22079-2.
- Adamson, L. B., and Frick, J. E. (2009). The Still Face: A History of a Shared Experimental Paradigm. *Infancy*, 451–473. doi: 10.1207/S15327078IN0404_01.
- Akaike, K. (1979). What is the Information Criterion AIC? *Surikagaku*, *153*, 5–11.
- Alonso, A., McDorman, S. A., and Romeo, R. R. (2024). How parent-child brain-to-brain synchrony can inform the study of child development. *Child Development Perspectives*, *18*(1), 26–35. doi: 10.1111/cdep.12494.
- Atila, F., Alimardani, M., Kawamoto, T., and Hiraki, K. (2023). Mother-child inter-brain synchrony during a mutual visual search task: A study of feedback valence and role. *Social Neuroscience*, *18*(4), 232–244. doi: 10.1080/17470919.2023.2228545.
- Atzaba-Poria, N., Deater-Deckard, K., and Bell, M. A. (2017). Mother-child interaction: Links between mother and child frontal electroencephalograph asymmetry and negative behavior. *Child Development*, *88*(2), 544–554. doi: 10.1111/cdev.12583.
- Atzil, S., Hendler, T., Zagoory-Sharon, O., Winetraub, Y., and Feldman, R. (2012). Synchrony and specificity in the maternal and the paternal brain: relations to oxytocin and vasopressin. *Journal of the American Academy of Child and Adolescent Psychiatry*, *51*(8), 798–811. doi: 10.1016/j.jaac.2012.06.008.
- Ayrolles, A., Brun, F., Chen, P., Djalovski, A., Beauxis, Y., Delorme, R., Bourgeron, T., Dikker, S., and Dumas, G. (2021). HyPyP: a Hyperscanning Python Pipeline for inter-brain connectivity analysis. *Social Cognitive and Affective Neuroscience*, *16*(1-2), 72–83. doi: 10.1093/scan/nsaa141.
- Azhari, A., Bizzego, A., and Esposito, G. (2022). Parent-child dyads with greater parenting stress exhibit less synchrony in posterior areas and more synchrony in frontal areas of the prefrontal cortex during shared play. *Social Neuroscience*, *17*(6), 520–531. doi: 10.1080/17470919.2022.2162118.
- Babiloni, F., Babiloni, C., Carducci, F., Romani, G. L., Rossini, P. M., Angelone, L. M., and Cincotti, F. (2004). Multimodal integration of EEG and MEG data: a simulation study with variable signal-to-noise ratio and number of sensors. *Human Brain Mapping*, *22*(1), 52–62. doi: 10.1002/hbm.20011.
- Baccalá, L. A., and Sameshima, K. (2001). Partial directed coherence: a new concept in neural structure determination. *Biological Cybernetics*, *84*(6), 463–474. doi: 10.1007/PL00007990.
- Baccalá, L. A., and Sameshima, K. (2021). Partial directed coherence: twenty years on some history and an appraisal. *Biological Cybernetics*, *115*(3), 195–204. doi: 10.1007/s00422-021-00880-y.
- Bánki, A., Köster, M., Cichy, R. M., and Hoehl, S. (2024). Communicative signals during joint attention promote neural processes of infants and caregivers. *Developmental Cognitive Neuroscience*, *65*, 101321. doi: 10.1016/j.dcn.2023.101321.

- Behrens, K. Y., Haltigan, J. D., and Bahm, N. I. G. (2016). Infant attachment, adult attachment, and maternal sensitivity: revisiting the intergenerational transmission gap. *Attachment and Human Development, 18*(4), 337–353. doi: 10.1080/14616734.2016.1167095.
- Bevilacqua, D., Davidesco, I., Wan, L., Chaloner, K., Rowland, J., Ding, M., Poeppel, D., and Dikker, S. (2019). Brain-to-Brain Synchrony and Learning Outcomes Vary by Student-Teacher Dynamics: Evidence from a Real-world Classroom Electroencephalography Study. *Journal of Cognitive Neuroscience, 31*(3), 401–411. doi: 10.1162/jocn_a_01274.
- Billeci, L., Riva, V., Capelli, E., Grumi, S., Pili, M.P., Cassa, M., Siri, E., Roberti, E., Borgatti, R., and Provenzi, L. (2024). 2-Brain Regulation for Improved Neuroprotection during Early Development (2-BRAINED): a translational hyperscanning research project. *Frontiers in Psychology, 15*, 1516616. doi: 10.3389/fpsyg.2024.1516616.
- Bi, X., Cui, H., and Ma, Y. (2023). Hyperscanning Studies on Interbrain Synchrony and Child Development: A Narrative Review. *Neuroscience, 530*, 38–45. doi: 10.1016/j.neuroscience.2023.08.035.
- Botero, M., Langley, H. A., and Venta, A. (2020). The untenable omission of touch in maternal sensitivity and attachment research. *Infant and Child Development, 29*(2), e2159. doi: 10.1002/icd.2159.
- Brambilla, M., Grumi, S., Manfredini, V., Pettenati, G., and Provenzi, L. (2023). Parent Infant Coding System, PICS. Version 4.0, Unpublished manual.
- Bråten, S. (1998). Intersubjective Communication and Emotion in Early Ontogeny. *Cambridge University Press*.
- Braungart-Rieker, J. M., Garwood, M. M., Powers, B. P., and Wang, X. (2001). Parental sensitivity, infant affect, and affect regulation: predictors of later attachment. *Child Development, 72*(1), 252–270. doi: 10.1111/1467-8624.00277.
- Brazelton, T. B., Tronick, E., Adamson, L., Als, H., and Wise, S. (1975). Early mother-infant reciprocity. *Ciba Foundation Symposium, 33*, 137–154. doi: 10.1002/9780470720158.ch9.
- Brooker, B. H., and Donald, M. W. (1980). Contribution of the speech musculature to apparent human EEG asymmetries prior to vocalization. *Brain and Language, 9*(2), 226–245. doi: 10.1016/0093-934X(80)90143-1.
- Burgess, A. P. (2013). On the interpretation of synchronization in EEG hyperscanning studies: a cautionary note. *Frontiers in Human Neuroscience, 7*, 881. doi: 10.3389/fnhum.2013.00881.
- Burle, B., Spieser, L., Roger, C., Casini, L., Hasbroucq, T., and Vidal, F. (2015). Spatial and temporal resolutions of EEG: Is it really black and white? A scalp current density view. *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology, 97*(3), 210–220. doi: 10.1016/j.ijpsycho.2015.05.004.
- Canolty, R. T., and Knight, R. T. (2010). The functional role of cross-frequency coupling. *Trends in Cognitive Sciences, 14*(11), 506–515. doi: 10.1016/j.tics.2010.09.001.
- Capitani, E., Lorenzini, C., Biuzzi, A., Alaimo, L., and Nante, N. (2023). Factors influencing the first thousand days of life. The importance of Nurturing Care. *Journal of Preventive Medicine and Hygiene, 64*(2), E172–E177. doi: 10.15167/2421-4248/jpmh2023.64.2.2850.

- Cassani, R., & Falk, T. H. (2019). Spectrotemporal modeling of biomedical signals: Theoretical foundation and applications. In *Encyclopedia of Biomedical Engineering* (pp. 144–163). Elsevier. doi: 10.1016/B978-0-12-801238-3.99993-8.
- Chang, C.-Y., Hsu, S.-H., Pion-Tonachini, L., and Jung, T.-P. (2020). Evaluation of Artifact Subspace Reconstruction for Automatic Artifact Components Removal in Multi-Channel EEG Recordings. *IEEE Transactions on Bio-Medical Engineering*, *67*(4), 1114–1121. doi: 10.1109/TBME.2019.2930186.
- Chiarion, G., Sparacino, L., Antonacci, Y., Faes, L., and Mesin, L. (2023). Connectivity Analysis in EEG Data: A Tutorial Review of the State of the Art and Emerging Trends. *Bioengineering (Basel, Switzerland)*, *10*(3). doi: 10.3390/bioengineering10030372.
- Choi, J. W., and Kim, K. H. (2018). Methods for functional connectivity analysis. In *Biological and Medical Physics, Biomedical Engineering* (pp. 125–145). Springer Singapore. doi: 10.1007/978-981-13-0908-3_6.
- Coan, J. A., and Allen, J. J. B. (2004). Frontal EEG asymmetry as a moderator and mediator of emotion. *Biological Psychology*, *67*(1-2), 7–49. doi: 10.1016/j.biopsycho.2004.03.002.
- Cohn, J. F., and Elmore, M. (1988). Effect of contingent changes in mothers' affective expression on the organization of behavior in 3-month-old infants. *Infant Behavior and Development*, *11*(4), 493–505. doi: 10.1016/0163-6383(88)90008-2.
- Cohn, J. F., and Tronick, E. Z. (1987). Mother–infant face-to-face interaction: The sequence of dyadic states at 3, 6, and 9 months. *Developmental Psychology*, *23*(1), 68–77. doi: 10.1037/0012-1649.23.1.68.
- Conradt, E., and Ablow, J. (2010). Infant physiological response to the still-face paradigm: contributions of maternal sensitivity and infants' early regulatory behavior. *Infant Behavior and Development*, *33*(3), 251–265. doi: 10.1016/j.infbeh.2010.01.001.
- Coppola, G., Aureli, T., Grazia, A., and Ponzetti, S. (2016). Reunion patterns in the still-face paradigm as predicted by maternal sensitivity and dyadic coordination. *Infancy: The Official Journal of the International Society on Infant Studies*, *21*(4), 453–477. doi: 10.1111/inf.12115.
- Czeszumski, A., Eustergerling, S., Lang, A., Menrath, D., Gerstenberger, M., Schuberth, S., Schreiber, F., Rendon, Z. Z., and König, P. (2020). Hyperscanning: A Valid Method to Study Neural Inter-brain Underpinnings of Social Interaction. *Frontiers in Human Neuroscience*, *14*, 39. doi: 10.3389/fnhum.2020.00039.
- Davidson, R. J. (1993). Cerebral asymmetry and emotion: Conceptual and methodological conundrums. *Cognition and Emotion*, *7*(1), 115–138. doi: 10.1080/02699939308409180.
- Davidson, R. J. (2004). What does the prefrontal cortex “do” in affect: perspectives on frontal EEG asymmetry research. *Biological Psychology*, *67*(1-2), 219–233. doi: 10.1016/j.biopsycho.2004.03.008.
- Debnath, R., Buzzell, G. A., Morales, S., Bowers, M. E., Leach, S. C., and Fox, N. A. (2020). The Maryland analysis of developmental EEG (MADE) pipeline. *Psychophysiology*, *57*(6), e13580. doi: 10.1111/psyp.13580.

- Delorme, A., and Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods*, *134*(1), 9–21. doi: 10.1016/j.jneumeth.2003.10.009.
- DiCorcia, J. A., Snidman, N., Sravish, A. V., and Tronick, E. (2016). Evaluating the nature of the still-face effect in the double face-to-face still-face paradigm using different comparison groups. *Infancy: The Official Journal of the International Society on Infant Studies*, *21*(3), 332–352. doi: 10.1111/infa.12123.
- DiCorcia, J. A., and Tronick, E. (2011). Quotidian resilience: exploring mechanisms that drive resilience from a perspective of everyday stress and coping. *Neuroscience and Biobehavioral Reviews*, *35*(7), 1593–1602. doi: 10.1016/j.neubiorev.2011.04.008.
- Dikker, S., Michalareas, G., Oostrik, M., Serafimaki, A., Kahraman, H. M., Struiksma, M. E., and Poeppel, D. (2021). Crowdsourcing neuroscience: Inter-brain coupling during face-to-face interactions outside the laboratory. *NeuroImage*, *227*, 117436. doi: 10.1016/j.neuroimage.2020.117436.
- Dimigen, O. (2020). Optimizing the ICA-based removal of ocular EEG artifacts from free viewing experiments. *NeuroImage*, *207*, 116117. doi: 10.1016/j.neuroimage.2019.116117.
- Djalovski, A., Dumas, G., Kinreich, S., and Feldman, R. (2021). Human attachments shape interbrain synchrony toward efficient performance of social goals. *NeuroImage*, *226*, 117600. doi: 10.1016/j.neuroimage.2020.117600.
- Donoghue, T., Schaworonkow, N., and Voytek, B. (2022). Methodological considerations for studying neural oscillations. *The European Journal of Neuroscience*, *55*(11-12), 3502–3527. doi: 10.1111/ejn.15361.
- Dumas, G. (2011). Towards a two-body neuroscience. *Communicative and Integrative Biology*, *4*(3), 349–352. doi: 10.4161/cib.4.3.15110.
- Dumas, G., Nadel, J., Soussignan, R., Martinerie, J., and Garnero, L. (2010). Inter-brain synchronization during social interaction. *PloS One*, *5*(8), e12166. doi: 10.1371/journal.pone.0012166.
- Endevelt-Shapira, Y., Djalovski, A., Dumas, G., and Feldman, R. (2021). Maternal chemosignals enhance infant-adult brain-to-brain synchrony. *Science Advances*, *7*(50), eabg6867. doi: 10.1126/sciadv.abg6867.
- Endevelt-Shapira, Y., and Feldman, R. (2023). Mother-Infant Brain-to-Brain Synchrony Patterns Reflect Caregiving Profiles. *Biology*, *12*(2). doi: 10.3390/biology12020284.
- Feldman, R. (2007). Parent-infant synchrony and the construction of shared timing; physiological precursors, developmental outcomes, and risk conditions. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, *48*(3-4), 329–354. doi: 10.1111/j.1469-7610.2006.01701.x.
- Feldman, R. (2017). The Neurobiology of Human Attachments. *Trends in Cognitive Sciences*, *21*(2), 80–99. doi: 10.1016/j.tics.2016.11.007.
- Feldman, R. (2020). What is resilience: an affiliative neuroscience approach. *World Psychiatry: Official Journal of the World Psychiatric Association (WPA)*, *19*(2), 132–150. doi: 10.1002/wps.20729.

- Feldman, R., Granat, A., Pariente, C., Kanety, H., Kuint, J., and Gilboa-Schechtman, E. (2009). Maternal depression and anxiety across the postpartum year and infant social engagement, fear regulation, and stress reactivity. *Journal of the American Academy of Child and Adolescent Psychiatry*, *48*(9), 919–927. doi: 10.1097/CHI.0b013e3181b21651.
- Feldman, R., Greenbaum, C. W., and Yirmiya, N. (1999). Mother-infant affect synchrony as an antecedent of the emergence of self-control. *Developmental Psychology*, *35*(1), 223–231. doi: 10.1037//0012-1649.35.1.223.
- Feldman, R., Greenbaum, C. W., Yirmiya, N., and Mayes, L. C. (1996). Relations between cyclicity and regulation in mother-infant interaction at 3 and 9 months and cognition at 2 years. *Journal of Applied Developmental Psychology*, *17*(3), 347–365. doi: 10.1016/S0193-3973(96)90031-3.
- Feldman, R., Magori-Cohen, R., Galili, G., Singer, M., and Louzoun, Y. (2011). Mother and infant coordinate heart rhythms through episodes of interaction synchrony. *Infant Behavior and Development*, *34*(4), 569–577. doi: 10.1016/j.infbeh.2011.06.008.
- Feldman, R., Singer, M., and Zagoory, O. (2010). Touch attenuates infants' physiological reactivity to stress. *Developmental Science*, *13*(2), 271–278. doi: 10.1111/j.1467-7687.2009.00890.x.
- Feldstein, S., Jaffe, J., Beebe, B., Crown, C. L., Jasnow, M., Fox, H., and Gordon, S. (1993). Coordinated interpersonal timing in adult-infant vocal interactions: A cross-site replication. *Infant Behavior and Development*, *16*(4), 455–470. doi: 10.1016/0163-6383(93)80004-R.
- Frith, U., and Frith, C. (2001). The biological basis of social interaction. *Current Directions in Psychological Science*, *10*(5), 151–155. doi: 10.1111/1467-8721.00137.
- Gabard-Durnam, L. J., Mendez Leal, A. S., Wilkinson, C. L., and Levin, A. R. (2018). The Harvard Automated Processing Pipeline for Electroencephalography (HAPPE): Standardized Processing Software for Developmental and High-Artifact Data. *Frontiers in Neuroscience*, *12*, 97. doi: 10.3389/fnins.2018.00097.
- Garrison, K. A., Scheinost, D., Finn, E. S., Shen, X., and Constable, R. T. (2015). The (in)stability of functional brain network measures across thresholds. *NeuroImage*, *118*, 651–661. doi: 10.1016/j.neuroimage.2015.05.046.
- Gasser, T., Sroka, L., and Möcks, J. (1985). The transfer of EOG activity into the EEG for eyes open and closed. *Electroencephalography and Clinical Neurophysiology*, *61*(2), 181–193. doi: 10.1016/0013-4694(85)91058-2.
- Georgieva, S., Lester, S., Noreika, V., Yilmaz, M. N., Wass, S., and Leong, V. (2020). Toward the Understanding of Topographical and Spectral Signatures of Infant Movement Artifacts in Naturalistic EEG. *Frontiers in Neuroscience*, *14*, 352. doi: 10.3389/fnins.2020.00352.
- Gianino, A., and Tronick, E. Z. (2013). The Mutual Regulation Model : The Infant's Self and Interactive Regulation and Coping and Defensive Capacities. *In Stress and Coping Across Development* (1st Edition, pp. 47–68). Psychology Press. doi: 10.4324/9781315825489-4.
- Goldenholz, D. M., Ahlfors, S. P., Hämäläinen, M. S., Sharon, D., Ishitobi, M., Vaina, L. M., and Stufflebeam, S. M. (2009). Mapping the signal-to-noise-ratios of cortical sources in magnetoencephalography and electroencephalography. *Human Brain Mapping*, *30*(4), 1077–1086. doi: 10.1002/hbm.20571.

- Goldstein, P., Weissman-Fogel, I., Dumas, G., and Shamay-Tsoory, S. G. (2018). Brain-to-brain coupling during handholding is associated with pain reduction. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(11), E2528–E2537. doi: 10.1073/pnas.1703643115.
- Granger, C. W. J. (1969). Investigating causal relations by econometric models and cross-spectral methods. *Econometrica: Journal of the Econometric Society*, *37*(3), 424. doi: 10.2307/1912791.
- Grosse Wiesmann, C., Schreiber, J., Singer, T., Steinbeis, N., and Friederici, A. D. (2017). White matter maturation is associated with the emergence of Theory of Mind in early childhood. *Nature Communications*, *8*, 14692. doi: 10.1038/ncomms14692.
- Gusella, J. L., Muir, D., and Tronick, E. Z. (1988). The effect of manipulating maternal behavior during an interaction on three- and six-month-olds' affect and attention. *Child Development*, *59*(4), 1111–1124. doi: 10.2307/1130278.
- Haartsen, R., van der Velde, B., Jones, E. J. H., Johnson, M. H., and Kemner, C. (2020). Using multiple short epochs optimises the stability of infant EEG connectivity parameters. *Scientific Reports*, *10*(1), 12703. doi: 10.1038/s41598-020-68981-5.
- Haley, D. W., Handmaker, N. S., and Lowe, J. (2006). Infant stress reactivity and prenatal alcohol exposure. *Alcoholism, Clinical and Experimental Research*, *30*(12), 2055–2064. doi: 10.1111/j.1530-0277.2006.00251.x.
- Hamilton, A. F. de C. (2021). Hyperscanning: Beyond the Hype. *Neuron*, *109*(3), 404–407. doi: 10.1016/j.neuron.2020.11.008.
- Ham, J., and Tronick, E. (2006). Infant resilience to the stress of the still-face: infant and maternal psychophysiology are related. *Annals of the New York Academy of Sciences*, *1094*, 297–302. doi: 10.1196/annals.1376.038.
- Hill, K. E., Neo, W. S., Hernandez, A., Hamrick, L. R., Kelleher, B. L., and Foti, D. (2020). Intergenerational Transmission of Frontal Alpha Asymmetry Among Mother-Infant Dyads. *Biological Psychiatry. Cognitive Neuroscience and Neuroimaging*, *5*(4), 420–428. doi: 10.1016/j.bpsc.2019.12.003.
- Hirata, M., Ikeda, T., Kikuchi, M., Kimura, T., Hiraishi, H., Yoshimura, Y., and Asada, M. (2014). Hyperscanning MEG for understanding mother-child cerebral interactions. *Frontiers in Human Neuroscience*, *8*, 118. doi: 10.3389/fnhum.2014.00118.
- Hoehl, S., Bánki, A., Brzozowska, A., Carollo, A., Kostorz, K., Nguyen, T., Pletti, C., Reisner, S., Schäfer, V. T., Schaetz, C., and Tünte, M. R. (2025). A developmental framework of interpersonal neural synchrony. *Developmental Review*, *78*(101234), 101234. doi: 10.1016/j.dr.2025.101234.
- Jean, A. D. L., Stack, D. M., and Arnold, S. (2014). Investigating maternal touch and infants' self-regulatory behaviours during a modified Face-to-Face Still-Face with touch procedure. *Infant and Child Development*, *23*(6), 557–574. doi: 10.1002/icd.1870.
- Jamieson, S. (2004). Identifying precursors of attachment in the Still-Face Paradigm.
- Jia, H. (2019). Connectivity Analysis. In *EEG Signal Processing and Feature Extraction* (pp. 241–266). Springer Singapore. doi: 10.1007/978-981-13-9113-2_12.

- Kanoga, S., Nakanishi, M., and Mitsukura, Y. (2016). Assessing the effects of voluntary and involuntary eyeblinks in independent components of electroencephalogram. *Neurocomputing*, *193*, 20–32. doi: 10.1016/j.neucom.2016.01.057.
- Kaye, K., and Fogel, A. (1980). The temporal structure of face-to-face communication between mothers and infants. *Developmental Psychology*, *16*(5), 454–464. doi: 10.1037/0012-1649.16.5.454.
- Kayhan, E., Matthes, D., Marriott Haresign, I., Bánki, A., Michel, C., Langeloh, M., Wass, S., and Hoehl, S. (2022). DEEP: A dual EEG pipeline for developmental hyperscanning studies. *Developmental Cognitive Neuroscience*, *54*, 101104. doi: 10.1016/j.dcn.2022.101104.
- Kay, M., Elkin, L., Higgins, J. J., and Wobbrock, J. O. (2025). mjskay/ARTool: ARTool 0.11.2. Zenodo. doi: 10.5281/ZENODO.594511.
- Killeen, L. A., and Teti, D. M. (2012). Mothers' frontal EEG asymmetry in response to infant emotion states and mother-infant emotional availability, emotional experience, and internalizing symptoms. *Development and Psychopathology*, *24*(1), 9–21. doi: 10.1017/S0954579411000629.
- Kivijärvi, M., Voeten, M. J. M., Niemelä, P., Räihä, H., Lertola, K., and Piha, J. (2001). Maternal sensitivity behavior and infant behavior in early interaction. *Infant Mental Health Journal*, *22*(6), 627–640. doi: 10.1002/imhj.1023.
- Koul, A., Ahmar, D., Iannetti, G. D., and Novembre, G. (2023). Spontaneous dyadic behavior predicts the emergence of interpersonal neural synchrony. *NeuroImage*, *277*, 120233. doi: 10.1016/j.neuroimage.2023.120233.
- Krzczykowski, J. E., Van Lieshout, R. J., and Schmidt, L. A. (2022). Transacting brains: testing an actor-partner model of frontal EEG activity in mother-infant dyads. *Development and Psychopathology*, *34*(3), 969–980. doi: 10.1017/S0954579420001558.
- Kumaravel, V. P., Farella, E., Parise, E., and Buiatti, M. (2022). NEAR: An artifact removal pipeline for human newborn EEG data. *Developmental Cognitive Neuroscience*, *54*, 101068. doi: 10.1016/j.dcn.2022.101068.
- Lachaux, J. P., Rodriguez, E., Martinerie, J., and Varela, F. J. (1999). Measuring phase synchrony in brain signals. *Human brain mapping*, *8*(4), 194–208. doi: 10.1002/(SICI)1097-0193(1999)8:4<194::AID-HBM4>3.0.CO;2-C.
- Laganaro, M., and Perret, C. (2011). Comparing electrophysiological correlates of word production in immediate and delayed naming through the analysis of word age of acquisition effects. *Brain Topography*, *24*(1), 19–29. doi: 10.1007/s10548-010-0162-x.
- Lamb, M. E., Morrison, D. C., and Malkin, C. M. (1987). The development of infant social expectations in face-to-face interaction: A longitudinal study. *Merrill-Palmer Quarterly (1982-)*, *241*-254. <https://www.jstor.org/stable/23086332>.
- Leach, S. C., Morales, S., Bowers, M. E., Buzzell, G. A., Debnath, R., Beall, D., and Fox, N. A. (2020). Adjusting ADJUST: Optimizing the ADJUST algorithm for pediatric data using geodesic nets. *Psychophysiology*, *57*(8), e13566. doi: 10.1111/psyp.13566.
- Leclère, C., Viaux, S., Avril, M., Achard, C., Chetouani, M., Missonnier, S., and Cohen, D. (2014). Why synchrony matters during mother-child interactions: a systematic review. *PloS One*, *9*(12), e113571. doi: 10.1371/journal.pone.0113571.

- Legerstee, M., and Markova, G. (2007). Intentions make a difference: infant responses to still-face and modified still-face conditions. *Infant Behavior and Development*, *30*(2), 232–250. doi: 10.1016/j.infbeh.2007.02.008.
- Leong, V., Byrne, E., Clackson, K., Georgieva, S., Lam, S., and Wass, S. (2017). Speaker gaze increases information coupling between infant and adult brains. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(50), 13290–13295. doi: 10.1073/pnas.1702493114.
- Leong, V., Noreika, V., Clackson, K., Georgieva, S., Brightman, L., Nutbrown, R., Fujita, S., Neale, D., and Wass, S. (2019). Mother-infant interpersonal neural connectivity predicts infants' social learning. Preprint.
- Levy, J., Goldstein, A., and Feldman, R. (2019). The neural development of empathy is sensitive to caregiving and early trauma. *Nature Communications*, *10*(1), 1905. doi: 10.1038/s41467-019-09927-y.
- Levy, J., Lankinen, K., Hakonen, M., and Feldman, R. (2021). The integration of social and neural synchrony: a case for ecologically valid research using MEG neuroimaging. *Social Cognitive and Affective Neuroscience*, *16*(1-2), 143–152. doi: 10.1093/scan/nsaa061.
- Lin, J.-F. L., Imada, T., Meltzoff, A. N., Hiraishi, H., Ikeda, T., Takahashi, T., Hasegawa, C., Yoshimura, Y., Kikuchi, M., Hirata, M., Minabe, Y., Asada, M., and Kuhl, P. K. (2023). Dual-MEG interbrain synchronization during turn-taking verbal interactions between mothers and children. *Cerebral Cortex (New York, N.Y.: 1991)*, *33*(7), 4116–4134. doi: 10.1093/cercor/bhac330.
- Makeig, S., Bell, A., Jung, T.-P., and Sejnowski, T. J. (1995). *Independent Component Analysis of Electroencephalographic Data. Advances in Neural Information Processing Systems*, *8*. https://proceedings.neurips.cc/paper_files/paper/1995/file/754dda4b1ba34c6fa89716b85d68532b-Paper.pdf.
- Malmberg, L.-E., Lewis, S., West, A., Murray, E., Sylva, K., and Stein, A. (2016). The influence of mothers' and fathers' sensitivity in the first year of life on children's cognitive outcomes at 18 and 36 months. *Child: Care, Health and Development*, *42*(1), 1–7. doi: 10.1111/cch.12294.
- Mantis, I., Stack, D. M., Ng, L., Serbin, L. A., and Schwartzman, A. E. (2014). Mutual touch during mother-infant face-to-face still-face interactions: influences of interaction period and infant birth status. *Infant Behavior and Development*, *37*(3), 258–267. doi: 10.1016/j.infbeh.2014.04.005.
- Markova, G., Nguyen, T., and Hoehl, S. (2019). Neurobehavioral Interpersonal Synchrony in Early Development: The Role of Interactional Rhythms. *Frontiers in Psychology*, *10*, 2078. doi: 10.3389/fpsyg.2019.02078.
- Marriott Haresign, I., Phillips, E. A. M., Whitehorn, M., Goupil, L., Noreika, V., Leong, V., and Wass, S. V. (2022). Measuring the temporal dynamics of inter-personal neural entrainment in continuous child-adult EEG hyperscanning data. *Developmental Cognitive Neuroscience*, *54*, 101093. doi: 10.1016/j.dcn.2022.101093.
- Marshall, P. J., Bar-Haim, Y., & Fox, N. A. (2002). Development of the EEG from 5 months to 4 years of age. *Clinical neurophysiology*, *113*(8), 1199-1208. doi: 10.1016/S1388-2457(02)00163-3.

- Mastergeorge, A. M., Paschall, K., Loeb, S. R., and Dixon, A. (2014). The Still-Face Paradigm and bidirectionality: associations with maternal sensitivity, self-esteem and infant emotional reactivity. *Infant Behavior and Development*, *37*(3), 387–397. doi: 10.1016/j.infbeh.2014.05.006.
- Mayes, L. C., and Carter, A. S. (1990). Emerging social regulatory capacities as seen in the still-face situation. *Child Development*, *61*(3), 754–763. doi: 10.1111/j.1467-8624.1990.tb02818.x.
- Mayes, L. C., Carter, A. S., Egger, H. L., and Pajer, K. A. (1991). Reflections on stillness: mothers' reactions to the still-face situation. *Journal of the American Academy of Child and Adolescent Psychiatry*, *30*(1), 22–28. doi: 10.1097/00004583-199101000-00004.
- McFarland, D. H., Fortin, A. J., and Polka, L. (2020). Physiological measures of mother-infant interactional synchrony. *Developmental Psychobiology*, *62*(1), 50–61. doi: 10.1002/dev.21913.
- Meltzoff, A. N., and Decety, J. (2004). What imitation tells us about social cognition: a rapprochement between developmental psychology and cognitive neuroscience. In *The Neuroscience of Social Interaction* (pp. 109–130). Oxford University PressOxford. doi: 10.1098/rstb.2002.1261.
- Mesman, J., Linting, M., Joosen, K. J., Bakermans-Kranenburg, M. J., and van Ijzendoorn, M. H. (2013). Robust patterns and individual variations: stability and predictors of infant behavior in the still-face paradigm. *Infant Behavior and Development*, *36*(4), 587–598. doi: 10.1016/j.infbeh.2013.06.004.
- Mesman, J., van IJzendoorn, M. H., and Bakermans-Kranenburg, M. J. (2009). The many faces of the Still-Face Paradigm: A review and meta-analysis. *Developmental Review: DR*, *29*(2), 120–162. doi: 10.1016/j.dr.2009.02.001
- Miller, J. G., Vrtička, P., Cui, X., Shrestha, S., Hosseini, S. M. H., Baker, J. M., and Reiss, A. L. (2019). Inter-brain synchrony in mother-child dyads during cooperation: An fNIRS hyperscanning study. *Neuropsychologia*, *124*, 117–124. doi: 10.1016/j.neuropsychologia.2018.12.021.
- Minagawa, Y., Hata, M., Yamamoto, E., Tsuzuki, D., and Morimoto, S. (2023). Inter-brain synchrony during mother-infant interactive parenting in 3-4-month-old infants with and without an elevated likelihood of autism spectrum disorder. *Cerebral Cortex (New York, N.Y. : 1991)*, *33*(24), 11609–11622. doi: 10.1093/cercor/bhad395.
- Mitsven, S. G., Prince, E. B., Messinger, D. S., Tenenbaum, E. J., Sheinkopf, S. J., Tronick, E. Z., Seifer, R., and Lester, B. M. (2022). Testing the mid-range model: Attachment in a high risk sample. *Developmental Science*, *25*(3), e13185. doi: 10.1111/desc.13185.
- Mognon, A., Jovicich, J., Bruzzone, L., and Buiatti, M. (2011). ADJUST: An automatic EEG artifact detector based on the joint use of spatial and temporal features. *Psychophysiology*, *48*(2), 229–240. doi: 10.1111/j.1469-8986.2010.01061.x.
- Montague, P. R., Berns, G. S., Cohen, J. D., McClure, S. M., Pagnoni, G., Dhamala, M., Wiest, M. C., Karpov, I., King, R. D., Apple, N., and Fisher, R. E. (2002). Hyperscanning: simultaneous fMRI during linked social interactions. *NeuroImage*, *16*(4), 1159–1164. doi:10.1006/nimg.2002.1150.
- Montirosso, R., Borgatti, R., Trojan, S., Zanini, R., and Tronick, E. (2010). A comparison of dyadic interactions and coping with still-face in healthy pre-term and full-term infants. *The British Journal of Developmental Psychology*, *28*(Pt 2), 347–368. doi: 10.1348/026151009X416429.

- Montirosso, R., and Provenzi, L. (2015). Implications of epigenetics and stress regulation on research and developmental care of preterm infants. *Journal of Obstetric, Gynecologic, and Neonatal Nursing : JOGNN*, *44*(2), 174–182. doi: 10.1111/1552-6909.12559.
- Moore, T., Arefadib, N., Deery, A., and West, S. (2017). The first thousand days: an evidence paper.
- Moszkowski, R. (2004). Infant Touching Behaviour During Mother-infant Face-to-face Interactions.
- Müller, M., Zietlow, A.-L., Tronick, E., and Reck, C. (2015). What Dyadic Reparation Is Meant to Do: An Association with Infant Cortisol Reactivity. *Psychopathology*, *48*(6), 386–399. doi: 10.1159/000439225.
- Murray, L., and Trevarthen, C. (1986). The infant's role in mother-infant communications. *Journal of Child Language*, *13*(1), 15–29. doi:10.1017/S0305000900000271
- Muthukumaraswamy, S. D. (2013). High-frequency brain activity and muscle artifacts in MEG/EEG: a review and recommendations. *Frontiers in Human Neuroscience*, *7*, 138. doi: 10.3389/fnhum.2013.00138.
- Neel, M. L., Jeanvoine, A., Kjeldsen, C. P., and Maitre, N. L. (2025). Mother-Infant Dyadic Neural Synchrony Measured Using EEG Hyperscanning and Validated Using Behavioral Measures. *Children (Basel, Switzerland)*, *12*(2). doi: 10.3390/children12020115.
- Nguyen, T., Abney, D. H., Salamander, D., Bertenthal, B. I., and Hoehl, S. (2021). Proximity and touch are associated with neural but not physiological synchrony in naturalistic mother-infant interactions. *NeuroImage*, *244*, 118599. doi: 10.1016/j.neuroimage.2021.118599.
- Nguyen, T., Schleihauf, H., Kayhan, E., Matthes, D., Vrtička, P., and Hoehl, S. (2020). The effects of interaction quality on neural synchrony during mother-child problem solving. *Cortex*, *124*, 235–249. doi: 10.1016/j.cortex.2019.11.020.
- Nguyen, T., Zimmer, L., and Hoehl, S. (2023). Your turn, my turn. Neural synchrony in mother-infant proto-conversation. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *378*(1875), 20210488. doi: 10.1098/rstb.2021.0488.
- Nolte, G., Bai, O., Wheaton, L., Mari, Z., Vorbach, S., and Hallett, M. (2004). Identifying true brain interaction from EEG data using the imaginary part of coherency. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, *115*(10), 2292–2307. doi: 10.1016/j.clinph.2004.04.029.
- Noreika, V., Georgieva, S., Wass, S., and Leong, V. (2020). 14 challenges and their solutions for conducting social neuroscience and longitudinal EEG research with infants. *Infant Behavior and Development*, *58*, 101393. doi: 10.1016/j.infbeh.2019.101393.
- Nunez, P. L., and Pilgreen, K. L. (1991). The spline-Laplacian in clinical neurophysiology: a method to improve EEG spatial resolution. *Journal of Clinical Neurophysiology: Official Publication of the American Electroencephalographic Society*, *8*(4), 397–413.
- O'Connor, T. G. (2003). Early experiences and psychological development: conceptual questions, empirical illustrations, and implications for intervention. *Development and Psychopathology*, *15*(3), 671–690. doi:10.1017/S0954579403000336.

- Oostenveld, R., Fries, P., Maris, E., and Schoffelen, J.-M. (2011). FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Computational Intelligence and Neuroscience*, 2011, 156869. doi: 10.1155/2011/156869.
- Oppenheimer, J. E., Measelle, J. R., Laurent, H. K., and Ablow, J. C. (2013). Mothers' vagal regulation during the Still-Face Paradigm: normative reactivity and impact of depression symptoms. *Infant Behavior and Development*, 36(2), 255–267. doi: 10.1016/j.infbeh.2013.01.003.
- Orehova, E. V., Stroganova, T. A., Posikera, I. N., and Elam, M. (2006). EEG theta rhythm in infants and preschool children. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, 117(5), 1047–1062. doi: 10.1016/j.clinph.2005.12.027.
- Palva, J. M., and Palva, S. (2018). Functional integration across oscillation frequencies by cross-frequency phase synchronization. *The European Journal of Neuroscience*, 48(7), 2399–2406. doi: 10.1111/ejn.13767.
- Pereda, E., Quiroga, R. Q., and Bhattacharya, J. (2005). Nonlinear multivariate analysis of neurophysiological signals. *Progress in Neurobiology*, 77(1-2), 1–37. doi: 10.1016/j.pneurobio.2005.10.003.
- Perone, S., Gartstein, M. A., and Anderson, A. J. (2020). Dynamics of frontal alpha asymmetry in mother-infant dyads: Insights from the Still Face Paradigm. *Infant Behavior and Development*, 61, 101500. doi: 10.1016/j.infbeh.2020.101500.
- Pezzulo, G., Donnarumma, F., and Dindo, H. (2013). Human sensorimotor communication: a theory of signaling in online social interactions. *PloS One*, 8(11), e79876. doi: 10.1371/journal.pone.0079876.
- Pezzulo, G., Donnarumma, F., Dindo, H., D'Ausilio, A., Konvalinka, I., and Castelfranchi, C. (2019). The body talks: Sensorimotor communication and its brain and kinematic signatures. *Physics of Life Reviews*, 28, 1–21. doi: 10.1016/j.plrev.2018.06.014.
- Phillips, E. A. M., Goupil, L., Whitehorn, M., Bruce-Gardyne, E., Csolsim, F. A., Marriott-Haresign, I., and Wass, S. V. (2023). Proactive or reactive? Neural oscillatory insight into the leader-follower dynamics of early infant-caregiver interaction. *Proceedings of the National Academy of Sciences of the United States of America*, 120(15), e2122481120. doi: 10.1073/pnas.2122481120.
- Pili, M.P., Capelli, E., Billeci, L., Riva, V., Cassa, M., Cremaschi, G., Procissi, G., Siri, E., Bachi, L., Merazzi, D., Ghirardello, S., and Provenzi, L. Follow the leader and shift: Changes in Parent-Infant Partial Directed Coherence after a Perturbation of Contingent Interaction. *Under review*.
- Pili, M. P., Provenzi, L., Billeci, L., Riva, V., Cassa, M., Siri, E., Procissi, G., Roberti, E., and Capelli, E. (2025). Exploring the impact of manual and automatic EEG pre-processing methods on interpersonal neural synchrony measures in parent-infant hyperscanning studies. *Journal of Neuroscience Methods*, 417, 110400. doi: 10.1016/j.jneumeth.2025.110400.
- Pion-Tonachini, L., Kreutz-Delgado, K., and Makeig, S. (2019). ICLLabel: An automated electroencephalographic independent component classifier, dataset, and website. *NeuroImage*, 198, 181–197. doi: 10.1016/j.neuroimage.2019.05.026.
- Portable EEG for Neuroscience Research. (n.d.). mBrainTrain. Retrieved from <https://mbraintrain.com/>.

- Posit team (2024). RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. Retrieved from <http://www.posit.co/>.
- Pratt, M., Goldstein, A., Levy, J., and Feldman, R. (2017). Maternal Depression Across the First Years of Life Impacts the Neural Basis of Empathy in Preadolescence. *Journal of the American Academy of Child and Adolescent Psychiatry*, *56*(1), 20–29.e3. doi: 10.1016/j.jaac.2016.10.012.
- Procissi, G., Capelli, E., Pili, M.P., Bachi, L., Riva, V., Billeci, L., Provenzi, L. (2025). Imaginary Coherence in Parent-Infant EEG Hyperscanning: Neural and Behavioral Synchrony in Social Interactions. *IEEE International Conference on Metrology for eXtended Reality, Artificial Intelligence and Neural Engineering (IEEE MetroXRINE 2025), Ancona, Italy. In press.*
- Provenzi, L., Borgatti, R., Menozzi, G., and Montiroso, R. (2015). A dynamic system analysis of dyadic flexibility and stability across the Face-to-Face Still-Face procedure: application of the State Space Grid. *Infant Behavior and Development*, *38*, 1–10. doi: 10.1016/j.infbeh.2014.10.001.
- Provenzi, L., Fumagalli, M., Bernasconi, F., Sirgiovanni, I., Morandi, F., Borgatti, R., and Montiroso, R. (2017). Very Preterm and Full-Term Infants' Response to Socio-Emotional Stress: The Role of Postnatal Maternal Bonding. *Infancy: The Official Journal of the International Society on Infant Studies*, *22*(5), 695–712. doi: 10.1111/inf.12175.
- Provenzi, L., Giorda, R., Fumagalli, M., Brambilla, M., Mosca, F., Borgatti, R., and Montiroso, R. (2019). Telomere length and salivary cortisol stress reactivity in very preterm infants. *Early Human Development*, *129*, 1–4. doi: 10.1016/j.earlhumdev.2018.12.002.
- Provenzi, L., Giusti, L., and Montiroso, R. (2016). Do infants exhibit significant cortisol reactivity to the Face-to-Face Still-Face paradigm? A narrative review and meta-analysis. *Developmental Review: DR*, *42*, 34–55. doi: 10.1016/j.dr.2016.07.001.
- Provenzi, L., Roberti, E., and Capelli, E. (2023). Envisioning translational hyperscanning: how applied neuroscience might improve family-centered care. *Social Cognitive and Affective Neuroscience*, *18*(1). doi: 10.1093/scan/nsac061.
- Provenzi, L., Scotto di Minico, G., Giusti, L., Guida, E., and Müller, M. (2018). Disentangling the Dyadic Dance: Theoretical, Methodological and Outcomes Systematic Review of Mother-Infant Dyadic Processes. *Frontiers in Psychology*, *9*, 348. doi: 10.3389/fpsyg.2018.00348.
- Redcay, E., and Schilbach, L. (2019). Using second-person neuroscience to elucidate the mechanisms of social interaction. *Nature Reviews. Neuroscience*, *20*(8), 495–505. doi: 10.1038/s41583-019-0179-4.
- Reindl, V., Gerloff, C., Scharke, W., and Konrad, K. (2018). Brain-to-brain synchrony in parent-child dyads and the relationship with emotion regulation revealed by fNIRS-based hyperscanning. *NeuroImage*, *178*, 493–502. doi: 10.1016/j.neuroimage.2018.05.060.
- Rosenblum, K. L., McDonough, S., Muzik, M., Miller, A., and Sameroff, A. (2002). Maternal representations of the infant: associations with infant response to the still face. *Child Development*, *73*(4), 999–1015. doi: 10.1111/1467-8624.00453.
- RStudio team (2023). RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. Retrieved from <http://www.rstudio.com/>.
- Salter Ainsworth, M. D., Blehar, M. C., Waters, E., and Wall, S. N. (2015). Patterns of Attachment: A Psychological Study of the Strange Situation. Psychology Press. doi: 10.4324/9780203758045.

