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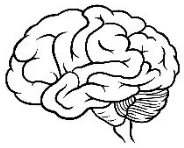
Department of Brain and Behavioral Sciences (DBBS)
MSc in Psychology, Neuroscience and Human Sciences

Parent-Infant Interpersonal Neural Synchronization and Social Gaze Direction during the Face-to-Face Still-Face Paradigm



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Introduction

In the first year of my master's degree, I took a course in Developmental Psychobiology taught by Professor Livio Provenzi. His approachable manner and the insights he shared during the first class immediately got my attention. He spoke about the dynamic nature of human beings and the early interactions between parents and infants, illustrating his points with scientific evidence that traces back to the earliest years of human history. He mentioned that "we are born to be wired," a concept that resonated deeply with me. During one of his classes, another researcher, Sarah Nazzari, joined to discuss the polyvagal theory. I remember being excited to hear about this theory because I was already familiar with it from my academic interests, but had never heard it explained by a professor in the class. After her class, I felt compelled to ask them if I could join their lab. Both Sarah Nazzari and Livio Provenzi were incredibly kind and supportive; Sarah encouraged me to send Livio an email to express my interest. From that moment, I began following their work closely, reading their articles, and staying in contact with them even before officially joining their lab.

What convinced me most about their lab was their commitment to integrating behavioral outcomes with evidence from related fields like neuroscience and biological psychology, demonstrating a truly multidisciplinary approach. As a psychologist, this alignment of interests was precisely what brought me to Italy and into this master's degree program, and it also shaped the choice of my thesis topic.

This thesis explores brain synchronization between parents and infants using a technique called hyperscanning, which I learned about through this project. Hyperscanning involves simultaneously recording brain signals from two or more individuals, allowing us to study their neural interactions in real time. It was a valuable experience that taught me how to implement

EEG, observe the dynamics of working with parents and infants, and pay attention to every detail during data analysis.

While writing this thesis, I began by discussing foundational studies on infant behavior, setting the stage for understanding the complexities of mother-infant interactions. I explored key theories related to these interactions, such as Mutual Regulation Model, and highlighted experimental works that have investigated these concepts. From there, I went into the brain signals and provided an overview of EEG as a method for measuring neural activity. I further explained the hyperscanning technique, which allows for the simultaneous recording of brain activity from multiple individuals, and reviewed relevant studies that have applied hyperscanning in the context of parent-infant interactions. My goal was to build a comprehensive framework that connects the behavioral, theoretical, and neuroscientific aspects of this field.

Abstract

Parent-infant social interactions and the corresponding brain activities is associated to sociocognitive development and this investigation is crucial for enhancing the quality of communication in parent-infant daily lives. Our study sought to understand how Interpersonal Neural Synchronization (INS) is affected by disruptions in parent-infant interactions, specifically focusing on how the INS in the theta band is associated to infant behavior (specifically gaze and emotional state) before and after the disruption. We conducted a study using the Face-to-Face Still-Face (FFSF) paradigm with 20 parent-infant dyads to examine infant gaze and emotional state as behavioral variables and explore their relationship with INS in the theta band. We used hyperscanning technique, and infant behaviors were coded after the data acquisition. Our findings showed significant changes in infant behavior (gaze and emotional state) across the episodes of the FFSF paradigm. Specifically, we observed a significant association between gaze and INS during the play episode but not during the reunion episode. However, we did not find the same result in INS in the theta band when comparing the emotional state between these episodes. These findings suggest that disruptions in parent-infant interactions can significantly alter theta band synchronization and that understanding these changes is crucial for developing interventions in early developmental contexts.

Keywords: *hyperscanning, interpersonal neural synchronization, EEG, parent-infant interaction, still-face*

Chapter 1

Dyadic Interactions

1.1. History of infant research

The study of dyadic, dynamic parent-infant interactions, particularly mother-infant interactions, began with the recognition of the critical role of infants in research. This interest originally stemmed from observations of abnormal development in infants due to maternal deprivation. In the early 1900s, studies on hospitalism gained significant attention among researchers. The term "hospitalism" was first used in Britain to highlight the adverse effects of prolonged stays in hospitals, such as infections and high mortality rates, particularly in infants (Rowold, 2018). In these hospitals, infants were placed in individual wards, isolated from each other and their parents. There was no playtime, and their only interaction was with nurses who provided food (Spitz, 1946). Infants, especially those under six months old, exhibited symptoms such as listlessness, relative immobility, unresponsiveness to stimuli like smiles, failure to gain weight, pallor, and infrequent crying (Bakwin, 1949). These concerning conditions drew the attention of many pediatricians, psychologists, and doctors, leading to increased observation and study of hospital settings.

Harry Bakwin (1894-1973) was one of the pioneers in researching the effects of hospitalism. His study at New York’s Bellevue Hospital was particularly notable due to its high infant mortality rate, which was attributed to malnutrition and infection. Bakwin advocated for a policy change at the hospital, emphasizing the importance of maternal involvement. Under the new policy, mothers were allowed to visit, play with, and cuddle their babies. The results were dramatic: despite a slight increase in infection rates, the infant mortality rate decreased from 30-35% to less than 10% (Van Der Horst & Van Der Veer, 2008).

Following Bakwin’s study, one of the most influential studies on infant development was conducted by René Spitz, known as the hospitalism study, which demonstrated the substantial impact of maternal deprivation on infant development. Spitz observed infants in a foundling home and a nursery, comparing the effects of different caregiving environments on the children's development.

In the foundling home, infants were deprived of maternal care, love, and stimulation, resulting in significant and irreversible damage. These infants were kept in a ward, but after they reached fifteen months of age, their environmental conditions improved. They were moved to larger, sunny rooms and each infant had at least three to four nurses to interact with, along with opportunities to interact with other infants (Spitz, 1946).

Spitz conducted a follow-up study when these children were between two and four years old, assessing their development in three key areas: bodily movement, handling materials, and adaptation to environmental demands. The results showed that the deterioration in these children’s development continued despite the improved conditions. More specifically, five out of twenty-one children were incapable of any locomotion (bodily movement), twelve out of twenty-one children could not eat alone with a spoon (handling materials), and six of the children were not toilet trained in any way (adaptation to environmental demands) (Spitz, 1946).

Spitz's study highlights that the first fifteen months of life are critical for development, and that maternal care plays a vital role in this process. It suggests that early deprivation can cause lasting developmental issues, and improving conditions later may not fully reverse the damage. Today, we understand that the early years of life are crucial for development, and maternal care is essential for healthy growth.

Following René Spitz's hospitalism study, the topics of maternal separation and deprivation gained considerable attention (Van Der Horst & Van Der Veer, 2008). John Bowlby, in his report to the World Health Organization (WHO), emphasized the profound emotional and behavioral turmoil that young children experience when separated from their mothers, and the significant improvements observed upon reunion (Bowlby, 1951).

As a result, studies on parent-infant interactions became crucial for understanding human development. Since those early years, research on mother-infant interactions has evolved, allowing researchers to conduct experimental studies that confirm or challenge previous clinical observations. Bowlby (1951) noted in his paper, "The evidence on which these views are based is largely clinical in origin. Immensely valuable though this evidence is, it is unfortunately neither systematic nor statistically controlled, and so has frequently met with skepticism from those not engaged in child psychiatry."

Building on these early