- Santamaria, L., Noreika, V., Georgieva, S., Clackson, K., Wass, S., and Leong, V. (2020). Emotional valence modulates the topology of the parent-infant inter-brain network. *NeuroImage*, 207, 116341. doi: 10.1016/j.neuroimage.2019.116341.
- Schüler, C., Berger, P., and Grosse Wiesmann, C. (2024). Predicting Theory of Mind in children from the infant connectome. In bioRxiv. doi: 10.1101/2024.05.22.595346.
- Schwarz, G. (1978). Estimating the dimension of a model. *The annals of statistics*, 461-464.
- Shen, C. (2015). Analysis of detrended time-lagged cross-correlation between two nonstationary time series. *Physics Letters. A*, 379(7), 680–687. doi: 10.1016/j.physleta.2014.12.036.
- Sherilyn Adler, S., and Tiffany, F. (2024). Alteration of mother and infant behavior and heart rate during a still-face perturbation of face-to-face interaction. In *Emotion and Early Interaction* (pp. 57–82). Routledge.
- Smith, E. E., Reznik, S. J., Stewart, J. L., and Allen, J. J. B. (2017). Assessing and conceptualizing frontal EEG asymmetry: An updated primer on recording, processing, analyzing, and interpreting frontal alpha asymmetry. *International Journal of Psychophysiology: Official Journal of the International Organization of Psychophysiology*, 111, 98–114. doi: 10.1016/j.ijpsycho.2016.11.005.
- Srinivasan, R. (1999). Methods to improve the spatial resolution of EEG. *International journal of bioelectromagnetism*, 1(1), 102-111.
- Srinivasan, R., Nunez, P. L., Tucker, D. M., Silberstein, R. B., and Cadusch, P. J. (1996). Spatial sampling and filtering of EEG with spline laplacians to estimate cortical potentials. *Brain Topography*, 8(4), 355–366. doi: 10.1007/BF01186911.
- Stack, D. M., and LePage, D. E. (1996). Infants' sensitivity to manipulations of maternal touch during face-to-face interactions. *Social Development (Oxford, England)*, 5(1), 41–55. doi: 10.1111/j.1467-9507.1996.tb00071.x.
- Stack, D. M., and Muir, D. W. (1990). Tactile stimulation as a component of social interchange: New interpretations for the still-face effect. *The British Journal of Developmental Psychology*, 8(2), 131–145. doi: 10.1111/j.2044-835X.1990.tb00828.x.
- Stack, D. M., and Muir, D. W. (1992). Adult tactile stimulation during face-to-face interactions modulates five-month-Olds' affect and attention. *Child Development*, 63(6), 1509. doi: 10.1111/j.1467-8624.1992.tb01711.x.
- Stam, C. J., Nolte, G., and Daffertshofer, A. (2007). Phase lag index: assessment of functional connectivity from multi channel EEG and MEG with diminished bias from common sources. *Human Brain Mapping*, 28(11), 1178–1193. doi: 10.1002/hbm.20346.
- Stern, D. N. (2018). *The Interpersonal World of the Infant: A View from Psychoanalysis and Developmental Psychology*. Routledge. doi: 10.4324/9780429482137.
- Swain, J. E., Lorberbaum, J. P., Kose, S., and Strathearn, L. (2007). Brain basis of early parent-infant interactions: psychology, physiology, and in vivo functional neuroimaging studies. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, 48(3-4), 262–287. doi: 10.1111/j.1469-7610.2007.01731.x.
- Swider-Cios, E., Turk, E., Levy, J., Beeghly, M., Vroomen, J., and van den Heuvel, M. I. (2024). The association of maternal-infant interactive behavior, dyadic frontal alpha asymmetry, and

- maternal anxiety in a smartphone-adapted still face paradigm. *Developmental Cognitive Neuroscience*, 66, 101352. doi: 10.1016/j.dcn.2024.101352.
- Swingler, M. M., Perry, N. B., Calkins, S. D., and Bell, M. A. (2014). Maternal sensitivity and infant response to frustration: the moderating role of EEG asymmetry. *Infant Behavior and Development*, 37(4), 523–535. doi: 10.1016/j.infbeh.2014.06.010.
- The jamovi project (2024). jamovi (Version 2.5) [Computer Software]. Retrieved from <https://www.jamovi.org>.
- The Mathworks Inc. (2024). MATLAB version: 9.13.0 (R2024a). Natick, Massachusetts: The Mathworks Inc. Retrieved from <https://www.mathworks.com>.
- The Observer XT. (n.d.). Noldus; Noldus Information Technology. Retrieved August 20, 2025, from <https://noldus.com/observer-xt-human>.
- Toda, S., and Fogel, A. (1993). Infant response to the still-face situation at 3 and 6 months. *Developmental psychology*, 29(3), 532.
- Tognoli, E., Lagarde, J., DeGuzman, G. C., and Kelso, J. A. S. (2007). The phi complex as a neuromarker of human social coordination. *Proceedings of the National Academy of Sciences of the United States of America*, 104(19), 8190–8195. doi: 10.1073/pnas.0611453104.
- Toppi, J., Borghini, G., Petti, M., He, E. J., De Giusti, V., He, B., Astolfi, L., and Babiloni, F. (2016). Investigating Cooperative Behavior in Ecological Settings: An EEG Hyperscanning Study. *PloS One*, 11(4), e0154236. doi: 10.1371/journal.pone.0154236.
- Tronick, E., Als, H., Adamson, L., Wise, S., and Brazelton, T. B. (1978). The infant's response to entrapment between contradictory messages in face-to-face interaction. *Journal of the American Academy of Child Psychiatry*, 17(1), 1–13. doi: 10.1016/S0002-7138(09)62273-1.
- Tronick, E. (2005). Why is connection with others so critical? The formation of dyadic states of consciousness and the expansion of individuals' states of consciousness: coherence governed selection and the co-creation of meaning out of messy meaning making.
- Tronick, E. (2007). *The Neurobehavioral and Social-Emotional Development of Infants and Children*. New York, NY: WW Norton and Company.
- Tronick, E. Z. (2018). Emotions and emotional communication in infants. In *Parent-Infant Psychodynamics* (pp. 35–53). Routledge. doi: 10.4324/9780429478154-5.
- Tronick, E. Z., and Cohn, J. F. (1989). Infant-mother face-to-face interaction: age and gender differences in coordination and the occurrence of miscoordination. *Child Development*, 60(1), 85–92. doi: 10.2307/1131074.
- Tsolisou, D. (2023). EEG connectivity analysis in infants: A Beginner's Guide on Preprocessing and Processing Techniques. *Brain Science Advances*, 9(4), 242–274. doi: 10.26599/BSA.2023.9050025.
- Turk, E., Endevelt-Shapira, Y., Feldman, R., van den Heuvel, M. I., and Levy, J. (2022). Brains in Sync: Practical Guideline for Parent-Infant EEG During Natural Interaction. *Frontiers in Psychology*, 13, 833112. doi: 10.3389/fpsyg.2022.833112.

- Urbain, C., Vogan, V. M., Ye, A. X., Pang, E. W., Doesburg, S. M., and Taylor, M. J. (2016). Desynchronization of fronto-temporal networks during working memory processing in autism. *Human Brain Mapping, 37*(1), 153–164. doi: 10.1002/hbm.23021.
- van der Velde, B., Haartsen, R., and Kemner, C. (2019). Test-retest reliability of EEG network characteristics in infants. *Brain and Behavior, 9*(5), e01269. doi: 10.1002/brb3.1269.
- Vinck, M., Oostenveld, R., van Wingerden, M., Battaglia, F., and Pennartz, C. M. A. (2011). An improved index of phase-synchronization for electrophysiological data in the presence of volume-conduction, noise and sample-size bias. *NeuroImage, 55*(4), 1548–1565. doi: 10.1016/j.neuroimage.2011.01.055.
- Wass, S. V., Noreika, V., Georgieva, S., Clackson, K., Brightman, L., Nutbrown, R., Covarrubias, L. S., and Leong, V. (2018). Parental neural responsivity to infants' visual attention: How mature brains influence immature brains during social interaction. *PLoS Biology, 16*(12), e2006328. doi: 10.1371/journal.pbio.2006328.
- Weinberg, M. K., and Tronick, E. Z. (1996). Infant affective reactions to the resumption of maternal interaction after the still-face. *Child Development, 67*(3), 905–914. doi: 10.1111/j.1467-8624.1996.tb01772.x.
- Weinberg, M. K., Tronick, E. Z., Cohn, J. F., and Olson, K. L. (1999). Gender differences in emotional expressivity and self-regulation during early infancy. *Developmental Psychology, 35*(1), 175–188.
- Widmann, A., Schröger, E., and Maess, B. (2015). Digital filter design for electrophysiological data--a practical approach. *Journal of Neuroscience Methods, 250*, 34–46. doi: 10.1016/j.jneumeth.2014.08.002.
- Winkler, I., Debener, S., Müller, K.-R., and Tangermann, M. (2015). On the influence of high-pass filtering on ICA-based artifact reduction in EEG-ERP. *Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual International Conference, 2015*, 4101–4105. doi: 10.1109/EMBC.2015.7319296.
- Wobbrock, J. O., Findlater, L., Gergle, D., and Higgins, J. J. (2011, May 7). The aligned rank transform for nonparametric factorial analyses using only anova procedures. *Proceedings of the SIGCHI Conference on Human Factors in Computing Systems. CHI '11: CHI Conference on Human Factors in Computing Systems, Vancouver BC Canada*. doi: 10.1145/1978942.1978963.
- Xie, W., Mallin, B. M., and Richards, J. E. (2018). Development of infant sustained attention and its relation to EEG oscillations: an EEG and cortical source analysis study. *Developmental Science, 21*(3), e12562. doi: 10.1111/desc.12562.
- Zakharov, I., Adamovich, T., Tabueva, A., Ismatullina, V., and Malykh, S. (2021). The effect of density thresholding on the EEG network construction. *Journal of Physics. Conference Series, 1727*(1), 012009. doi: 10.1088/1742-6596/1727/1/012009
- Zamm, A., Loehr, J. D., Vesper, C., Konvalinka, I., Kappel, S. L., Heggli, O. A., Vuust, P., & Keller, P. E. (2024). A practical guide to EEG hyperscanning in joint action research: from motivation to implementation. *Social Cognitive and Affective Neuroscience, 19*(1). doi: 10.1093/scan/nsae026.

Zimmermann, M., Schultz-Nielsen, K., Dumas, G., & Konvalinka, I. (2024). Arbitrary methodological decisions skew inter-brain synchronization estimates in hyperscanning-EEG studies. *Imaging Neuroscience*, 2, 1-19. doi: 10.1162/imag_a_00350.

APPENDIX

The appendix contains the original articles from which Chapter 2, Chapter 3 and Chapter 4 were adapted.

Chapter 2

Billeci, L., Riva, V., Capelli, E., Grumi, S., Pili, M.P., Cassa, M., Siri, E., Roberti, E., Borgatti, R., and Provenzi, L. (2024). 2-Brain Regulation for Improved Neuroprotection during Early Development (2-BRAINED): a translational hyperscanning research project. *Frontiers in Psychology*, 15, 1516616. doi: 10.3389/fpsyg.2024.1516616.

Appendix contains the original article.

Chapter 3

Pili, M. P., Provenzi, L., Billeci, L., Riva, V., Cassa, M., Siri, E., Procissi, G., Roberti, E., and Capelli, E. (2025). Exploring the impact of manual and automatic EEG pre-processing methods on interpersonal neural synchrony measures in parent-infant hyperscanning studies. *Journal of Neuroscience Methods*, 417, 110400. doi: 10.1016/j.jneumeth.2025.110400.

Appendix contains the original article.

Chapter 4

Pili, M.P., Capelli, E., Billeci, L., Riva, V., Cassa, M., Cremaschi, G., Procissi, G., Siri, E., Bachi, L., Merazzi, D., Ghirardello, S., and Provenzi, L. Follow the leader and shift: Changes in Parent-Infant Partial Directed Coherence after a Perturbation of Contingent Interaction. *Under review*.

Appendix contains the manuscript sent to NeuroImage on October 9th, 2025 and the Supplementary Materials.



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2-Brain Regulation for Improved Neuroprotection during Early Development (2-BRAINED): a translational hyperscanning research project

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Introduction: Very preterm (VPT) birth is a major risk condition for child development and parental wellbeing, mainly due to multiple sources of stress (e.g., separation and pain exposure) during the neonatal intensive care unit (NICU) stay. Early video-feedback (VF) interventions proved effective in promoting VOT infants' development and parental wellbeing. Electroencephalography (EEG) hyperscanning allows the assessment of brain-to-brain co-regulation during live interaction between infants and parents, offering promising insights into the mechanisms behind the interactive benefits of early VF interventions.

Goals: This study aimed to compare indices of brain-to-brain co-regulation between dyads of full-term (FT) and VPT infants interacting with their mothers and investigate the effect of an early post-discharge VF intervention on the brain-to-brain co-regulation indices of VPT dyads.

Methods and analysis: VPT and FT dyads will be enrolled at birth, and the former will be randomly allocated to one of two arms: VF intervention or care as usual. Short-term effectiveness will be assessed through ratings of mother–infant interaction videotaped before and after the VF intervention or care as usual. Mothers of VPT and FT infants will report on their mental state, parenting stress and bonding, and infant temperament and sensory profile at 3 and 6 months (corrected age, CA). At 9 months CA, all dyads will participate in a lab-based EEG-hyperscanning paradigm to assess brain-to-brain co-regulation through phase-locking value (PLV) and other explorative indices.

Ethics and dissemination: This study was funded by the Italian Ministry of Health and received approval by the Ethics Committee of Pavia (Italy) and participating hospitals. Research findings will be reported in scientific publications, presented at international conferences, and disseminated to the general public.

Study registration number: GR-2021-12375213 (Italian Ministry of Health registry).

KEYWORDS

EEG, hyperscanning, infant, parent, preterm, synchrony, video-feedback

Introduction

Very preterm (VPT) birth is a major challenge for healthcare systems worldwide (Beam et al., 2020), representing the leading cause of long-lasting chronic diseases in childhood and child mortality (Ohuma et al., 2023). While VPT infants are exposed to heightened medical risk and several stress sources from the neonatal intensive care unit (NICU) environment (Cong et al., 2017; Provenzi et al., 2018), their parents also may face critical levels of psychological distress leading to increased risk for depression, anxiety, and parenting stress in the postpartum period (Caporali et al., 2020).

Notably, biobehavioral dysregulation patterns have been observed in dyads of VPT infants and their caregivers in the first months of life (Jean and Stack, 2012; Montiroso et al., 2010; Neugebauer et al., 2022; Provenzi et al., 2019), suggesting that VPT birth and NICU-related stress may affect child development and parental adjustment by altering critical processes of dyadic co-regulation during the first 1,000 days (Feldman, 2006; Linnér and Almgren, 2020). Early interventions that promote parent–infant closeness may help foster the establishment of similar psychobiological co-regulatory processes (Ionio et al., 2021; Lordier et al., 2019; Mörelus et al., 2015; Welch and Ludwig, 2017), providing buffering and protective benefits for both child development and parental wellbeing (Burke, 2018; He et al., 2021; Thomson et al., 2020).

Video-feedback (VF) interventions are well-validated parenting support programs that focus on parent–infant closeness, promote parental sensitivity, facilitate co-regulatory processes, and provide neuroprotective effects for child development (Ballidin et al., 2018; Poslawsky et al., 2015; Provenzi et al., 2020; Tryphonopoulos and Letourneau, 2020). VF interventions include a diverse range of procedures and methodologies aimed at promoting positive parenting. They capitalize on allowing parents to observe themselves and their interactions with their infant “from the outside,” thereby facilitating mentalization and reflective functions (Leyton et al., 2019; Riva Crugnola et al., 2021). Although different theoretical and methodological VF approaches have been described (Provenzi et al., 2020), previous research highlighted benefits for child development and the quality of parent–child interaction in different clinical contexts and populations, including preterm infants and their caregivers (Barlow et al., 2016; Hoffenkamp et al., 2015). More recently, a clinical trial by Pisoni et al. (2021) highlighted long-term improvement in the developmental quotient scores of 24-month-old VPT infants of age following a VF intervention, adding to the evidence that remote video consultation may be an effective home care approach (Hägi-Pedersen et al., 2021; Suir et al., 2022).

Hyperscanning is a relatively recent approach to the study of brain-to-brain co-regulation in live interactive partners using different electrophysiological and neuroimaging techniques (Bi et al., 2023; Nguyen et al., 2020). By simultaneously recording multiple brains' activity, hyperscanning allows the acquisition of neurophysiological measures of human dyadic or group-based neurophysiological coordination (Czeszumski et al., 2020). Among the different available techniques, EEG offers special advantages when the hyperscanning paradigm is applied to pediatric and even newborn populations. Wireless EEG devices are relatively non-invasive and allow for freedom of movement, making them ideal for studying mother–infant interactions in both laboratory and ecological settings during the first months of life. By using EEG-hyperscanning paradigms, Leong et al.

(2017) highlighted how gaze direction during face-to-face interactions between adults and 8-month-old infants affects patterns of dyadic neural connectivity. Similarly, different patterns of theta power fluctuations were observed when 12-month-old infants play solo in the presence of the caregiver or when they actively interact together (Wass et al., 2018). More recently, the phase-locking values (PLVs) indicating the strength of brain-to-brain co-regulation in theta and alpha frequency bands were computed during 7-month-old infants' interaction with the caregiver compared to an adult stranger (Endevelt-Shapira et al., 2021). The study reported greater inter-brain attunement when infants were interacting with the mother, despite the addition of maternal chemo-signals in the setting of infant–stranger interaction attenuated the significant difference.

As the field of parent–infant hyperscanning research is rapidly growing, the accumulating knowledge is contributing to a pivotal epistemic and theoretical shift in developmental neurosciences from a mono-personal account to a strongly relational perspective (Dumas, 2011). The application of hyperscanning paradigms to the study of parent–infant brain-to-brain co-regulation in at-risk and clinical pediatric populations holds promises to acquire innovative data on the mechanisms by which the early caregiving environment fosters and promotes child neurodevelopment (Provenzi et al., 2023). Moreover, as specific indices of brain-to-brain co-regulation become validated in typical development, they may also be embedded into a novel set of neurobehavioral markers to assess the benefit of early interventions. Such *translational hyperscanning* vision has been recently framed in the affective neuroscience literature (Provenzi et al., 2023), yet there is a lack of research on the brain-to-brain co-regulation of VPT infants and their parents and on the potentially beneficial neuroprotective effects of early parenting interventions in this population.

Study AIMS

General and specific aims

The *2-Brain Regulation to Achieve Improved Neuroprotection during Early Development* (2-BRAINED) research project is funded by the Italian Ministry of Health under the Ricerca Finalizzata 2021 program (research line: Giovani Ricercatori, project code: GR-2021-12375213). It is aimed to assess brain-to-brain co-regulation patterns in dyads of VPT infants and their caregivers and further explore how an early VF intervention may facilitate specific inter-brain regulatory indices.

The first specific aim (Aim 1) is to assess the presence of statistically significant differences in a set of brain-to-brain co-regulation indices—primarily, PLV measure—between dyads of caregivers and VPT compared to FT infants. Previous research gave evidence of specific markers of lower co-regulation in behavioral synchrony (Montiroso et al., 2010), physiological coupling (Feldman and Eidelman, 2007; Porges et al., 2019), and neuroendocrine attunement (Provenzi et al., 2019) in dyads of VPT infants during the first year of life. Consistently, we hypothesized that VPT infants and their caregivers would show less strong brain-to-brain co-regulation indices compared to dyads of FT counterparts at 9 months (corrected age for prematurity, CA).

The second specific aim (Aim 2) is to investigate the effect of an early VF intervention for parents of VPT infants on the selected

indices of brain-to-brain co-regulation at 9 months CA. By comparing EEG-hyperscanning-derived indices of inter-brain coupling between dyads of VPT infants exposed to the VF intervention and dyads exposed to care as usual during the 3 months following NICU discharge, we hypothesize to describe greater brain-to-brain co-regulation in the former group.

Additional exploratory aims

The longitudinal nature of this study and the possibility to collect a multi-layer set of data for what pertains to the neurobehavioral development of FT and VPT infants as well as the parenting environment during the first year of life allow us to set the stage for a number of exploratory analyses that will further guide future spin-off studies stemming from the 2-BRAINED research project. In this study, we highlight five main exploratory aims that appear relevant for future translational research in the field of affective neuroscience and developmental psychobiology.

First, the availability of behavioral and EEG physiological data from the 9-month interactive procedure (see below, *Study design and procedures*) will allow for the exploration of patterns of bio-neurophysiological coupling within dyads. Previous research produced limited evidence for the presence of correlations between specific interactive behaviors and EEG signaling during interactive tasks (Liu et al., 2018). Similarly, in VPT infants and their caregivers, the presence of a matched coupling or overlapping regulatory profiles between interactive behaviors and neuroendocrine or physiological oscillations is debated (Provenzi et al., 2019). This study will provide a suitable data setup to further explore the presence of significant coupling between interactive behaviors and neurophysiological brain activity in typically developing and at-risk pediatric populations.

Second, it will be possible to explore how brain-to-brain co-regulation in typically developing FT infants and their caregivers is affected by different dimensions that characterize infants' development (e.g., sensory profile and temperament) and the caregiving environment (e.g., affective symptoms, parenting stress, and parent–infant bonding). It is well known that different behavioral indices of caregiver–infant co-regulation (e.g., matching, synchrony, and dyadic repair) (Provenzi et al., 2018) are shaped by individual characteristics and contributions by parent and infant behavior. For instance, Fuertes et al. (2006) have suggested that infant temperament may play a critical role in the emergence of early attachment patterns and co-regulation of socio-emotional stress in full-term infants. Similarly, maternal affective symptoms may result in different dyadic organizations of critical behaviors signaling reciprocal attention and socio-emotional availability, such as gaze direction (Lotzin et al., 2015) and emotional cues (Bigelow et al., 2018).

Third, the role of sensory profile and environmental sensitivity to sensory inputs is recognized as an important contributor to child socio-emotional stress regulation (Greven et al., 2019; Lionetti et al., 2018). Previous research has highlighted how infants with diverse sensory profiles (e.g., sensation seekers or passive encoders) may exhibit differences in their resting state EEG activity (Pierce et al., 2021). Additionally, sensory reactivity in FT and VPT infants at 12 months has been found to be associated with later behavioral problems in toddlerhood (Maitre et al., 2020). The role of infants' sensory profile in setting the stage for different gradients of

brain-to-brain co-regulation is yet to be explored, and such investigation may shed light on genetic-informed individual differences in the early establishment of parent–infant relationship.

Fourth, consistent with the previous exploratory goal, it can be speculated that VPT infants—due to early adverse sensory stimulations during the NICU stay (Aita et al., 2013; Pineda et al., 2019)—may exhibit specifically altered profiles of sensory regulation compared to FT counterparts. Niutanen et al. (2020) recently conducted a review of the literature highlighting how VPT infants may exhibit abnormal regulation of sensory inputs with consequences for sensory-motor integration and stress regulation. As the sensory regulation profile of VPT infants may be partially learned from attempts to adapt to the NICU, the present study may also help identify how early alterations in the sensory environment influence the emergence of precocious forms of brain-to-brain co-regulation with the caregiver.

Finally, by collecting quantitative data on the parents' experience of the NICU hospitalization—including both psychological stress and perceived support from the staff—it will be possible to estimate how caring for parents' wellbeing during the NICU stay may promote later electrophysiological caregiver–infant attunement. Previous research has highlighted that mothers of VPT infants may exhibit lower sensitivity to their infants' facial and bodily cues (Butti et al., 2018). However, their brain reactivity to emotional pictures of their own VPT infants appears heightened compared to that of FT infants' mothers (Montirosso et al., 2017). These preliminary findings suggest that the brains of VPT infants' caregivers may process interactive-salient stimuli differently, potentially influenced by the stressful experience of NICU hospitalization and early parent–infant separation. The present study will allow us to study how the brain activity of VPT infants' mothers processes relevant social cues during real-life face-to-face interactions, further contributing to understanding how caregivers' brain adapts to preterm birth and hospitalization. In this context, NICU-related stress and perceived support from staff could be considered potential moderators of the caregivers' EEG activity when interacting with their VPT infant—with relevant consequences for the observed brain-to-brain co-regulation.

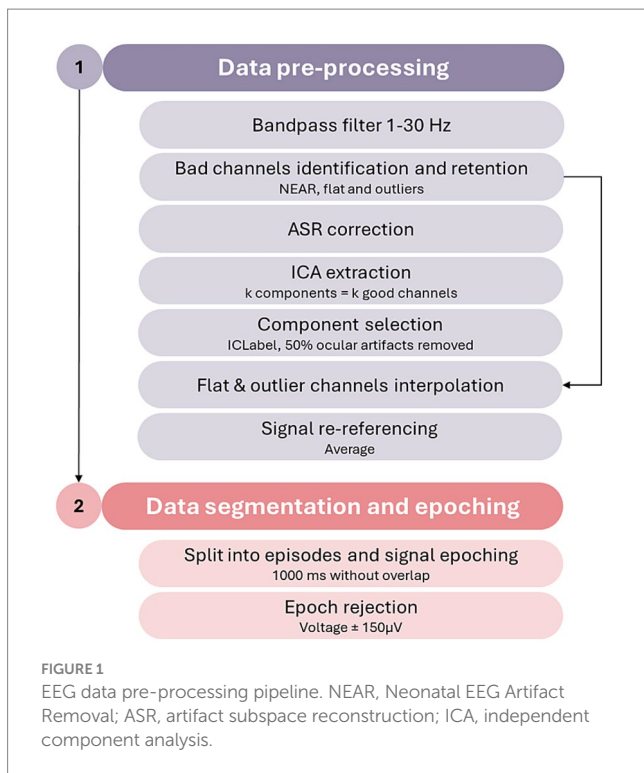
Methods and procedures

Study design and procedures

The 2-BRAINED project is a randomized-controlled trial (RCT) with three arms. The first arm includes VPT infants and their caregivers randomly allocated to the intervention arm (VPT-VF). The second arm includes VPT infants and their caregivers randomly allocated to the care as usual arm (VPT-CU). This arm will act as a control group matched to preterm conditions of VPT-VF. The third arm includes FT infants and their caregivers and will act as an additional control group unmatched by preterm conditions. Both VPT-CU and FT arms will receive no VF intervention.

Population, enrollment, and arm allocation

VPT and FT infants will be enrolled at birth by contacting their caregivers within the first 48 h after delivery. Informed consent will be obtained. VPT infants will be considered eligible in the presence of the following conditions, as reported in medical charts: gestational age below 35 weeks, absence of major brain lesions as documented by



cerebral ultra-sound, no neuro-sensory deficits including retinopathy of prematurity (ROP) equal or above stage 2, absence of genetic syndrome, or malformations involving the central nervous system. FT infants will be considered eligible if they meet the following conditions: a gestational age of 37 weeks or more, are healthy, and show no evident signs of neurodevelopmental risk or morbidities. For both groups, exclusion criteria will include single-parent families, parental age under 18 years, lack of Italian language mastery, and the presence of documented psychiatric disorders.

Study timeline

The 2-BRAINED study features five data collection waves (see Figure 1). The VF intervention is delivered after wave T1 (NICU discharge) and before wave T2 (3 months CA) to subjects allocated to the VPT-VF arm. The EEG-hyperscanning task will occur for all subjects at wave T4 (9 months CA) and will feature the videotaping of mother–infant interaction according to a modified Face-to-Face Still-Face (FFSF) procedure (Tronick et al., 1978) and the simultaneous EEG data collection from both the interactive partner. At each wave, parents will receive questionnaires by email using REDCap.¹

VF intervention

Remote videotaping

Before (T1) and after (T2) the VF intervention sessions for participants allocated to VPT-VF—and at the same timepoints for participants allocated to VPT-CU—a 15-min mother–infant interaction will be videotaped remotely. Before videotaping,

mothers will be asked to position the webcam or smartphone to have the widest possible view of the play area and see the entire body of both the mother and the infant. The interaction paradigm includes 10-min unrestrained face-to-face play followed by a 6-min FFSF procedure (Tronick et al., 1978) as described here: During the 2-min Play episode, mothers will be asked to play with the infant as they usually do (e.g., the infant can stay in an infant seat or on a carpet); during the 2-min Still-Face episode, mothers will be asked to interrupt any communication and maintain a still, poker face while keeping eye-contact with their infant; unconstrained interaction will be resumed during the 2-min Reunion episode.

Intervention details

The remote VF intervention has been adapted according to previous research from our group (Grumi et al., 2021). It comprises six weekly 1-h sessions organized in two subsequent phases: four *sharing the focus* sessions and two *integration* sessions. *Sharing the focus* sessions are dedicated to the discussion between the psychologist and the mother of specific themes related to parenting and parent–infant interaction: physical stimulation, responsiveness, teaching, and parenting experience (see Table 1). During these sessions, a purposively trained psychologist invites the mother to jointly review and discuss brief clips obtained from the pre-intervention videotaped interaction, usually starting from potential curiosity, comments, or requests from the mother herself. The goal of the *sharing of the focus* sessions is to develop insights about the infants' behavioral signals, how to respond contingently and appropriately, how to promote emotion regulation, and how to sustain cognitive and behavioral achievements. In the subsequent two *integration* sessions, the mother plays with the infant while the psychologist provides guidance based on insights co-developed during the previous four sessions. The goal is to promote a pragmatic translation of the insights into interactive skills.

FFSF procedure

At 9 months (CA for VPT participants), mothers and infants will take part in a FFSF procedure in the laboratory. The FFSF will include three episodes: During the Play episode (2 min), mothers and infants will interact face-to-face avoiding the use of toys and pacifier; during the Still-Face episode (1 min), mothers will be asked to interrupt any communication toward the infant, to maintain a still, poker face, while maintaining eye-contact; and during the Reunion episode (2 min), unconstrained interaction will be resumed. The procedure has been previously adopted to assess biobehavioral dimensions (Provenzi et al., 2019; Provenzi et al., 2017) and physiological underpinnings (Montirosso et al., 2010; Mantis et al., 2014) of socio-emotional stress regulation in VPT infants. The entire procedure will be videotaped for the offline coding of specific maternal and infant interactive behaviors (see *Measures* for details).

Neurophysiological procedures

EEG data acquisition will occur at 500 Hz sampling frequency during the 9-month FFSF procedure employing the Smarting Pro (mBrainTrain, Belgrade, Serbia) system equipped with two 32-channel EEG caps featuring wireless Bluetooth connection between the amplifiers and the mBrainTrain Streamer software installed on two separate laptops. The laptops receiving data will be linked to each other via a network cable to ensure synced data collection. The use of wireless EEG caps will allow greater flexibility and comfort for participating dyads.

¹ <https://www.project-redcap.org/>

TABLE 1 Description of the thematic focus of the four *sharing the focus* sessions of the video-feedback (VF) intervention.

Thematic cluster	Key topic	Goal
Sensory stimulation and regulation	Sensory channels	Highlighting infant preference or avoidance of specific sensory channels and stimuli.
	Intensity of stimulation	Regulating intensity of physical stimulation and understanding infant sensory thresholds.
	Affective social touch	Facilitating parental affective touch to promote infant state regulation, postural stability, and attention orientation.
Nurturing and sensitive caregiving	Parental sensitivity	Promoting parental perception, interpretation, and appropriate responsiveness to infant communicative signals.
	Sense of agency	Detecting and supporting the infant's interactive initiatives (e.g., vocalizations and attention orienting).
	Exploration and safety	Supporting the infant exploration of the environment and building safety and trust in parental secure base.
	Rhythm and reparation	Facilitating the emergence of a proto-conversational rhythm in the dyad and supporting reparative actions of interactive perturbations.
Cognitive sensitivity and scaffolding	Attention skills	Supporting and scaffolding infant sustained and focused attention to the physical and social environment.
	Modeling and guidance	Providing a model to foster observational learning and the functional use of tools and toys.
	Proximal development zone	Improving caregiver awareness of the cognitive abilities of the infant to make appropriate play proposals and support infant emerging abilities.
Parenting experience and mental state	Mind-mindedness	Improving awareness about parental representations of the infant mind and keeping high levels of curiosity about infant behaviors.
	Self-care and self-regulation	Highlighting the importance of parental psychological wellbeing and reflective functions; promoting parental psychological self-care and compassion.
	Self-efficacy	Strengthening the caregiver's sense of efficacy as a parent and nourishing trust in the parent's own experience and mental representations of the infant.

Upon arrival, the infant will be familiarized with the setting: A play mat and toys will be available to aid in acclimatization to the environment. The researchers will debrief parents with a comprehensive explanation of the study's aims and procedures. Cap sizes will be selected to fit participants' head circumference. The caps fitting process will commence with the caregiver to ensure greater infant comfort and familiarity with the equipment. The conductive gel will be applied to optimize signal conductivity and minimize artifacts.

Measures

Demographic (e.g., parental age, parental job, and parental educational level), neonatal (e.g., gestational age, birth weight, and Apgar score), and clinical variables (e.g., NICU length of stay and minor morbidities) will be obtained from medical charts. Parent-report questionnaires are summarized and described in Table 2. As for behavioral coding purposes, the videotapes obtained from two cameras during the lab FFSF procedure will be edited offline using Movavi Video Suite 2020 software and a single synced video showing both frontal views of the caregiver and the infant's face, hands, and torso will be produced. Videos will be micro-analytically coded for infants' and caregivers' target interactive behaviors according to an adaptation of the Parent-Infant Coding Scheme (PICS, Version 4.0; Brambilla et al., 2023) as reported in Table 2. PICS codes will be computed as a percentage of time for each FFSF episode (Table 3).

Plan of EEG data elaboration

Pre-processing pipeline

Dyadic EEG data will be pre-processed with a fully automated pipeline built using the MATLAB-based (The MathWorks Inc., 2024) interacting toolbox EEGLAB (Delorme and Makeig, 2004). A brief description of the main pre-processing steps is available in Figure 2.

The parent and infant signals will be pre-processed separately with the same steps and parameters for both. First, data filtering will be performed with the application of a bandpass 1–30 Hz filter as the planned analyses (see below) will be conducted on the lower (theta and alpha) frequency bands. Subsequently, flat and outlier channels will be detected using the Neonatal EEG Artifact Removal (NEAR) plugin (Kumaravel et al., 2022) and retained (i.e., put in a separate temporary matrix) for later interpolation. Dyads in which at least one of the members displays a signal with more than 15% ($N > 5$) of flagged channels will be excluded from further analyses. The EEG signal from all non-flagged channels will undergo noise correction through the artifact subspace reconstruction (ASR; Chang et al., 2020), with burst criterion (k) set at 10; subsequently, analysis of the components of the signal will be performed through the independent component analysis (ICA; runica function with default settings), producing as many components as the number of good channels. The resulting components will be then classified through the ICLabel (Pion-Tonachini et al., 2019) plugin. Every component flagged as having a 50% or more probability of being an ocular artifact will be rejected. At this stage, the matrix containing the flat and outlier channels will be re-merged with the EEG matrix, and the bad channels

TABLE 2 Details of questionnaires included in the study.

Construct	Questionnaire	Reference	Item N	Likert scale	Description	Study wave(s)
Parental NICU-related stress	Parental Stressor Scale—NICU (PSS-NICU)	Miles et al. (1993)	46	5-point	Three main factor scores representing stress related to infants' appearance, environmental sights and sounds, and parental role alteration	T1 (only VPT)
Sensory profile	Sensory Profile-2 (SP-2)	Dunn (2014)	54	5-point	The infant version (0–6 months) identifies 5 sensory patterns. The toddler version (7–35 months) identifies four sensory patterns.	T2, T3, T4
Anxiety symptoms	State–Trait Anxiety Inventory (STAI-Y)	Spielberger et al. (1983)	40	4-point	One trait score representing a tendency to feel anxiety and one state score representing the present levels of anxiety	T2, T4
Depression symptoms	Beck Depression Inventory (BDI-II)	Beck et al. (1996)	21	4-point	Global score representing a quantitative appreciation of the severity of symptoms of depression	T2, T4
Parenting stress	Parenting Stress Index—Short Form (PSI-SF)	Abidin et al. (2006)	36	5-point	Three subscale scores addressing parental distress, parent–child dysfunctional interaction, and stress related to difficult child behavior. A global score is also obtained.	T2, T3, T4
Temperament	Infant Behavior Questionnaire-Revised (IBQ-R) very short form	Gartstein and Rothbart (2003)	37	7-point	Three subscale factor scores addressing negative affectivity, surgency, and regulatory capacity.	T2, T3, T4

VPT, very preterm; NICU, neonatal intensive care unit.

will be interpolated through spherical interpolation using the pre-processed signal. The signal will be then re-referenced to the average signal of the channels and split into three different sets containing each phase of the experimental procedure (Play, Still-Face, and Reunion): Each of these sets will be subsequently segmented into 1,000 ms epochs avoiding overlaps. Bad data segments containing residual artifacts in each of the three phases will be identified. All segments in which at least one of the target channels used for estimates of dyadic co-regulation (see below) displays a voltage exceeding $\pm 150 \mu\text{V}$ (Debnath et al., 2020) will be marked as rejected. The rejected epochs in the infant's and parent's signals will be merged to obtain the final pool of rejected epochs for the dyad. This ensures that all the rejected epochs for one interactive member of the dyad will be similarly mirrored for the other partner. Dyadic data will undergo

further analyses if their signal contains at least 30 good epochs in both the Play and Reunion phases; if this criterion is not met, their signal will undergo manual epoch rejection performed by an expert EEG coder. In case after the manual epoch rejection, the dyad will result having less than 30 good merged epochs in at least one of the FFSEF episodes, the signal will be excluded from further analyses.

Estimating indices of inter-brain co-regulation

Several inter-brain synchronization indices have been proposed so far to estimate the coupling between two brains (Czeszumski et al., 2020). Since there is still debate on the appropriateness of each inter-brain synchronization measure, we plan to compute and compare several indices (see Table 4). To further check for spurious findings and gather stronger evidence that the obtained co-regulation

TABLE 3 Selection of codes from the Parent-Infant Coding Scheme (PICS, Version 4.0).

Variable	Levels	Description
A. Both interactive partners		
Emotional state	Negative	Clear display of negative emotionality (e.g., eyes, mouth, general movements of the face or the body, and other vocal or non-vocal signals) including fussing and crying.
	Neutral	No clear display of negative or positive emotionality.
	Positive	Clear display of positive emotionality (e.g., eyes, mouth, general movements of the face or the body, and other vocal or non-vocal signals) including smiles and laughs.
Gaze direction	Face-directed	Attention focus is on the interactive partner's face
	Object-directed	Attention focus is on the interactive partner body (e.g., hands and torso) or other objects.
	Avoiding	The subject is actively avoiding eye contact as displayed by head and body movements/posture.
Approach/withdrawal	Withdrawal	Evident leaning backward and/or turning the head away to avoid interaction
	Neutral	No evident backward or forward movements.
	Approach	Evident leaning forward and/or reaching forward to engage in interactive behaviors.
B. Parental-specific codes		
Vocal inputs	No voice	No vocal productions.
	Negative	Vocal comments that convey explicit critique or rejection of infants' behaviors or state.
	Pragmatic	Vocal comments that are finalized to modify or instruct the interactive partner's cognitive state, such as requests, attention-getting, and explanations.
	Social	Vocal comments that convey playful and social engagement such as singing, laughing, and playing nursery rhymes.
	Nurturing	Vocal comments that express appreciation or acceptance of infants' behaviors or state or are finalized to soothe infants' stress. These also include mind-related comments (e.g., "you think" and "you want") and mirroring of infants' communicative bids.
Tactile inputs	No touch	No tactile stimulations.
	Negative	Tactile stimulations that clearly appear intrusive and/or provoke or increase a negative emotionality state in the interactive partner.
	Pragmatic	Tactile stimulations that are finalized to modify or instruct the interactive partner postures or movements in the environment, such as holding, shadowing, and attention-getting.
	Social	Tactile stimulations that convey playful and social engagement such as tickling, squeezing, and any other appropriate entertaining tactile stimulations that are fast-paced, dynamic, repetitive, and/or characterized by quick cinematic features.
	Nurturing	Tactile stimulations that are finalized to soothe or regulate the behavioral state of the interactive partners. These include stroking, kissing, massaging, and any other appropriate tactile stimulations with clear regulatory functions and conveying a sense of affective closeness.

The complete coding manual is available upon request to the corresponding author.

estimations are not artifact production, we will compare the synchronization indices obtained from the real dyads to surrogate data generated by randomly pairing mothers and infants from different dyads.

The computation of synchronization measures will preferentially occur considering homologous channels for the sake of interpretability and computational costs. However, as an exploratory analysis, we will also compute synchronization between non-homologous channels because we can hypothesize that synchronization tasks between mother and infant can involve different brain areas in the two actors (Endevelt-Shapira et al., 2021).

Regarding the frequency bands on which the synchronization measures will be computed, we will mainly consider alpha and theta. Indeed, these frequencies have been found to be involved in parent-infant social tasks, with theta fluctuations linked to changes in shared

attention during joint play of parent and infant (Wass et al., 2018), enhancement of alpha and theta power linked to changes in directed gaze (Leong et al., 2017), and fluctuations in alpha band linked to changes in emotional states of mother and child (Santamaria et al., 2020).

Moreover, since we are interested in the dynamic evolution of brain-to-brain synchronization, we plan to evaluate the trend of each synchronization index over time (i.e., over the epochs). In particular, we are interested in the change between an asynchronous to a synchronous state, which is defined as reparation. Reparation is a dyadic process in which unmatched dyadic states are transformed into matched dyadic states. We will compute the rate and the latency of reparation considering the EEG synchronization indices as previously performed in synchronized behaviors assessment (Provenzi et al., 2015).

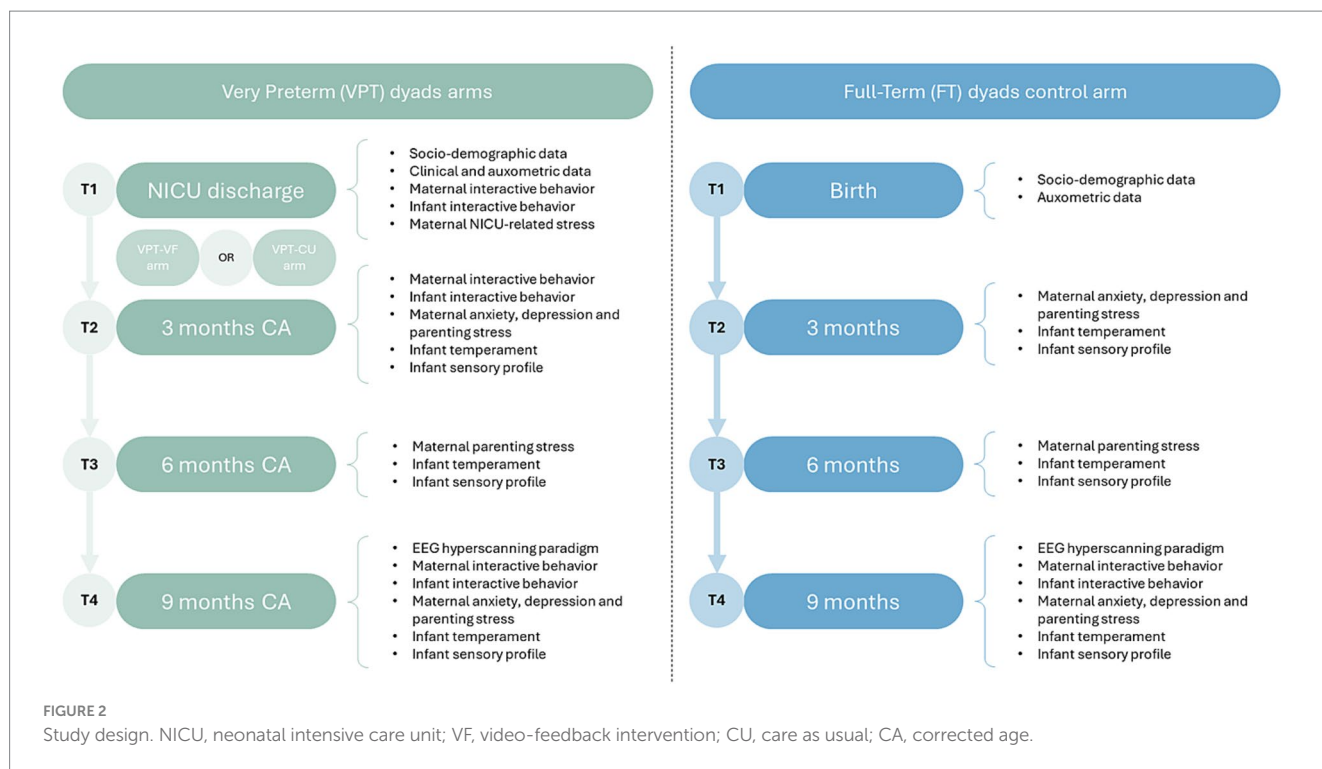


TABLE 4 Indices of inter-brain co-regulation adopted in the 2-BRAINED study.

Index	Description	Notes	Reference
Phase-Locking Value (PLV)	Frequency-specific transients of phase locking independent of amplitude. The value ranges from 0 to 1: values closer to 0 indicate random signals with unsynchronized phases; values closer to 1 indicate stronger coupling between the two signals.	While previous studies focused mainly on infant frequency bands, cross-frequency PLV indices will be obtained for the purposes of the 2-BRAINED study.	Lachaux et al. (1999); Canolty and Knight (2010)
Imaginary Coherence (ICoh)	Computed through spectral density (power) of each participant and cross-spectral density between them to estimate the average phase difference and consistency of phase difference synchronization.	ICoh is expressed as a complex number: the real part represents how much the coherence is driven by instantaneous interactions; the imaginary part shows how much the coherence is based on lagged interactions.	Dikker et al. (2021); Turk et al. (2022)
Amplitude-Amplitude Coupling (AAC)	Expressed as Pearson's correlation coefficient between normalized power time courses of the two signals.	Amplitude coupling was suggested as a valid alternative to phase coupling for three main reasons: amplitude changes are more easily estimated; amplitude modulations are more extensively characterized across EEG studies; amplitude modulations are more sensitive to neural coupling phenomena non-detectable with other phase-related measures.	Haresign et al. (2022); Koul et al. (2023)

Statistical power and sample size estimates

The sample size has been estimated according to over-arching Aim 2, setting parameters as follows: medium effect size, $f = 0.25$, $\alpha = 0.05$, $\beta = 0.20$, number of groups = 3 (VPT-VF, VPT-CU, and FT). The procedure yielded a total sample size of 159 subjects (53 subjects per RCT arm). Nonetheless, considering the longitudinal nature of the study and the attrition rate related to EEG tasks with infants, an oversampling of $n = 80$ (~ +50%) subjects per RCT arm was planned to secure the minimum sample size for appropriately powered statistical analyses.

Plan of statistical analyses

Preliminary analyses

Specific aim 1

General linear models (GLMs) will be carried out to compare VPT and FT dyadic brain-to-brain co-regulation indices during the experimental procedure phases. Theoretically relevant (e.g., gestational age) and statistically identified (e.g., any variable significantly linked with the outcome variables) confounding variables will be controlled for in the analytical model.

Specific aim 2

Separate analyses of variance (ANOVA) will be used with dyadic brain-to-brain co-regulation indices as the dependent variable and groups (FT, VPT-VF, and VPT-CU) as the independent variable. Theoretically relevant (e.g., gestational age) and statistically identified (e.g., any variable significantly linked with the outcome variables) confounding variables will be controlled for in the analytical model.

Additional aims

Models to track early developmental trajectories will be estimated in Mplus by latent class growth analysis with inter-individual variations in time of assessment and mixed-effect linear models with repeated measures to assess group differences in rates of temperament, emotional, and sensory profiles.

Discussion

The present protocol describes an RCT study that aims to assess the benefits of an early post-discharge video-feedback intervention to enhance and promote both parental and VPT infants' outcomes. By collecting dual-source EEG data in a hyper-scanning paradigm and from face-to-face real-time interactions between parents and infants, the study also aims at providing estimations of the effects of such intervention not only for the individual adjustment of caregivers and infants but also for the emergence of dyadic co-regulatory biobehavioral processes. Such co-regulation profiles are meant to be critical indicators of a nurturing caregiving environment during the first months of life fostering affective wellbeing and stress resilience (Feldman, 2020; Levy and Feldman, 2019).

Sources of bias and mitigation strategies

The heterogeneity of VPT infants' conditions should not be underestimated. Even in the absence of severe comorbidities and brain injuries, the experience of NICU hospitalization might be very different for each infant and their parents. To avoid extreme variations, the gestational age range will be constrained between 28 and 35 weeks. Moreover, stress related to the NICU environment will be evaluated and quantified with a well-validated questionnaire (Miles et al., 1993). Selection issues might affect random allocation plans in RCT arms. The allocation to VPT-VF and VPT-CU arms will occur by using an automatically generated list of binary codes that will be consecutively matched with the enrolled families across consecutive sampling. This will reduce the risk of self-selection. To further avoid confounding by infant sex and assure sex distribution balancing, the random allocation will be stratified by infant sex by *post-hoc* controls every 20 enrollments. Parental gender will also be unconstrained, inviting the primary caregiver—and not explicitly the mother—to participate in the study, VF intervention, and observational procedures. EEG procedural steps and artifacts might easily result in the loss of subjects in a longitudinal study; a 50% oversampling was planned to achieve the minimum sample size for adequately powered statistical analyses.

Expected results and impact

The project represents a translational application of the emerging field of hyper-scanning in developmental neuroscience (Provenzi et al., 2023). As neuroscience is moving toward a radical shift in considering interpersonal exchanges as the primary unit of analysis and observation (Leong et al., 2019; Hoehl and Markova, 2018), clinical applications are meant to be implemented to innovate healthcare. Previous proposals have been advanced to apply such a bi-personal neuroscientific approach to the field of adult psychiatry (Saul et al., 2022) and child development (Nguyen et al., 2020).

In this study, we aim to innovate the field of family-centered care in pediatric settings by embedding a cutting-edge approach to the study of parent–infant interaction and co-regulation processes into well-validated approaches to parental support and child development promotion. As the video-feedback intervention is well-acknowledged for its beneficial implications for parental wellbeing, child development, and quality of the early parent–child relationship (Fukkink et al., 2011; Montirosso et al., 2020), it represents an elite clinical setting to test the advantages of new neuroscientific-inspired metrics that specifically focus on the assessment of brain-to-brain co-regulatory processes. In this context, the present study has multifaceted implications.

From a scientific perspective, this study will provide first-of-a-kind quantitative estimations of inter-brain coupling and co-regulation in a sample of VPT infants and their caregivers. While previous research has highlighted functional and structural alterations in VPT infants' brains (Dereymaeker et al., 2017; Hüppi et al., 1998; Pittet et al., 2019), very little is known about VPT neurophysiological functioning in real-life settings. Moreover, the study will provide insights into “how much” inter-brain synchrony should be expected in typical and atypical developmental trajectories. As medium levels of attunement and matching have been suggested to be optimal in terms of behavioral co-regulation during the first months (Provenzi et al., 2016), similar expectations appear to be plausible for what pertains to inter-brain coupling.

From a clinical point of view, the 2-BRAINED project is expected to produce evidence of the efficacy of an early intervention for VPT infants and their parents that is delivered after NICU discharge. This is a critical window for continuity of care as parents transition from potentially high-quality family-centered care during NICU stay to a lack of appropriate and tailored support at home (Lundqvist et al., 2019; Provenzi et al., 2016). From this perspective, this study will explore the efficacy of an intervention aimed at granting continuity of care from the hospital to the house, extending and empowering family-centered care for VPT infants' parents.

From a translational neuroscience perspective, the study will also offer an unprecedented opportunity to obtain first-hand dyadic neurophysiological target outcomes of well-validated early family-centered VF intervention. While the road to developing qualitative and quantitative measures of the effectiveness and efficacy of such family-centered interventions is yet to be fully implemented, the integration of behavioral, self-report, and neurobiological markers is promising for future advances.

Patient and public involvement

Active engagement of families will be pursued through public-dedicated web/social communications, digital content, and newsletters describing the achieved goals and implications of the study. Parental associations and professional orders will be engaged through online webinars to capitalize on the data obtained from the present study to further fuel a culture of family-centered care for preterm infants and their parents.

Ethics and dissemination

Ethics, privacy, and data management

The Ethics Committee Pavia and collaborating partners granted approval for the study on 16 February 2023 (protocol number: 0008588/23) and officially launched on 24 April 2023 (GR-2021-12375213). All procedures align with the ethical principles outlined in the Declaration of Helsinki for research involving human subjects, ensuring no harm to participants. The study intervention offers additional opportunities for families without altering standard mother–infant care programs. Infants will undergo planned diagnostic and therapeutic interventions at the child neurology and psychiatric unit IRCCS Mondino Foundation, Pavia, Italy, and the Scientific Institute IRCCS *E. Medea*, Bosisio Parini, Italy.

Data management will occur in accordance with the General Data Protection Regulation (Regulation 2016/279, commonly known as GDPR), to guarantee the privacy and the security of the gathered data. In this sense, all the infants' parents will sign an informed consent module after that the study's aims and modalities will be clearly explained and eventual doubts will be solved. Each subject will be assigned a code, and data will be stored in a pseudonymized form. After the period of conservation (25 years), data will be made completely anonymous. In line with the open science principle, the anonymized data collected for the study will be published on a publicly accessible “repository” (i.e., Zenodo) to promote the dissemination of research results with a view to furthering the research itself and the scientific community.

Dissemination

The dissemination strategy involves presenting findings at national and international scientific meetings, publishing in developmental psychology journals, and engaging in outreach activities with families and healthcare specialists. This aims to promote early family-centered intervention and share insights with the wider public.

Ethics statement

The studies involving humans were approved by Comitato Etico, Policlinico San Matteo, Pavia (Italy). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

LB: Methodology, Supervision, Writing – original draft, Writing – review & editing. VR: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. EC: Writing – original draft, Writing – review & editing. SG: Writing – original draft, Writing – review & editing. MP: Writing – original draft, Writing – review & editing. MC: Writing – original draft, Writing – review & editing. ES: Writing – original draft, Writing – review & editing. ER: Writing – original draft, Writing – review & editing. RB: Writing – original draft, Writing – review & editing. LP: Conceptualization, Funding acquisition, Methodology, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author LP declared that he was an editorial board member of *Frontiers*, at the time of submission. This had no impact on the peer review process and the final decision.

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

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References

- Abidin, R., Flens, J. R., and Austin, W. G. (2006). *The parenting stress index*. Lawrence Erlbaum Associates Publishers.
- Aita, M., Johnston, C., Goulet, C., Oberlander, T. F., and Snider, L. (2013). Intervention minimizing preterm infants' exposure to NICU light and noise. *Clin. Nurs. Res.* 22, 337–358. doi: 10.1177/1054773812469223
- Ballidin, S., Fisher, P. A., and Wirtberg, I. (2018). Video feedback intervention with children: a systematic review. *Res. Soc. Work. Pract.* 28, 682–695. doi: 10.1177/1049731516671809
- Barlow, J., Sembi, S., and Underdown, A. (2016). Pilot RCT of the use of video interactive guidance with preterm babies. *J. Reprod. Infant Psychol.* 34, 511–524. doi: 10.1080/02646838.2016.1217404
- Beam, A. L., Fried, I., Palmer, N., Agniel, D., Brat, G., Fox, K., et al. (2020). Estimates of healthcare spending for preterm and low-birthweight infants in a commercially insured population: 2008–2016. *J. Perinatol.* 40, 1091–1099. doi: 10.1038/s41372-020-0635-z
- Beck, A. T., Steer, R. A., and Brown, G. K. (1996). “Beck depression inventory–II.” Psychological assessment. Unpublished manual.
- Bi, X., Cui, H., and Ma, Y. (2023). Hyperscanning studies on interbrain synchrony and child development: a narrative review. *Neuroscience* 530, 38–45. doi: 10.1016/j.neuroscience.2023.08.035
- Bigelow, A. E., Beebe, B., Power, M., Stafford, A.-L., Ewing, J., Egleson, A., et al. (2018). Longitudinal relations among maternal depressive symptoms, maternal mind-mindedness, and infant attachment behavior. *Infant Behav. Dev.* 51, 33–44. doi: 10.1016/j.infbeh.2018.02.006
- Brambilla, M., Grumi, S., Manfredini, V., Pettenati, G., and Provenzi, L. (2023). *Parent-Infant Coding System, PICS*. Version 4.0, Unpublished manual.
- Burke, S. (2018). Systematic review of developmental care interventions in the neonatal intensive care unit since 2006. *J. Child Health Care* 22, 269–286. doi: 10.1177/1367493517753085
- Butti, N., Montiroso, R., Borgatti, R., and Urgesi, C. (2018). Maternal sensitivity is associated with configural processing of infant's cues in preterm and full-term mothers. *Early Hum. Dev.* 125, 35–45. doi: 10.1016/j.earlhumdev.2018.08.018
- Canolty, R. T., and Knight, R. T. (2010). The functional role of cross-frequency coupling. *Trends Cogn. Sci.* 14, 506–515.
- Caporali, C., Pisoni, C., Gasparini, L., Ballante, E., Zecca, M., Orcesi, S., et al. (2020). A global perspective on parental stress in the neonatal intensive care unit: a meta-analytic study. *J. Perinatol.* 40, 1739–1752. doi: 10.1038/s41372-020-00798-6
- Chang, C.-Y., Hsu, S.-H., Pion-Tonachini, L., and Jung, T.-P. (2020). Evaluation of artifact subspace reconstruction for automatic artifact components removal in Multi-Channel EEG recordings. *IEEE Trans. Biomed. Eng.* 67, 1114–1121. doi: 10.1109/TBME.2019.2930186
- Cong, X., Wu, J., Vittner, D., Xu, W., Hussain, N., Galvin, S., et al. (2017). The impact of cumulative pain/stress on neurobehavioral development of preterm infants in the NICU. *Early Hum. Dev.* 108, 9–16. doi: 10.1016/j.earlhumdev.2017.03.003
- Czeszumski, A., Eustergelring, S., Lang, A., Menrath, D., Gerstenberger, M., Schuberth, S., et al. (2020). Hyperscanning: a valid method to study neural inter-brain underpinnings of social interaction. *Front. Hum. Neurosci.* 14:39. doi: 10.3389/fnhum.2020.00039
- Debnath, R., Buzzell, G. A., Morales, S., Bowers, M. E., Leach, S. C., and Fox, N. A. (2020). The Maryland analysis of developmental EEG (MADE) pipeline. *Psychophysiology* 57:e13580. doi: 10.1111/psyp.13580
- Delorme, A., and Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J. Neurosci. Methods* 134, 9–21. doi: 10.1016/j.jneumeth.2003.10.009
- Dereymaeker, A., Pillay, K., Vervisch, J., De Vos, M., Van Huffel, S., Jansen, K., et al. (2017). Review of sleep-EEG in preterm and term neonates. *Early Hum. Dev.* 113, 87–103. doi: 10.1016/j.earlhumdev.2017.07.003
- Dikker, S., Michalareas, G., Oostrik, M., Serafimaki, A., Kahraman, H. M., Struiksma, M. E., et al. (2021). Crowdsourcing neuroscience: inter-brain coupling during face-to-face interactions outside the laboratory. *NeuroImage*, 227:117436.
- Dumas, G. (2011). Towards a two-body neuroscience. *Commun. Integ. Biol.* 4, 349–352. doi: 10.4161/cib.4.3.15110
- Dunn, W. (2014). *Sensory Profile 2 manual*. San Antonio, TX: Pearson.
- Endevelt-Shapira, Y., Djalovski, A., Dumas, G., and Feldman, R. (2021). Maternal chemosignals enhance infant-adult brain-to-brain synchrony. *Sci. Adv.* 7:eabg6867. doi: 10.1126/sciadv.abg6867
- Feldman, R. (2006). From biological rhythms to social rhythms: physiological precursors of mother-infant synchrony. *Dev. Psychol.* 42, 175–188. doi: 10.1037/0012-1649.42.1.175
- Feldman, R. (2020). What is resilience: an affiliative neuroscience approach. *World Psychiatry* 19, 132–150. doi: 10.1002/wps.20729
- Feldman, R., and Eidelman, A. I. (2007). Maternal postpartum behavior and the emergence of infant–mother and infant–father synchrony in preterm and full-term infants: the role of neonatal vagal tone. *Dev. Psychobiol.* 49, 290–302. doi: 10.1002/dev.20220
- Fuertes, M., Santos, P. L. D., Beeghly, M., and Tronick, E. (2006). More than maternal sensitivity shapes attachment: infant coping and temperament. *Ann. N. Y. Acad. Sci.* 1094, 292–296. doi: 10.1196/annals.1376.037
- Fukking, R. G., Trienekens, N., and Kramer, L. J. C. (2011). Video feedback in education and training: putting learning in the picture. *Educ. Psychol. Rev.* 23, 45–63. doi: 10.1007/s10648-010-9144-5
- Gartstein, M. A., and Rothbart, M. K. (2003). Studying infant temperament via the revised infant behavior questionnaire. *Infant Behav. Dev.* 26, 64–86.
- Greven, C. U., Lionetti, F., Booth, C., Aron, E. N., Fox, E., Schendan, H. E., et al. (2019). Sensory processing sensitivity in the context of environmental sensitivity: a critical review and development of research agenda. *Neurosci. Biobehav. Rev.* 98, 287–305. doi: 10.1016/j.neubiorev.2019.01.009
- Grumi, S., Borgatti, R., and Provenzi, L. (2021). Supporting parenting at home-empowering rehabilitation through engagement (SPHERE): study protocol for a randomised control trial. *BMJ Open* 11:e051817. doi: 10.1136/bmjopen-2021-051817
- Hägi-Pedersen, M.-B., Kronborg, H., and Norlyk, A. (2021). Knowledge of mothers and fathers' experiences of the early in-home care of premature infants supported by video consultations with a neonatal nurse. *BMC Nurs.* 20:54. doi: 10.1186/s12912-021-00572-9
- Haresign, I. M., Phillips, E. A. M., Whitehorn, M., Goupil, L., Noreika, V., Leong, V., et al. (2022). Measuring the temporal dynamics of inter-personal neural entrainment in continuous child-adult EEG hyperscanning data. *Dev. Cogn. Neurosci.* 54:101093.
- He, F. B., Axelin, A., Ahlqvist-Björkroth, S., Raikola, S., Löyttyniemi, E., and Lehtonen, L. (2021). Effectiveness of the close collaboration with parents intervention on parent-infant closeness in NICU. *BMC Pediatr.* 21:28. doi: 10.1186/s12887-020-02474-2
- Hoehl, S., and Markova, G. (2018). Moving developmental social neuroscience toward a second-person approach. *PLoS Biol.* 16:e3000055. doi: 10.1371/journal.pbio.3000055
- Hoffenkamp, H. N., Tooten, A., Hall, R. A. S., Braeken, J., Eliëns, M. P. J., Vingerhoets, A. J. J. M., et al. (2015). Effectiveness of hospital-based video interaction guidance on parental interactive behavior, bonding, and stress after preterm birth: a randomized controlled trial. *J. Consult. Clin. Psychol.* 83, 416–429. doi: 10.1037/a0038401
- Hüppi, P. S., Maier, S. E., Peled, S., Zientara, G. P., Barnes, P. D., Jolesz, F. A., et al. (1998). Microstructural development of human newborn cerebral white matter assessed in vivo by diffusion tensor magnetic resonance imaging. *Pediatr. Res.* 44, 584–590. doi: 10.1203/00006450-199810000-00019
- Ionio, C., Ciuffo, G., and Landoni, M. (2021). Parent-infant skin-to-skin contact and stress regulation: a systematic review of the literature. *Int. J. Environ. Res. Public Health* 18:4695. doi: 10.3390/ijerph18094695
- Jean, A. D. L., and Stack, D. M. (2012). Full-term and very-low-birth-weight preterm infants' self-regulating behaviors during a still-face interaction: influences of maternal touch. *Infant Behav. Dev.* 35, 779–791. doi: 10.1016/j.infbeh.2012.07.023
- Koul, A., Ahmar, D., Iannetti, G. D., and Novembre, G. (2023). Spontaneous dyadic behavior predicts the emergence of interpersonal neural synchrony. *NeuroImage*, 277:120233.
- Kumaravel, V. P., Farella, E., Parise, E., and Buiatti, M. (2022). NEAR: an artifact removal pipeline for human newborn EEG data. *Dev. Cogn. Neurosci.* 54:101068. doi: 10.1016/j.dcn.2022.101068
- Lachaux, J. P., Rodriguez, E., Martinerie, J., and Varela, F. J. (1999). Measuring phase synchrony in brain signals. *Hum. Brain Mapp.* 8, 194–208.
- Leong, V., Byrne, E., Clackson, K., Georgieva, S., Lam, S., and Wass, S. (2017). Speaker gaze increases information coupling between infant and adult brains. *Proc. Natl. Acad. Sci. USA* 114, 13290–13295. doi: 10.1073/pnas.1702493114
- Leong, V., Noreika, V., Clackson, K., Georgieva, S., Brightman, L., Nutbrown, R., et al. (2019). Mother-infant interpersonal neural connectivity predicts infants' social learning. doi: 10.1101/2019.03.20.2930186
- Levy, J., and Feldman, R. (2019). Synchronous interactions Foster empathy. *J. Exp. Neurosci.* 13:1179069519865799. doi: 10.1177/1179069519865799
- Leyton, F., Olhaverly, M., Alvarado, R., Rojas, G., Dueñas, L., Downing, G., et al. (2019). Video feedback intervention to enhance parental reflective functioning in primary caregivers of inpatient psychiatric children: protocol for a randomized feasibility trial. *Trials* 20:20. doi: 10.1186/s13063-019-3310-y
- Linnér, A., and Almgren, M. (2020). Epigenetic programming—the important first 1000 days. *Acta Paediatr.* 109, 443–452. doi: 10.1111/apa.15050
- Lionetti, F., Aron, A., Aron, E. N., Burns, G. L., Jagiellowicz, J., and Pluess, M. (2018). Dandelions, tulips and orchids: evidence for the existence of low-sensitive, medium-sensitive and high-sensitive individuals. *Transl. Psychiatry* 8:24. doi: 10.1038/s41398-017-0090-6
- Liu, D., Liu, S., Liu, X., Zhang, C., Li, A., Jin, C., et al. (2018). Interactive brain activity: review and Progress on EEG-based Hyperscanning in social interactions. *Front. Psychol.* 9:9. doi: 10.3389/fpsyg.2018.01862

- Lordier, L., Meskaldji, D.-E., Grouiller, F., Pittet, M. P., Vollenweider, A., Vasung, L., et al. (2019). Music in premature infants enhances high-level cognitive brain networks. *Proc. Natl. Acad. Sci. USA* 116, 12103–12108. doi: 10.1073/pnas.1817536116
- Lotzin, A., Lu, X., Kriston, L., Schiborr, J., Musal, T., Romer, G., et al. (2015). Observational tools for measuring parent-infant interaction: a systematic review. *Clin. Child. Fam. Psychol. Rev.* 18, 99–132. doi: 10.1007/s10567-015-0180-z
- Lundqvist, P., Weis, J., and Sivberg, B. (2019). Parents' journey caring for a preterm infant until discharge from hospital-based neonatal home care—a challenging process to cope with. *J. Clin. Nurs.* 28, 2966–2978. doi: 10.1111/jocn.14891
- Maitre, N. L., Key, A. P., Slaughter, J. C., Yoder, P. J., Neel, M. L., Richard, C., et al. (2020). Neonatal multisensory processing in preterm and term infants predicts sensory reactivity and internalizing tendencies in early childhood. *Brain Topogr.* 33, 586–599. doi: 10.1007/s10548-020-00791-4
- Mantis, I., Stack, D. M., Ng, L., Serbin, L. A., and Schwartzman, A. E. (2014). Mutual touch during mother-infant face-to-face still-face interactions: influences of interaction period and infant birth status. *Infant Behav. Dev.* 37, 258–267. doi: 10.1016/j.infbeh.2014.04.005
- Miles, M. S., Funk, S. G., and Carlson, J. (1993). Parental Stressor Scale: neonatal intensive care unit. *Nurs. Res.* 42, 148–152. doi: 10.1097/00006199-199305000-00005
- Montirosso, R., Arrigoni, F., Casini, E., Nordio, A., De Carli, P., Di Salle, F., et al. (2017). Greater brain response to emotional expressions of their own children in mothers of preterm infants: an fMRI study. *J. Perinatol.* 37, 716–722. doi: 10.1038/jp.2017.2
- Montirosso, R., Borgatti, R., Trojan, S., Zanini, R., and Tronick, E. (2010). A comparison of dyadic interactions and coping with still-face in healthy pre-term and full-term infants. *Br. J. Dev. Psychol.* 28, 347–368. doi: 10.1348/026151009x416429
- Montirosso, R., Rosa, E., Giorda, R., Fazzi, E., Orcesi, S., Cavallini, A., et al. (2020). Early parenting intervention – biobehavioral outcomes in infants with neurodevelopmental disabilities (EPI-BOND): study protocol for an Italian multicentre randomised controlled trial. *BMJ Open* 10:e035249. doi: 10.1136/bmjopen-2019-035249
- Mörelus, E., Örténstrand, A., Theodorsson, E., and Frostell, A. (2015). A randomised trial of continuous skin-to-skin contact after preterm birth and the effects on salivary cortisol, parental stress, depression, and breastfeeding. *Early Hum. Dev.* 91, 63–70. doi: 10.1016/j.earlhumdev.2014.12.005
- Neugebauer, C., Oh, W., McCarty, M., and Mastergeorge, A. M. (2022). Mother–infant dyadic synchrony in the NICU context. *Adv. Neonatal Care* 22:170. doi: 10.1097/ANC.0000000000000855
- Nguyen, T., Bánki, A., Markova, G., and Hoehl, S. (2020). Studying parent-child interaction with hyperscanning. *Prog. Brain Res.* 254, 1–24. doi: 10.1016/b.pbr.2020.05.003
- Niutanan, U., Harra, T., Lano, A., and Metsäranta, M. (2020). Systematic review of sensory processing in preterm children reveals abnormal sensory modulation, somatosensory processing and sensory-based motor processing. *Acta Paediatr.* 109, 45–55. doi: 10.1111/apa.14953
- Ohuma, E. O., Moller, A.-B., Bradley, E., Chakwera, S., Hussain-Alkhateeb, L., Lewin, A., et al. (2023). National, regional, and global estimates of preterm birth in 2020, with trends from 2010: a systematic analysis. *Lancet* 402, 1261–1271. doi: 10.1016/S0140-6736(23)00878-4
- Pierce, S., Kadlaskar, G., Edmondson, D. A., McNally Keehn, R., Dydak, U., and Keehn, B. (2021). Associations between sensory processing and electrophysiological and neurochemical measures in children with ASD: an EEG-MRS study. *J. Neurodev. Disord.* 13:5. doi: 10.1186/s11689-020-09351-0
- Pineda, R., Raney, M., and Smith, J. (2019). Supporting and enhancing NICU sensory experiences (SENSE): defining developmentally-appropriate sensory exposures for high-risk infants. *Early Hum. Dev.* 133, 29–35. doi: 10.1016/j.earlhumdev.2019.04.012
- Pion-Tonachini, L., Kreutz-Delgado, K., and Makeig, S. (2019). ICLabel: an automated electroencephalographic independent component classifier, dataset, and website. *NeuroImage* 198, 181–197. doi: 10.1016/j.neuroimage.2019.05.026
- Pisoni, C., Provenzi, L., Moncecchi, M., Caporali, C., Naboni, C., Stronati, M., et al. (2021). Early parenting intervention promotes 24-month psychomotor development in preterm children. *Acta Paediatr.* 110, 101–108. doi: 10.1111/apa.15345
- Pittet, M. P., Vasung, L., Huppi, P. S., and Merlini, L. (2019). Newborns and preterm infants at term equivalent age: a semi-quantitative assessment of cerebral maturity. *NeuroImage Clin.* 24:102014. doi: 10.1016/j.nicl.2019.102014
- Porges, S. W., Davila, M. I., Lewis, G. F., Kolacz, J., Okonmah-Obazee, S., Hane, A. A., et al. (2019). Autonomic regulation of preterm infants is enhanced by family nurture intervention. *Dev. Psychobiol.* 61, 942–952. doi: 10.1002/dev.21841
- Poslawsky, I. E., Naber, F. B., Bakermans-Kranenburg, M. J., van Daalen, E., van Engeland, H., and van IJzendoorn, M. H. (2015). Video-feedback intervention to promote positive parenting adapted to autism (VIPP-AUTI): a randomized controlled trial. *Autism* 19, 588–603. doi: 10.1177/1362361314537124
- Provenzi, L., Barello, S., Fumagalli, M., Graffigna, G., Sirgiovanni, I., Savarese, M., et al. (2016). A comparison of maternal and paternal experiences of becoming parents of a very preterm infant. *J. Obstet. Gynecol. Neonatal. Nurs.* 45, 528–541. doi: 10.1016/j.jogn.2016.04.004
- Provenzi, L., Casini, E., de Simone, P., Reni, G., Borgatti, R., and Montirosso, R. (2015). Mother–infant dyadic repairation and individual differences in vagal tone affect 4-month-old infants' social stress regulation. *J. Exp. Child Psychol.* 140, 158–170. doi: 10.1016/j.jecp.2015.07.003
- Provenzi, L., Fumagalli, M., Bernasconi, F., Sirgiovanni, I., Morandi, F., Borgatti, R., et al. (2017). Very preterm and full-term infants' response to socio-emotional stress: the role of postnatal maternal bonding. *Infancy* 22, 695–712. doi: 10.1111/inf.12175
- Provenzi, L., Giorda, R., Fumagalli, M., Brambilla, M., Mosca, F., Borgatti, R., et al. (2019). Telomere length and salivary cortisol stress reactivity in very preterm infants. *Early Hum. Dev.* 129, 1–4. doi: 10.1016/j.earlhumdev.2018.12.002
- Provenzi, L., Giusti, L., Caglia, M., Rosa, E., Mascheroni, E., and Montirosso, R. (2020). Evidence and open questions for the use of video-feedback interventions with parents of children with neurodevelopmental disabilities. *Front. Psychol.* 11:11. doi: 10.3389/fpsyg.2020.01374
- Provenzi, L., Giusti, L., Fumagalli, M., Frigerio, S., Morandi, F., Borgatti, R., et al. (2019). The dual nature of hypothalamic-pituitary-adrenal axis regulation in dyads of very preterm infants and their mothers. *Psychoneuroendocrinology* 100, 172–179. doi: 10.1016/j.psyneuen.2018.10.007
- Provenzi, L., Guida, E., and Montirosso, R. (2018). Preterm behavioral epigenetics: a systematic review. *Neurosci. Biobehav. Rev.* 84, 262–271. doi: 10.1016/j.neubiorev.2017.08.020
- Provenzi, L., Olson, K. L., Montirosso, R., and Tronick, E. (2016). Infants, mothers, and dyadic contributions to stability and prediction of social stress response at 6 months. *Dev. Psychol.* 52, 1–8. doi: 10.1037/dev0000072
- Provenzi, L., Roberti, E., and Capelli, E. (2023). Envisioning translational hyperscanning: how applied neuroscience might improve family-centered care. *Soc. Cogn. Affect. Neurosci.* 18:nsac061. doi: 10.1093/scan/nsac061
- Riva Crugnola, C., Ierardi, E., Peruta, V., Miotoli, M., and Albizzati, A. (2021). Video-feedback attachment based intervention aimed at adolescent and young mothers: effectiveness on infant-mother interaction and maternal mind-mindedness. *Early Child Dev. Care* 191, 475–489. doi: 10.1080/03004430.2019.1652172
- Santamaria, L., Noreika, V., Georgieva, S., Clackson, K., Wass, S., and Leong, V. (2020). Emotional valence modulates the topology of the parent-infant inter-brain network. *NeuroImage* 207:116341. doi: 10.1016/j.neuroimage.2019.116341
- Saul, M. A., He, X., Black, S., and Charles, F. (2022). A two-person neuroscience approach for social anxiety: a paradigm with interbrain synchrony and neurofeedback. *Front. Psychol.* 12:12. doi: 10.3389/fpsyg.2021.568921
- Spielberger, C. D. (1983). *State-Trait Anxiety Inventory for Adults (STAI-AD)*. APA PsychTests.
- Suir, I., Oosterhaven, J., Boonzaaijer, M., Nuysink, J., and Jongmans, M. (2022). The AIMS home-video method: parental experiences and appraisal for use in neonatal follow-up clinics. *BMC Pediatr.* 22:338. doi: 10.1186/s12887-022-03398-9
- Thomson, G., Flacking, R., George, K., Feeley, N., Haslund-Thomsen, H., De Coen, K., et al. (2020). Parents' experiences of emotional closeness to their infants in the neonatal unit: a meta-ethnography. *Early Hum. Dev.* 149:105155. doi: 10.1016/j.earlhumdev.2020.105155
- Tronick, E., Als, H., Adamson, L., Wise, S., and Brazelton, T. B. (1978). The infant's response to entrapment between contradictory messages in face-to-face interaction. *J. Am. Acad. Child Psychiatry* 17, 1–13. doi: 10.1016/s0002-7138(09)62273-1
- Tryphonopoulos, P. D., and Letourneau, N. (2020). Promising results from a video-feedback interaction guidance intervention for improving maternal-infant interaction quality of depressed mothers: a feasibility pilot study. *Can. J. Nurs. Res.* 52, 74–87. doi: 10.1177/0844562119892769
- Turk, E., Endevelt-Shapira, Y., Feldman, R., van den Heuvel, M. I., and Levy, J. (2022). Brains in sync: Practical guideline for parent–infant EEG during natural interaction. *Front. Psychol.* 13:833112.
- Wass, S. V., Noreika, V., Georgieva, S., Clackson, K., Brightman, L., Nutbrown, R., et al. (2018). Parental neural responsivity to infants' visual attention: how mature brains influence immature brains during social interaction. *PLoS Biol.* 16:e2006328. doi: 10.1371/journal.pbio.2006328
- Welch, M. G., and Ludwig, R. J. (2017). Calming cycle theory and the co-regulation of oxytocin. *Psychodyn. Psychiatry* 45, 519–540. doi: 10.1521/pdps.2017.45.4.519



Exploring the impact of manual and automatic EEG pre-processing methods on interpersonal neural synchrony measures in parent-infant hyperscanning studies

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ABSTRACT

Background: Electroencephalograph (EEG) hyperscanning allows studying Interpersonal Neural Synchrony (INS) between two or more individuals across different social conditions, including parent-infant interactions. Signal pre-processing is crucial to optimize computation of INS estimates; however, few attempts have been made at comparing the impact of different dyadic EEG data pre-processing methods on INS estimates.

New methods: EEG data collected on 31 mother-infant dyads (8–10 months) engaged in a Face-to-Face Still-Face Procedure were pre-processed with two versions of the same pipeline, the “automated” and the “manual”. Cross-frequency PLV in the theta (3–5 Hz, 4–7 Hz) and alpha (6–9 Hz, 8–12 Hz) frequency bands were computed after automated and manual pre-processing and compared through Pearson’s correlations and Repeated Measures ANOVAs.

Results: PLVs computed in the theta, but not alpha, frequency band were significantly higher after automated pre-processing than after manual pre-processing. Moreover, the automated pipeline rejected a significantly lower percentage of ICs and epochs compared to the manual pipeline.

Comparison with existing methods: While no direct comparison with existing dyadic EEG data pre-processing pipelines was made, this is the first study assessing the impact of different methodological decisions, particularly of the degree of pre-processing automatization, on cross-frequency PLV computed on a dataset of parent-infant dyads.

Conclusions: Non-directional phase-based INS indexes such as the PLV seem to be affected by the degree of automatization of the pre-processing pipeline. Future research should strive for standardization of dyadic EEG pre-processing methods.

1. Introduction

Hyperscanning is defined as the simultaneous recording of brain activity in two or more subjects. It can be performed using a variety of techniques, such as functional magnetic resonance imaging (fMRI; Montague et al., 2002), magnetoencephalography (MEG; Hirata et al., 2014; Czeszumski et al., 2020), functional near-infrared spectroscopy (fNIRS; Miller et al., 2019) and, finally, electroencephalography (EEG; Toppi et al., 2016). In the last decades, hyperscanning has been proposed as a paradigm to investigate Interpersonal Neural Synchronization

(INS), the temporal alignment of neural activity between two or more interactive partners (Koul et al., 2023), with the use of diverse indexes computed following different signal properties (e.g. phase, amplitude) and/or theoretical assumptions (directional or non-directional synchronization). Among these different indexes used to measure INS, Phase-Locking Value (PLV; Lachaux et al., 1999) is one of the most commonly used in hyperscanning research due to its aptness at capturing the rapid flow of information between two social agents (Burgess, 2013). Given its reputation as a privileged standpoint from which to observe the unfolding of social exchanges (Roberti et al.,

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2023), the hyperscanning paradigm has been used to investigate the neurophysiological underpinnings of social engagement during different types of interactions, among which early parent-infant interactions (Nguyen et al., 2020).

In developmental research, EEG is one of the most used techniques to perform hyperscanning studies due to its aptness at studying INS in the usually fast-paced and dynamic naturalistic interactions (Turk et al., 2022). Notably, the literature on developmental psychology has seen an increase in studies using EEG hyperscanning to investigate dyadic INS (Atilla et al., 2023; Atzaba-Poria et al., 2017; Leong et al., 2017; Krzeczowski et al., 2022; Endevelt-Shapira et al., 2021; Leong et al., 2019; Perone et al., 2020; Santamaria et al., 2020; Wass et al., 2018). Practical guidelines on how to collect EEG data and estimate INS in parent-infant dyads have been established on recent publications (for a review see Turk et al., 2022) as an attempt to reach common ground and ensure the reproducibility and comparability of results.

A fundamental step to estimate INS in EEG hyperscanning studies is signal pre-processing. The practical guidelines written by Turk et al. (2022) recommend applying a manual or semi-automated pre-processing routine which includes visual inspection of the raw data and removal of flat channels, data re-referencing, data filtering, interpolation or removal of spurious channels, manual artifacts rejection, Independent Component Analysis (ICA) or wavelet analysis, manual or automated artifacts rejection and segmentation of the signal into epochs; in addition, recently published pipelines, such as DEEP (Kayhan et al., 2022), MADE (Debnath et al., 2020) or the MATLAB-based HAPPE (Gabard-Durnam et al., 2018) provide standardized (DEEP) and/or fully automatized methods (HAPPE, MADE) to pre-process developmental EEG data. Despite this, a standardization of the pre-processing routine in this field seems to still be lacking. The majority of the authors of parent-infant EEG hyperscanning studies (see Atilla et al., 2023; Leong et al., 2017; Endevelt-Shapira et al., 2021; Santamaria et al., 2020; Wass et al., 2018) seem to have opted for custom scripts for EEG data pre-processing, generating discrepancies among the literature in three main aspects: 1. the order in which the pre-processing steps are performed; 2. the parameters set for each step, and 3. the degree of automatization of the whole process. While it is known that different settings on some of the most commonly used pre-processing steps such as the type of filter (Widmann et al., 2015; Winkler et al., 2015), ICA algorithm (Dimigen, 2020), and other automatized algorithms for noise correction such as Artifact Subspace Reconstruction (ASR; Chang et al., 2018) may differently impact the properties of the EEG signal, the degree of automatization of the pipeline may pose an additional challenge to the reproducibility of the findings reported in developmental EEG hyperscanning research. Indeed, semi-automated pre-processing methods were especially used in the first parent-infant EEG hyperscanning studies (Leong et al., 2017; Leong et al., 2019; Santamaria et al., 2020) and their usage was also recommended in dyadic EEG hyperscanning guidelines (Turk et al., 2022). However, discrepancies in artifact rejection stemming from individual sensitivity of the coder may produce variations in the pre-processed signals that, in turn, could impact INS estimates to an unknown degree. Automatized pre-processing methods, on the other hand, may minimize the issue of individual differences in the pre-processing steps and sensitivity of the coders; however, while more recent studies pre-processed dyadic EEG data using an automatized approach (Atilla et al., 2023; Endevelt-Shapira et al., 2021; Swider-Cios et al., 2024), attempts at establishing standardized and automatized dyadic EEG data pre-processing routines are still rare in the literature (Kayhan et al., 2022). Moreover, artifact rejection based on visual inspection is still a diffused practice in dyadic EEG hyperscanning studies (Kayhan et al., 2022). For these reasons, it is crucial to understand whether different methodological decisions taken during the EEG signal pre-processing, particularly the degree of automatization of the pre-processing pipeline, have an impact on INS estimates.

In the present study, we aimed at comparing the performance of a

fully automated pipeline for dual parent-infant EEG data with the one of a more traditional, “manual” dual EEG pre-processing method. As the names suggest, the two pipelines differed exclusively in their degree of automatization, whereas the order and thresholds of the specific steps were the same for both (see par. 2.2., *Data pre-processing*). Our interest was in two main outcomes: the main pre-processing outputs (e.g. number of interpolated channels, type and number of rejected independent components, rejected epochs) and INS estimates. For the purposes of this study and for its predominant use in parent-infant EEG hyperscanning studies (Atilla et al., 2023; Leong et al., 2017; Leong et al., 2019; Santamaria et al., 2020), the PLV was used as the only proxy of INS.

By increasing the knowledge on the intricacies of data pre-processing and artifact correction in dyadic EEG data, we aim at encouraging developmental researchers to reach a common ground in regards to the modalities of EEG data pre-processing in this field and thus increase the comparability and reproducibility of the results that will be obtained in future studies.

2. Methods

2.1. EEG data acquisition

Dual EEG data was taken from a dataset of 31 mother-infant (8–10 months; 16 males and 15 females) dyads who had their brain activity recorded through the EEG hyperscanning paradigm (Dumas, 2011; Montague et al., 2002) while they were engaged in a Face-to-Face Still-Face Procedure (FFSF; Tronick et al., 1978). Further information in regards to the recruitment strategy, inclusion and exclusion criteria are available on Supplementary Materials 1 (par. S1.1). The FFSF comprised of three episodes: a 2-min face-to-face play interaction (Play), a 1-min period of maternal still face (Still-Face), and a 2-min face-to-face play interaction (Reunion). The timing of episode switches was guided by the experimenters by providing verbal cues to the caregiver while pressing a key to send a marker to the EEG recording. The FFSF is a standard procedure which allows the observation of infants' socio-emotional competence and ability to cope with stress emotion regulation strategies (Montiroso et al., 2010) and has been previously used in dyadic EEG studies (Gartstein, 2020; Perone et al., 2020; Swider-Cios et al., 2024).

The EEG equipment of choice was the Smarting Pro (mBrainTrain, 2024), featuring two 32-channels caps and amplifiers connected via Bluetooth to the mBrainTrain Streamer program installed on two different computers, one for each member of the dyad. Brain activity was recorded on the infant's computer, which was connected to the parent's computer through a LAN cable. Sampling rate was set at 250 Hz.

2.2. Data pre-processing

EEG data was pre-processed on the MATLAB-based (The MathWorks Inc., 2024) interacting toolbox EEGLAB ver. 2024.0 (Delorme and Makeig, 2004) using two different custom pipelines: the “automated” pipeline, which required no control from the coder, and the “manual” pipeline, in which the coder was directly involved in the processes of channel interpolation, independent components (ICs) rejection and visual inspection of the signal. A summarization of the main steps of the two pipelines is presented in Fig. 1.

Both pipelines were built according to previous guidelines on dyadic EEG data pre-processing (Debnath et al., 2020; Kayhan et al., 2022; Turk et al., 2022), and differed from one another exclusively on their degree of automatization in the following steps: detection of bad channels, IC selection, epoch rejection. In both pipelines, the infants' and the parents' signals were pre-processed separately, the infant first and the parent last.

Data filtering. Both the automated and the manual pipeline applied

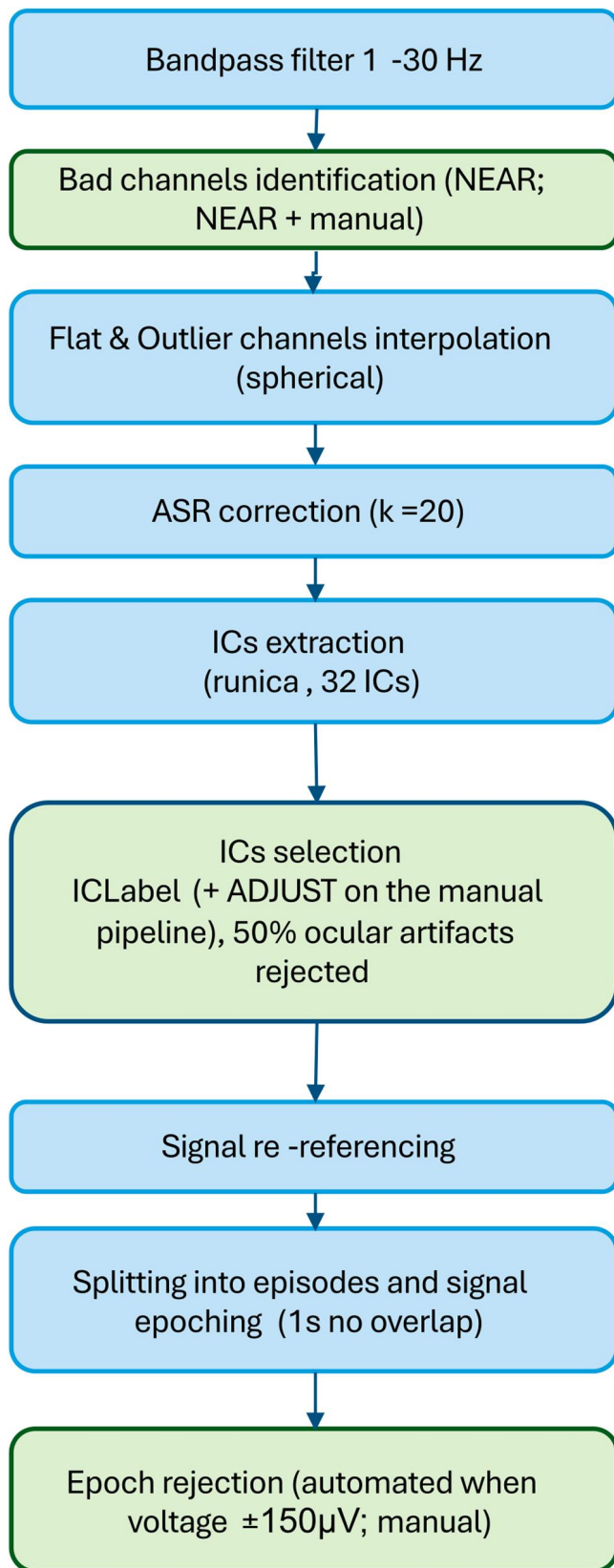


Fig. 1. Comparison between the two pre-processing pipelines. Steps in which the degree of automatization between the two pipelines was different are displayed in green.

the default EEGLAB Hamming windowed sinc FIR bandpass filter to the signals, with 1 Hz and 30 Hz as the lower and upper limits respectively. This was in line with the frequency bands of choice for the INS analyses (theta and alpha; see par. 2.3.).

Detection of bad channels. In both pipelines, flat and outlier channel detection was performed using the Neonatal EEG Artifact Removal (NEAR) plugin, which has been validated in EEG data acquired both in adults and in infants aged 8–10 months (Kumaravel et al., 2022). In the automated pipeline, NEAR was the only method for detection of bad channels, whereas in the manual pipeline, the usage of NEAR was followed by a visual inspection of the signal by the coder, who checked the output of NEAR to either confirm it or to detect additional flat or outlier channels to interpolate.

Channel interpolation. In both pipelines, the channels that were previously detected as flat or outliers were interpolated using the spherical spline method. Interpolation of the channels with bad signal was performed on the whole recording rather than on the specific segments in which they resulted as flat or outliers.

Noise correction. Both pipelines proceeded with noise correction using the ASR algorithm (Chang et al., 2019). In our pipelines, ASR was set in correction mode in order to preserve the temporal synchronization of the two signals, and deployed a cutoff parameter (k) at the default value of $k = 20$. The cutoff parameter k is crucial in determining the sensitivity of the noise correction process: every segment of data that exceeds k is considered artifactual and thus corrected into EEG-like data (Kumaravel et al., 2022). Consequently, a low k (below 20 in the adult population) will result in a stricter noise correction, whereas a high k (over 30 in the adult population) will result in a looser noise correction.

Independent Component Analysis (ICA). Analysis of the components of the signals was performed through ICA in both pipelines using the default Infomax ICA algorithm (*runica*). In both the automated and the manual pipeline, *runica* produced as many components as the total number of channels ($N = 32$). Components were then categorized using the ICLabel plugin ver. 1.6 (Pion-Tonachini et al., 2019) and subsequently inspected for removal following different criteria for each pipeline. In the automated pipeline, the inspection was entirely automatized, and ICs were rejected from the signal if they displayed a probability of 50 % or higher of being an ocular artifact. In the manual pipeline, on the other hand, the inspection was performed by the coder after a preliminary assessment of the artifactual ICs performed with the ADJUST plugin ver. 1.1.1 (Mognon et al., 2011). The coder was required to 1. check the ICs flagged by ADJUST to confirm or reject the output; and 2. manually check the remaining ICs and reject those displaying a probability higher than 50 % of containing ocular artifacts. Both ICLabel and ADJUST have been trained exclusively on adult EEG data; however, while ADJUST demonstrated slightly inferior performance in labeling infant EEG ICs compared to algorithms specifically designed for infant data, ICLabel's performance on infant EEG data was found to be non-inferior to that of infant-specific IC labeling plugins such as Adjusted ADJUST (Leach et al., 2020).

Signal re-referencing. In both pipelines, re-referencing was performed using the average signal of the channels.

Signal segmentation and epoching. In both pipelines, the signal was segmented into the three episodes of the experimental procedure: Play (120 s), Still-Face (60 s), Reunion (120 s). Each episode was subsequently epoched in 1 s epochs with no overlap coherently with previous EEG hyperscanning studies (Ayrolles et al., 2021; Bevilacqua et al., 2019; Goldstein et al., 2018).

Bad epochs rejection. After epoching, the Play and Reunion episodes were inspected for residual artifacts. At this stage, the signal acquired during the Still-Face was not included due to the larger percentage of artifacts that were expected to be found in this specific episode compared to the Play and Reunion. In the automated pipeline, the process was entirely automatized: the script automatically flagged all the epochs in which at least one of the fourteen target channels for PLV computation (see par. 2.3.) displayed a voltage exceeding $\pm 150\mu\text{V}$

(parameter used in [Debnath et al., 2020](#)). In the manual pipeline, on the other hand, the process was performed by the coder, who would visually inspect the signal and flag all the epochs in which at least one of the fourteen target channels for PLV computation displayed either a voltage exceeding $\pm 150\mu\text{V}$ or residual artifacts. In both pipelines, all epochs flagged in one member of the dyad were automatically flagged in the other member to maintain the temporal synchronization of the signals.

2.3. INS indexes - PLV

PLV is a measure of frequency-specific transients of phase locking independent of amplitude ([Lachaux et al., 1999](#)) which has been widely used in EEG hyperscanning studies with developmental samples ([Atilla et al., 2023](#); [Leong et al., 2017](#); [Leong et al., 2019](#); [Santamaria et al., 2020](#)). Values range from [0 1], where $\text{PLV} = 0$ indicates random signals with unsynchronized phases and $\text{PLV} = 1$ indicates stronger coupling between the two signals. In the present study, PLV was computed using MATLAB Fieldtrip ([Oostenveld et al., 2011](#)) custom scripts (see Supplementary Materials 1, par. S1.2, for details). The frequency bands of choice were the theta and the alpha ([Haartsen et al., 2020](#); [van der Velde et al., 2019](#)) due to their respective association with changes in directed gaze during joint and individual play ([Wass et al., 2018](#)) and in changes in emotional states during the interaction ([Perone et al., 2020](#); [Santamaria et al., 2020](#)). Considering the differences in frequency peaks between the infant and the adult brain ([Noreika et al., 2020](#)), we opted for a cross-frequency approach, computing theta and alpha PLVs using both the infant thresholds (3–5 Hz for theta and 6–9 Hz for alpha; [Marshall et al., 2002](#); [van der Velde et al., 2019](#)) and the adult thresholds (4–7 Hz for theta and 8–12 Hz for alpha; [Hill et al., 2020](#)).

Following a previous work ([Santamaria et al., 2020](#)), fourteen ($N = 14$) out of 32 total channels were considered for the computation, seven of which were frontal (F3, F4, Fz, FC1, FC2, FC5, FC6) and seven were central (C3, C4, Cz, CP1, CP2, CP5, CP6). As illustrated in [Fig. 2](#), the computation of the PLV was performed between homologous channels, resulting in a total of $N = 14$ different PLVs, one per channel. Single-channel PLV would be then grouped according to their region (frontal, central) and averaged in order to obtain a mean frontal and a mean central PLV per episode (Play, Reunion) and frequency band (theta, alpha).

2.4. Data quality assessment

To ensure that the quality of our data was adequate for the computation of the PLV, signal-to-noise ratio (SNR) was computed on the raw parent and infant signals before undergoing data pre-processing. SNR was calculated through a MATLAB EEGLAB custom script that, coherently with previous works ([Babiloni et al., 2004](#); [Donoghue et al., 2021](#); [Goldenholz et al., 2009](#)), used the spectral method to estimate the ratio between the power of the signal and the power of the noise for each channel. In our script, the signal was operationalized as the power displayed in the theta (3–5 Hz for the infants and 4–7 Hz for the parents) and the alpha (6–9 Hz for the infant and 8–12 Hz for the parents) frequency bands, whereas the noise was equal to the power of the white noise (≥ 100 Hz for both infants and parents, considering that frequency

bands of neural activity are defined up to 95 Hz; see [Koul et al., 2023](#)). After computing the power of the signal and the power of the noise using the “bandpower” function, we calculated the ratio between them in each channel (flat and outlier channels were momentarily interpolated for this purpose) and transformed the results into decibels. SNR values for each channel were then averaged in order to obtain a single global SNR value for each subject. Positive SNR values are regarded as indicative of a higher power in the “signal” and negative values indicate a higher power in the bands that are considered “noise”. No standardized cut-off for SNR values is present for studies including real EEG from parent-infant interactions; however, previous works deploying real and simulated data of adult EEG signals recommend a threshold up to 10 dB ([Babiloni et al., 2004](#)), so that oscillatory measures such as instantaneous phase and frequency ([Donoghue et al., 2021](#)) can be reliably estimated.

2.5. Statistical analysis

Data analysis was performed using Jamovi version 2.3.28 ([The jamovi project, 2024](#) n.d.). The graphs representing the mean SNR values of our signals were built with RStudio version 2023.12.1 ([RStudio Team 2023](#)). To explore the impact of the different methodological choices taken during the two different pre-processing methods on PLVs, the following plan of analysis was performed. Descriptive statistics and Student’s t tests for paired samples were applied to detect potential differences between the two pipelines on specific outputs of the data pre-processing, namely number of interpolated channels per signal, type of ICs, percentage of rejected ICs and epochs per episode. After computation of PLV on the signals pre-processed with the two different pipelines, Pearson’s correlations and repeated measures ANOVA accounting for Episode (Play, Reunion) and Pipeline (Automated, Manual) were computed for each Region (Frontal, Central) and frequency band (theta, alpha) to compare the impact of the different pre-processing methods on this index.

3. Results

3.1. SNR estimation

Global SNR values for the parents’ and infants’ signals during Play (A) and Reunion (B) episodes are showcased in [Fig. 3](#). All subjects revealed positive SNR values in both episodes, indicating a good quality of the data used for PLV computation. However, SNRs in the infants’ signals were found to be significantly higher compared to the ones computed in the parents’ signals in both episodes ($F(1,30) = 579.47$; $p < 0.001$). Indeed, SNRs computed in the infants’ signals ranged [21.86 33.9] in the Play episode (mean global value: 28.06 ± 3.40 dB) and [22.54 34.4] in the Reunion (mean global value: 27.64 ± 3.40 dB); the parents’ signals, on the other hand, had significantly lower SNRs, with values ranging [3.05 18.7] in the Play episode (mean global value: 9.74 ± 3.84 dB) and values ranging [4.90 23.7] in the Reunion episode (mean global value: 11.10 ± 4.01 dB). Despite these differences, all the infants’ and the majority of the parents’ signals showcased a SNR that exceeded the threshold of 10 dB, meaning that our dataset was adequate for a reliable computation of the PLV in our frequency bands of interest.

3.2. Data pre-processing outputs

A summary of the descriptive statistics, along with the results of the statistical analyses, is available on [Table 1](#).

3.2.1. Interpolated channels

As shown in [Table 1](#), we have compared the performance of the two pipelines during channel interpolation based on two parameters: overall number of interpolated channels (out of $N = 32$) and number of interpolated “target” channels for PLV computation (out of $N = 14$). In both

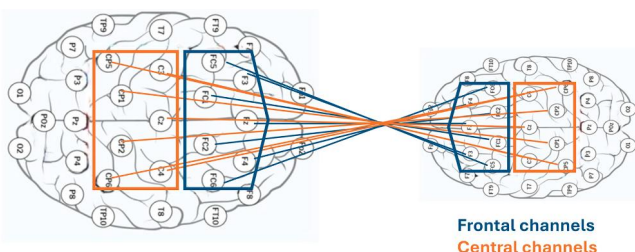


Fig. 2. Channels of interest for phase-based INS estimates.

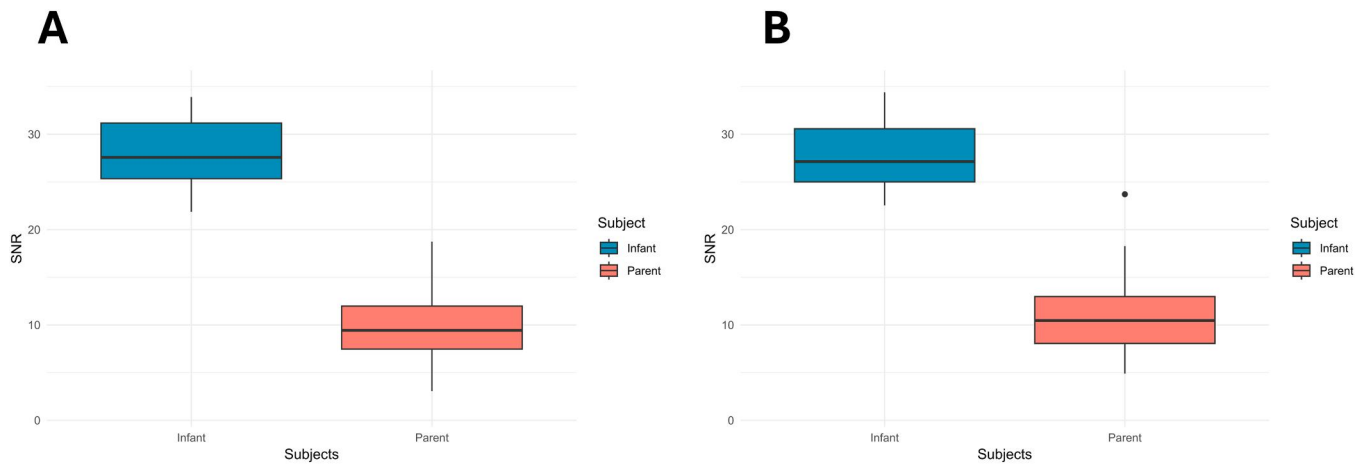


Fig. 3. Mean SNR values of the raw infants' and parents' signals during the Play (A) and Reunion (B) episodes.

Table 1

Descriptive statistics and paired samples Student's t tests comparing the performance of the two pipelines (automated to manual).

Parameter	Mean ± SD (range) - Automated	Mean ± SD (range) - Manual	Student's t	p	C.I.
N interpolated channels (Parent)	2.44 ± 2.02 (0–8)	2.27 ± 2.02 (0–8)	1.64	0.110	−0.04; 0.39
N target interpolated channels (Parent)	0.15 ± 0.36 (0–1)	0.15 ± 0.36 (0–1)	NA	NA	NA
N interpolated channels (Infant)	1.65 ± 1.65 (0–6)	1.88 ± 1.84 (0–6)	−1.49	0.147	−0.56; 0.09
N target interpolated channels (Infant)	0.18 ± 0.58 (0–3)	0.35 ± 0.81 (0–3)	−1.79	0.083	−0.38; 0.02
Perc. removed components (Parent)	7.74 ± 6.27 % (0–33.33 %)	21.98 ± 12.67 % (3.13–46.88 %)	−6.48	< 0.001	−18.7; −9.75
Perc. removed components (Infant)	4.25 ± 2.37 % (0–12.5 %)	14.92 ± 11.52 % (0–28.13 %)	−5.24	< 0.001	−14.8; −6.51
Merged rejected epochs (Play)	5.10 ± 11.27 (0–55)	20.71 ± 19.07 (1–74)	−6.27	< 0.001	−20.7; −10.53
Merged rejected epochs (Reunion)	6.45 ± 12.23 (0–54)	22.55 ± 21.65 (2–85)	−5.04	< 0.001	−22.6; −9.57

Note. Significant comparisons are reported in bold.

these parameters and in both the parents' and the infants' signals, the performance of the two pipelines did not significantly differ from one another, as demonstrated by the non-significant p-values yielded by the paired samples Student's t test (see Table 1). The number of overall interpolated channels ranged [0 8] (0–25 %) for the parents' signals and [0 6] (0–18.75 %) for the infants' signals, whereas the number of interpolated target channels ranged [0 1] for parents (0–3.13 %) for parents and [0 3] (0–16.67 %) for infants' signals.

3.2.2. Type of ICs

Figs. 4 and 5 illustrate the ICs detected by ICLabel during each pre-processing method in the infants' (Fig. 4) and the parents' (Fig. 5) signals. Results of Student's t tests comparing the results of the automated and manual pre-processing pipelines are showcased in Table 2.

Figs. 4 and 5 show how the distributions of the ICs in the infants' and the parents' signals are slightly different from one another. While the infants' signals showcased a majority of "Brain" ICs both after

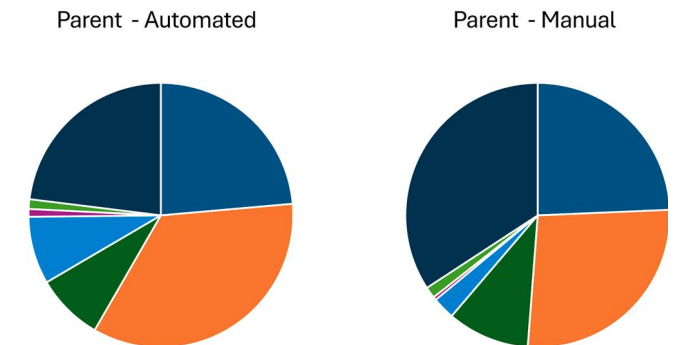


Fig. 5. Comparison of ICLabel results between the two pipelines for the parents' signal.

automated and manual pre-processing, followed by "Muscle" and "Other", "Eye", and then "Heart", "Channel noise" and "Line noise", the parents' signals displayed a majority of either "Muscle" and "Other" ICs, followed by "Brain", "Eye", and then "Heart", "Channel noise" and "Line noise".

In the infants' signals, ICLabel ran in the automated pipeline found significantly more "Muscle" ($t(30) = 2.59; p = 0.014$), "Heart" ($t(30) = 2.10; p = 0.044$) and "Line Noise" ($t(30) = 2.06; p = 0.048$) ICs, as well as significantly less "Other" ICs ($t(30) = -2.07; p = 0.048$), compared to the manual pipeline. In the parents' signals, on the other hand, ICLabel ran in the automated pipeline found significantly more "Heart" ($t(30) = 2.89; p = 0.007$) ICs and significantly less "Eye" ($t(30) = -2.58; p = 0.015$) and "Other" ($t(30) = -2.96; p = 0.006$) ICs compared to the manual pipeline.

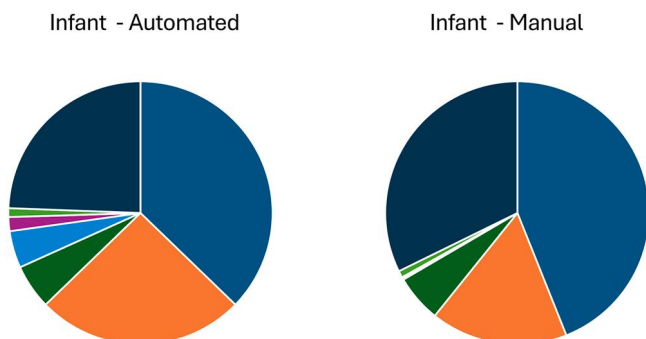


Fig. 4. Comparison of ICLabel results between the two pipelines for the infants' signal.

Table 2

Paired samples Student's t tests comparing the type of ICs resulting from ICLabel for each pipeline (automated to manual).

Component	Infant		Parent	
	Student's t	p-value (C.I.)	Student's t	p-value (C.I.)
Brain	-1.89	0.067 (-13.56; 0.49)	-0.35	0.731 (-4.97; 3.53)
Muscle	2.59	0.014 (1.88; 15.66)	1.99	0.056 (-0.22; 15.91)
Eye	-0.39	0.697 (-1.81; 1.22)	-2.58	0.015 (-3.26; -0.38)
Heart	2.10	0.044 (0.12; 8.53)	2.89	0.007 (1.63; 9.43)
Line noise	2.06	0.048 (0.015; 3.21)	1.45	0.158 (-0.22; 1.29)
Channel noise	0.63	0.536 (-0.59; 1.13)	-0.34	0.735 (-1.44; 1.03)
Other	-2.07	0.048 (-15.41; -0.09)	-2.96	0.006 (-18.88; -3.45)

Note. Significant comparisons are reported in bold.

3.2.3. Rejected ICs

We also explored potential differences in performance of the two pipelines in terms of percentage of removed ICs. Paired samples Student's t test highlighted significant differences in the percentage of ICs rejected by the two pipelines both in the parents' ($t(30) = -6.48$; $p < 0.001$) and the infants' signals ($t(30) = -5.24$; $p < 0.001$), with the manual pipeline removing 2.84 times more ICs in the parents' signals and 3.51 times more ICs in the infants' signals compared to the automated one (see Table 1).

3.2.4. Rejected epochs

Another parameter of interest when preliminarily comparing the performance of the two pipelines is the number of rejected (merged) epochs in the Play and Reunion episodes, considering that this step was performed by EEGLAB in the automated pipeline and by a human coder in the manual pipeline using comparable exclusion criteria for bad epochs.

All recordings contained at least 30 (25 %) good epochs in both Play and Reunion episodes, ensuring sufficient data to compute PLVs. Significant differences emerged in the number of epochs rejected by the two pipelines both in the Play ($t(30) = -6.27$; $p < 0.001$) and Reunion ($t(30) = -5.04$; $p < 0.001$) episodes: more specifically, the manual pipeline tended to reject 16 more epochs both in the Play and Reunion episodes (see Table 1).

3.3. PLV

Supplementary material 2 (S2) comprehensively reports, for each dyad, the PLVs computed in each episode (Play, Reunion), region (Frontal, Central) and frequency band (theta, alpha) after automated and manual pre-processing, together with the number of epochs used for the computation. Results on theta and alpha PLVs are reviewed separately in the following paragraphs.

3.3.1. Theta band

Central tendency measures for theta PLVs computed in the Play and Reunion episodes, as well as the correlations matrices comparing the PLVs obtained after automated and manual pre-processing, are available respectively on Tables 3 and 4. Fig. 6 plots the trends of the two pipelines across episodes in the frontal (A) and central (B) channels.

PLVs computed after automated and manual pre-processing yielded comparable values: PLVs after automated pre-processing ranged [0.254 0.329], whereas PLVs after manual pre-processing ranged [0.247 0.334]. Pearson's correlations across homologous episodes and regions were all significant, positive and strong (see Table 4). Repeated

Table 3

Descriptive statistics comparing the PLVs calculated on the theta and alpha frequency bands after automated and manual pre-processing across episodes and regions.

Variable	Mean \pm SD (range) - Automated	Mean \pm SD (range) - Manual
PLV theta - Play - Frontal	0.294 \pm 0.003 (0.254-0.329)	0.282 \pm 0.017 (0.263-0.334)
PLV theta - Play - Central	0.289 \pm 0.003 (0.260-0.324)	0.278 \pm 0.017 (0.250-0.341)
PLV theta - Reunion - Frontal	0.295 \pm 0.003 (0.270-0.328)	0.279 \pm 0.016 (0.259-0.329)
PLV theta - Reunion - Central	0.293 \pm 0.003 (0.270-0.322)	0.276 \pm 0.019 (0.247-0.329)
PLV alpha - Play - Frontal	0.206 \pm 0.002 (0.188-0.235)	0.206 \pm 0.011 (0.184-0.239)
PLV alpha - Play - Central	0.210 \pm 0.002 (0.191-0.232)	0.211 \pm 0.009 (0.190-0.229)
PLV alpha - Reunion - Frontal	0.207 \pm 0.002 (0.190-0.238)	0.208 \pm 0.012 (0.189-0.239)
PLV alpha - Reunion - Central	0.212 \pm 0.002 (0.190-0.244)	0.211 \pm 0.013 (0.185-0.241)

measures ANOVA revealed a significant effect of Pipeline, but not of Episode, both in the frontal ($F(1,30) = 42.17$; $p < 0.001$) and central ($F(1,30) = 56.84$; $p < 0.001$) channels. In addition, PLV computed on central channels exhibited a significant Episode*Pipeline effect ($F(1,30) = 5.86$; $p = 0.022$). The findings indicate that, although the PLVs computed on the theta frequency band were strongly and significantly correlated with one another across the two pipelines, the automated pipeline tended to produce significantly higher theta PLVs in the frontal and central channels compared to the manual pipeline.

3.3.2. Alpha band

Results on the analyses - central tendency measures, Pearson's correlations - conducted on PLVs calculated on the alpha frequency band are available respectively on Tables 3 and 5. Fig. 7 plots the trends of the two pipelines across episodes in the frontal (A) and central (B) channels.

PLVs computed after automated and manual pre-processing yielded comparable values, with PLVs ranging [0.184 0.241] after automated pre-processing and PLVs ranging [0.185 0.247] after manual pre-processing. Pearson's correlations across homologous episodes and regions were all significant, positive and strong (see Table 6). Repeated measures ANOVA revealed no significant effects of Episode, Pipeline, or Episode*Pipeline interaction both in the frontal and central channels (see Table 7).

4. Discussion

To investigate the impact of different methodological decisions during dyadic EEG data pre-processing, we have pre-processed the signal of 31 mother-infant dyads with two pipelines - the "automated" and the "manual" pipelines, featuring the same pre-processing steps, but different degrees of automatization. After computing SNR of the raw signals and after pre-processing them with each of the two pipelines, we have computed cross-frequency PLVs in the theta (3-5 & 6-9 Hz) and alpha (6-9 & 8-12 Hz) frequency bands and compared the values resulting after each pre-processing method. The two pipelines showcase significant differences both in the outputs they generated (particularly type of ICs and rejected ICs and epochs) and in the way they affected PLVs. The automated pipeline tended to preserve the original signals by rejecting less ICs and epochs, and produced significantly higher PLVs in the theta frequency band compared to the manual pipeline. Despite this, the automated pipeline tended to contain more ICs labeled as various sources of noise such as "Heart" and "Line Noise", whereas the manual pipeline contained more ICs labeled as ocular artifacts or "Other". No significant differences were found between the two pipelines in terms of interpolated channels, ICs labeled as brain activity, and PLVs computed

Table 4

Correlation matrix (Pearson's r) comparing the PLVs calculated on the theta frequency band after automated and manual pre-processing across episodes and regions.

	Manual PLV theta – Play - Frontal	Manual PLV theta – Play – Central	Manual PLV theta – Reunion - Frontal	Manual PLV theta – Reunion - Central
Automated PLV theta – Play - Frontal	r = 0.746 *** p < 0.001	r = 0.678 *** p < 0.001	r = 0.606 *** p < 0.001	r = 0.571 *** p < 0.001
Automated PLV theta – Play – Central	r = 0.597 *** p < 0.001	r = 0.730 *** p < 0.001	r = 0.488 ** p = 0.005	r = 0.576 *** p < 0.001
Automated PLV theta – Reunion - Frontal	r = 0.528 ** p = 0.002	r = 0.561 ** p = 0.001	r = 0.575 *** p < 0.001	r = 0.592 *** p < 0.001
Automated PLV theta – Reunion - Central	r = 0.513 ** p = 0.003	r = 0.583 *** p < 0.001	r = 0.568 *** p < 0.001	r = 0.750 *** p < 0.001

Note: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001. Correlations between PLVs computed on homologous episodes and regions across pipelines are written in bold.

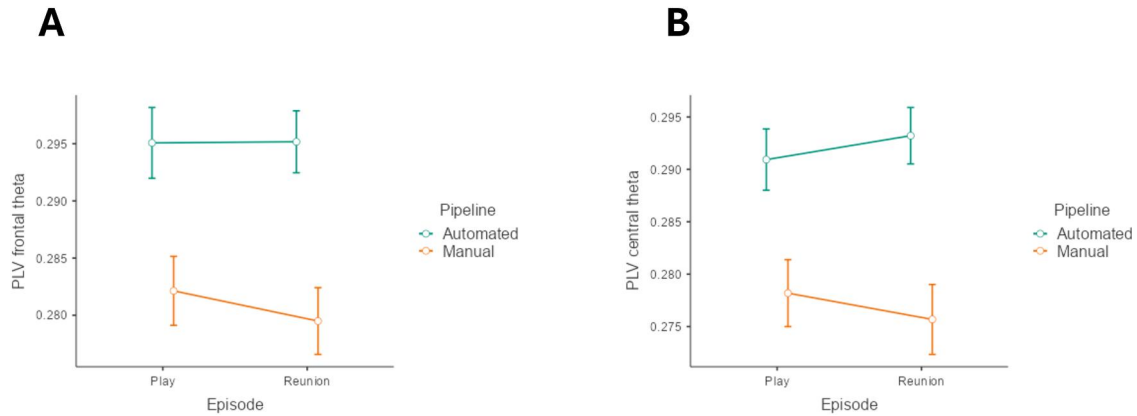


Fig. 6. Trends of the PLVs calculated across episodes (Play, Reunion) in the frontal (A) and central (B) channels in the theta frequency band.

Table 5

Correlation matrix (Pearson's r) comparing the PLVs calculated on the alpha frequency band after automated and manual pre-processing across episodes and regions.

	Manual PLV alpha – Play - Frontal	Manual PLV alpha – Play – Central	Manual PLV alpha – Reunion - Frontal	Manual PLV alpha – Reunion - Central
Automated PLV alpha – Play - Frontal	r = 0.879 *** p < 0.001	r = 0.693 *** p < 0.001	r = 0.613 *** p < 0.001	r = 0.701 *** p < 0.001
Automated PLV alpha – Play – Central	r = 0.650 *** p < 0.001	r = 0.824 *** p < 0.001	r = 0.523 ** p = 0.003	r = 0.733 *** p < 0.001
Automated PLV alpha – Reunion - Frontal	r = 0.597 *** p < 0.001	r = 0.569 *** p < 0.001	r = 0.928 *** p < 0.001	r = 0.709 *** p < 0.001
Automated PLV alpha – Reunion - Central	r = 0.670 *** p < 0.001	r = 0.644 *** p < 0.001	r = 0.690 *** p < 0.001	r = 0.900 *** p < 0.001

Note: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001. Correlations between PLVs computed on homologous episodes and regions across pipelines are written in bold.

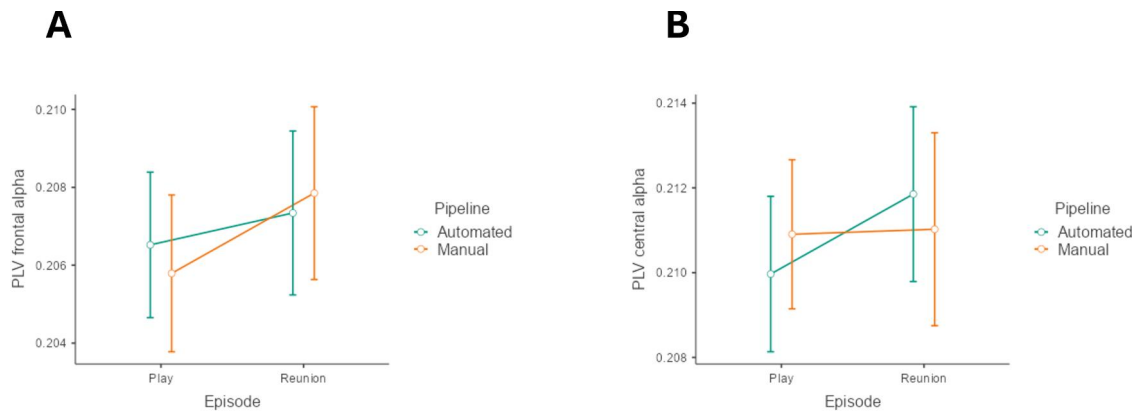


Fig. 7. Trends of the PLVs calculated across episodes (Play, Reunion) in the frontal (A) and central (B) channels in the alpha frequency band.

Table 6

Results of the repeated measures ANOVA accounting for Episode (Play, Reunion) and Pipeline (Automated, Manual) conducted on the theta PLVs for each region of interest (Frontal, Central).

ble		df	F	p-value
PLV theta – Frontal	Episode	1,30	0.618	0.438
	Pipeline	1,30	42.166	< 0.001
	Episode*Pipeline	1,30	2.085	0.159Varia
PLV theta - Central	Episode	1,30	0.003	0.955
	Pipeline	1,30	56.838	< 0.001
	Episode*Pipeline	1,30	5.863	0.022

Note. Significant effects are reported in bold.

Table 7

Results of the repeated measures ANOVA accounting for Episode (Play, Reunion) and Pipeline (Automated, Manual) conducted on the alpha PLVs for each region of interest (Frontal, Central).

Variable		df	F	p-value
PLV alpha – Frontal	Episode	1,30	0.026	0.874
	Pipeline	1,30	0.715	0.404
	Episode*Pipeline	1,30	1.148	0.292
PLV alpha - Central	Episode	1,30	0.505	0.483
	Pipeline	1,30	0.005	0.942
	Episode*Pipeline	1,30	1.550	0.223

in the alpha frequency band. Moreover, PLVs produced by the two pipelines were highly correlated in both frequency bands of interest. These results suggest that phase-based INS estimates such as the PLV seem to be affected, although only partially, by the choice of using a manual or automated pre-processing pipeline.

To the authors' knowledge, this is the first study comparing different methodological decisions during dyadic EEG data pre-processing using real dyadic EEG data including infants. We will break down each result and implication of our findings, to then proceed with analyzing the potential implications and limitations of the present study.

4.1. Differences in pre-processing outputs

Our analyses revealed that the automated pipeline tended to label significantly more ICs labeled as "Muscle" (infants' signals only), "Heart" (infant and parent signals) and "Line Noise" (infants' signals only) compared to the manual pre-processing; conversely, it tended to label significantly less ICs labeled as "Eye" (parents' signals only) and "Other" (infant and parent signals) compared to the manual pipeline. In addition, the automated pipeline rejected significantly less ICs and epochs per episode compared to the manual one. No significant differences across pipelines emerged when looking at the number of interpolated channels and the percentage of ICs labeled as "Brain" activity.

The origin of the differences in the types of ICs produced by the two pre-processing methods is not clear, especially considering that the two pre-processing pipelines have no additional differences apart from their levels of automatization. One possible explanation for this result is that the slight discrepancies between the two pipelines at the bad channel detection and interpolation level, although not statistically significant, could have still determined significant changes in ICA decomposition. Notably, differences in the sets of interpolated channels were more common in the infants' signals ($N = 8$) compared to the parents' signals ($N = 3$), which is consistent with the differences in the IC ratios across pipelines being more frequent in the formers compared to the latter. In the parents' signals with discrepancies in the set of channels interpolated by the two pipelines, the automated pipeline tended to systematically interpolate more channels than the manual one, which could have in turn reduced the global detection rate of some artifactual ICs such as ocular artifacts and those labelled as "Other". The opposite trend was found, however, in the infants' signals with discrepant channel

interpolation across pipelines, since the majority of the signals ($N = 5$) had more interpolated channels in the manual pipeline compared to the automated one (although the remaining three displayed the opposite trend). This could explain, for instance, the tendency of the automated pipeline to detect significantly more artifactual ICs (e.g. muscular, cardiac and "line noise" artifacts) compared to the manual pipeline; despite this, further investigation is needed to assess the impact of channel interpolation on ICA decomposition. This applies especially to the parents' data, since ICLabel as well as ADJUST were validated on an adult population (Leach et al., 2020; Mognon et al., 2011; Pion-Tonachini et al., 2019), whereas discrepancies in the IC classification in infant data are not unlikely due to the structural and functional differences between the infant and the adult brain (Noreika et al., 2020). Although in fact ICLabel was found to have a comparable performance to IC-classification plugins built specifically for infant EEG data such as adjusted-ADJUST (Leach et al., 2020), it is possible for it to underperform when dealing with EEG data recorded on different subjects such as infants (Leach et al., 2020; Pion-Tonachini et al., 2019).

Notably, in the parents' EEG recordings, the automated pipeline was found to label significantly less ICs as ocular artifacts compared to the manual pipeline, whereas this was not true for the infants' EEG data (see par. 3.2.2.). This difference could provide a partial explanation to the tendency of the manual pipeline to reject more ICs in the parents' signals compared to the automated one; however, the majority of the discrepancies in the ICs rejection process between the two pipelines could be easily ascribed to the level of automatization, analogously to the differences in the number of rejected epochs per episode. In addition, the manual pipeline implemented the use of the ADJUST plugin to support the coder in ICs rejection, which is trained not only to reject eye blinks and vertical and horizontal eye movements, but also to detect generic discontinuities in the signals (e.g. impedance fluctuations or electronic devices interferences; Mognon et al., 2011). It is plausible to think that these characteristics of the ADJUST plugin may have constituted an additional reason behind the tendency of the manual pipeline to reject significantly more ICs than the automated pre-processing.

Overall, these findings suggest that the degree of automatization of a dual EEG pre-processing pipeline has a significant impact on the type of extracted ICs and, consequently, on its capacity to detect and reject a various number of artifacts such as motion-related artifacts, eye movements and general discontinuities of the EEG signals. An automated pre-processing method has been found to be more conservative of the original signal properties and thus reject less ICs and epochs compared to a human coder, who can be more prone to reject ICs and epochs that display general discontinuities or motion-related artifacts. While these findings provide insights that could aid researchers in choosing between a manual or automated pre-processing method, it does not provide evidence on the ideal thresholds and parameters that should be used when processing EEG data recorded on parent-infant dyads. Considering that this is the first study comparing the performance of two different dual EEG data pre-processing methods, however, further evidence is needed to support these findings and their respective interpretations.

4.2. Pipeline-dependent changes in PLVs

Together with their differences in terms of rejected ICs and epochs, the automated and the manual pipelines seemed to affect cross-frequency PLVs in different fashions. Repeated measures ANOVA revealed that theta PLVs computed in the frontal (Fig. 6A) and central (Fig. 6B) channels after automated pre-processing were significantly higher than those computed after the manual pre-processing: However, no significant differences were displayed by the alpha PLVs (both in frontal and central regions) between the two pipelines; moreover, Pearson's correlations among theta and alpha PLVs calculated after automated and manual pre-processing in homologous episodes (Play, Reunion) and regions (Frontal, Central) were all positive, strong and significant.

PLV is one of the most widely used measures of INS in EEG hyperscanning research due to its aptness at capturing the dynamic flow of information between two social agents during interactions; despite this, the literature provides evidence on the proneness of this index to spurious hyperconnections (i.e. between non-matched participants; Burgess, 2013) and on its tendency to inflate with shorter epoching (Zimmermann et al., 2023). While our results cannot provide a direct support to these claims, it is clear that the choice of an automated or manual pre-processing pipeline has, to some degree, affected our INS estimates, particularly those that were computed on the infant and adult theta frequency band. We propose two potential explanations as to why only the theta PLVs displayed a significant effect of the Pipeline factor.

Framework 1 - contamination from ocular artifacts. In our study, the automated and the manual pipelines were made to reject ICs that showcased a high probability (50 % or more) of being an ocular artifact. According to our analyses, the manual pipeline tended to label significantly more ICs as ocular artifacts in the parents' signals and, in general, rejected significantly more ICs in both infants and parents compared to the automated one. Notably, previous works assessing ocular activity in the adult brain have consistently detected these types of artifacts in the lower frequencies, including adult delta (0.5–4 Hz) and theta (4–7 Hz; Gasser et al., 1985; Kanoga et al., 2016), but not alpha. It is thus possible that, contrary to the alpha PLVs, the PLVs computed in the theta frequency band were inflated by some leftover ocular artifacts in the parents' signals that remained undetected by ICLabel during automated, but not manual, pre-processing.

Framework 2 - different signal conservation rates. According to our analyses, the manual pipeline tended to reject significantly more ICs and epochs in both members of the dyad (infant and parent) compared to the automated one. While this could have implied that the manual pipeline had a lower probability than the automated one to contain residual noise – e.g. ocular artifacts – that could have inflated PLVs in the theta band, it could have also inadvertently removed portions of the signal that displayed theta oscillations, thus leading to lower PLVs in this frequency band compared to those computed after automated pre-processing. Notably, while ICA is a reliable method to separate brain sources from stereotypical artifacts (e.g. eye movement, muscle artifacts), mixed ICs containing both brain and artifactual data are not uncommon (Winkler et al., 2015), including ICs with mixed brain and ocular sources. Consequently, it is possible that the manual pipeline, being stricter in terms of ICs and epoch rejection, could have rejected more portions of the signal in which vertical and horizontal eye movements were mixed with theta oscillations stemming from brain activity, something that may not have happened with alpha oscillations due to the location of ocular artifacts at lower frequencies.

Comprehensively, our findings seem to suggest that choosing an automated or manual pre-processing method, although equal in terms of order of the specific steps and parameters chosen to minimize artifacts, has a significant impact on phase-based INS measures such as the PLV. However, as we have no access to the "ground truth" PLVs of our datasets, we have no means to assess whether one pipeline was more fitting than the other for dealing with dyadic EEG data. Moreover, as there is limited evidence regarding the impact of the degree of automatization of EEG data pre-processing pipelines on INS estimates, EEG technicians and other experts in the field are encouraged to take a critical approach to the proposed interpretations and to test the reproducibility of our findings through further methodological studies. It is also recommended that future studies make comparisons not only between pipelines with different degrees of automatization, but also between pipelines with different pre-processing steps and thresholds in order to determine how different pipelines impact the signal quality and, subsequently, INS estimates in dyadic EEG data.

4.3. Towards the use of automated pre-processing pipelines for dyadic EEG data processing

Overall, the significant differences revealed by our analyses in the theta PLVs obtained after automated and manual pre-processing suggest the need for further research and systematization of EEG data pre-processing collected in parent-infant dyads. A potential path towards this endeavor could be the employment of fully automatized dyadic EEG pre-processing methods accounting for the structural and functional differences between the adult and the infant brain. Employing an automated pipeline for EEG data pre-processing would have several advantages such as increased rapidity of analysis and enhanced reproducibility of the findings. Rapidity of analysis is a significant perk when dealing with dual EEG data, especially when dealing with large datasets: manually pre-processing one dyad, especially with pipelines containing highly time-consuming steps such as ICA decomposition and epoch rejection, may take up to one hour per dyad, whereas our automated pre-processing pipeline, for instance, pre-processes at least two more dyads in the same time frame. This would allow EEG hyperscanning researchers not only to obtain the results of their data collections in a reasonable time frame, but also to make significant advances on the field of EEG data pre-processing methodology by exploring the impact of different pre-processing parameters on the signals. Regarding reproducibility, it is clear that a fully automated pre-processing pipeline, with parameters and thresholds set in advance, is less prone to subjective decision making compared to a manual pipeline requiring an expert coder for IC and epoch rejection. Employing a highly reproducible pipeline may be an invaluable asset in hyperscanning research, especially when considering the dearth of reproducibility studies addressing data analysis techniques in this field of research (Kayhan et al., 2022). However, we recognize that this study represents nothing more than a small contribution towards this endeavor. Notably, we have no means to reliably assess whether our automated pipeline improved SNR in a significantly more adequate way compared to our manual pre-processing; in addition, we do not know whether our findings regarding automated and manual pre-processing pipelines can be replicated with INS indexes based on different signal properties such as power amplitude (e.g. amplitude envelope coupling). Phase and amplitude are indeed known for being inter-independent properties of the EEG signal, and although there are methodological works challenging this claim (Zimmermann et al., 2023), it remains important to assess whether phase and amplitude-based INS indexes are affected in similar or different ways by the type of pre-processing method and by different steps and thresholds. We therefore highlight the need for further evidence regarding the feasibility of fully automated pre-processing in developmental EEG hyperscanning studies with the use of control measures such as SNR and power analysis in the frequency bands of interest in the field such as theta and alpha, as well as the investigation of the impact of different pre-processing methods on INS indexes based on other signal properties such as amplitude.

5. Study Limitations

Despite the presented findings and implications, the present study has notable limitations, including the exclusive use of the PLV as INS index, the absence of quality assessment on the signal modified by the two pipelines such as SNR on the selected frequency bands, lack of control measures of our INS synchrony of choice (PLV), and the absence of comparison of our automated pipeline with other automated and open-source pipelines (e.g. DEEP, HAPPE, MADE).

5.1. Usage of one INS index as outcome measure

As previously mentioned in the paper, the only INS index used as a metric to understand the impact of different pre-processing methods on dyadic EEG data was the PLV, calculated as co-variance of phase

between the activity recorded on the parent and the infant on homologous channels. This constitutes a significant limitation to our findings, especially considering that an increasing number of hyperscanning studies is employing other and more robust measures of INS such as the Circular Correlation (CCorr; Burgess, 2013), the Partial Directed Coherence (PDC; Leong et al., 2017; Santamaria et al., 2020) and the Amplitude-Amplitude Coupling using the envelope (AAC; Koul et al., 2023; Palva and Palva, 2018). While the CCorr is a phase-based INS estimate like the PLV and measures the circular co-variance between the observed and the expected phase of the two signals (Burgess, 2013), PDC and AAC are based on different signal properties and theoretical assumptions. While all these indexes are optimized for minimizing spurious correlations, we cannot exclude that the use of different methodological choices may impact these indexes differently as seen in the paper of Zimmermann et al. (2023). Future studies comparing different dual EEG data pre-processing methods should therefore use at least two INS indexes as outcome measures, possibly based on different signal properties (phase, amplitude) and/or different theoretical assumptions (directional vs non-directional synchronization).

5.2. No quality assessment of the pre-processed signal (SNR)

A second limitation to our findings is the fact that, while we have performed a quality check of the raw signals through computation of SNR, we did not assess potential differences on the degree in which SNR was improved after automated and after manual pre-processing. While we can assume that both pipelines have increased SNR from its baseline levels through filtering and artifacts rejection, the literature does not provide any standardized procedure on how to reliably estimate SNR in dyadic EEG data after signal pre-processing due to a lack of consensus of what portions of data should be defined as “noise”. Considering how SNR has been found to positively impact phase-based INS measures such as PLV and CCorr in Zimmermann and colleagues’ methodological work (Zimmermann et al., 2023), as well as how failure to properly conduct some of the earliest pre-processing steps such as data filtering, artifact rejection and re-referencing may lead to lower SNR due to the introduction of new artifactual data into the signal (Chiarion et al., 2023), we posit that computation of SNR should be implemented as one of the control measures of signal quality before and after pre-processing, together with other procedures such as dyads shuffling aimed at ensuring that the synchrony values obtained after data processing reflect interaction-driven neural synchronization rather than, for instance, sensorimotor communication (Pezzulo et al., 2013; Pezzulo et al., 2019). Advantages of SNR computation after data pre-processing include a better understanding of how different methodological decisions could affect signal quality in both adult and infant EEG, which in turn would promote an informed standardization of dyadic EEG data pre-processing routines.

5.3. No control measures on the INS index of choice

While the present study was not aimed at validating the PLV as the gold standard for mother-infant INS studies, it is important to point out that we did not implement control measures such as dyads shuffling to reliably assess the nature of the INS patterns reported in this work. It has been indeed argued that, while it has been extensively used to estimate INS during parent-infant interactions (Atilla et al., 2023; Atzaba-Poria et al., 2017; Leong et al., 2017; Krzeczkowski et al., 2022; Endevelt-Shapira et al., 2021; Leong et al., 2019; Perone et al., 2020; Santamaria et al., 2020; Wass et al., 2018), PLV could rather reflect the similarities in frequency of oscillation between the two subjects or, simply put, the fact that the two members of the dyad are engaged in the same task during neural data acquisition (Burgess, 2013). Notably, the present work includes SNR as a control measure for the adequacy of our dataset for PLV computation; despite this, usage of control measures such as dyads shuffling in both methodological works and dyadic EEG

hyperscanning studies is highly recommended.

5.4. No comparison with other validated pipelines (DEEP, HAPPE, MADE)

It is also important to note that the performance of our automated and manual pipelines was not compared to the one of other open-source pre-processing methods such as DEEP (Kayhan et al., 2022), MADE (Debnath et al., 2020) or HAPPE (Gabard-Durnam et al., 2018). By comparing the performance of multiple pre-processing pipelines, it would have been possible to assess more precisely which steps of the pre-processing make the most significant difference in INS estimates such as PLV or other indexes based on other signal properties.

6. Conclusions

Despite these limitations, the present study delivers preliminary evidence on the importance of the choice of the pre-processing method when analyzing dyadic EEG data, and highlights once more the need for standardization of the EEG data pre-processing methods in developmental hyperscanning research in terms of degree of automatization of noise correction, as well as order of the steps and their specific thresholds. To achieve this, it is recommended that EEG experts and developmental neuroscientists conduct further methodological studies on dyadic EEG data pre-processing methods, something that could potentially lead establishment of best practices and the creation of validated and reproducible automated or semi-automated pre-processing tools. Furthermore, we highlight the importance of assessing the impact of different methodological decisions on INS estimates based on other signal properties (e.g. amplitude) or theoretical assumptions (e.g. directional synchronization).

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CRedit authorship contribution statement

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.jneumeth.2025.110400](https://doi.org/10.1016/j.jneumeth.2025.110400).

Data availability

Data will be made available on request.

References

- Atilla, F., Alimardani, M., Kawamoto, T., Hiraki, K., 2023. Mother-child inter-brain synchrony during a mutual visual search task: a study of feedback valence and role. *Soc. Neurosci.* 18 (4), 232–244. <https://doi.org/10.1080/17470919.2023.2228545>.
- Atzaba-Poria, N., Deater-Deckard, K., Bell, M.A., 2017. Mother-child interaction: links between mother and child frontal electroencephalograph asymmetry and negative behavior. *Child Dev.* 88 (2), 544–554. <https://doi.org/10.1111/cdev.12583>.
- Ayrolles, A., Brun, F., Chen, P., Djalovski, A., Beauxis, Y., Delorme, R., Bourgeron, T., Dikker, S., Dumas, G., 2021. HyPyP: a hyperscanning python pipeline for inter-brain connectivity analysis. *Soc. Cogn. Affect. Neurosci.* 16 (1–2), 72–83. <https://doi.org/10.1093/scan/nsaa141>.
- Babiloni, F., Babiloni, C., Carducci, F., Romani, G.L., Rossini, P.M., Angelone, L.M., Cincotti, F., 2004. Multimodal integration of EEG and MEG data: a simulation study with variable signal-to-noise ratio and number of sensors. *Hum. Brain Mapp.* 22 (1), 52–62. <https://doi.org/10.1002/hbm.20011>.
- Bevilacqua, D., Davidesco, I., Wan, L., Chaloner, K., Rowland, J., Ding, M., Poeppel, D., Dikker, S., 2019. Brain-to-brain synchrony and learning outcomes vary by student-teacher dynamics: evidence from a real-world classroom electroencephalography study. *J. Cogn. Neurosci.* 31 (3), 401–411. https://doi.org/10.1162/jocn_a_01274.
- Burgess, A.P., 2013. On the interpretation of synchronization in EEG hyperscanning studies: a cautionary note. *Front. hum. neurosci.* 7 (881).
- Chang, C.Y., Hsu, S.H., Pion-Tonachini, L., Jung, T.P., 2018. Evaluation of artifact subspace reconstruction for automatic EEG artifact removal (July). In 2018 40th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). IEEE, pp. 1242–1245. <https://doi.org/10.1109/EMBC.2018.8512547> (July).
- Chang, C.Y., Hsu, S.H., Pion-Tonachini, L., Jung, T.P., 2019. Evaluation of artifact subspace reconstruction for automatic artifact components removal in multi-channel EEG recordings. *IEEE Trans. Biomed. Eng.* 67 (4), 1114–1121. <https://doi.org/10.1109/TBME.2019.2930186>.
- Chiarion, G., Sparacino, L., Antonacci, Y., Faes, L., Mesin, L., 2023. Connectivity analysis in EEG data: a tutorial review of the state of the art and emerging trends. *Bioengineering* 10 (3), 372. <https://doi.org/10.3390/bioengineering10030372>.
- Czeszumski, A., Eustergering, S., Lang, A., Menrath, D., Gerstenberger, M., Schuberth, S., Schreiber, F., Rendon, Z.Z., König, P., 2020. Hyperscanning: a valid method to study neural inter-brain underpinnings of social interaction. *Front. Hum. Neurosci.* 14, 39. <https://doi.org/10.3389/fnhum.2020.00039>.
- Debnath, R., Buzzell, G.A., Morales, S., Bowers, M.E., Leach, S.C., Fox, N.A., 2020. The Maryland analysis of developmental EEG (MADE) pipeline. *Psychophysiology* 57 (6), e13580. <https://doi.org/10.1111/psyp.13580>.
- Delorme, A., Makeig, S., 2004. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J. Neurosci. Methods* 134 (1), 9–21. <https://doi.org/10.1016/j.jneumeth.2003.10.009>.
- van der Velde, B., Haartsen, R., Kemner, C., 2019. Test-retest reliability of EEG network characteristics in infants. *Brain Behav.* 9 (5), e01269. <https://doi.org/10.1002/brb3.1269>.
- Dimigen, O., 2020. Optimizing the ICA-based removal of ocular EEG artifacts from free viewing experiments. *NeuroImage* 207, 116117. <https://doi.org/10.1016/j.neuroimage.2019.116117>.
- Donoghue, T., Schaworonkow, N., Voytek, B., 2021. Methodological considerations for studying neural oscillations. *Nat. Neurosci.* 24 (11), 1541–1549. <https://doi.org/10.1038/s41593-021-00961-0>.
- Dumas, G., 2011. Towards a two-body neuroscience. *Commun. Integr. Biol.* 4 (3), 349–352. <https://doi.org/10.4161/cib.4.3.15110>.
- Endevelt-Shapira, Y., Djalovski, A., Dumas, G., Feldman, R., 2021. Maternal chemosignals enhance infant-adult brain-to-brain synchrony. *Sci. Adv.* 7 (50), eabg6867. <https://doi.org/10.1126/sciadv.abg6867>.
- Gabard-Durnam, L.J., Mendez Leal, A.S., Wilkinson, C.L., Levin, A.R., 2018. The Harvard Automated Processing Pipeline for Electroencephalography (HAPPE): standardized processing software for developmental and high-artifact data. *Front. Neurosci.* 12, 316496. <https://doi.org/10.3389/fnins.2018.00097>.
- Gartstein, M.A., 2020. Frontal electroencephalogram (EEG) asymmetry reactivity: exploring changes from baseline to still face procedure response. *Int. J. Behav. Dev.* 44 (3), 193–204. <https://doi.org/10.1177/0165025419850899>.
- Gasser, T., Sroka, L., Möcks, J., 1985. The transfer of EOG activity into the EEG for eyes open and closed. *Electroencephalogr. Clin. Neurophysiol.* 61 (2), 181–193. [https://doi.org/10.1016/0013-4694\(85\)91058-2](https://doi.org/10.1016/0013-4694(85)91058-2).
- Goldenholz, D.M., Ahlfors, S.P., Hämäläinen, M.S., Sharon, D., Ishitobi, M., Vaina, L.M., Stufflebeam, S.M., 2009. Mapping the signal-to-noise-ratios of cortical sources in magnetoencephalography and electroencephalography. *Hum. Brain Mapp.* 30 (4), 1077–1086. <https://doi.org/10.1002/hbm.20571>.
- Goldstein, P., Weissman-Fogel, I., Dumas, G., Shamay-Tsoory, S.G., 2018. Brain-to-brain coupling during handholding is associated with pain reduction. *Proc. Natl. Acad. Sci. USA* 115 (11), E2528–E2537. <https://doi.org/10.1073/pnas.1703643115>.
- Haartsen, R., van der Velde, B., Jones, E.J., Johnson, M.H., Kemner, C., 2020. Using multiple short epochs optimises the stability of infant EEG connectivity parameters. *Sci. Rep.* 10, 1–13. <https://doi.org/10.1038/s41598-020-68981-5>.
- Hill, K.E., Neo, W.S., Hernandez, A., Hamrick, L.R., Kelleher, B.L., Foti, D., 2020. Intergenerational transmission of frontal alpha asymmetry among mother-infant dyads. *Biol. Psychiatry.: Cogn. Neurosci. Neuroimaging* 5 (4), 420–428. <https://doi.org/10.1016/j.bpsc.2019.12.003>.
- Hirata, M., Ikeda, T., Kikuchi, M., Kimura, T., Hiraishi, H., Yoshimura, Y., Asada, M., 2014. Hyperscanning MEG for understanding mother-child cerebral interactions. *Front. Hum. Neurosci.* 8, 118. <https://doi.org/10.3389/fnhum.2014.00118>.
- Kanoga, S., Nakanishi, M., Mitsukura, Y., 2016. Assessing the effects of voluntary and involuntary eyeblinks in independent components of electroencephalogram. *Neurocomputing* 193, 20–32. <https://doi.org/10.1016/j.neucom.2016.01.057>.
- Kayhan, E., Matthes, D., Haresign, I.M., Bánki, A., Michel, C., Langeloh, M., Wass, S., Hoehl, S., 2022. DEEP: A dual EEG pipeline for developmental hyperscanning studies. *Dev. Cogn. Neurosci.* 54, 101104. <https://doi.org/10.1016/j.dcn.2022.101104>.
- Koul, A., Ahmar, D., Iannetti, G.D., Novembre, G., 2023. Spontaneous dyadic behavior predicts the emergence of interpersonal neural synchrony. *NeuroImage* 277, 120233. <https://doi.org/10.1016/j.neuroimage.2023.120233>.
- Krzeczkowski, J.E., Van Lieshout, R.J., Schmidt, L.A., 2022. Transacting brains: testing an actor-partner model of frontal EEG activity in mother-infant dyads. *Dev. Psychopathol.* 34 (3), 969–980. <https://doi.org/10.1017/S0954579420001558>.
- Kumaravel, V.P., Farella, E., Parise, E., Buiatti, M., 2022. NEAR: an artifact removal pipeline for human newborn EEG data. *Dev. Cogn. Neurosci.* 54, 101068. <https://doi.org/10.1016/j.dcn.2022.101068>.
- Lachaux, J.P., Rodriguez, E., Martinerie, J., Varela, F.J., 1999. Measuring phase synchrony in brain signals. *Hum. Brain Mapp.* 8 (4), 194–208. [https://doi.org/10.1002/\(SICI\)1097-0193\(1999\)8:4<194::AID-HBM4>3.0.CO;2-C](https://doi.org/10.1002/(SICI)1097-0193(1999)8:4<194::AID-HBM4>3.0.CO;2-C).
- Leach, S.C., Morales, S., Bowers, M.E., Buzzell, G.A., Debnath, R., Beall, D., Fox, N.A., 2020. Adjusting ADJUST: optimizing the ADJUST algorithm for pediatric data using geodesic nets. *Psychophysiology* 57 (8), e13566. <https://doi.org/10.1111/psyp.13566>.
- Leong, V., Byrne, E., Clackson, K., Georgieva, S., Lam, S., Wass, S., 2017. Speaker gaze increases information coupling between infant and adult brains. *Proc. Natl. Acad. Sci.* 114 (50), 13290–13295. <https://doi.org/10.1073/pnas.1702493114>.
- Leong, V., Noreika, V., Clackson, K., Georgieva, S., Brightman, R., Nutbrown, R., Fujita, S., Neale, D., & Wass, S. (2019). Mother-infant interpersonal neural connectivity predicts infants' social learning. Preprint.
- Marshall, P.J., Bar-Haim, Y., Fox, N.A., 2002. Development of the EEG from 5 months to 4 years of age. *Clin. Neurophysiol.* 113 (8), 1199–1208. [https://doi.org/10.1016/s1388-2457\(02\)00163-3](https://doi.org/10.1016/s1388-2457(02)00163-3).
- mBrainTrain. (2024). Smarting Wireless EEG. Retrieved from (<https://mbraintrain.com/smarting-wireless-eeeg/>).
- Miller, J.G., Vrtička, P., Cui, X., Shrestha, S., Hosseini, S.H., Baker, J.M., Reiss, A.L., 2019. Inter-brain synchrony in mother-child dyads during cooperation: an fNIRS hyperscanning study. *Neuropsychologia* 124, 117–124. <https://doi.org/10.1016/j.neuropsychologia.2018.12.021>.
- Mognon, A., Jovicich, J., Bruzzone, L., Buiatti, M., 2011. ADJUST: an automatic EEG artifact detector based on the joint use of spatial and temporal features. *Psychophysiology* 48 (2), 229–240. <https://doi.org/10.1111/j.1469-8986.2010.01061.x>.
- Montague, P.R., Berns, G.S., Cohen, J.D., McClure, S.M., Pagnoni, G., Dhamala, M., Wiest, M.C., Karvov, I., King, R.D., Apple, N., Fisher, R.E., 2002. Hyperscanning: simultaneous fMRI during linked social interactions. *NeuroImage* 16 (4), 1159–1164. <https://doi.org/10.1006/nimg.2002.1150>.
- Montrosso, R., Borgatti, R., Trojan, S., Zanini, R., Tronick, E., 2010. A comparison of dyadic interactions and coping with still-face in healthy pre-term and full-term infants. *Br. J. Dev. Psychol.* 28 (2), 347–368. <https://doi.org/10.1348/026151009X416429>.
- Nguyen, T., Bánki, A., Markova, G., Hoehl, S., 2020. Studying parent-child interaction with hyperscanning. *Prog. Brain Res.* 254, 1–24. <https://doi.org/10.1016/bs.pbr.2020.05.003>.
- Noreika, V., Georgieva, S., Wass, S., Leong, V., 2020. 14 challenges and their solutions for conducting social neuroscience and longitudinal EEG research with infants. *Infant Behav. Dev.* 58, 101393. <https://doi.org/10.1016/j.infbeh.2019.101393>.
- Oostenveld, R., Fries, P., Maris, E., Schoffelen, J.M., 2011. FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput. Intell. Neurosci.* 2011 (1), 156869. <https://doi.org/10.1155/2011/156869>.
- Palva, J.M., Palva, S., 2018. Functional integration across oscillation frequencies by cross-frequency phase synchronization. *Eur. J. Neurosci.* 48 (7), 2399–2406. <https://doi.org/10.1111/ejn.13767>.
- Perone, S., Gartstein, M.A., Anderson, A.J., 2020. Dynamics of frontal alpha asymmetry in mother-infant dyads: Insights from the Still Face Paradigm. *Infant Behav. Dev.* 61, 101500. <https://doi.org/10.1016/j.infbeh.2020.101500>.
- Pezzulo, G., Donnarumma, F., Dindo, H., 2013. Human sensorimotor communication: a theory of signaling in online social interactions. *PLoS One* 8 (11), e79876. <https://doi.org/10.1371/journal.pone.0079876>.
- Pezzulo, G., Donnarumma, F., Dindo, H., D'Ausilio, A., Konvalinka, I., Castelfranchi, C., 2019. The body talks: sensorimotor communication and its brain and kinematic signatures. *Phys. Life Rev.* 28, 1–21. <https://doi.org/10.1016/j.plrev.2018.06.014>.
- Pion-Tonachini, L., Kreutz-Delgado, K., Makeig, S., 2019. ICLABEL: an automated electroencephalographic independent component classifier, dataset, and website. *NeuroImage* 198, 181–197. <https://doi.org/10.1016/j.neuroimage.2019.05.026>.
- Roberti, E., Capelli, E., Provenzi, L., 2023. Envisioning translational hyperscanning: how applied neuroscience might improve family-centered care. *Soc. Cogn. Affect. Neurosci.* 18 (1), nsac061. <https://doi.org/10.1093/scan/nsac061>.
- RStudio Team, 2023. RStudio: Integrated Development for R. RStudio, PBC, Boston, MA (URL <http://www.rstudio.com/>).

- Santamaria, L., Noreika, V., Georgieva, S., Clackson, K., Wass, S., Leong, V., 2020. Emotional valence modulates the topology of the parent-infant inter-brain network. *NeuroImage* 207, 116341. <https://doi.org/10.1016/j.neuroimage.2019.116341>.
- Swider-Cios, E., Turk, E., Levy, J., Beeghly, M., Vroomen, J., van den Heuvel, M.I., 2024. The association of maternal-infant interactive behavior, dyadic frontal alpha asymmetry, and maternal anxiety in a smartphone-adapted still face paradigm. *Dev. Cogn. Neurosci.* 66, 101352. <https://doi.org/10.1016/j.dcn.2024.101352>.
- The jamovi project (2024). jamovi (Version 2.5) [Computer Software]. Retrieved from (<https://www.jamovi.org>).
- The Mathworks Inc. (2024). MATLAB version: 9.13.0 (R2024a). Natick, Massachusetts: The Mathworks Inc. Retrieved from (<https://www.mathworks.com>).
- Toppi, J., Borghini, G., Petti, M., He, E.J., De Giusti, V., He, B., Astolfi, L., Babiloni, F., 2016. Investigating cooperative behavior in ecological settings: an EEG hyperscanning study. *PloS One* 11 (4), e0154236. <https://doi.org/10.1371/journal.pone.0154236>.
- Tronick, E., Als, H., Adamson, L., Wise, S., Brazelton, T.B., 1978. The infant's response to entrapment between contradictory messages in face-to-face interaction. *J. Am. Acad. Child Psychiatry* 17 (1), 1–13. [https://doi.org/10.1016/s0002-7138\(09\)62273-1](https://doi.org/10.1016/s0002-7138(09)62273-1).
- Turk, E., Endevelt-Shapira, Y., Feldman, R., van den Heuvel, M.I., Levy, J., 2022. Brains in sync: practical guideline for parent–infant EEG during natural interaction. *Front. Psychol.* 13, 833112. <https://doi.org/10.3389/fpsyg.2022.833112>.
- Wass, S.V., Noreika, V., Georgieva, S., Clackson, K., Brightman, L., Nutbrown, R., Santamaria, L., Leong, V., 2018. Parental neural responsivity to infants' visual attention: How mature brains influence immature brains during social interaction. *PLoS Biol.* 16 (12), e2006328. <https://doi.org/10.1371/journal.pbio.2006328>.
- Widmann, A., Schröger, E., Maess, B., 2015. Digital filter design for electrophysiological data—a practical approach. *J. Neurosci. Methods* 250, 34–46. <https://doi.org/10.1016/j.jneumeth.2014.08.002>.
- Winkler, I., Debener, S., Müller, K.R., Tangermann, M., 2015. On the influence of high-pass filtering on ICA-based artifact reduction in EEG-ERP (August). In 2015 37th annual international conference of the IEEE engineering in medicine and biology society (EMBC). IEEE, pp. 4101–4105. <https://doi.org/10.1109/EMBC.2015.7319296> (August).
- Zimmermann, M., Schultz-Nielsen, K., Dumas, G., Konvalinka, I., 2023. Arbitrary methodological decisions skew inter-brain synchronization estimates in hyperscanning-EEG studies. Preprint. <https://doi.org/10.31234/osf.io/h8gx2>.

Follow the leader and shift: Changes in Parent-Infant Partial Directed Coherence after a Perturbation of Contingent Interaction

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ABSTRACT

Background. Biobehavioral co-regulation processes with the caregiver shapes infants' socio-cognitive and emotional development. Parents and infants are believed to co-regulate their neural activity during social interactions, a phenomenon known as interpersonal neural synchrony (INS). While INS is expected to be predominantly of the mutual kind, we hypothesize that an interactive perturbation might change the structure of the dyadic INS, leading it to become directed/sequential.

Methods. A sample of 48 parent-infant dyads (infant mean age 9 months) engaged in an adapted Face-to-Face Still-Face (FFSF) procedure while their brain activity was recorded via the electroencephalograph (EEG) hyperscanning paradigm. Partial directed coherence (PDC) in the *alpha* and *theta* bands was measured both in the infant-to-parent (I->P) and parent-to-infant (P->I) directions. Infant negative emotionality and gaze aversion were coded and quantified as percentage of time across the FFSF, as well as percentage of maternal affective touch during Play and Reunion.

Results. *Alpha* PDC was predominantly infant-led during both Play and Reunion; however, a significant reduction in the number of I->P connections was observed in the subgroup of dyads with high infant negative emotionality. *Theta* PDC was also predominantly infant-led during Play, while in the Reunion, dyads displaying high infant gaze aversion or high maternal affective touch displayed a significant increase in the number of P->I-dominated inter-neural connections.

Conclusions. Our results suggest that the INS in the caregiver-infant dyad is sensitive to interactive ruptures, so that direction of reciprocal influence is shaped as the dual dynamic system is engaged in regulating perturbations of expected contingencies.

Keywords: EEG; Hyperscanning; Parent; Infant; Interpersonal Neural Synchrony; Still-Face; Partial Directed Coherence

Highlights

- Parents and infants can establish synchrony according to a lead and lag model.
- Dyadic partial directed coherence may change after an interactive perturbation.
- EEG signal of 48 parent-infant dyads was acquired during an adapted FFSF.
- Infant-led and parent-led PDC was computed in *alpha* (6-12 Hz) and *theta* (3-7 Hz).
- Changes in PDC directionality occurred in the high negativity subgroups.

1. INTRODUCTION

In the first thousand days of life, humans are particularly sensitive to the quality of the caregiving environment, which has a crucial role in shaping their lifelong well-being as well as their socio-emotional and cognitive development ([Berretta et al. 2021](#); [Capitani et al. 2023](#); [Feldman 2015](#)). Particularly, the infants' relationship with their primary caregiver (the mother in the majority of modern societies and cultures) is fundamental in promoting social bonding, emotion regulation and communicative abilities ([Bi et al. 2023](#); [Feldman 2007](#)). Starting as early as the third month of life, infants and their caregivers are able to temporally align their behavioral as well as biological states, such as heart rate, hormonal levels and neural activities, reaching what has been called "biobehavioral synchrony" ([Bi et al. 2023](#); [Feldman 2007](#)). While the term "synchrony" seems to allude to a perfect match between the biobehavioral states of the dyad, researchers argue that infants and their parents "synchronize" through cycles of matched and mismatched affective states at a relatively predictable rate ([Provenzi et al. 2018](#)).

This continuous cycle of mismatches and reparations, as well as its association with infant development, can be observed through the Face-to-Face Still Face Paradigm (FFSF; ([Tronick et al. 1978](#))), which presents an experimentally induced interactive perturbation during a free play interaction between parents and their infants. A meta-analytical work on the "still-face effect" in infants aged 0-9 months ([Mesman et al. 2009](#)) revealed that, from baseline to reunion, infants showcase a significant increase in negative affect and a subsequent decrease in positive affect, but no significant differences in gaze towards the mother and neutral affect. Infant sex and age acted as moderator for positive affect and gaze respectively, with male infants having the strongest decrease in positive affect from baseline to reunion, and infants aged 6-9 months displaying the strongest increase in gaze to the mother from baseline to reunion. These results highlight that, while some dyadic behaviors such as social gaze and positive affect may go through a reparation process after an interactive disruption, infants may also showcase a carryover (still-face) effect even after the end of the interactive perturbation and the resumption of the dyadic exchange. Naturally, maternal behavior also plays a pivotal role in determining

the effectiveness of the reparation process. Several studies have shown that maternal affective touch, which is considered a central component of the global mother-infant communication system and a component of constructs such as maternal sensitivity and reciprocity ([Botero et al. 2020](#); [Endevelt-Shapira and Feldman 2023](#); [Feldman et al. 2010](#)), is associated with reduction of the still-face effect ([Stack and Muir 1990](#); [Stack and Muir 1992](#)). Moreover, maternal affective touch was found to contribute to co-regulation during mother-infant interactions, as well as to attenuate infant stress reactivity ([Feldman et al. 2010](#)) and increase parasympathetic activity ([Madden-Rusnak et al. 2025](#)).

In the last decade, there has been an increasing interest in observing the neurophysiological correlates of biobehavioral synchrony, a construct that has been called Interpersonal Neural Synchrony (INS; [Koul et al. 2023](#)). Dual neural data for INS computation is often acquired using the electroencephalograph (EEG) hyperscanning paradigm, an innovative and child-friendly technique that allows the simultaneous recording of brain activity of two interactive partners both in experimental and ecological settings ([Turk et al. 2022](#)). In the last decade, the body of research on parent-infant EEG hyperscanning has been steadily growing, and highlighted associations between INS and dyadic behaviors such as shared attention during joint play ([Wass et al. 2018](#)), mutual gaze ([Leong et al. 2019](#)), valence of emotional feedback ([Santamaria et al. 2020](#)), as well as between INS and maternal biobehavioral cues such as maternal sensitivity ([Endevelt-Shapira and Feldman 2023](#)), maternal chemosignals ([Endevelt-Shapira et al. 2021](#)), and maternal visual and tactile stimulation ([Neel et al. 2025](#)). Most of these studies have operationalized INS using concurrent, or non-directed, connectivity metrics, which reflect the matching of neurobehavioral states during the social interaction ([Turk et al. 2022](#)). Such metrics include mainly phase-based metrics such as the phase-locking value (PLV; [Lachaux et al. 1999](#)) and the weighted phase-lag index (wPLI; [Vinck et al. 2011](#)); however, concurrent INS can be measured also with amplitude- or coherence-based measures such as Imaginary Coherence ([Dikker et al., 2021](#); [Nolte et al., 2004](#)) and amplitude-amplitude coupling ([Palva and Palva 2018](#)). Although underrepresented in the current literature, sequential (or directed) INS metrics, such as Partial Directed Coherence (PDC; [Baccalá and Sameshima 2021](#)) and Time-lagged cross-correlations ([Wass et al. 2018](#)) are believed to

better reflect the time-lagged nature of dyadic interactions ([Turk et al. 2022](#)). Sequential INS is based on the assumption that the neurobehavioral activity of one member of the dyad is “framing”, or “causing” the neurobehavioral patterns showcased by the other member of the dyad, a pattern that has been observed multiple times in a strictly behavioral level during ecological observational studies ([Cohn and Elmore 1988](#); [Cohn and Tronick 1987](#); [Feldstein et al. 1993](#); [Kaye and Fogel 1980](#)). At the neural level, Santamaria and colleagues ([2020](#)) have observed that, when mothers displayed positive emotionality towards a toy in front of their ten-month-old infants, mother-to-infant directed INS measured via PDC in the *alpha* band (6-9 Hz) was significantly stronger compared to the one measured during displays of negative emotionality. Similarly, Wass and colleagues ([2018](#)) have explored the relationship between parents’ neural responsivity and infant attention focus during joint versus solo play, highlighting how, during joint play, infants were more attentive when their parents were more neurally responsive to them.

Despite its potential to highlight the effect of an interactive perturbation on biobehavioral synchrony, no published EEG hyperscanning study, to date, has assessed INS before and after a FFSF procedure. Considering how the FFSF has been found to disrupt some dyadic behaviors that contribute to both concurrent and sequential INS, such as positive emotionality and attention focus ([Mesman et al. 2009](#)), it would be valuable to explore the effects of this experimental paradigm at the neural level, particularly if and how the Still-Face episode has an impact on the time-lagged dyadic dance that mothers and infants establish during ecological social interactions. In line with previous research on parent-infant sequential behavioral synchrony ([Feldman 2007](#); [Feldman et al. 1996](#); [Feldman et al. 1999](#)) and on the still-face effect (see [Mesman et al. 2009](#) for an overview), we aim at testing the following experimental hypotheses. Our first hypothesis (H1) is that, during the baseline (play) episode of the FFSF procedure, sequential INS between parents and their 9-month-old infants will follow a bidirectional pattern, meaning that we expect no significant differences between the infant-led INS (I->P, infant leading and parent following) and the parent-led INS values (P->I, parent leading and infant following). This hypothesis is supported by previous behavioral studies on sequential parent-infant behavioral

synchrony, which showcased a prevalence of mutual synchrony among parents and infants aged 9 months due to infants reaching the phase of intersubjectivity ([Feldman 2007](#); [Feldman et al. 1996](#); [Feldman et al. 1999](#)). After the interactive perturbation (reunion episode), we hypothesize (H2) that the dyads whose infants showcase higher percentages of negative affect or gaze aversion or whose parents showcased higher percentages of affective touch will display a shift in the direction of INS, going from bidirectional to parent-led (P->I). This hypothesis is based on the assumption that parents whose infants showcase a marked still-face effect, characterized by numerous instances of negative emotionality and/or gaze aversion, will actively try to bring their infant back to a state of positive engagement, thus leading the dyadic interaction both on a behavioral and neural level.

2. METHODS

2.1. Participants

Our sample consisted of 74 infants (age range 8-11 months) and their primary caregivers (their mothers) enrolled as control subjects for the [\[masked for peer review\]](#) project, a longitudinal RCT study assessing the neuroprotective role of an early video-feedback intervention in very preterm infants (see [\[masked for peer review\]](#) for details on the recruitment strategy). Inclusion criteria for this specific group were the following. For infants, gestational age of 37 weeks or more, absence of known medical, genetic, or neurological conditions, and absence of major complications in pregnancy and/or delivery likely to affect brain development. For parents, age of 18 years or more, proficiency in the [\[masked for peer review\]](#) language, absence of psychiatric disorders. All participating dyads were adequately briefed on the experimental procedure and provided informed consent. The informed consent for the infant was provided by both the participating parent and the other parental figure.

2.2. Procedure

The study aligns with the principles of the declaration of Helsinki and was approved by the Ethics Committee of the [\[masked for peer review\]](#) on February 16th, 2023 (protocol number: [\[masked for peer review\]](#)). A graphical summary of the experimental procedure, as well as of the INS and behavioral analyses, is provided in Figure 1.

When the infants reached 9±2 months of age, the participating dyads were invited to one of our labs at [masked for peer review] and [masked for peer review] to engage in an adapted Face-to-Face Still Face Procedure (FFSF; [Tronick et al. 1978](#)) while their brain activity was recorded through the EEG hyperscanning paradigm. The EEG equipment of choice was the Smarting Pro 32, a wireless dual EEG system developed by mBrainTrain (<https://mbraintrain.com/>) which features two EEG caps with 32 embedded channels positioned according to the 10-20 international system and their respective portable amplifiers. Said amplifiers are connected via Bluetooth to two different laptops, one visualizing the signal of the parent and the other the signal of the infant. The two laptops are connected via a LAN cable in order to record the neurophysiological data from a single device (the infant's laptop) and save it in a single file. The sampling rate was set at 500 Hz.

The experimental setting was prepared according to the guidelines provided by ([Turk et al. 2022](#)). After giving some time to the infant to acclimate to the new environment and after illustrating the procedure to the parent, the participants were fitted the EEG caps in the following order: parent first, infant second. Conductive gel was applied on the participants' scalp in order to keep the channel impedances under 20kΩ (10kΩ for the channels of interest, see *2.4. Synchrony analysis*) and ensure good signal quality. As soon as the participants were sitting in front of each other at a distance of approximately 30-50 cm, with the infant sitting on an age-appropriate highchair, the experimental procedure began. The adapted FFSF consisted of the following episodes: Play (2 min), in which the parent was asked to play with their infant "as usual" and possibly without the use of toys; Still-Face (1 min) in which the parent was asked to stop interacting with the infant and look at him/her with an emotionless expression; Reunion (2 min), in which the parent was asked to resume playing with their infant with the same modalities of the Play episode. The FFSF procedure is a validated experimental paradigm which has been extensively used to assess the behavioral ([Jean and Stack 2012](#); [Provenzi et al. 2017](#)) and neurophysiological ([Mantis et al. 2014](#); [Montirosso et al. 2010](#); [Perone et al. 2020](#); [Swider-Cios et al. 2024](#)) correlates of emotion regulation. In light of the previous reports of the significant number of infants unable to complete the task ([Liu et al. 2020](#)), the Still-Face episode, conventionally lasting two minutes, was shortened to one

minute in order to increase the retention rate of the neural and behavioral data. The participants were videotaped through the whole procedure with two video cameras, one pointing towards the infant and the other towards the parent, for offline behavioral coding (see section 2.5. *Behavioral coding*).

Please put Figure 1 here.

2.3. EEG data pre-processing

EEG data pre-processing was performed using an automated ad-hoc pipeline developed in the MATLAB (version R2024a; [The Mathworks 2024a](#)) interacting toolbox EEGLAB ([Delorme and Makeig 2004](#)). The pipeline was built in accordance with previous guidelines for artifact minimization in dual parent-infant EEG data ([Turk et al. 2022](#)) as well as with previously validated instruments such as DEEP ([Kayhan et al. 2022](#)) and HAPPE ([Gabard-Durnam et al. 2018](#)). The pre-processing was the same for the infants' and parents' signals and followed these steps: data filtering with a Hamming windowed sinc FIR bandpass 1-30 Hz filter, flat and outlier channel detection via the Neonatal EEG Artifact Removal (NEAR; [Kumaravel et al. 2022](#)) plugin, noise correction via Artifact Subspace Reconstruction ([Chang et al. 2020](#)) with cutoff parameter $k = 10$, Independent Component Analysis (ICA; Infomax algorithm) and independent component (IC) classification with the ICLabel plugin ([Pion-Tonachini et al. 2019](#)), rejection of ocular ICs ($\geq 50\%$ of probability of the IC being an eye blink or a vertical/horizontal eye movement), bad channel interpolation via the spherical spline method, signal re-referencing to the average reference, data segmentation into the three episodes of the FFSF, data epoching in 1s epochs with no overlap, and automated epoch rejection of all the epochs in which at least one channel of interest displayed a voltage exceeding $\pm 150\mu\text{V}$. Epochs that were rejected in one member of the dyad would be rejected in the other member of the dyad as well in order to maintain the temporal synchronization of the neural data. A more detailed description of the pre-processing pipeline can be found in previous works from our group ([\[masked for peer review\]](#); [\[masked for peer review\]](#)).

Dyads were considered viable for synchrony analysis if they met the following criteria: less than 6 (19%) interpolated channels, 30 or more ($\geq 25\%$) good (merged) epochs per episode. Among the 48 dyads

included in the final sample, the number of interpolated channels ranged [0 4] (mean = 0.90, SD = 1.08) for the parent signals and [0 5] (mean = 1.08, SD = 1.40) for the infant signals, whereas the number of rejected epochs was in average 6.08 (SD = 12.35, range [0 61]) in the Play episode and 9.33 (SD = 16.82, range [0 72]) in the Reunion episode.

2.4. Synchrony analysis

Since our aim was to investigate whether parent-infant INS followed a sequential pattern, we chose to compute INS using the Partial Directed Coherence (PDC; [Baccalá and Sameshima 2021](#)). PDC is the frequency domain equivalent of Granger causality ([Granger 1969](#)) and measures the directed influences between a pair of signals in a multivariate dataset ([Baccalá and Sameshima 2001](#); [Choi and Kim 2018](#); [Santamaria et al. 2020](#)). Assuming that the simultaneously recorded m channel signals $x(t) = [x_1(t), \dots, x_m(t)]^T$ can be described by a multivariate vector autoregressive (MVAR) model as follows:

$$x(t) = \sum_{n=1}^p A_n x(t-n) + e(t) \quad (1)$$

where p is the model order, A_n is the matrix of model coefficients at time lag n , and $e(t) = [e_1(t), \dots, e_m(t)]^T$ is a multivariate Gaussian white noise with mean equal to zero and covariance matrix Σ . Transforming said MVAR model by Fourier Transform (FT), we obtain:

$$\bar{A}(f) = I - A(f) = [\bar{a}_1(f) \bar{a}_2(f) \dots \bar{a}_m] \quad (2)$$

where $A(f)$ is the FT of the model coefficients and $\bar{a}_{i,j}(f)$ is the i, j th element of $\bar{A}(f)$. From the transformed model coefficients, the PDC can be computed as

$$PDC_{i \rightarrow j}(f) = \frac{\bar{a}_{i,j}(f)}{\sqrt{\bar{a}_j^H(f) \bar{a}_j(f)}} \quad (3)$$

where H indicates the transpose and complex conjugate operator. PDC is thus a normalized measure able to quantify the relative strength of the influence of the signal x_i on the signal x_j at frequency f ([Choi and Kim 2018](#); [Santamaria et al. 2020](#)). While it does not have a predetermined range of values, higher

PDC values indicate a higher probability for the connection between signal x_i and signal x_j to be directed (Choi and Kim 2018). When both the feedback and feedforward connections between signal x_i and signal x_j are strong, it is likely that they are influencing each other in a bidirectional fashion (Pereda et al. 2005).

In the present study, PDC was computed through a custom script that calls functions from the MATLAB toolbox Fieldtrip (Oostenveld et al. 2011). To minimize the effect of volume conduction as well as the amount of data contained in the MVAR modeling, inter-neural PDC was computed across a subset of 6 channels, two of which frontal (F3, F4), two central (C3, C4) and the remaining two posterior (P3, P4; see Figure 1). Notably, these channels have yielded significant INS values in previous studies, including those that computed INS using the PDC (Leong et al. 2017; Santamaria et al. 2020). For what concerns the frequencies of interest, we chose to compute PDC in the *theta* and *alpha* frequency bands due to their association with cognitive (Leong et al. 2017; Wass et al. 2018) and socio-emotional processing (Endevelt-Shapira et al. 2021; Endevelt-Shapira and Feldman 2023; Santamaria et al. 2020). To account for the differences in the adult and infant neural rhythms (Noreika et al. 2020) while following the original mathematical formula of the PDC that does not allow the use of a “true” cross-frequency approach to INS (Turk et al. 2022), we chose to consider as *theta* frequency the neural activity comprised between 3 and 7 Hz, and the *alpha* frequency all the neural activity comprised between 6 and 12 Hz. These broadened *theta* and *alpha* thresholds allowed us to include both the adult rhythms (4-7 Hz for *theta* and 8-12 Hz for *alpha*) and the infant rhythms (3-5 Hz for *theta* and 6-9 Hz for *alpha*).

Another important parameter to choose when computing PDC is the model order p . In line with previous literature (Jia 2019; Santamaria et al. 2020), our custom script selects the model order by an iterative process that computes the lowest best fitting value according to the Akaike Information Criterion (AIC; Akaike 1979) and the Bayesian Information Criterion (BIC; Schwarz 1978). In our dataset, the optimal model order for 44 out of 48 dyads was consistently $p = 5$ both during Play and during Reunion, a value consistent with a previous work that computed PDC in a sample of mother-infant dyads (Santamaria et al. 2020). Regarding the remaining four dyads, two ($N = 2$) of them consistently reported a model order

different than 5 in both FFSF episodes ($p = 4$ for one dyad and $p = 6$ for the other), whereas the remaining two ($N = 2$) dyads displayed a different model order across FFSF episodes, with $p = 5$ in the Play episode and $p = 4$ or 7 in the Reunion.

2.5. Behavioral coding

Videos of the FFSF procedure were microanalytically coded for interactive behaviors according to an adaptation of the Parent-Infant Coding Scheme (PICS ver. 4.0; Brambilla et al. 2023, unpublished manual). A complete list of the behaviors of interest is provided in our previously published study protocol [masked for peer review]. Behavioral coding was performed via the Noldus The Observer TX software ([The Observer XT](#)) by two undergraduate students and two research assistants trained in the usage of our coding scheme. Behaviors of interest were coded for each member of the dyad and for each episode and measured as the percentage of time spent showcasing said behavior. For the purposes of this study, we considered only the following behaviors: percentage of infant negative emotionality during the SF episode, percentage of infant gaze aversion during the SF episode, and percentage of maternal affective touch during the Reunion episode. A definition of the behaviors of interest is provided in Supplementary Materials.

2.6. Statistical analysis

Analyses were performed on RStudio 2023.12.1 (Posit team 2024). Due to the Shapiro-Wilk test revealing a non-normal distribution of the variables of interest, non-parametric tests were deemed as more suitable for statistical precision. The following plan of analysis was followed.

Preliminary analyses – behavioral data. To explore potential behavioral changes across the FFSF episodes, a non-parametric repeated measures ANOVA (Friedman test) was run on the three behavioral variables of interest (see above) to test the effect of the Episode (Play, SF, Reunion) factor. Subsequently, in order to explore potential differences in sequential INS (as measured by PDC) according to the percentage of time spent displaying the behaviors of interest, dyads were median-split according to their scores in the following variables: infant gaze aversion during the SF episode, infant

negative emotionality during the SF episode, and maternal affective touch during the Reunion episode. For each of these variables, dyads who displayed scores higher than the median were included in the “high” group, whereas dyads who displayed scores lower than the median were included in the “low” group. The sample size for each behavioral “high” and “low” subgroup was $N = 24$.

Main analyses. For each frequency band and channel combination, an Aligned Rank Transform Analysis of Variance (ART ANOVA; [Wobbrock et al. 2011](#)) was run on the PDC values with the following factors: Episode (Play vs Reunion), Direction (I->P vs P->I) and Behavior (high vs low; one model per behavioral variable). Alongside the effect of the single factors, interaction effects were also explored, including two-way effects (Episode*Direction, Episode*Behavior and Direction*Behavior) as well as the three-way interaction between Episode, Direction and Behavior. ART ANOVA is a statistical test for factorial non-parametric analysis that relies on a pre-processing step that first “aligns” the data for each effect before assigning ranks, averaged when ties occur ([Wobbrock et al. 2011](#)). For the purposes of our study, ART ANOVA was deemed as the most appropriate statistical test for non-parametric samples due to its capacity to handle repeated measures and multivariate study designs ([Wobbrock et al. 2011](#)). In RStudio, ART ANOVA was run using the package ARTool ([Kay et al. 2025](#); [Wobbrock et al. 2011](#)). Results were controlled for multiple comparisons via False Discovery Rate (FDR; Benjamini-Hochberg method), with $\alpha = 0.05$.

Post-hoc analyses. We were also interested in exploring whether the impact of the Episode and the Direction factors (as single factors) was different according to the percentage of time spent by the dyads displaying one of the behaviors of interest. Thus, for each frequency band, channel combination and behavior of interest, we ran Wilcoxon tests for paired samples on each subsample (separately for low and high dyads) in order to compare the PDC values according to the Episode (Play vs Reunion) and then to the Direction (I->P vs P->I). Results were controlled for multiple comparisons via False Discovery Rate (FDR; Benjamini-Hochberg method), with $\alpha = 0.05$. After identifying the number of connections displaying a dominant direction, we ran a two proportions Fisher exact test (fisher.test function in RStudio) in order to explore potential episode-related changes in direction dominance across episodes

(Play vs Reunion). The two-proportions Fisher exact test was performed for each frequency band (*alpha*, *theta*), PDC direction (I->P, P->I) and behavioral subgroup (low and high infant gaze aversion, low and high infant negative emotionality, low and high maternal affective touch).

Sensitivity analyses. The statistical tests performed to assess the changes in parent-infant PDC in function of the FFSF episode and direction of connectivity were previously ran on the whole sample in order to explore potential between episode and between direction changes in PDC regardless of the behavioral group. In addition, we checked the percentage of overlap between the low and high subgroups of the three behaviors of interest to better assess the extent of the interaction between the three behaviors of interest, as well as its impact on parent-infant PDC fluctuations.

2.7. *Data reduction*

Among the 74 dyads, a total of 26 dyads were excluded from the final sample for the following reasons: failure to complete the experimental procedure (N = 12), failure to acquire the neural data on the infant (N = 10), technical issues with the EEG equipment (N = 1) and inadequate EEG signal for synchrony analyses (N = 3; among which N = 2 for 6 or more interpolated channels of interest and N = 1 for less than 30 good epochs per episode). Thus, the final sample consisted of 48 parent-infant dyads.

3. RESULTS

Our final sample included 48 parent-infant dyads (mean infant age at assessment = 9 months and 15 days, range 8-11 months; 28 males and 20 females), all pertaining to the full-term control arm of the study (mean gestational age 39.43 weeks). The participating parents were, in all dyads, the biological mother of the infant (mean parent age at assessment = 34.65 ± 3.74 years). Table 1 provides the central tendency measures of the global PDC values.

Please put Table 1 here.

3.1. Behavioral Still-face effect

The Friedman test revealed a significant effect of the Episode factor for infant gaze aversion ($\chi^2 = 32.2$; $df = 2$; $p < 0.001$) and infant negative emotionality ($\chi^2 = 32.1$; $df = 2$; $p < 0.001$), but not for maternal affective touch (see Figure 2). Post-hoc analyses highlighted a significantly higher percentage of infant gaze aversion during SF compared to both Play (Durbin-Conover statistic = 6.19; $p < 0.001$) and Reunion (Durbin-Conover statistic = 5.70; $p < 0.001$), as well as a significantly lower percentage of infant negative emotionality during Play compared to both SF (Durbin-Conover statistic = 6.56; $p < 0.001$) and Reunion (Durbin-Conover statistic = 5.05; $p < 0.001$). The findings, overall, indicate the presence of a still-face effect in our sample. Supplementary Materials (S1, “Behavioral data”) report additional information regarding the behavioral characteristics of our sample, including the distribution of the dyads in the low and high behavioral subgroups.

Please put Figure 2 here.

3.2. PDC changes in function of episode, direction and behavior

The full results of the ART ANOVA for each frequency band and channel combination are reported in Supplementary Materials (S2, “Full statistical report”, Table S2.1) for both *alpha* and *theta* band. Whenever connections would display significant effects for more than one factor, priority was given to the factor (or interaction factor) with the highest F statistic and lowest p-value (called dominant factor).

3.2.1. Alpha band (6-12 Hz)

The majority of the channel combinations (19/36) had direction as the dominant significant factor ($F \geq 1.973$; $p < 0.05$), followed by episode*gaze aversion (dominant in 4/36 channel combinations; $F \geq 4.971$; $p < 0.05$), episode*direction (dominant in 3/36 channel combinations; $F \geq 4.952$; $p < 0.05$) and episode*negative emotionality (dominant in 2/36 channel combinations; $F \geq 5.174$; $p < 0.05$). PDC in 2/36 channel combinations showed a dominant direct effect of negative emotionality ($F \geq 5.740$; $p < 0.05$), and 1/36 channel combinations showed a dominance of the three-way effect between episode, direction and negative emotionality ($F = 13.28$; $p \leq 0.001$). 5/36 channel combinations showcased no significant effect of any single or interaction factors on PDC. However, after controlling for multiple comparisons, only the single effect of direction maintained statistical significance for 18/36

connections ($F \geq 6.392$; $p_{\text{adj}} < 0.05$), whereas 1/36 connections maintained a significant interaction effect of episode and gaze aversion ($F = 14.471$; $p_{\text{adj}} = 0.045$). The remaining 17 channel combinations showcased no effect of any of the single and interaction effects after controlling for multiple comparisons.

3.2.2. *Theta band (3-7 Hz)*

The majority of the channel combinations (19/36) had direction as the dominant significant factor ($F \geq 0.687$; $p < 0.05$), followed by episode*direction (dominant in 3/36 channel combinations; $F \geq 5.826$; $p < 0.05$) and episode*negative emotionality (dominant in 2/36 channel combinations; $F \geq 6.023$; $p < 0.05$). Significant two-way interaction effects between episode and affective touch (dominant in 1/36 channel combinations; $F = 5.83$; $p = 0.02$) and episode and gaze aversion (dominant in 1/36 channel combinations; $F = 8.74$; $p = 0.005$) have been found, as well as a three-way interaction between episode, direction and all the three behavioral variables (dominant in 1/36 channel combinations for interaction with affective touch, 1/36 for interaction with gaze aversion and 1/36 for interaction with negative emotionality; $F \geq 4.279$; $p < 0.05$). One channel combination displayed a dominance of the single effect of episode ($F = 4.30$; $p = 0.044$), whereas the remaining 6/36 channel combinations showcased no significant effect of any single or interaction factor on PDC. After controlling for multiple comparisons, only the single effect of direction maintained statistical significance for 17/36 channel combinations ($F \geq 6.762$; $p_{\text{adj}} < 0.05$), including 1/36 connections that maintained a significant interaction effect of episode and gaze aversion factors ($F = 14.939$; $p_{\text{adj}} = 0.045$). The remaining 18 channel combinations showcased no effect of any of the single and interaction effects after controlling for multiple comparisons.

3.3. *Inter-episode differences (Play vs Reunion) in PDC in low vs high behavioral subgroups*

3.3.1. *Alpha band (6-12 Hz)*

Among the two available directions (I->P and P->I), P->I is the PDC direction that displays a greater amount of significant episode*behavior interactions. I->P PDC, on the other hand, displays only limited episode*behavior interactions. Inter-episode changes in PDC values in our sample, however, do not

survive control for multiple comparisons via FDR. Statistics are reported in Supplementary Materials (S2, “Full statistical report”, Table S2.2).

Whole sample. In the whole sample of 48 dyads, 1/36 I->P connections showcased a significantly higher *alpha* PDC in the Play episode compared to Reunion ($W = 812$; mean difference = 0.016; $p = 0.021$), whereas 6/36 P->I connections displayed an opposite pattern (stronger PDC in the Reunion compared to Play; $W \leq 395$; mean difference ≤ -0.006 ; $p < 0.05$). After correction for multiple comparisons (FDR), no significant changes in I->P and P->I PDC were observed from Play to Reunion. Results are illustrated in Figure 3.

Gaze aversion. In the subgroup of dyads whose infants displayed low gaze aversion during the SF episode, I->P PDC in the *alpha* band was significantly stronger in the Play episode (compared to Reunion) in five (5/36) channel combinations ($W \geq 227$; mean difference ≥ 0.013 ; $p < 0.05$). The opposite trend, i.e. stronger PDC in the Reunion compared to the Play episode, was found in the P->I direction for 3/36 channel combinations ($W \leq 76$; mean difference ≤ -0.004 ; $p < 0.05$). When considering the subgroup of dyads whose infants displayed high gaze aversion during the SF episode, both I->P and P->I direction showcased stronger PDC in the Reunion compared to the Play episode, although the effect was more evident in the P->I direction (2/36 channel combinations in I->P vs 8/36 channel combinations in P->I PDC; $W \leq 80$; mean difference ≤ -0.01 ; $p < 0.05$). All these effects did not survive FDR correction.

Negative emotionality. In the subgroup of dyads whose infants displayed low negative emotionality during the SF episode, changes in I->P and P->I PDC were very limited. 1/36 I->P connections were significantly stronger in the Play episode compared to Reunion (1/36; $W = 234$; mean difference = 0.028; $p = 0.015$), whereas the opposite pattern was observed for P->I PDC (1/36 connections were significantly stronger in the Reunion episode compared to Play; $W = 79$; mean difference = -0.006; $p = 0.042$). In the subgroup of dyads whose infants displayed high negative emotionality during the SF episode, the effect of episode was more pronounced in P->I PDC compared to I->P. More specifically, 2/36 I->P connections were significantly stronger in Play compared to Reunion ($W \geq 232$; mean difference ≥ 0.017 ; $p < 0.025$), whereas in the P->I direction, PDC was significantly stronger in Reunion

compared to Play in 17/36 channel combinations ($W \leq 79$; mean difference ≤ -0.008 ; $p < 0.05$). All these effects did not survive FDR correction.

Affective touch. In the subgroup of dyads whose parents displayed low percentages of affective touch during the Reunion episode, changes in PDC strength from Play to Reunion were limited both in I->P and P->I directions. 1/36 I->P connections were significantly stronger in the Play compared to the Reunion episode ($W = 63$; mean difference = -0.021 ; $p = 0.012$); however, an equal amount of I->P connections (1/36) was significantly stronger in Reunion compared to Play ($W = 223$; mean difference = 0.020 ; $p = 0.037$). In addition, 2/36 P->I connections were significantly stronger in Reunion compared to Play ($W \leq 78$; mean difference ≤ -0.003 ; $p < 0.05$). In the subgroup of dyads whose parents displayed high percentages of affective touch during the Reunion episode, the effect of episode was more marked in P->I PDC compared to I->P PDC. Particularly, 1/36 I->P connections were significantly stronger in the Play compared to Reunion episode ($W = 227$; mean difference = 0.017 ; $p = 0.027$), whereas for P->I PDC, 9/36 connections were significantly stronger in the Reunion compared to Play episode ($W \leq 81$; mean difference ≤ -0.014 ; $p < 0.05$). All these effects, however, did not survive FDR correction.

Please put Figure 3 here.

3.2. Theta band (3-7 Hz)

Similarly to the *alpha* band, the PDC direction that displays a greater amount of significant episode*behavior interactions is the P->I. Despite this, between-episodes differences in I->P and P->I PDC did not survive FDR correction. Statistics are reported in Supplementary Materials (S2, “Full statistical report”, Table S2.3).

Whole sample. In the whole sample of 48 dyads, 3/36 I->P connections displayed a significantly higher *theta* PDC in the Play episode compared to Reunion ($W \geq 784$; mean difference ≥ 0.01 ; $p < 0.05$), whereas 1/36 I->P ($W = 386$; mean difference = -0.007 ; $p = 0.038$) and 4/36 P->I connections ($W \leq 394$; mean difference ≤ -0.004 ; $p < 0.05$) showcased a significantly stronger PDC in the Reunion compared to Play. After correction for multiple comparisons (FDR), all significant changes in PDC from Play to Reunion lost statistical significance. Results are illustrated in Figure 4.

Gaze aversion. In the subgroup of dyads whose infants displayed low gaze aversion during the SF episode, the effect of episode was significant only in a limited number of I->P connections. More specifically, 4/36 I->P connections showcased a significantly higher PDC in the Play compared to Reunion episode ($W \geq 220$; mean difference ≥ 0.013 ; $p < 0.05$), whereas only 1/36 showcased the opposite pattern (stronger PDC in Reunion compared to Play; $W = 74$; mean difference = -0.009 ; $p = 0.029$). In the subgroup of dyads whose infants displayed high gaze aversion during the SF episode, however, an opposite pattern could be observed: the effect of episode was significant only in a limited amount of P->I connections. Particularly, 7/36 connections displayed stronger PDC in the Reunion compared to the Play episode ($W \leq 78$; mean difference ≤ -0.012 ; $p < 0.05$). All these effects did not survive FDR correction.

Negative emotionality. In the subgroup of dyads whose infants displayed low negative emotionality during the SF episode, significant changes in I->P and P->I PDC were limited. In the I->P direction, 2/36 connections showcased a stronger PDC in the Play compared to Reunion episode ($W \geq 220$; mean difference ≥ 0.016 ; $p < 0.05$), whereas for the P->I direction, 1/36 connections showcased a significantly stronger PDC in the Play compared to Reunion ($W = 231$; mean difference = 0.020 ; $p = 0.019$) and 1/36 connections displayed an opposite pattern (stronger PDC in Reunion; $W = 77$; mean difference = -0.011 ; $p = 0.037$). In the subgroup of dyads whose infants displayed high negative emotionality during the SF episode, on the other hand, changes in PDC across episodes were more pronounced in the P->I direction. Only 2/36 I->P connections had a significantly stronger PDC in the Play compared to Reunion episode ($W \geq 234$; mean difference ≥ 0.009 ; $p < 0.025$), whereas PDC in the P->I direction was significantly stronger in Reunion compared to Play episode for 11/36 connections ($W \leq 79$; mean difference ≤ -0.009 ; $p < 0.05$). None of these effects, however, survived FDR correction.

Affective touch. In the subgroup of dyads whose parents displayed low percentages of affective touch during the Reunion episode, changes in PDC across episodes were limited both in the I->P and P->I directions. Regarding the I->P direction, 2/36 connections displayed a significantly stronger PDC in the Play compared to Reunion episode ($W \geq 224$; mean difference ≥ 0.017 ; $p < 0.05$); however, an equal amount of connections (2/36) showcased the opposite pattern (stronger in Reunion; $W \leq 81$; mean

difference ≤ -0.011 ; $p < 0.05$). Regarding the P->I direction, only 1/36 connections displayed a significantly stronger PDC in the Reunion compared to the Play episode ($W = 74$; mean difference = -0.013 ; $p = 0.029$). When considering the subgroup of dyads whose parents displayed high percentages of affective touch during the Reunion episode, changes in PDC strength across episodes were significant only in the P->I direction, in which 7/36 connections displayed stronger PDC in the Reunion episode compared to Play ($W \leq 78$; mean difference ≤ -0.016 ; $p < 0.05$). None of these effects survived FDR correction.

Please put Figure 4 here.

3.4. Intra-episode differences (I->P vs P->I connectivity) in PDC in low vs high behavioral subgroups

4.1. Alpha band (6-12 Hz)

Both during Play and Reunion episodes, PDC seemed to be significantly stronger in the I->P direction compared to the P->I direction. However, the ratio of connections that display stronger I->P/P->I PDC has been shown to change according to specific maternal and infant behaviors during the SF episode and Reunion. Statistics are shown in Supplementary Materials (S2, “Full statistical report”, Table S2.4). Results are summarized in Figure 5.

Whole sample. Paired Wilcoxon tests conducted on the whole sample revealed a clear pattern of I->P directionality in our sample during both Play and Reunion. Specifically, 22/36 connections during Play ($W \geq 818$; mean difference ≥ 0.009 ; $p_{\text{adj}} < 0.05$) and 13/36 during Reunion ($W \geq 814$; mean difference ≥ 0.015 ; $p_{\text{adj}} < 0.05$) displayed a significantly higher PDC in the I->P compared to the P->I direction. Conversely, only 0/36 during Play and 2/36 connections during Reunion ($W \leq 329$; mean difference ≤ -0.016 ; $p_{\text{adj}} < 0.025$). 2 proportion z-test revealed no significant differences between proportions of I->P and P->I-dominated connections from Play to Reunion. A graphical representation of the results is available in Figure 5A.

Gaze aversion. Dyads with low infant gaze aversion and those with high gaze aversion showcased similar directionality patterns across episodes. Results are summarized in Figure 5B. In the subgroup of low infant gaze aversion dyads, 13/36 connections during the Play episode ($W \geq 241$; mean difference ≥ 0.016 ; $p_{\text{adj}} < 0.05$) and 6/36 in the Reunion ($W \geq 240$; mean difference ≥ 0.029 ; $p_{\text{adj}} < 0.05$) displayed a

significantly higher PDC in the I->P direction compared to P->I; conversely, no connection displayed significantly stronger PDC in the P->I direction compared to I->P in both FFSF episodes. A similar pattern was observed in the subgroup of high infant gaze aversion dyads, where 12/36 channel combinations during Play ($W \geq 241$; mean difference ≥ 0.020 ; $p_{\text{adj}} < 0.05$) and 6/36 during Reunion ($W \geq 240$; mean difference ≥ 0.022 ; $p_{\text{adj}} < 0.05$) displayed a significantly higher I->P PDC compared to the P->I counterpart. Only one channel combination (1/36) displayed a significantly higher PDC in the P->I direction compared to I->P in the Reunion, but not in the Play episode ($W = 50$; mean difference = -0.025 ; $p_{\text{adj}} = 0.022$). The inter-episode changes in I->P PDC detected in both subgroups were not statistically significant.

Negative emotionality. The PDC directionality patterns across Play and Reunion differed according to the percentage of infant negative emotionality displayed during the SF episode. More specifically, in the low subgroup, 9/36 connections during the Play episode ($W \geq 249$; mean difference ≥ 0.033 ; $p_{\text{adj}} < 0.025$) and 7/36 during the Reunion ($W \geq 244$; mean difference ≥ 0.023 ; $p_{\text{adj}} < 0.05$) displayed significantly stronger I->P PDC compared to P->I, whereas no connections during Play and 1/36 during Reunion ($W = 61$; mean difference = -0.022 ; $p_{\text{adj}} = 0.044$) were significantly stronger in the P->I direction. Conversely, in the high negative emotionality subsample, 17/36 connections during the Play ($W \geq 240$; mean difference ≥ 0.015 ; $p_{\text{adj}} < 0.05$) were found to showcase a significantly stronger I->P PDC, with a reduction to 5/36 ($W \geq 241$; mean difference ≥ 0.030 ; $p_{\text{adj}} < 0.05$) during the Reunion episode. This change was observed to be statistically significant ($p = 0.004$; 95% CI: 1.58-21.95), whereas the same change was not significant in the subgroup of low negative emotionality dyads. Connections with significantly stronger PDC in the P->I direction, on the other hand, went from 0/36 during Play to 1/36 during Reunion ($W = 62$; mean difference = -0.021 ; $p_{\text{adj}} = 0.048$), and the change in proportions was not significant. Results are summarized in Figure 5C.

Affective touch. Similarly to the results reported on infant gaze aversion, splitting the sample in low and high affective touch dyads did not highlight behavior-dependent differences in PDC directionality. Results are summarized in Figure 5D. In the subgroup of low affective touch dyads, 17/36 connections in Play ($W \geq 238$; mean difference ≥ 0.019 ; $p_{\text{adj}} < 0.05$) and 10/36 in Reunion ($W \geq 239$; mean difference

≥ 0.025 ; $p_{\text{adj}} < 0.05$) displayed a significantly stronger PDC in the I->P direction compared to P->I. As for the subgroup of dyads with high percentages of parental affective touch, the I->P direction dominance was less pronounced. 8/36 during Play ($W \geq 245$; mean difference ≥ 0.021 ; $p_{\text{adj}} < 0.05$) and 2/36 during Reunion ($W \geq 252$; mean difference ≥ 0.027 ; $p_{\text{adj}} < 0.025$) displayed a significantly higher PDC in the I->P direction, whereas no connections during Play and 1/36 connections during Reunion ($W = 40$; mean difference = -0.031 ; $p_{\text{adj}} = 0.01$) were found to have a higher PDC in the P->I direction. In both low and high affective touch subsamples, changes in proportion of I->P and P->I-dominated connections did not reach statistical significance.

Please put Figure 5 here.

3.4.2. *Theta band (3-7 Hz)*

In the *theta* frequency band, the impact of infant and maternal behavior during the FFSF on PDC directionality was more pronounced compared to the *alpha* band, with more evident and significant differences in the high percentage subgroups compared to the low percentage ones. Results are described in Supplementary Materials (S2, “*Full statistical report*”, Table S2.5). A graphic representation of the findings is available in Figure 6.

Whole sample. Analyses conducted on the whole sample revealed different patterns of direction dominance in *theta* PDC across Play and Reunion. Results are illustrated in Figure 6A. A total of 7/36 connections during Play ($W \geq 841$; mean difference ≥ 0.015 ; $p_{\text{adj}} < 0.025$) and 2/36 during Reunion ($W \geq 851$; mean difference = 0.012 ; $p_{\text{adj}} < 0.025$) displayed a significantly higher PDC in the I->P direction compared to P->I. Moreover, 7/36 connections during the Play episode ($W \leq 336$; mean difference ≤ -0.011 ; $p_{\text{adj}} < 0.025$) and 9/36 during Reunion ($W \leq 364$; mean difference ≤ -0.018 ; $p_{\text{adj}} < 0.05$) showcased significantly stronger PDC in the P->I direction. No significant changes in proportion of I->P and P->I-dominated connections from Play to Reunion were observed.

Gaze aversion. PDC directionality across Play and Reunion episodes followed similar patterns for low and high infant gaze aversion dyads; however, dyads pertaining to the high infant gaze aversion subgroup displayed slightly stronger shifts in directionality across episodes. Results are illustrated in Figure 6B. In the subgroup of low infant gaze aversion dyads, 5/36 connections during Play ($W \geq 243$;

mean difference ≥ 0.021 ; $p_{\text{adj}} < 0.05$) were significantly stronger in the I->P direction compared to P->I, a number that was reduced to 0/36 during Reunion; conversely, connections with significantly stronger PDC in the P->I direction went from 2/36 during Play ($W \leq 62$; mean difference ≤ -0.017 ; $p_{\text{adj}} < 0.05$) to 5/36 during Reunion ($W \leq 59$; mean difference ≤ -0.016 ; $p_{\text{adj}} < 0.05$). These changes in I->P and P->I connection ratios did not reach statistical significance. Regarding the subgroup of dyads with high percentage of infant gaze aversion, 4/36 connections displayed a significantly stronger PDC in the I->P direction in the Play episode ($W \geq 247$; mean difference ≥ 0.016 ; $p_{\text{adj}} < 0.05$), a number that decreased to 0/36 during Reunion. The increase was not significant. Connections displaying a significantly stronger P->I, on the other hand, went from 0/36 during Play to 6/36 during Reunion ($W \leq 60$; mean difference ≤ -0.026 ; $p_{\text{adj}} < 0.05$); this increase was found to be statistically significant ($p = 0.025$; 95% CI: 0-0.78).

Negative emotionality. Different PDC directionality patterns across Play and Reunion were shown by the subgroup of low infant negative emotionality and high infant negative emotionality dyads. Results are illustrated in Figure 6C. For the group of low negative emotionality, 6/36 connections during Play ($W \leq 60$; mean difference ≤ -0.017 ; $p_{\text{adj}} < 0.05$) and 4/36 during Reunion ($W \leq 61$; mean difference ≤ -0.017 ; $p_{\text{adj}} < 0.05$) were found to display a significantly stronger PDC in the P->I direction, whereas 4/36 connections during Play ($W \geq 239$; mean difference ≥ 0.015 ; $p_{\text{adj}} < 0.05$) and 1/36 during Reunion ($W = 246$; mean difference = 0.018; $p_{\text{adj}} = 0.029$) showcased an opposite pattern (I->P stronger than P->I PDC). These changes across the two FFSF episodes were not statistically significant. In the subgroup of high negative emotionality dyads, 7/36 connections ($W \geq 244$; mean difference ≥ 0.013 ; $p_{\text{adj}} < 0.05$) were found to display a significantly stronger PDC in the I->P direction during Play, a number that was significantly reduced to 0/36 during Reunion ($p = 0.011$; 95% CI: 1.61-Inf). Conversely, 0/36 connections during Play and 5/36 during Reunion ($W \leq 56$; mean difference ≤ -0.028 ; $p_{\text{adj}} < 0.05$) displayed a significantly stronger PDC in the P->I direction. Said inter-episode ratio change was, however, non-significant.

Affective touch. The subgroup of high maternal affective touch dyads displayed the largest changes in PDC directionality across episodes compared to the low affective touch subgroup. Results are

illustrated in Figure 6D. In the low parental affective touch dyads, 4/36 connections during Play ($W \geq 246$; mean difference ≥ 0.027 ; $p_{\text{adj}} < 0.05$) and 4/36 during Reunion ($W \geq 241$; mean difference ≥ 0.018 ; $p_{\text{adj}} < 0.05$) displayed a significantly stronger PDC in the I->P direction, whereas 1/36 connections during Play ($W = 55$; mean difference = -0.018 ; $p_{\text{adj}} = 0.032$) and 3/36 during Reunion ($W \leq 60$; mean difference ≤ -0.018 $p_{\text{adj}} < 0.05$) showcased the opposite pattern. Inter-episode changes in I->P and P->I PDC-dominated connections were not statistically significant. As for the high parental affective touch dyads, 3/36 connections during Play ($W \geq 248$; mean difference ≥ 0.013 ; $p_{\text{adj}} \leq 0.025$) and 0/36 during Reunion showcased a significantly higher I->P PDC. Conversely, 1/36 connections during Play ($W = 62$; mean difference = -0.014 ; $p_{\text{adj}} = 0.048$) and 10/36 during the Reunion episode ($W \leq 61$; mean difference ≤ -0.021 ; $p_{\text{adj}} < 0.05$) displayed a higher P->I PDC, with the increase from Play to Reunion being statistically significant ($p = 0.006$; 95% CI: 0.002-0.60).

Please put Figure 6 here.

4. DISCUSSION

We have collected dual EEG data from 48 parent-infant dyads during an adapted FFSF procedure and computed I->P and P->I PDC in order to explore if and how dyadic lead and follow dynamics changed after an interactive perturbation. We were also interested in understanding to what extent individual differences in how the dyads responded to the FFSF procedure associated with I->P and P->I PDC across Play and Reunion episodes. Our findings indicate that, in our sample, a still-face effect occurred at the behavioral level, with a significant increase in infant negative emotionality during SF and Reunion compared to Play and a significant increase in infant gaze aversion during the SF compared to Play and Reunion. At the neural level, inter-episode changes in I->P and P->I PDC in the *alpha* (6-12 Hz) and *theta* (3-7 Hz) frequency bands were evident only in the subgroup of dyads displaying higher-than-the-median percentages of infant gaze aversion and negative emotionality during the SF episode, as well as of maternal affective touch during the Reunion. In our sample, direction of connectivity (I->P vs P->I) was the factor that best explained the changes in parent-infant PDC, indicating that neurobehavioral synchrony may have been established in a sequential fashion. Post-hoc analyses conducted in the subgroups of dyads with low versus high percentages of infant gaze aversion, infant negative

emotionality and maternal affective touch complemented the results obtained from the ART ANOVA, highlighting differences in the interplay between PDC direction and dyadic behavior among the two frequencies of interest. More specifically, changes in *alpha* PDC directionality were exclusively driven by the degree of infant negative emotionality during the SF episode, with high percentages (>36.78% of total time, corresponding to 22s or more) being associated with a significant decrease in the proportion of I->P-dominated connectivity from Play to Reunion. Changes in *theta* PDC directionality, on the other hand, were related to all the behavioral variables of interest; specifically, high percentages of infant negative emotionality during the SF (>36.78% of total time) were associated with a significant decrease in the proportion of I->P-dominated connections from Play to Reunion, whereas high percentages of infant gaze aversion during the SF (>66.01% of total time, corresponding to 39s or more) and maternal affective touch during the Reunion (>28.96% of total time, corresponding to 34s or more) were related to a significant increase of the proportion of P->I-dominated connections from Play to Reunion. In light of this, our experimental hypotheses (H1 and H2, see *Introduction*) were partially supported for *theta* PDC and refuted for *alpha* PDC.

4.1. The dyadic “lead and follow” dynamic in *alpha* versus *theta* frequencies

Contrary to our expectations, parent-infant PDC in the *alpha* band seemed to follow a clear sequential pattern during the Play episode, featuring the infant in the leading role and the parent in the follower one. In this frequency band and episode, dyads from our sample displayed a remarkably higher number of I->P-directed connections compared to the P->I counterparts, which were almost non-existent. These results are in contrast with previous observations of behavioral synchrony in parents and their 9-month-old infants, where synchrony was prevalently of the mutual (bidirectional) type due to the emergence of intersubjectivity ([Feldman 2007](#); [Feldman et al. 1996](#); [Feldman et al. 1999](#)). Interestingly, our *alpha* PDC results are more consistent with the findings on parent-infant dyads with younger infants (3-8 months), where the synchrony structure was found to be of the lagged/sequential type and featuring the infant as the “leader” ([Feldman et al. 1996](#); [Feldman et al. 1999](#)). Our findings on *alpha* PDC are also in contrast with Santamaria and colleagues’ findings of a significantly stronger PDC in the P->I condition compared to I->P ([Santamaria et al. 2020](#)), although this difference could be ascribed to

the characteristics of the study design. Conversely, trends of parent-infant *theta* PDC during the FFSF procedure seemed to be more in line with our experimental hypotheses, as well as with the current literature on parent-infant sequential biobehavioral synchrony. Both in the full sample and in the median-split subsamples, parent-infant *theta* PDC during the Play episode seemed to follow a prevalently bidirectional pattern (or otherwise a weakly infant-led pattern in the subgroup of high infant negative emotionality and gaze aversion), which then changed into a directed, parent-led type of connectivity during the Reunion episode. This change in PDC directionality, however, was evident only in the whole sample of 48 dyads, as well as in the subsamples of high affective touch dyads and high gaze aversion dyads. (see Results), whereas no significant changes in *theta* PDC directionality were found in the low behavioral subsamples.

One possible explanation to the different results showcased by *alpha* and *theta* PDC could lie in the different functional roles of these frequency bands during dyadic interaction, and how these roles relate to the potential causes of the still-face effect. It is generally accepted that the distressing nature of the FFSF procedure can be ascribed to the violation of social expectations, and particularly the violation of the expectations regarding maternal availability ([DiCorcia et al. 2016](#); [Legerstee and Markova 2007](#); [Mesman et al. 2009](#)). Notably, INS in the *theta* frequency band has been previously associated with attentional and cognitive components of the dyadic interaction, including changes in shared attention during joint play ([Wass et al. 2018](#)) and changes in directed gaze ([Leong et al. 2017](#); [Turk et al. 2022](#)), and *theta* activity in the infant brain has been associated with attention to social stimulation ([Orekhova et al. 2006](#)). While parent-infant INS in the *alpha* frequency band has also been related to changes in directed gaze in a previous study ([Leong et al. 2017](#)), this band has been often associated with changes in emotional states ([Atzaba-Poria et al. 2017](#); [Krzeczkowski et al. 2022](#); [Perone et al. 2020](#); [Swider-Cios et al. 2024](#); [Turk et al. 2022](#)) as well as valence of maternal emotional feedback ([Santamaria et al. 2020](#)). It is thus possible that, due to the more cognitive nature of the FFSF procedure, the *theta* band may have better captured the potentially disruptive effect of the interactive perturbation on I->P PDC, whereas PDC in the *alpha* band could have been more sensitive to other behavioral characteristics of the dyads such as emotional valence (see below). Notably, the hypothesis of the sensitivity of the *alpha* band to

the emotional states of the dyad could also explain the clear directional pattern of *alpha* PDC (favouring I->P direction) during the Play episode, considering that negative emotionality during that episode was significantly lower compared to the subsequent FFSF episodes. In the same fashion, the decrease in I->P directionality during the Reunion episode could be ascribed to the significant increase in infant negative emotionality during this episode, which may have partially disrupted the I->P connectivity strength.

4.2. The neural underpinnings of the still-face effect

The dyads from our sample showcased a clear still-face effect at the behavioral level, with a significant increase in infant negative emotionality from Play to SF which carried over during the Reunion episode. Conversely, infant gaze aversion increased from Play to SF and returned to baseline levels during Reunion, whereas no significant differences in percentages of maternal affective touch were found across episodes. The findings are consistent with Mesman and colleagues' meta-analytical work, which reported a significant increase in negative emotionality from Play to SF and Reunion and no significant changes in other dyadic behaviors such as social gaze from Play to Reunion in infants aged 0-9 months ([Mesman et al. 2009](#)).

At the neural level, changes in PDC did not seem to be driven by the FFSF procedure per se, but rather by the interplay between dyadic behavior and PDC directionality (I->P-dominant or P->I-dominant) across the FFSF episodes. While episode-dependent changes in I->P and P->I PDC (*alpha*, *theta*) did not reach statistical significance after correction for multiple comparisons, more robust changes in *alpha* and *theta* PDC were detected when assessing the direction (I->P versus P->I) changes from Play to Reunion in the subsample of dyads displaying high percentages of the behaviors of interest. More specifically, the number of connections with significantly higher I->P PDC in both *alpha* and *theta* frequency bands underwent a significant reduction from Play to Reunion in the subgroup of dyads with high negative emotionality during the SF episode, with I->P connections going from 17/36 to 5/36 for the *alpha* frequency band and from 7/36 to 0/36 in the *theta* frequency band. Conversely, the number of connections with significantly higher P->I PDC in the *theta* band, but not *alpha*, showcased a significant increase from Play to Reunion in the subgroup of dyads with high infant gaze aversion and in the

subgroup with high maternal affective touch. In the high infant gaze aversion subgroup, P->I connections went from 0/36 during Play to 6/36 during Reunion, whereas in the high maternal affective touch subgroup, P->I connections went from 1/36 during Play to 10/36 during Reunion. Our findings suggest that the still-face effect is not a strictly biobehavioral phenomenon – on the contrary, interactive perturbations produce significant changes in sequential parent-infant INS, particularly in the ratio of I->P and P->I directional connectivity across the different FFSF episodes. These changes in parent-infant directional connectivity are particularly evident in infants showcasing high behavioral displays of distress such as high negative emotionality and high gaze aversion, whereas in dyads with limited behavioral displays of distress, changes in PDC directionality patterns were negligible. There are two possible interpretations of these findings, which are not mutually exclusive. Parents of infants with high-intensity displays of distress during the SF, such as fussing, crying, negative emotional expressions, or even lack of social gaze, could have felt an increased need to soothe their baby and have him/her re-engaged in a positive exchange during the Reunion episode. This could have resulted in the parent being more proactive in the social exchange, leading to a shift to P->I *theta* PDC (in the subgroup of high infant gaze aversion), or to a reduction of the I->P connections in the high infant negative emotionality subgroup (both *theta* and *alpha* frequency), from Play to Reunion. Simultaneously, infants showcasing high negative emotionality and/or high gaze aversion during the SF could have felt a higher need of co-regulating with their parents, making them more receptive to the parent's proposals and thus decreasing the strength of their leading role in the interactive exchange compared to the Play episode. Contrarily, we can hypothesize that the dyads whose infants had low behavioral displays of distress required less effort to co-regulate and return to the previous levels of engagement, leading to negligible changes in PDC directionality patterns from Play to Reunion.

A separate interpretation can be made for the findings on the impact of maternal affective touch on *theta* PDC directionality. Together with its role in the reduction of the still-face effect ([see Stack and Muir 1990; Stack and Muir 1992](#)), maternal affective touch was previously reported to direct the infants' attention to the mother's hands ([Stack and Muir 1990](#)), to be temporally organized with infants' affect and attention ([Jean et al. 2014](#)), and also to associate with higher concurrent parent-infant Circular

correlation (a concurrent INS metric measuring phase synchronization between two signals, see [Burgess 2013](#) for details) when accompanied by direct maternal eye contact ([Neel et al. 2025](#)). Our results suggest that maternal affective touch could be a cue through which parent-infant INS, and particularly INS in the P->I direction, is established. This could be especially relevant in the Reunion compared to the Play episode, since the distress experienced during the SF episode could make them more receptive to the parental co-regulating strategies, thus changing the INS directionality compared to the baseline levels. It is worth noting that, in our sample, maternal affective touch did not seem to be reactive to the infants' behavior during the SF episode. Notably, we did not find significant differences in the percentage of time dedicated to affective touch from Play to Reunion, and the majority of the mothers displaying high affective touch during the Reunion episode (17/24) also displayed high levels of affective touch during the Play episode. It is possible that, for the parents from our sample, affective touch was just a way for them to positively engage with their infant regardless of his/her emotional state, and that the association between this behavior and P->I PDC changes in the *theta* band could indeed be ascribed to an increased infant receptiveness to parental co-regulating attempts during the Reunion episode compared to Play.

While maternal affective touch has been associated with the construct of maternal sensitivity ([Botero et al. 2020](#); [Endevelt-Shapira and Feldman 2023](#); [Feldman et al. 2010](#)), we have not assessed whether parents showing higher percentages of affective touch scored also high in maternal sensitivity scales. Interestingly, levels of maternal sensitivity during dyadic interactions showcased a positive association with parent-infant concurrent INS as measured by the wPLI ([Endevelt-Shapira and Feldman 2023](#)), meaning that there could be a potential link between *theta* INS, maternal sensitivity and maternal affective touch. Further studies on parent-infant biobehavioral synchrony are needed to assess the interplay between these three variables.

4.3. The complementing roles of *alpha* and *theta* PDC

Our study revealed how directionality of *alpha* and *theta* PDC was associated with different dyadic behaviors throughout the FFSF procedure. Changes in *alpha* PDC, as measured in the frequencies comprised between 6 and 12 Hz, were found to be exclusively associated with high infant negative

emotionality during the SF episode, whereas changes in *theta* PDC, measured in the frequencies going from 3 to 7 Hz, were found to be associated with high display of all the behaviors of interest. In the current EEG hyperscanning literature, *alpha* fluctuations in mother-infant dyads were conventionally associated with valence of the emotional feedback ([Santamaria et al. 2020](#)), changes of emotional states ([Atzaba-Poria et al. 2017](#); [Krzeczkowski et al. 2022](#); [Perone et al. 2020](#); [Swider-Cios et al. 2024](#); [Turk et al. 2022](#)), and more recently with visual and tactile stimulation ([Neel et al. 2025](#)), whereas *theta* INS was frequently associated with changes in shared attention during joint play ([Wass et al. 2018](#)), maternal sensitivity and maternal intrusiveness ([Endevelt-Shapira and Feldman 2023](#)) as well as emotional processing ([Endevelt-Shapira et al. 2021](#)). Our findings seem to align with this distinction, further supporting the different but complementing roles of *alpha* and *theta* INS in ecological dyadic interactions. It is worth noting, however, that while both *alpha* and *theta* have been repeatedly found to be associated with dyadic behaviors, researchers argue that INS measured in the *theta* band could be less reliable than the *alpha* counterpart due to the overlap between the *theta* frequency (4-7 Hz) and ocular artifacts ([Gasser et al. 1985](#); [Kanoga et al. 2016](#)), whereas *alpha* has been found to be less affected by artifactual activity, including facial myogenic and speech artifacts ([Georgieva et al. 2020](#); [Laganaro and Perret 2011](#); [Muthukumaraswamy 2013](#); [Santamaria et al. 2020](#)). Additionally, a recent methodological work highlighted significant fluctuations in parent-infant concurrent INS in the *theta*, but not *alpha*, band according to the level of pipeline automatization ([Pili et al. 2025](#)), reinforcing the hypothesis that *theta* INS may be more susceptible to myogenic artifacts compared to *alpha*. As for our study, the robustness of the associations between *theta* PDC and the behavior displayed by our dyads during the FFSF procedure is supported by the fact that the findings reported for the *theta* band were largely replicated in the *alpha* band. This includes not only the significant effect of the direction factor in the main analyses via ART ANOVA, but also the non-significant impact of the episode factor in I->P and P->I PDC strength and the significant reduction of the proportion of I->P connections from Play to Reunion in the subgroup of dyads with high infant negative emotionality. Nonetheless, further studies are needed to ascertain the association between *alpha* and *theta* PDC with socioemotional processing, as the majority of the current studies focus on concurrent INS.

4.4. The potential involvement of frontal areas in sequential INS

It is worth noting that the vast majority of the connections showcasing a significant I->P or P->I directed pattern started from a frontal channel (F3, F4) and arrived at either a central (C3, C4) or posterior (P3, P4) channel. This is true for both *theta* and *alpha* frequency bands, for both Play and Reunion episodes, and for both whole and median-split samples (see Figures 5 and 6). While it is known that EEG presents a low spatial resolution, as the current generated by the brain sources is blurred by resisting layers such as the cerebrospinal fluid, the skull and the scalp ([Burle et al. 2015](#); [Makeig et al. 1995](#); [Srinivasan et al. 1996](#); [Srinivasan 1999](#)), it is still relevant to discuss how our findings compare with the existing literature about the neural correlates of parent-infant INS, and particularly with the body of evidence collected from functional near infrared spectroscopy (fNIRS) and magnetoencephalography (MEG) hyperscanning. Previous studies conducted on parent-infant and parent-child dyads have indeed highlighted the involvement of frontal regions, such as the bilateral lateral and median prefrontal cortex (lPFC and mPFC) ([Nguyen et al. 2020](#); [Nguyen et al. 2021](#)) and the inferior frontal gyrus (IFG) ([Lin et al. 2023](#)), in establishing INS during interactive and cooperative tasks. The PFC has been found to be implied in all the processes related to the engagement in a mutual interaction, including the detection of communicative signals directed towards the self, mentalization and reward ([Nguyen et al. 2021](#); [Redcay and Schilbach 2019](#)), as well as higher order cognitive functions such as attentional regulation, working memory, regulation of the emotional experiences and planning of self-initiated social behaviors ([Azhari et al. 2022](#)). Similarly, the IFG is part of the “mirror neuron” network and has been associated with the detection of communicative signals related to social interaction, and particularly to the verbal components of social communication ([Lin et al. 2023](#); [Redcay and Schilbach 2019](#)). In their study, Endevelt-Shapira and Feldman ([Endevelt-Shapira and Feldman 2023](#)) argued that parent-infant INS could involve the activation of the frontotemporal network, which is implied in processes of social cognition and empathy ([Atzil et al. 2012](#); [Frith and Frith 2001](#)). Notably, several studies have reported a joint involvement of frontal and temporal areas, the latter including the temporo-parietal junction ([Minagawa et al. 2023](#)) and the right angular gyrus ([Lin et al. 2023](#)). While we were not able to assess the involvement of the frontotemporal network due to the exclusion of the temporal channels from the

analysis (which was motivated by their higher presence of myogenic speech confounds in these peripheral channels; see [Brooker and Donald 1980](#)), the consistent role of the frontal channels as the “sending hub” of directed parent-infant INS may be indicative of the infants’ and parents’ intention of initiating and maintaining social behavior. As for the involvement of central and posterior channels as the “receiving hubs”, the result could be in line with a previous EEG hyperscanning study which highlighted a significant connection between the maternal right central area (channel C4) and the infant right occipito-temporal area (channel P8) ([Endevelt-Shapira et al. 2021](#)). In the adult brain, activity in the centroparietal regions has been proposed as a neuromarker for social coordination during spontaneous inter-neural coupling ([Dumas et al. 2010](#); [Tognoli et al. 2007](#)), whereas in the infant brain, there is evidence of the inferior parietal cortex, and particularly the inferior parietal lobule, being involved in the processing of the similarities and differences between the self and the other, as well as to imitation and the recognition of being imitated ([Meltzoff and Decety 2004](#)). Again, we have no means to determine which were the brain sources underlying the activity recorded from the central and posterior channels of choice; however, the involvement of these channels as the “receiving hubs” both in parents and infants may underlie their intention of being receptive of each other’s behaviors and communicative signals. Evidence from further studies on parent-infant sequential INS is needed in order to support the involvement of a fronto-parieto-occipital network during dyadic lead and follow social dynamics.

5. LIMITATIONS AND IMPLICATIONS

The present study contains several limitations, which restrict the generalizability and interpretability of the obtained results.

The first limitation relates to the enlarged and partially overlapped thresholds used to select the *theta* and *alpha* frequency bands in our study. While our intention was to adopt the assumption that parents and infants have different peaks for the same frequency bands ([Noreika et al. 2020](#); [Turk et al. 2022](#)) while strictly following Baccalà and Sameshima’s original formulation of the PDC ([Baccalà and Sameshima 2021](#)), it should also be considered that our thresholds partially overlap with frequencies other than *theta* and *alpha* in the adult and infant brain respectively. More specifically, the 3-7 Hz

interval for the *theta* band partially overlaps with the adult *delta* waves (1-3 Hz; [Koul et al. 2023](#)) and the infant *alpha* (6-9 Hz; [Xie et al. 2018](#)). In the same fashion, the 6-12 Hz interval for the *alpha* band partially overlaps with the adult *theta* (4-8 Hz; [Koul et al. 2023](#)) and the infant *beta* frequency (9-14 Hz; [Xie et al. 2018](#)). While a previous work has encouraged the use of a cross-frequency approach when dealing with parent-infant INS ([Turk et al. 2022](#)), it is important to note that the majority of the studies in this field ([Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#); [Leong et al. 2017](#); [Leong et al. 2019](#); [Neel et al. 2025](#); [Santamaria et al. 2020](#); [Wass et al. 2018](#)) still work under the assumption that parents and infants showcase the same frequency peaks during social interaction, which are conventionally set at 4-7 Hz for *theta* ([Endevelt-Shapira et al. 2021](#); [Endevelt-Shapira and Feldman 2023](#)) and at 6-9 Hz for *alpha* ([Neel et al. 2025](#); [Santamaria et al. 2020](#)). Conduction of further studies utilizing the “conventional” *theta* and *alpha* thresholds are encouraged in order to assess the robustness of the results presented in the current work.

Another important limitation lies in the lack of a significance threshold for our PDC values. While we tried to compensate for this limitation by assessing the presence of significant differences between the I->P and P->I PDC and correcting for multiple comparisons, we did not use any data-driven or pre-selected threshold to determine whether our INS estimates were strong enough to describe a truly causal relationship between the parents’ and the infants’ signals. Notably, Santamaria and colleagues ([Santamaria et al. 2020](#)) have employed proportional thresholding to eliminate spurious PDC connections in their study, and while this method is expected to lead to more stable networks metrics ([Garrison et al. 2015](#)), other researchers have risen concerns regarding the robustness of this methodology due to significant changes in global connectivity measures in function to the chosen threshold ([Adamovich et al. 2022](#); [Tsolisou 2023](#); [Zakharov et al. 2021](#)). Future research on parent-infant PDC ought to address this limitation and propose robust thresholds through which sequential INS can confidently describe a “causal relationship” between the signal of the leading partner and the one of the following partner.

Lastly, our coding system, including the use of the median split to divide our sample in “high” and “low” display of the behaviors of interest, might constitute another limitation of our study. While previous

studies employing concurrent INS metrics have reported significant associations between parent-infant INS and specific dyadic behaviors such as mutual gaze ([Leong et al. 2017](#); [Leong et al. 2019](#)) and positive emotional valence ([Santamaria et al. 2020](#)), we cannot exclude the interplay between different sensory stimuli (visual, tactile, auditory) in shaping the mutual neural coordination between parents and their infants. Interestingly, a recent study from Neel and colleagues ([Neel et al. 2025](#)) described a significant increase in INS measured through the CCorr in the *alpha* band when maternal tactile stimulation was added to visual stimulation in 3-month-old infants, indicating that parent-infant INS could also be fostered by multimodal sensory input rather than only by single behavioral cues. Moreover, our categorization of infant and parental behavior into “high” and “low” rates was highly dependent on the individual characteristics of our dyads, with median percentages of negative emotionality during SF and maternal affective touch during Reunion not even reaching fifty percent. This meant that, for instance, some of the “high negative emotionality” infants displayed behaviors that were coded as negative emotionality for less than thirty seconds during the SF episode, and the same applies to mothers who were in the “high affective touch” group (affectionately touching their infant for less than a minute during the Reunion episode). While it is more unlikely that an infant displaying truly high negative emotionality during the SF episode can complete the FFSF and provide adequate EEG data, we encourage researchers in the developmental neuroscience field who are interested in investigating changes in parent-infant INS in function to behavior to adopt more robust methods to define subsamples based on the behavior duration, such as rating scales. Moreover, we encourage to investigate the impact of multimodal sensory stimulation in fostering parent-infant sequential INS, as well as the impact of macroscopic dyadic behaviors, such as maternal sensitivity and maternal intrusiveness.

6. CONCLUSIONS

Even with the aforementioned limitations, the present study has several implications, including being the first study to assess parent-infant sequential INS in an ecological setting including an interactive perturbation. It provides novel insights into the “lead-and-follow” mechanisms of parent-infant interaction, and how parents and infants negotiate their roles after an interactive perturbation.

Moreover, it highlights the complementary role of *alpha* and *theta* INS during parent-infant interaction, and how these two frequencies are related to different dyadic behaviors. If consistently replicated in future studies, these findings could open the possibility of utilizing PDC as a neuromarker for parent-infant co-regulation in typical and at-risk conditions.

7. REFERENCES

- Adamovich, T., Zakharov, I., Tabueva, A., & Malykh, S. (2022). The thresholding problem and variability in the EEG graph network parameters. *Scientific Reports*, 12(1), 18659. <https://doi.org/10.1038/s41598-022-22079-2>.
- Akaike, K. (1979). What is the Information Criterion AIC? *Surikagaku*, 153, 5–11.
- Atzaba-Poria, N., Deater-Deckard, K., & Bell, M. A. (2017). Mother-Child Interaction: Links Between Mother and Child Frontal Electroencephalograph Asymmetry and Negative Behavior. *Child Development*, 88(2), 544–554. <https://doi.org/10.1111/cdev.12583>.
- Atzil, S., Hendler, T., Zagoory-Sharon, O., Winetraub, Y., & Feldman, R. (2012). Synchrony and specificity in the maternal and the paternal brain: relations to oxytocin and vasopressin. *Journal of the American Academy of Child and Adolescent Psychiatry*, 51(8), 798–811. <https://doi.org/10.1016/j.jaac.2012.06.008>.
- Azhari, A., Bizzego, A., & Esposito, G. (2022). Parent-child dyads with greater parenting stress exhibit less synchrony in posterior areas and more synchrony in frontal areas of the prefrontal cortex during shared play. *Social Neuroscience*, 17(6), 520–531. <https://doi.org/10.1080/17470919.2022.2162118>.
- Baccalá, L. A., & Sameshima, K. (2001). Partial directed coherence: a new concept in neural structure determination. *Biological Cybernetics*, 84(6), 463–474. <https://doi.org/10.1007/PL00007990>.
- Baccalá, L. A., & Sameshima, K. (2021). Partial directed coherence: twenty years on some history and an appraisal. *Biological Cybernetics*, 115(3), 195–204. <https://doi.org/10.1007/s00422-021-00880-y>.
- Berretta, E., Cutuli, D., Laricchiuta, D., & Petrosini, L. (2021). From animal to human epigenetics. In *Developmental Human Behavioral Epigenetics* (pp. 27–58). Elsevier. <https://doi.org/10.1016/B978-0-12-819262-7.00002-7>.
- Billeci, L., Riva, V., Capelli, E., Grumi, S., Paola Pili, M., Cassa, M., Siri, E., Roberti, E., Borgatti, R., & Provenzi, L. (2024). 2-Brain Regulation for Improved Neuroprotection during Early Development (2-

BRAINED): a translational hyperscanning research project. *Frontiers in Psychology*, 15, 1516616. <https://doi.org/10.3389/fpsyg.2024.1516616>.

Bi, X., Cui, H., & Ma, Y. (2023). Hyperscanning Studies on Interbrain Synchrony and Child Development: A Narrative Review. *Neuroscience*, 530, 38–45. <https://doi.org/10.1016/j.neuroscience.2023.08.035>.

Botero, M., Langley, H. A., & Venta, A. (2020). The untenable omission of touch in maternal sensitivity and attachment research. *Infant and Child Development*, 29(2), e2159. <https://doi.org/10.1002/icd.2159>.

Brambilla, M., Grumi, S., Manfredini, V., Pettenati, G., and Provenzi, L. (2023). Parent Infant Coding System, PICS. Version 4.0, Unpublished manual.

Brooker, B. H., & Donald, M. W. (1980). Contribution of the speech musculature to apparent human EEG asymmetries prior to vocalization. *Brain and Language*, 9(2), 226–245. [https://doi.org/10.1016/0093-934X\(80\)90143-1](https://doi.org/10.1016/0093-934X(80)90143-1).

Burgess, A. P. (2013). On the interpretation of synchronization in EEG hyperscanning studies: a cautionary note. *Frontiers in Human Neuroscience*, 7, 881. <https://doi.org/10.3389/fnhum.2013.00881>.

Burle, B., Spieser, L., Roger, C., Casini, L., Hasbroucq, T., & Vidal, F. (2015). Spatial and temporal resolutions of EEG: Is it really black and white? A scalp current density view. *International Journal of Psychophysiology : Official Journal of the International Organization of Psychophysiology*, 97(3), 210–220. <https://doi.org/10.1016/j.ijpsycho.2015.05.004>.

Capitani, E., Lorenzini, C., Biuzzi, A., Alaimo, L., & Nante, N. (2023). Factors influencing the first thousand days of life. The importance of Nurturing Care. *Journal of Preventive Medicine and Hygiene*, 64(2), E172–E177. <https://doi.org/10.15167/2421-4248/jpmh2023.64.2.2850>.

Chang, C.-Y., Hsu, S.-H., Pion-Tonachini, L., & Jung, T.-P. (2020). Evaluation of Artifact Subspace Reconstruction for Automatic Artifact Components Removal in Multi-Channel EEG Recordings. *IEEE*

Transactions on Bio-Medical Engineering, 67(4), 1114–1121.
<https://doi.org/10.1109/TBME.2019.2930186>.

Choi, J. W., & Kim, K. H. (2018). Methods for functional connectivity analysis. In *Biological and Medical Physics, Biomedical Engineering* (pp. 125–145). Springer Singapore. https://doi.org/10.1007/978-981-13-0908-3_6.

Cohn, J. F., & Elmore, M. (1988). Effect of contingent changes in mothers' affective expression on the organization of behavior in 3-month-old infants. *Infant Behavior & Development*, 11(4), 493–505. [https://doi.org/10.1016/0163-6383\(88\)90008-2](https://doi.org/10.1016/0163-6383(88)90008-2).

Cohn, J. F., & Tronick, E. Z. (1987). Mother–infant face-to-face interaction: The sequence of dyadic states at 3, 6, and 9 months. *Developmental Psychology*, 23(1), 68–77. <https://doi.org/10.1037/0012-1649.23.1.68>.

Delorme, A., & Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *Journal of Neuroscience Methods*, 134(1), 9–21. <https://doi.org/10.1016/j.jneumeth.2003.10.009>.

DiCorcia, J. A., Snidman, N., Sravish, A. V., & Tronick, E. (2016). Evaluating the nature of the still-face effect in the double face-to-face still-face paradigm using different comparison groups. *Infancy: The Official Journal of the International Society on Infant Studies*, 21(3), 332–352. <https://doi.org/10.1111/infa.12123>.

Dikker, S., Michalareas, G., Oostrik, M., Serafimaki, A., Kahraman, H. M., Struiksma, M. E., & Poeppel, D. (2021). Crowdsourcing neuroscience: Inter-brain coupling during face-to-face interactions outside the laboratory. *NeuroImage*, 227, 117436. <https://doi.org/10.1016/j.neuroimage.2020.117436>.

Dumas, G., Nadel, J., Soussignan, R., Martinerie, J., & Garnero, L. (2010). Inter-brain synchronization during social interaction. *PloS One*, 5(8), e12166. <https://doi.org/10.1371/journal.pone.0012166>.

Endevelt-Shapira, Y., Djalovski, A., Dumas, G., & Feldman, R. (2021). Maternal chemosignals enhance infant-adult brain-to-brain synchrony. *Science Advances*, 7(50), eabg6867. <https://doi.org/10.1126/sciadv.abg6867>.

Endevelt-Shapira, Y., & Feldman, R. (2023). Mother-Infant Brain-to-Brain Synchrony Patterns Reflect Caregiving Profiles. *Biology*, 12(2). <https://doi.org/10.3390/biology12020284>.

Feldman, R. (2007). Parent-infant synchrony and the construction of shared timing; physiological precursors, developmental outcomes, and risk conditions. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, 48(3-4), 329–354. <https://doi.org/10.1111/j.1469-7610.2006.01701.x>.

Feldman, R. (2015). Sensitive periods in human social development: New insights from research on oxytocin, synchrony, and high-risk parenting. *Development and Psychopathology*, 27(2), 369–395. <https://doi.org/10.1017/S0954579415000048>.

Feldman, R., Greenbaum, C. W., & Yirmiya, N. (1999). Mother-infant affect synchrony as an antecedent of the emergence of self-control. *Developmental Psychology*, 35(1), 223–231. <https://doi.org/10.1037//0012-1649.35.1.223>.

Feldman, R., Greenbaum, C. W., Yirmiya, N., & Mayes, L. C. (1996). Relations between cyclicity and regulation in mother-infant interaction at 3 and 9 months and cognition at 2 years. *Journal of Applied Developmental Psychology*, 17(3), 347–365. [https://doi.org/10.1016/S0193-3973\(96\)90031-3](https://doi.org/10.1016/S0193-3973(96)90031-3).

Feldman, R., Singer, M., & Zagoory, O. (2010). Touch attenuates infants' physiological reactivity to stress. *Developmental Science*, 13(2), 271–278. <https://doi.org/10.1111/j.1467-7687.2009.00890.x>.

Feldstein, S., Jaffe, J., Beebe, B., Crown, C. L., Jasnow, M., Fox, H., & Gordon, S. (1993). Coordinated interpersonal timing in adult-infant vocal interactions: A cross-site replication. *Infant Behavior & Development*, 16(4), 455–470. [https://doi.org/10.1016/0163-6383\(93\)80004-R](https://doi.org/10.1016/0163-6383(93)80004-R).

Frith, U., & Frith, C. (2001). The biological basis of social interaction. *Current Directions in Psychological Science*, 10(5), 151–155. <https://doi.org/10.1111/1467-8721.00137>.

Gabard-Durnam, L. J., Mendez Leal, A. S., Wilkinson, C. L., & Levin, A. R. (2018). The Harvard Automated Processing Pipeline for Electroencephalography (HAPPE): Standardized Processing Software for Developmental and High-Artifact Data. *Frontiers in Neuroscience*, 12, 97. <https://doi.org/10.3389/fnins.2018.00097>.

Garrison, K. A., Scheinost, D., Finn, E. S., Shen, X., & Constable, R. T. (2015). The (in)stability of functional brain network measures across thresholds. *NeuroImage*, 118, 651–661. <https://doi.org/10.1016/j.neuroimage.2015.05.046>.

Gasser, T., Sroka, L., & Möcks, J. (1985). The transfer of EOG activity into the EEG for eyes open and closed. *Electroencephalography and Clinical Neurophysiology*, 61(2), 181–193. [https://doi.org/10.1016/0013-4694\(85\)91058-2](https://doi.org/10.1016/0013-4694(85)91058-2).

Georgieva, S., Lester, S., Noreika, V., Yilmaz, M. N., Wass, S., & Leong, V. (2020). Toward the Understanding of Topographical and Spectral Signatures of Infant Movement Artifacts in Naturalistic EEG. *Frontiers in Neuroscience*, 14, 352. <https://doi.org/10.3389/fnins.2020.00352>.

Granger, C. W. J. (1969). Investigating causal relations by econometric models and cross-spectral methods. *Econometrica: Journal of the Econometric Society*, 37(3), 424. <https://doi.org/10.2307/1912791>.

Jean, A. D. L., & Stack, D. M. (2012). Full-term and very-low-birth-weight preterm infants' self-regulating behaviors during a Still-Face interaction: influences of maternal touch. *Infant Behavior & Development*, 35(4), 779–791. <https://doi.org/10.1016/j.infbeh.2012.07.023>.

Jean, A. D. L., Stack, D. M., & Arnold, S. (2014). Investigating maternal touch and infants' self-regulatory behaviours during a modified Face-to-Face Still-Face with touch procedure. *Infant and Child Development*, 23(6), 557–574. <https://doi.org/10.1002/icd.1870>.

Jia, H. (2019). Connectivity Analysis. In *EEG Signal Processing and Feature Extraction* (pp. 241–266). Springer Singapore. https://doi.org/10.1007/978-981-13-9113-2_12.

- Kanoga, S., Nakanishi, M., & Mitsukura, Y. (2016). Assessing the effects of voluntary and involuntary eyeblinks in independent components of electroencephalogram. *Neurocomputing*, 193, 20–32. <https://doi.org/10.1016/j.neucom.2016.01.057>.
- Kaye, K., & Fogel, A. (1980). The temporal structure of face-to-face communication between mothers and infants. *Developmental Psychology*, 16(5), 454–464. <https://doi.org/10.1037/0012-1649.16.5.454>.
- Kayhan, E., Matthes, D., Marriott Haresign, I., Bánki, A., Michel, C., Langeloh, M., Wass, S., & Hoehl, S. (2022). DEEP: A dual EEG pipeline for developmental hyperscanning studies. *Developmental Cognitive Neuroscience*, 54, 101104. <https://doi.org/10.1016/j.dcn.2022.101104>.
- Kay, M., Elkin, L., Higgins, J. J., & Wobbrock, J. O. (2025). *mjskay/ARTool: ARTool 0.11.2*. Zenodo. <https://doi.org/10.5281/ZENODO.594511>.
- Koul, A., Ahmar, D., Iannetti, G. D., & Novembre, G. (2023). Spontaneous dyadic behavior predicts the emergence of interpersonal neural synchrony. *NeuroImage*, 277, 120233. <https://doi.org/10.1016/j.neuroimage.2023.120233>.
- Krzeczkowski, J. E., Van Lieshout, R. J., & Schmidt, L. A. (2022). Transacting brains: testing an actor-partner model of frontal EEG activity in mother-infant dyads. *Development and Psychopathology*, 34(3), 969–980. <https://doi.org/10.1017/S0954579420001558>.
- Kumaravel, V. P., Farella, E., Parise, E., & Buiatti, M. (2022). NEAR: An artifact removal pipeline for human newborn EEG data. *Developmental Cognitive Neuroscience*, 54, 101068. <https://doi.org/10.1016/j.dcn.2022.101068>.
- Lachaux, J.-P., Rodriguez, E., Martinerie, J., & Varela, F. J. (1999). Measuring phase synchrony in brain signals. *Human Brain Mapping*, 8(4), 194–208. [https://doi.org/10.1002/\(SICI\)1097-0193\(1999\)8:4<194::AID-HBM4>3.0.CO;2-C](https://doi.org/10.1002/(SICI)1097-0193(1999)8:4<194::AID-HBM4>3.0.CO;2-C).

Laganaro, M., & Perret, C. (2011). Comparing electrophysiological correlates of word production in immediate and delayed naming through the analysis of word age of acquisition effects. *Brain Topography*, 24(1), 19–29. <https://doi.org/10.1007/s10548-010-0162-x>.

Legerstee, M., & Markova, G. (2007). Intentions make a difference: infant responses to still-face and modified still-face conditions. *Infant Behavior & Development*, 30(2), 232–250. <https://doi.org/10.1016/j.infbeh.2007.02.008>.

Leong, V., Byrne, E., Clackson, K., Georgieva, S., Lam, S., & Wass, S. (2017). Speaker gaze increases information coupling between infant and adult brains. *Proceedings of the National Academy of Sciences of the United States of America*, 114(50), 13290–13295. <https://doi.org/10.1073/pnas.1702493114>.

Leong, V., Noreika, V., Clackson, K., Georgieva, S., Brightman, L., Nutbrown, R., Fujita, S., Neale, D., & Wass, S. (2019). Mother-infant interpersonal neural connectivity predicts infants' social learning. Preprint.

Lin, J.-F. L., Imada, T., Meltzoff, A. N., Hiraishi, H., Ikeda, T., Takahashi, T., Hasegawa, C., Yoshimura, Y., Kikuchi, M., Hirata, M., Minabe, Y., Asada, M., & Kuhl, P. K. (2023). Dual-MEG interbrain synchronization during turn-taking verbal interactions between mothers and children. *Cerebral Cortex (New York, N.Y. : 1991)*, 33(7), 4116–4134. <https://doi.org/10.1093/cercor/bhac330>.

Liu, C. H., Zhang, E., Snidman, N., & Tronick, E. (2020). Infant affect response in the face-to-face still face among Chinese- and European American mother-infant dyads. *Infant Behavior & Development*, 60, 101469. <https://doi.org/10.1016/j.infbeh.2020.101469>.

Madden-Rusnak, A., Micheletti, M., Bailey, L., & de Barbaro, K. (2025). Soothing touch matters: Patterns of everyday mother-infant physical contact and their real-time physiological implications. *Infant Behavior & Development*, 78, 102021. <https://doi.org/10.1016/j.infbeh.2024.102021>.

Makeig, S., Bell, A., Jung, T.-P., & Sejnowski, T. J. (1995). Independent Component Analysis of Electroencephalographic Data. *Advances in Neural Information Processing Systems*, 8.

https://proceedings.neurips.cc/paper_files/paper/1995/file/754dda4b1ba34c6fa89716b85d68532b-Paper.pdf.

Mantis, I., Stack, D. M., Ng, L., Serbin, L. A., & Schwartzman, A. E. (2014). Mutual touch during mother-infant face-to-face still-face interactions: influences of interaction period and infant birth status. *Infant Behavior & Development*, 37(3), 258–267. <https://doi.org/10.1016/j.infbeh.2014.04.005>.

Meltzoff, A. N., & Decety, J. (2004). What imitation tells us about social cognition: a rapprochement between developmental psychology and cognitive neuroscience. In *The Neuroscience of Social Interaction* (pp. 109–130). Oxford University PressOxford. <https://doi.org/10.1098/rstb.2002.1261>.

Mesman, J., van IJzendoorn, M. H., & Bakermans-Kranenburg, M. J. (2009). The many faces of the Still-Face Paradigm: A review and meta-analysis. *Developmental Review: DR*, 29(2), 120–162. <https://doi.org/10.1016/j.dr.2009.02.001>.

Minagawa, Y., Hata, M., Yamamoto, E., Tsuzuki, D., & Morimoto, S. (2023). Inter-brain synchrony during mother-infant interactive parenting in 3-4-month-old infants with and without an elevated likelihood of autism spectrum disorder. *Cerebral Cortex (New York, N.Y. : 1991)*, 33(24), 11609–11622. <https://doi.org/10.1093/cercor/bhad395>.

Montirosso, R., Borgatti, R., Trojan, S., Zanini, R., & Tronick, E. (2010). A comparison of dyadic interactions and coping with still-face in healthy pre-term and full-term infants. *The British Journal of Developmental Psychology*, 28(Pt 2), 347–368. <https://doi.org/10.1348/026151009X416429>.

Muthukumaraswamy, S. D. (2013). High-frequency brain activity and muscle artifacts in MEG/EEG: a review and recommendations. *Frontiers in Human Neuroscience*, 7, 138. <https://doi.org/10.3389/fnhum.2013.00138>.

Neel, M. L., Jeanvoine, A., Kjeldsen, C. P., & Maitre, N. L. (2025). Mother-Infant Dyadic Neural Synchrony Measured Using EEG Hyperscanning and Validated Using Behavioral Measures. *Children (Basel, Switzerland)*, 12(2). <https://doi.org/10.3390/children12020115>.

Nguyen, T., Abney, D. H., Salamander, D., Bertenthal, B. I., & Hoehl, S. (2021). Proximity and touch are associated with neural but not physiological synchrony in naturalistic mother-infant interactions. *NeuroImage*, 244, 118599. <https://doi.org/10.1016/j.neuroimage.2021.118599>.

Nguyen, T., Schleihauf, H., Kayhan, E., Matthes, D., Vrtička, P., & Hoehl, S. (2020). The effects of interaction quality on neural synchrony during mother-child problem solving. *cortex*, 124, 235-249. <https://doi.org/10.1016/j.cortex.2019.11.020>.

Nolte, G., Bai, O., Wheaton, L., Mari, Z., Vorbach, S., & Hallett, M. (2004). Identifying true brain interaction from EEG data using the imaginary part of coherency. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, 115(10), 2292–2307. <https://doi.org/10.1016/j.clinph.2004.04.029>.

Noreika, V., Georgieva, S., Wass, S., & Leong, V. (2020). 14 challenges and their solutions for conducting social neuroscience and longitudinal EEG research with infants. *Infant Behavior & Development*, 58, 101393. <https://doi.org/10.1016/j.infbeh.2019.101393>.

Oostenveld, R., Fries, P., Maris, E., & Schoffelen, J.-M. (2011). FieldTrip: Open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Computational Intelligence and Neuroscience*, 2011, 156869. <https://doi.org/10.1155/2011/156869>.

Orekhova, E. V., Stroganova, T. A., Posikera, I. N., & Elam, M. (2006). EEG theta rhythm in infants and preschool children. *Clinical Neurophysiology: Official Journal of the International Federation of Clinical Neurophysiology*, 117(5), 1047–1062. <https://doi.org/10.1016/j.clinph.2005.12.027>.

Palva, J. M., & Palva, S. (2018). Functional integration across oscillation frequencies by cross-frequency phase synchronization. *The European Journal of Neuroscience*, 48(7), 2399–2406. <https://doi.org/10.1111/ejn.13767>.

Pereda, E., Quiroga, R. Q., & Bhattacharya, J. (2005). Nonlinear multivariate analysis of neurophysiological signals. *Progress in Neurobiology*, 77(1-2), 1–37. <https://doi.org/10.1016/j.pneurobio.2005.10.003>.

Perone, S., Gartstein, M. A., & Anderson, A. J. (2020). Dynamics of frontal alpha asymmetry in mother-infant dyads: Insights from the Still Face Paradigm. *Infant Behavior & Development*, 61, 101500. <https://doi.org/10.1016/j.infbeh.2020.101500>.

Pili, M. P., Provenzi, L., Billeci, L., Riva, V., Cassa, M., Siri, E., Procissi, G., Roberti, E., & Capelli, E. (2025). Exploring the impact of manual and automatic EEG pre-processing methods on interpersonal neural synchrony measures in parent-infant hyperscanning studies. *Journal of Neuroscience Methods*, 417, 110400. <https://doi.org/10.1016/j.jneumeth.2025.110400>.

Pion-Tonachini, L., Kreutz-Delgado, K., & Makeig, S. (2019). ICLabel: An automated electroencephalographic independent component classifier, dataset, and website. *NeuroImage*, 198, 181–197. <https://doi.org/10.1016/j.neuroimage.2019.05.026>.

Portable EEG for Neuroscience Research. (n.d.). mBrainTrain. Retrieved from <https://mbraintrain.com/>.

Posit team (2024). RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. Retrieved from <http://www.posit.co/>.

Provenzi, L., Fumagalli, M., Bernasconi, F., Sirgiovanni, I., Morandi, F., Borgatti, R., & Montiroso, R. (2017). Very Preterm and Full-Term Infants' Response to Socio-Emotional Stress: The Role of Postnatal Maternal Bonding. *Infancy: The Official Journal of the International Society on Infant Studies*, 22(5), 695–712. <https://doi.org/10.1111/infa.12175>.

Provenzi, L., Scotto di Minico, G., Giusti, L., Guida, E., & Müller, M. (2018). Disentangling the Dyadic Dance: Theoretical, Methodological and Outcomes Systematic Review of Mother-Infant Dyadic Processes. *Frontiers in Psychology*, 9, 348. <https://doi.org/10.3389/fpsyg.2018.00348>.

Redcay, E., & Schilbach, L. (2019). Using second-person neuroscience to elucidate the mechanisms of social interaction. *Nature Reviews. Neuroscience*, 20(8), 495–505. <https://doi.org/10.1038/s41583-019-0179-4>.

Santamaria, L., Noreika, V., Georgieva, S., Clackson, K., Wass, S., & Leong, V. (2020). Emotional valence modulates the topology of the parent-infant inter-brain network. *NeuroImage*, 207, 116341. <https://doi.org/10.1016/j.neuroimage.2019.116341>.

Schwarz, G. (1978). Estimating the dimension of a model. *The annals of statistics*, 461-464.

Srinivasan, R., Nunez, P. L., Tucker, D. M., Silberstein, R. B., & Cadusch, P. J. (1996). Spatial sampling and filtering of EEG with spline laplacians to estimate cortical potentials. *Brain Topography*, 8(4), 355–366. <https://doi.org/10.1007/BF01186911>.

Srinivasan, R. (1999). Methods to improve the spatial resolution of EEG. *International journal of bioelectromagnetism*, 1(1), 102-111.

Stack, D. M., & Muir, D. W. (1990). Tactile stimulation as a component of social interchange: New interpretations for the still-face effect. *The British Journal of Developmental Psychology*, 8(2), 131–145. <https://doi.org/10.1111/j.2044-835X.1990.tb00828.x>.

Stack, D. M., & Muir, D. W. (1992). Adult tactile stimulation during face-to-face interactions modulates five-month-Olds' affect and attention. *Child Development*, 63(6), 1509. <https://doi.org/10.1111/j.1467-8624.1992.tb01711.x>.

Swider-Cios, E., Turk, E., Levy, J., Beeghly, M., Vroomen, J., & van den Heuvel, M. I. (2024). The association of maternal-infant interactive behavior, dyadic frontal alpha asymmetry, and maternal anxiety in a smartphone-adapted still face paradigm. *Developmental Cognitive Neuroscience*, 66, 101352. <https://doi.org/10.1016/j.dcn.2024.101352>.

The Mathworks Inc. (2024). MATLAB version: 9.13.0 (R2024a). Natick, Massachusetts: The Mathworks Inc. Retrieved from <https://www.mathworks.com>.

The Observer XT. (n.d.). Noldus; Noldus Information Technology. Retrieved August 20, 2025, from <https://noldus.com/observer-xt-human>.

Tognoli, E., Lagarde, J., DeGuzman, G. C., & Kelso, J. A. S. (2007). The phi complex as a neuromarker of human social coordination. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(19), 8190–8195. <https://doi.org/10.1073/pnas.0611453104>.

Tronick, E., Als, H., Adamson, L., Wise, S., & Brazelton, T. B. (1978). The infant's response to entrapment between contradictory messages in face-to-face interaction. *Journal of the American Academy of Child Psychiatry*, *17*(1), 1–13. [https://doi.org/10.1016/S0002-7138\(09\)62273-1](https://doi.org/10.1016/S0002-7138(09)62273-1).

Tsolisou, D. (2023). EEG connectivity analysis in infants: A Beginner's Guide on Preprocessing and Processing Techniques. *Brain Science Advances*, *9*(4), 242–274. <https://doi.org/10.26599/BSA.2023.9050025>.

Turk, E., Endevelt-Shapira, Y., Feldman, R., van den Heuvel, M. I., & Levy, J. (2022). Brains in Sync: Practical Guideline for Parent-Infant EEG During Natural Interaction. *Frontiers in Psychology*, *13*, 833112. <https://doi.org/10.3389/fpsyg.2022.833112>.

Vinck, M., Oostenveld, R., van Wingerden, M., Battaglia, F., & Pennartz, C. M. A. (2011). An improved index of phase-synchronization for electrophysiological data in the presence of volume-conduction, noise and sample-size bias. *NeuroImage*, *55*(4), 1548–1565. <https://doi.org/10.1016/j.neuroimage.2011.01.055>.

Wass, S. V., Noreika, V., Georgieva, S., Clackson, K., Brightman, L., Nutbrown, R., Covarrubias, L. S., & Leong, V. (2018). Parental neural responsivity to infants' visual attention: How mature brains influence immature brains during social interaction. *PLoS Biology*, *16*(12), e2006328. <https://doi.org/10.1371/journal.pbio.2006328>.

Wobbrock, J. O., Findlater, L., Gergle, D., & Higgins, J. J. (2011, May 7). The aligned rank transform for nonparametric factorial analyses using only anova procedures. *Proceedings of the SIGCHI Conference on Human Factors in Computing Systems*. CHI '11: CHI Conference on Human Factors in Computing Systems, Vancouver BC Canada. <https://doi.org/10.1145/1978942.1978963>.

Xie, W., Mallin, B. M., & Richards, J. E. (2018). Development of infant sustained attention and its relation to EEG oscillations: an EEG and cortical source analysis study. *Developmental Science*, 21(3), e12562. <https://doi.org/10.1111/desc.12562>.

Zakharov, I., Adamovich, T., Tabueva, A., Ismatullina, V., & Malykh, S. (2021). The effect of density thresholding on the EEG network construction. *Journal of Physics. Conference Series*, 1727(1), 012009. <https://doi.org/10.1088/1742-6596/1727/1/012009>.

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Table 1. Grand central tendency measures of the PDC values for each frequency band, FFSF episode and direction of connectivity.

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Figure 5. Brain maps representing the ratio of I->P-dominant and P->I-dominant PDC in the *alpha* (6-12 Hz) frequency band. The analyses were performed on the whole sample (A) as well as for the median-split samples according to the level of infant gaze aversion during the SF (B), infant negative emotionality during the SF (C) and maternal affective touch during the Reunion (D). The arrows indicate the connections having a I->P dominance (gold) or a P->I dominance (blue) at a significance level of adjusted p -value ≤ 0.05 . Thickness of the connections represents the significance level (low thickness: $p_{\text{adj}} \leq 0.05$; medium thickness: $p_{\text{adj}} \leq 0.01$; high thickness: $p_{\text{adj}} \leq 0.001$). Square brackets indicate significant inter-episode changes in number of directed connections according to the two proportions Fisher exact test (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

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Follow the leader and shift: Changes in Parent-Infant Partial Directed Coherence after a Perturbation of Contingent Interaction

SUPPLEMENTARY MATERIAL

[Authors masked for peer review]

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SUPPLEMENTARY MATERIALS 1 (S1) – BEHAVIORAL DATA

This section contains the instructions of the microanalytical coding scheme used to extract the behaviors of the included dyads during the FFSF procedure (Table S1.1), the central tendency measures of the behaviors of interest (Table S1.2), as well as the distribution of the included dyads in the low and high behavioral subgroups.

Table S1.1. Description of the codes of our behaviors of interest. Adapted from [masked for peer review].

Variable	Levels	Description
INFANT-SPECIFIC CODES		
Emotional state	Negative	Clear display of negative emotionality (e.g., eyes, mouth, general movements of the face or the body, and other vocal or non-vocal signals) including fussing and crying.
Gaze direction	Object-directed/avoiding	The subject is actively avoiding eye contact as displayed by head and body movements/posture or looking at an inanimate object.
PARENT-SPECIFIC CODES		
Tactile inputs	Affective	Tactile stimulations that convey playful and social engagement such as tickling, squeezing, and any other appropriate entertaining tactile stimulations that are fast-paced, dynamic, repetitive, and/or characterized by quick cinematic features. This category also includes tactile stimulations that are finalized to soothe or regulate the behavioral state of the interactive partners, such as stroking, kissing, massaging, and any other appropriate tactile stimulations with clear regulatory functions and conveying a sense of affective closeness.

Table S1.2. Central tendency measures of the behavioral variables of interest per episode. Behaviors of interest included in the main and post-hoc analyses are highlighted in *italic and bold*.

EPISODE	MEAN ± SD (PERCENTAGE)	RANGE	MEDIAN
Infant gaze aversion			
Play	39.12 ± 22.58	8.75-97.09	36.82
SF	62.63 ± 22.15	6.83-98.46	66.01
Reunion	41.70 ± 23.89	0-87.70	37.5
Infant negative emotionality			
Play	10.30 ± 17.38	0-77.25	0
SF	37.91 ± 28.82	0-93.55	36.78
Reunion	31.91 ± 34.57	0-100	19.83
Maternal affective touch			
Play	33.03 ± 25.14	0-91.44	26.49
Reunion	36.34 ± 24.33	0-86.57	28.96

Figure S1.1. Distribution of the dyads according to the number of episodes in which the infant displayed high (as in higher than the median) gaze aversion. Legend: P = Play; SF = Still-Face; R = Reunion.

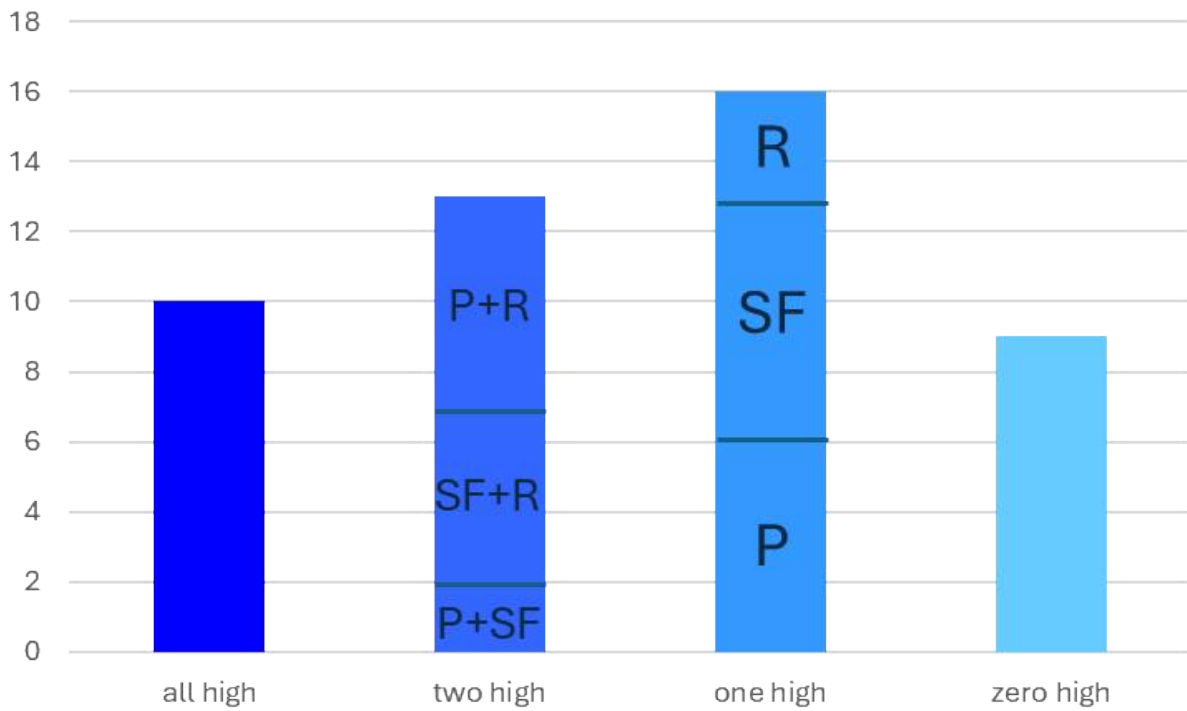


Table S1.3. Distribution of the dyads according to the number of episodes in which the infant displayed high (as in higher than the median) gaze aversion.

Distribution across FFSF episodes	N dyads
3/3 high	10
2/3 high	13
Play + SF	2
SF + Reunion	5
Play + Reunion	6
1/3 high	16
Play	6
SF	7
Reunion	3
0/3 high	9

Figure S1.2. Distribution of the dyads according to the number of episodes in which the infant displayed high (as in higher than the median) negative emotionality. Legend: P = Play; SF = Still-Face; R = Reunion.

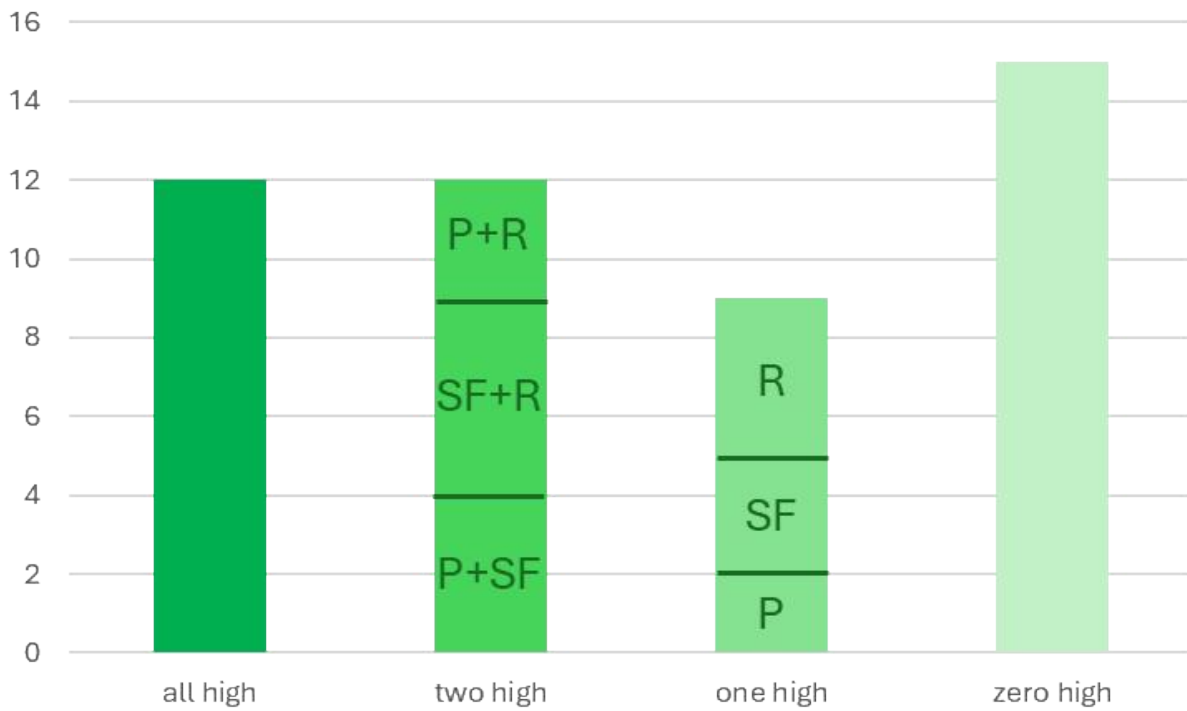


Table S1.4. Distribution of the dyads according to the number of episodes in which the infant displayed high (as in higher than the median) negative emotionality.

Distribution across FFSF episodes	N dyads
3/3 high	12
2/3 high	12
Play + SF	4
SF + Reunion	5
Play + Reunion	3
1/3 high	9
Play	2
SF	3
Reunion	4
0/3 high	15

Figure S1.3. Distribution of the dyads according to the number of episodes in which the mother displayed high (as in higher than the median) affective touch. Legend: P = Play; SF = Still-Face; R = Reunion.

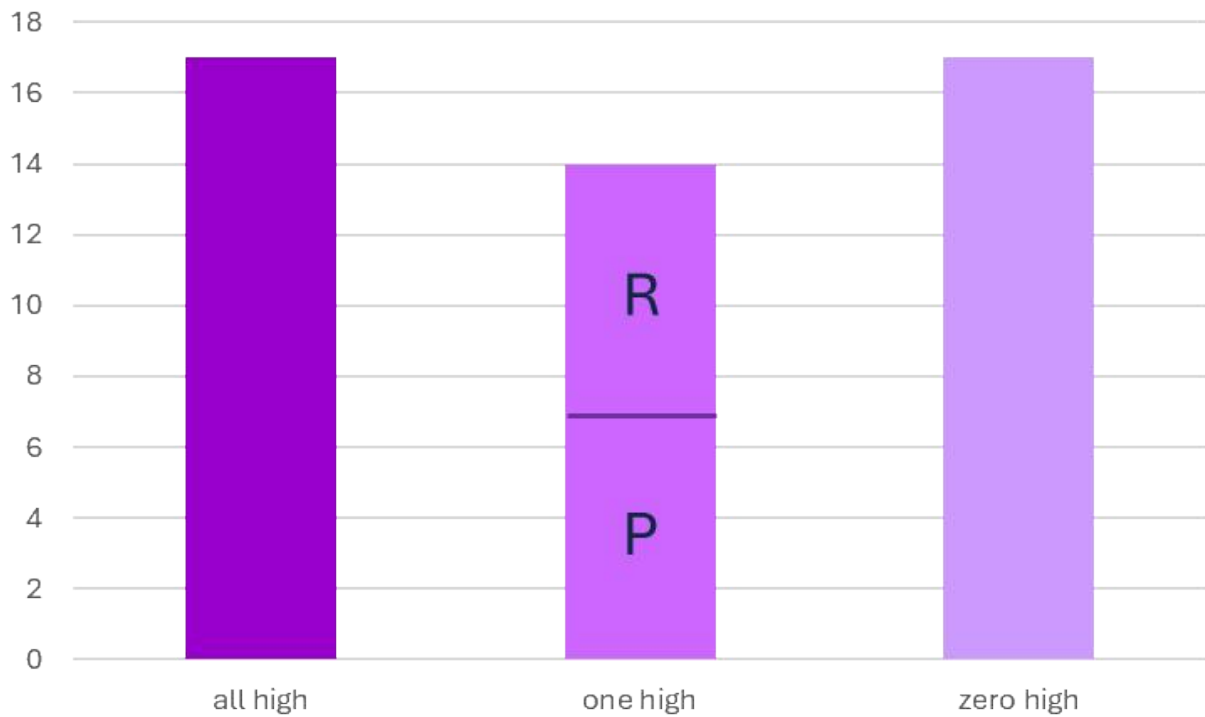


Table S1.5. Distribution of the dyads according to the number of episodes in which the mother displayed high (as in higher than the median) affective touch.

Distribution across FFSF episodes	N dyads
2/2 high	17
1/2 high	14
Play	7
Reunion	7
0/2 high	17

Table S1.6. Percentage of overlap across behavioral low and high subgroups. Legend: GA = Gaze aversion; NE = Negative emotionality; AT = Affective touch.

Groups	Low subgroup overlap	High subgroup overlap
GA + NE	50% (N = 12/24)	50% (N = 12/24)
GA + AT	50% (N = 12/24)	50% (N = 12/24)
NE + AT	54% (N = 13/24)	54% (N = 13/24)

SUPPLEMENTARY MATERIAL 2 (S2) – FULL STATISTICAL REPORT

This section contains the results of the ART ANOVA (Table S2.1) and of the paired samples Wilcoxon tests (Tables S2.2-S2.5) summarized in the Results section and in Figures 3-6 from the manuscript.

Table S2.1. F-statistics and p-values (raw and adjusted) of the ART ANOVA run on *alpha* and *theta* PDC. The table reports only the statistics of the factors with $p_{adj} \leq 0.05$.

Channel pair	ALPHA				THETA			
	Sign. factor	F	p (raw)	p (adjusted)	Sign. factor	F	p (raw)	p (adjusted)
F3-F3	Direction	8.859	0.005	0.012	None			
F3-F4	None				None			
F3-C3	Direction	64.504	< 0.001	< 0.001	Direction	17.460	< 0.001	< 0.001
F3-C4	Direction	57.493	< 0.001	< 0.001	Direction	13.859	< 0.001	0.002
F3-P3	Direction	34.355	< 0.001	< 0.001	Direction	7.296	0.01	0.021
F3-P4	Direction	43.026	< 0.001	< 0.001	Direction	15.182	< 0.001	0.001
F4-F3	Direction	8.156	0.006	0.016	None			
F4-F4	None				None			
F4-C3	Direction	62.937	< 0.001	< 0.001	Direction	21.383	< 0.001	< 0.001
F4-C4	Direction	48.482	< 0.001	< 0.001	Direction	15.463	< 0.001	< 0.001
F4-P3	Direction	42.731	< 0.001	< 0.001	Direction	13.852	< 0.001	< 0.001
F4-P4	Direction	33.022	< 0.001	< 0.001	Direction	12.222	0.001	0.003
C3-F3	None				Direction	19.876	< 0.001	< 0.001
C3-F4	None				Direction	23.793	< 0.001	< 0.001
C3-C3	Direction	15.984	< 0.001	0.001	None			
C3-C4	Direction	6.392	0.015	0.030	None			
C3-P3	None				None			
C3-P4	None				None			
C4-F3	None				Direction	11.743	0.001	0.004
C4-F4	None				Direction	18.806	< 0.001	< 0.001
C4-C3	Direction	15.330	< 0.001	0.001	None			
C4-C4	Direction	8.703	0.005	0.012	None			
C4-P3	Direction	7.681	0.008	0.018	None			
C4-P4	None				None			
P3-F3	None				Direction	16.410	< 0.001	< 0.001
P3-F4	Direction	11.451	0.001	0.004	Direction	48.833	< 0.001	< 0.001
P3-C3	Episode*Gaze aversion	14.471	< 0.001	0.045	None			
P3-C4	None				None			

P3-P3	None				None			
P3-P4	None				Direction	6.7618	0.012	0.027
P4-F3	None				Episode*Gaze aversion	14.939	< 0.001	0.045
P4-F4	None				Direction	25.201	< 0.001	< 0.001
P4-C3	Direction	13.211	0.001	0.002	None			
P4-C4	Direction	8.028	0.001	0.016	None			
P4-P3	None				None			
P4-P4	None				Direction	7.155	0.010	0.023

Table S2.2. Set of I->P and P->I connections with a significantly ($p_{raw} \leq 0.05$) different *alpha* (6-12 Hz) PDC strength from Play to Reunion according to the ranked sum Wilcoxon test for paired samples. Results were computed separately for each behavioral group (infant gaze aversion during the SF, infant negative emotionality during the SF, maternal affective touch during the Reunion) and for each level (low percentage vs high percentage), as well as on the whole sample of 48 parent-infant dyads. Channel pairs are always indicated with the infant channel first and the parent channel second. Mean difference > 0 designates connections in which PDC was significantly higher during Play compared to Reunion, whereas mean difference < 0 indicates connections in which PDC was significantly higher during Reunion compared to Play.

WHOLE SAMPLE					
Direction	Chan pair	W	Mean difference	p-value (raw)	Adjusted p value
I->P	F4-F3	812	0.016	0.021	0.347
P->I	C3-C3	395	-0.009	0.048	0.419
	P4-C4	328	-0.015	0.007	0.238
	C4-F3	339	-0.013	0.010	0.238
	F4-P3	369	-0.010	0.024	0.347
	P3-P3	345	-0.006	0.012	0.246
	F3-P4	314	-0.007	0.004	0.238
INFANT GAZE AVERSION SF (LOW SUBGROUP)					
I->P	F4-F3	241	0.027	0.008	0.285
	F4-P4	231	0.027	0.019	0.343
	C4-F4	227	0.016	0.027	0.381
	P3-C3	229	0.023	0.023	0.381
	P3-C4	232	0.013	0.018	0.343
P->I	C4-F3	73	-0.014	0.027	0.381
	P4-C4	74	-0.018	0.029	0.387
	P3-P3	76	-0.004	0.033	0.419
INFANT GAZE AVERSION SF (HIGH SUBGROUP)					
I->P	C3-F3	80	-0.010	0.046	0.429
	C3-F4	73	-0.011	0.027	0.381
P->I	F3-P4	26	-0.021	<0.001	0.110
	F3-C3	57	-0.028	0.007	0.268
	F4-C3	62	-0.020	0.011	0.302
	C4-P3	62	-0.021	0.011	0.302
	C4-F4	67	-0.017	0.016	0.343
	F4-P3	69	-0.018	0.019	0.343
	C3-C4	78	-0.022	0.039	0.421
	C4-P4	80	-0.010	0.046	0.429
INFANT NEGATIVE EMOTIONALITY SF (LOW SUBGROUP)					
I->P	F4-F3	234	0.028	0.015	0.333
P->I	F4-P3	79	-0.006	0.042	0.427
INFANT NEGATIVE EMOTIONALITY SF (HIGH SUBGROUP)					
I->P	F4-C3	243	0.025	0.007	0.268
	F4-P4	232	0.017	0.018	0.343
P->I	C4-F3	36	-0.019	<0.001	0.157
	P4-C4	45	-0.025	0.002	0.216
	C3-C3	47	-0.018	0.002	0.216
	P4-P4	49	-0.020	0.003	0.221
	P4-C3	51	-0.016	0.004	0.225
	C4-C4	55	-0.012	0.005	0.256
	P3-C3	60	-0.011	0.009	0.290

	F4-P4	60	-0.018	0.009	0.290
	P3-F3	63	-0.009	0.012	0.302
	F4-C4	66	-0.014	0.015	0.333
	F4-C3	68	-0.021	0.018	0.343
	C4-P4	70	-0.011	0.021	0.365
	F3-P4	72	-0.012	0.025	0.381
	F4-F3	73	-0.017	0.027	0.381
	P3-P3	76	-0.008	0.034	0.419
	P3-C4	79	-0.014	0.042	0.427
	C4-P3	79	-0.019	0.042	0.427
MATERNAL AFFECTIVE TOUCH REUNION (LOW SUBGROUP)					
I->P	F4-P4	223	0.020	0.037	0.421
	P4-C3	63	-0.021	0.012	0.302
P->I	P4-C4	63	-0.015	0.012	0.302
	F3-P4	78	-0.003	0.039	0.421
MATERNAL AFFECTIVE TOUCH REUNION (HIGH SUBGROUP)					
I->P	F3-P4	227	0.017	0.027	0.381
P->I	C4-F3	40	-0.025	<0.001	0.157
	C4-F4	59	-0.018	0.008	0.285
	P3-P3	68	-0.015	0.018	0.343
	P3-C4	72	-0.015	0.025	0.381
	F3-P3	76	-0.014	0.034	0.419
	F3-C4	78	-0.014	0.039	0.421
	C3-F3	80	-0.019	0.046	0.429
	F4-P3	80	-0.017	0.046	0.429
	C3-C4	81	-0.021	0.049	0.451

Table S2.3. Set of I->P and P->I connections with a significantly ($p_{raw} \leq 0.05$) different *theta* (3-7 Hz) PDC strength from Play to Reunion according to the ranked sum Wilcoxon test for paired samples. Results were computed separately for each behavioral group (infant gaze aversion during the SF, infant negative emotionality during the SF, maternal affective touch during the Reunion) and for each level (low percentage vs high percentage), as well as on the whole sample of 48 parent-infant dyads. Channel pairs are always indicated with the infant channel first and the parent channel second. Mean difference > 0 designates connections in which PDC was significantly higher during Play compared to Reunion, whereas mean difference < 0 indicates connections in which PDC was significantly higher during Reunion compared to Play.

WHOLE SAMPLE					
Direction	Chan pair	W	Mean difference	p-value (raw)	Adjusted p value
I->P	P4-C3	386	-0.007	0.038	0.419
	F4-C4	794	0.010	0.034	0.419
	F4-F3	809	0.011	0.023	0.347
	F4-P4	784	0.011	0.044	0.419
P->I	C4-P3	336	-0.015	0.009	0.238
	F4-P3	325	-0.014	0.006	0.238
	P3-P3	394	-0.004	0.047	0.419
	C4-P4	334	-0.011	0.008	0.238
INFANT GAZE AVERSION SF (LOW SUBGROUP)					
I->P	C3-F3	250	0.017	0.003	0.225
	F4-C4	248	0.019	0.004	0.225
	F4-F3	246	0.022	0.005	0.256
	P4-C3	74	-0.009	0.029	0.387
	P3-C3	220	0.013	0.046	0.429
INFANT GAZE AVERSION SF (HIGH SUBGROUP)					
P->I	F3-P4	41	-0.022	0.001	0.157
	C4-P3	47	-0.031	0.002	0.216
	C4-P4	49	-0.020	0.003	0.221
	F4-F4	65	-0.012	0.014	0.333
	F4-C3	66	-0.020	0.015	0.333
	F3-C3	73	-0.028	0.027	0.381
	C4-C4	78	-0.013	0.039	0.421
INFANT NEGATIVE EMOTIONALITY SF (LOW SUBGROUP)					
I->P	C3-P4	224	0.016	0.034	0.419
	F4-F3	220	0.018	0.046	0.429
P->I	P3-C3	231	0.020	0.019	0.343
	F4-P3	77	-0.011	0.037	0.421
INFANT NEGATIVE EMOTIONALITY SF (HIGH SUBGROUP)					
I->P	P4-F4	236	0.009	0.013	0.320
	F4-C3	234	0.017	0.015	0.333
P->I	P3-C3	55	-0.012	0.005	0.256
	C4-F3	58	-0.021	0.007	0.282
	C4-C4	67	-0.014	0.016	0.343
	C3-C3	69	-0.017	0.019	0.343
	P3-P3	71	-0.009	0.023	0.381
	C4-P4	73	-0.016	0.027	0.381

	F3-C3	74	-0.022	0.029	0.387
	F4-C4	77	-0.017	0.037	0.421
	P4-P4	78	-0.022	0.039	0.421
	P3-F3	79	-0.011	0.042	0.427
	P4-C4	79	-0.019	0.042	0.427
MATERNAL AFFECTIVE TOUCH REUNION (LOW SUBGROUP)					
I->P	P4-C3	36	-0.015	<0.001	0.157
	C3-P4	239	0.018	0.009	0.302
	F4-C4	224	0.017	0.034	0.419
	F3-P3	81	-0.011	0.049	0.451
P->I	P4-C4	74	-0.013	0.029	0.387
MATERNAL AFFECTIVE TOUCH REUNION (HIGH SUBGROUP)					
P->I	C4-P4	39	-0.021	<0.001	0.157
	C4-F3	52	-0.026	0.004	0.225
	P3-P3	57	-0.016	0.007	0.269
	F4-P3	62	-0.019	0.011	0.302
	C3-F3	77	-0.022	0.037	0.421
	C4-P3	77	-0.027	0.037	0.421
	C4-F4	78	-0.019	0.039	0.421

Table S2.4. Set of connections during Play and Reunion episodes with a significantly ($p_{adj} \leq 0.05$) different *alpha* (6-12 Hz) PDC strength between I->P and P->I directions according to the ranked sum Wilcoxon test for paired samples. Results were computed separately for each behavioral group (infant gaze aversion during the SF, infant negative emotionality during the SF, maternal affective touch during the Reunion) and for each level (low percentage vs high percentage), as well as on the whole sample of 48 parent-infant dyads. Channel pairs are always indicated with the infant channel first and the parent channel second. Mean difference > 0 designates connections with a significant I->P dominance, whereas mean difference < 0 indicates connections with a significant P->I dominance.

WHOLE SAMPLE					
Episode	Chan pair	W	Mean difference	p-value (raw)	Adjusted p value
Play	C3-C3	958	0.029	<0.001	<0.001
	C4-C3	968	0.027	<0.001	<0.001
	F3-C3	1117	0.052	<0.001	<0.001
	F4-C3	1090	0.058	<0.001	<0.001
	P3-C3	953	0.029	<0.001	<0.001
	P4-C3	877	0.015	0.003	0.009
	C3-C4	932	0.023	<0.001	0.001
	C4-C4	900	0.021	0.001	0.004
	F3-C4	1135	0.060	<0.001	<0.001
	F4-C4	1088	0.047	<0.001	<0.001
	P3-C4	818	0.009	0.018	0.042
	P4-C4	880	0.018	0.002	0.008
	F3-F3	847	0.021	0.007	0.020
	F4-F3	915	0.025	<0.001	0.003
	F3-F4	864	0.023	0.023	0.012
	C4-P3	850	0.021	0.007	0.019
	F3-P3	1028	0.033	<0.001	<0.001
	F4-P3	1036	0.036	<0.001	<0.001
	P3-P3	843	0.013	0.008	0.022
	P4-P3	824	0.012	0.015	0.037
F3-P4	1059	0.041	<0.001	<0.001	
F4-P4	1091	0.038	<0.001	<0.001	
Reunion	C3-C3	814	0.016	0.020	0.047
	C4-C3	835	0.015	0.011	0.027
	F3-C3	1032	0.052	<0.001	<0.001
	F4-C3	1031	0.044	<0.001	<0.001
	P3-C3	846	0.015	0.007	0.020
	P4-C3	873	0.021	0.003	0.010
	F3-C4	951	0.042	<0.001	<0.001
	F4-C4	968	0.034	<0.001	<0.001
	F3-F3	821	0.016	0.016	0.039
	P3-F4	240	-0.022	<0.001	0.001
	P4-F4	329	-0.016	0.007	0.020
	F3-P3	977	0.034	<0.001	<0.001
	F4-P3	959	0.028	<0.001	<0.001
	F3-P4	975	0.030	<0.001	<0.001
F4-P4	883	0.018	0.002	0.008	
INFANT GAZE AVERSION SF (LOW SUBGROUP)					
Play	C3-C3	242	0.033	0.007	0.038
	C4-C3	256	0.028	0.002	0.013
	F3-C3	267	0.058	<0.001	0.005
	F3-C4	268	0.057	<0.001	<0.001

	F3-P3	268	0.040	<0.001	0.005
	F3-P4	274	0.055	<0.001	0.002
	F4-C3	270	0.063	<0.001	0.004
	F4-C4	270	0.041	<0.001	0.004
	F4-F3	255	0.028	0.002	0.015
	F4-P3	271	0.042	<0.001	0.004
	F4-P4	274	0.044	<0.001	0.003
	P3-C3	250	0.038	0.003	0.022
	P3-P3	241	0.016	0.008	0.040
Reunion	F3-C3	258	0.061	0.001	0.012
	F3-C4	244	0.053	0.006	0.033
	F3-P3	253	0.047	0.002	0.018
	F4-C3	262	0.050	<0.001	0.008
	F4-C4	240	0.029	0.009	0.042
	F4-P3	262	0.031	<0.001	0.008
INFANT GAZE AVERSION SF (HIGH SUBGROUP)					
Play	C3-C3	246	0.024	0.005	0.029
	C3-C4	245	0.026	0.005	0.032
	F3-C3	300	0.047	<0.001	<0.001
	F3-C4	292	0.063	<0.001	<0.001
	F3-P3	251	0.026	0.003	0.021
	F3-P4	262	0.026	<0.001	0.008
	F4-C3	285	0.053	<0.001	<0.001
	F4-C4	287	0.053	<0.001	<0.001
	F4-P3	259	0.029	0.001	0.011
	F4-P4	279	0.033	<0.001	0.002
	P4-C3	241	0.020	0.008	0.040
	P4-C4	242	0.026	0.007	0.038
Reunion	F3-C3	269	0.042	<0.001	0.004
	F3-C4	240	0.032	0.009	0.042
	F3-P3	245	0.022	0.005	0.032
	F3-P4	274	0.029	<0.001	0.003
	F4-C3	262	0.039	<0.001	0.008
	F4-C4	249	0.039	0.004	0.024
	P3-F4	50	-0.025	0.003	0.022
INFANT NEGATIVE EMOTIONALITY SF (LOW SUBGROUP)					
Play	C4-C3	259	0.033	0.001	0.011
	F3-C3	273	0.044	<0.001	0.003
	F3-C4	283	0.063	<0.001	<0.001
	F3-P3	254	0.039	0.002	0.017
	F3-P4	249	0.033	0.004	0.024
	F4-C3	267	0.052	<0.001	0.005
	F4-C4	269	0.046	<0.001	0.004
	F4-P3	259	0.037	0.001	0.011
	F4-P4	271	0.035	<0.001	0.004
Reunion	F3-C3	265	0.065	<0.001	0.006
	F3-C4	249	0.050	0.004	0.024
	F3-P3	248	0.039	0.004	0.025
	F4-C3	275	0.054	<0.001	0.003
	F4-C4	249	0.033	0.004	0.024
	F4-P3	263	0.035	<0.001	0.008
	F4-P4	244	0.023	0.006	0.033
	P3-F4	61	-0.022	0.010	0.044
INFANT NEGATIVE EMOTIONALITY SF (HIGH SUBGROUP)					
	C3-C3	262	0.032	<0.001	<0.001

Play	C3-C4	248	0.025	<0.001	<0.001
	C4-C4	251	0.025	0.003	0.021
	F3-C3	293	0.061	<0.001	<0.001
	F3-C4	294	0.058	<0.001	<0.001
	F3-F4	243	0.025	0.007	0.035
	F3-P3	270	0.027	0.007	0.004
	F3-P4	285	0.048	<0.001	<0.001
	F4-C3	289	0.063	<0.001	<0.001
	F4-C4	287	0.048	<0.001	<0.001
	F4-F3	244	0.015	0.006	0.033
	F4-P3	269	0.035	<0.001	0.004
	F4-P4	284	0.042	<0.001	<0.001
	P3-C3	262	0.043	<0.001	0.008
	P3-P3	241	0.022	0.008	0.040
	P3-P4	240	0.021	0.009	0.042
	P4-C4	264	0.035	<0.001	0.007
Reunion	F3-C3	266	0.039	<0.001	0.006
	F3-P3	256	0.030	0.002	0.014
	F3-P4	280	0.039	<0.001	0.001
	F4-C3	248	0.035	0.004	0.025
	F4-C4	241	0.035	0.008	0.040
	P3-F4	62	-0.021	0.011	0.048
MATERNAL AFFECTIVE TOUCH REUNION (LOW SUBGROUP)					
Play	C3-C3	270	0.040	<0.001	0.004
	C3-C4	256	0.028	0.002	0.014
	C4-C3	260	0.033	<0.001	0.010
	C4-C4	281	0.037	<0.001	0.001
	C4-P3	244	0.033	0.006	0.033
	F3-C3	286	0.049	<0.001	<0.001
	F3-C4	299	0.074	<0.001	<0.001
	F3-P3	277	0.045	<0.001	0.002
	F3-P4	251	0.034	0.003	0.021
	F4-C3	291	0.070	<0.001	<0.001
	F4-C4	300	0.062	<0.001	<0.001
	F4-F3	252	0.040	0.003	0.020
	F4-P3	263	0.040	<0.001	0.008
	F4-P4	279	0.039	<0.001	0.002
	P3-C3	258	0.029	0.001	0.012
	P3-C4	238	0.019	0.011	0.048
P4-C4	241	0.019	0.008	0.040	
Reunion	C4-C3	239	0.030	0.010	0.044
	C4-P3	248	0.025	0.004	0.025
	F3-C3	274	0.063	<0.001	0.003
	F3-C4	255	0.061	0.002	0.015
	F3-F3	251	0.039	0.003	0.021
	F3-P3	268	0.048	<0.001	0.005
	F3-P4	241	0.033	0.008	0.040
	F4-C3	288	0.058	<0.001	<0.001
	F4-C4	265	0.052	<0.001	0.006
F4-P3	285	0.042	<0.001	<0.001	
MATERNAL AFFECTIVE TOUCH REUNION (HIGH SUBGROUP)					
Play	F3-C3	284	0.056	<0.001	<0.001
	C3-C4	277	0.047	<0.001	0.002
	F3-P3	245	0.021	0.005	0.032
	P3-P4	284	0.047	<0.001	<0.001

	F4-C3	263	0.046	<0.001	0.008
	F4-C4	251	0.032	0.003	0.021
	F4-P3	263	0.032	<0.001	0.008
	F4-P4	272	0.037	<0.001	0.003
Reunion	F3-C3	252	0.041	0.003	0.020
	F3-P4	257	0.027	0.001	0.013
	P3-F4	40	-0.031	<0.001	0.010

Table S2.5. Set of connections during Play and Reunion episodes with a significantly ($p_{adj} \leq 0.05$) different *theta* (3-7 Hz) PDC strength between I->P and P->I directions according to the ranked sum Wilcoxon test for paired samples. Results were computed separately for each behavioral group (infant gaze aversion during the SF, infant negative emotionality during the SF, maternal affective touch during the Reunion) and for each level (low percentage vs high percentage), as well as on the whole sample of 48 parent-infant dyads. Channel pairs are always indicated with the infant channel first and the parent channel second. Mean difference > 0 designates connections with a significant I->P dominance, whereas mean difference < 0 indicates connections with a significant P->I dominance.

WHOLE SAMPLE					
Episode	Chan pair	W	Mean difference	p-value (raw)	Adjusted p value
Play	F3-C3	886	0.021	0.002	0.007
	F4-C3	964	0.024	<0.001	<0.001
	F3-C4	1031	0.025	<0.001	<0.001
	F4-C4	1013	0.022	<0.001	<0.001
	C3-F3	336	-0.013	0.009	0.024
	P3-F3	288	-0.021	0.002	0.007
	P4-F3	305	-0.014	0.003	0.010
	C3-F4	299	-0.014	0.003	0.009
	C4-F4	306	-0.011	0.003	0.010
	P3-F4	279	-0.015	0.001	0.005
	P4-F4	258	-0.016	<0.001	0.002
	F4-P3	841	0.015	0.009	0.023
	F3-P4	884	0.016	0.002	0.007
	F4-P4	974	0.020	<0.001	<0.001
Reunion	F4-C3	872	0.012	0.003	0.010
	C3-F3	230	-0.026	<0.001	<0.001
	C4-F3	340	-0.023	0.010	0.026
	P3-F3	262	-0.026	<0.001	0.003
	P4-F3	254	-0.020	<0.001	0.002
	C3-F4	216	-0.029	<0.001	<0.001
	C4-F4	220	-0.022	<0.001	<0.001
	P3-F4	64	-0.031	<0.001	<0.001
	P4-F4	193	-0.022	<0.001	<0.001
	F3-P4	851	0.012	0.006	0.020
P4-P4	364	-0.018	0.021	0.049	
INFANT GAZE AVERSION SF (LOW SUBGROUP)					
Play	F3-C4	260	0.029	<0.001	0.010
	F3-P4	243	0.022	0.007	0.035
	F4-C3	246	0.032	0.005	0.029
	F4-C4	246	0.022	0.005	0.029
	F4-P4	256	0.021	0.002	0.014
	P3-F4	57	-0.019	0.007	0.035
	P4-F3	62	-0.017	0.011	0.048
Reunion	C3-F3	51	-0.018	0.004	0.024
	C3-F4	50	-0.031	0.003	0.022
	C4-F4	59	-0.016	0.008	0.040
	P3-F4	21	-0.033	<0.001	0.002
	P4-F4	51	-0.017	0.004	0.024
INFANT GAZE AVERSION SF (HIGH SUBGROUP)					
Play	F3-C4	265	0.021	<0.001	0.006
	F4-C3	250	0.016	0.003	0.022

	F4-C4	271	0.023	<0.001	0.004
	F4-P4	247	0.019	0.004	0.028
Reunion	C3-F3	60	-0.033	0.009	0.042
	C3-F4	56	-0.027	0.006	0.033
	C4-F4	55	-0.027	0.005	0.032
	P3-F4	13	-0.030	<0.001	<0.001
	P4-F3	50	-0.028	0.003	0.022
	P4-F4	47	-0.026	0.002	0.018
	INFANT NEGATIVE EMOTIONALITY SF (LOW SUBGROUP)				
Play	C3-F4	60	-0.017	0.009	0.042
	F3-C4	246	0.022	0.005	0.029
	F4-C3	239	0.019	0.010	0.044
	F4-C4	262	0.020	<0.001	0.008
	F4-P4	246	0.015	0.005	0.029
	P3-F3	60	-0.027	0.010	0.042
	P3-F4	46	-0.022	0.002	0.017
	P4-F3	59	-0.021	0.008	0.040
	P4-F4	26	-0.025	<0.001	0.003
	P4-P4	25	-0.030	<0.001	0.003
Reunion	C3-F4	61	-0.022	0.010	0.044
	F4-C3	246	0.018	0.005	0.029
	P3-F4	4	-0.034	<0.001	<0.001
	P4-F3	61	-0.017	0.010	0.044
	P4-P4	56	-0.020	0.006	0.033
INFANT NEGATIVE EMOTIONALITY SF (HIGH SUBGROUP)					
Play	F3-C3	253	0.027	0.002	0.018
	F3-C4	278	0.028	<0.001	0.002
	F3-P3	244	0.013	0.006	0.033
	F3-P4	259	0.020	0.001	0.011
	F4-C3	257	0.029	0.001	0.013
	F4-C4	256	0.025	0.002	0.014
	F4-P4	250	0.025	0.003	0.022
Reunion	C3-F3	56	-0.031	0.006	0.033
	C3-F4	48	-0.037	0.003	0.020
	C4-F4	40	-0.028	0.001	0.010
	P3-F4	27	-0.029	<0.001	0.003
	P4-F4	37	-0.028	<0.001	0.008
MATERNAL AFFECTIVE TOUCH REUNION (LOW SUBGROUP)					
Play	F3-C4	271	0.035	<0.001	0.004
	F4-C3	269	0.040	<0.001	0.004
	F4-C4	288	0.038	<0.001	<0.001
	F4-P4	246	0.027	0.005	0.029
	P4-F4	55	-0.018	0.005	0.032
Reunion	C3-F3	57	-0.018	0.007	0.035
	C3-F4	60	-0.027	0.009	0.042
	F3-C3	241	0.030	0.008	0.040
	F3-P3	241	0.032	0.008	0.040
	F4-C3	273	0.024	<0.001	0.003
	F4-P3	254	0.018	0.002	0.017
	P3-F4	13	-0.026	<0.001	<0.001
MATERNAL AFFECTIVE TOUCH REUNION (HIGH SUBGROUP)					
Play	C4-F4	62	-0.014	0.011	0.048
	F3-C4	256	0.016	0.002	0.014
	F3-P4	254	0.020	0.002	0.017
	F4-P4	248	0.013	0.004	0.025

Reunion	C3-F3	57	-0.034	0.007	0.035
	C3-F4	48	-0.032	0.003	0.020
	C4-F3	59	-0.037	0.008	0.040
	C4-F4	23	-0.030	<0.001	0.002
	P3-F3	61	-0.037	0.010	0.044
	P3-F4	19	-0.037	<0.001	0.001
	P3-P3	61	-0.021	0.010	0.044
	P4-C4	43	-0.028	0.001	0.013
	P4-F3	57	-0.028	0.007	0.035
	P4-F4	22	-0.024	<0.001	0.002

Figure S2.1. Heatmaps of I->P PDC in the *alpha* (6-12 Hz) frequency band measured in the median-split samples of dyads with low versus high infant gaze aversion during the SF (A), low versus high infant negative emotionality during the SF (B) and low versus high maternal affective touch during the Reunion (C).

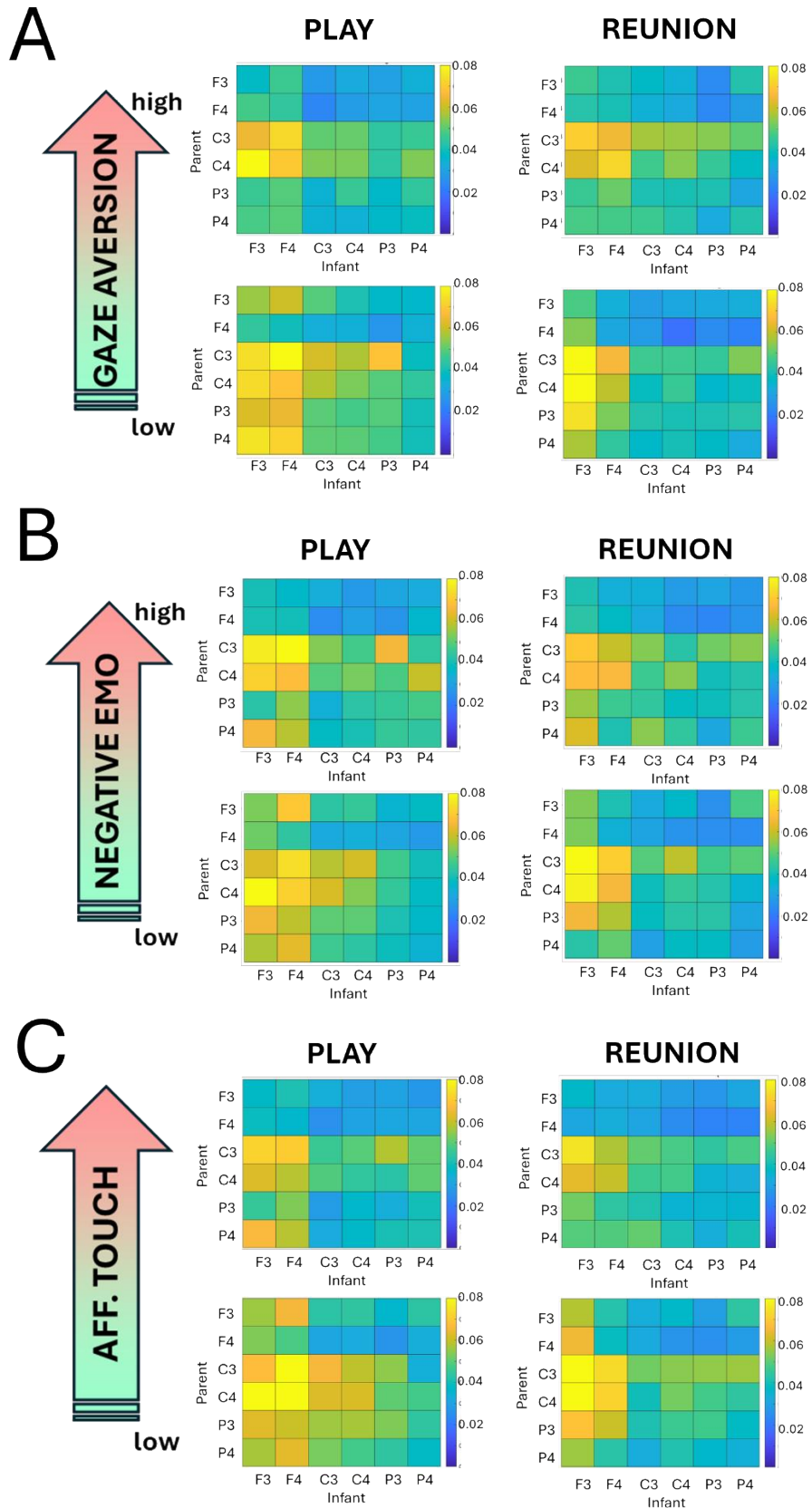


Figure S2.2. Heatmaps of P->I PDC in the *alpha* (6-12 Hz) frequency band measured in the median-split samples of dyads with low versus high infant gaze aversion during the SF (A), low versus high infant negative emotionality during the SF (B) and low versus high maternal affective touch during the Reunion (C).

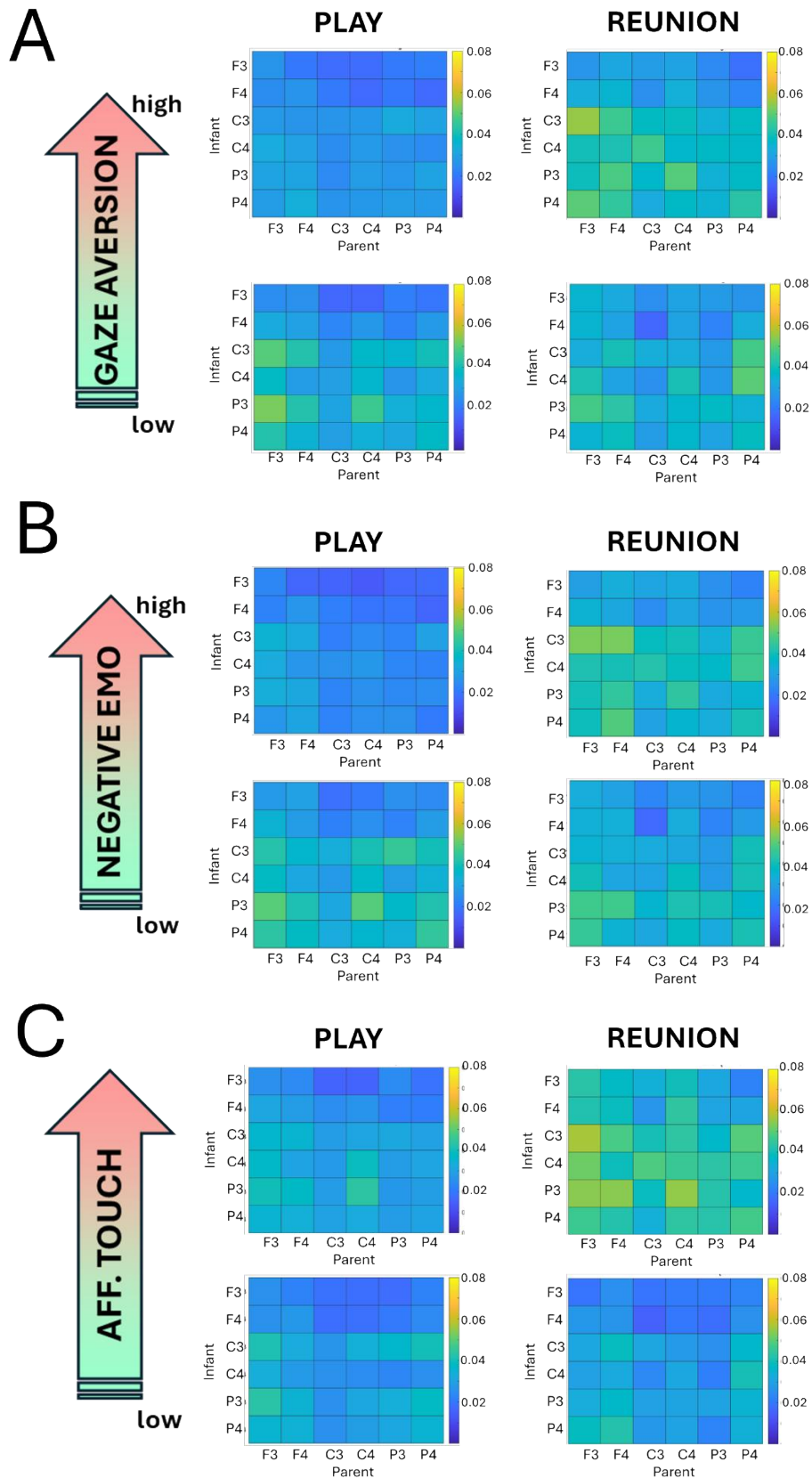


Figure S2.3. Heatmaps of I->P PDC in the *theta* (3-7 Hz) frequency band measured in the median-split samples of dyads with low versus high infant gaze aversion during the SF (A), low versus high infant negative emotionality during the SF (B) and low versus high maternal affective touch during the Reunion (C).

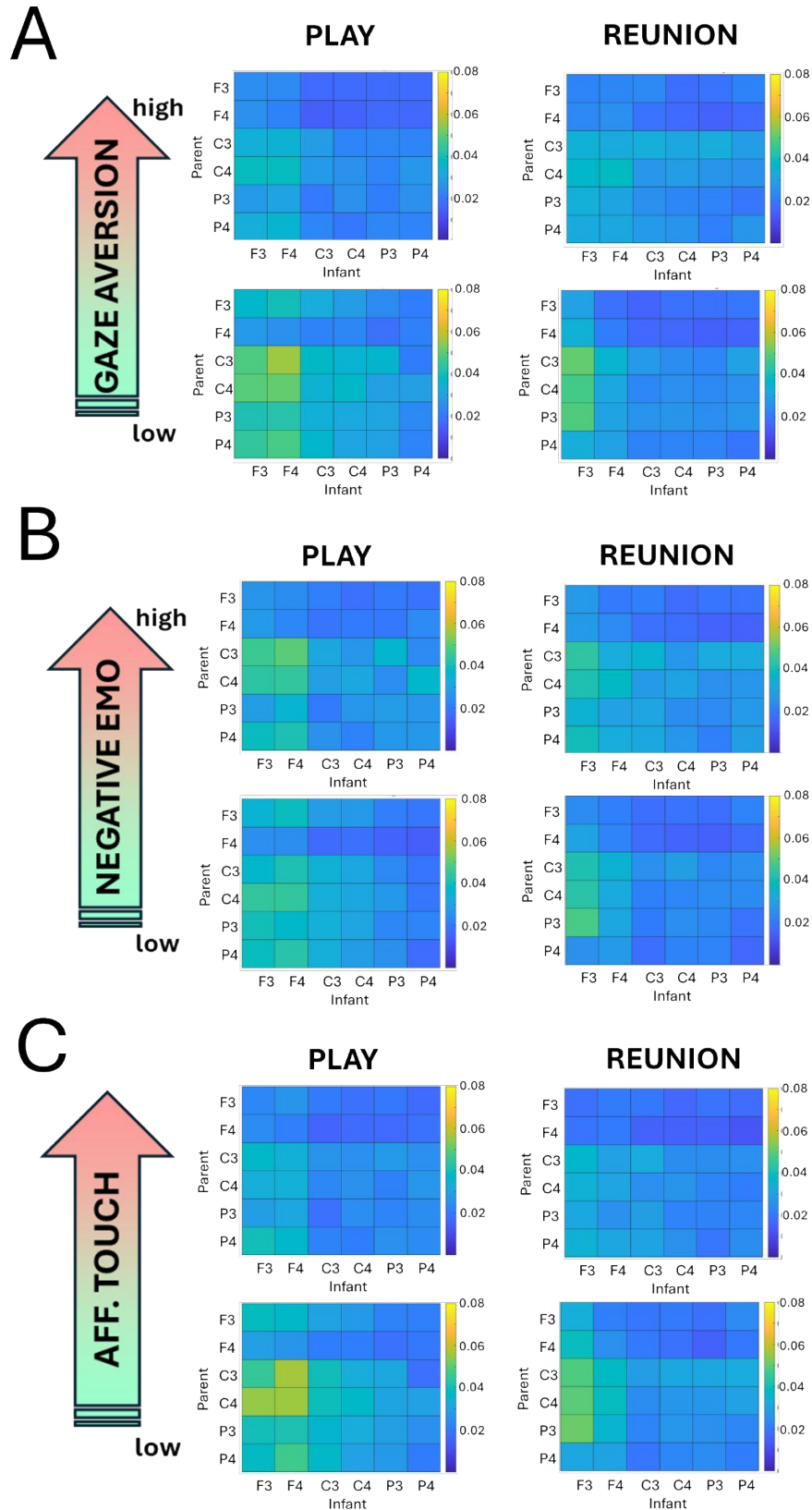


Figure S2.4. Heatmaps of P->I PDC in the *theta* (3-7 Hz) frequency band measured in the median-split samples of dyads with low versus high infant gaze aversion during the SF (A), low versus high infant negative emotionality during the SF (B) and low versus high maternal affective touch during the Reunion (C).

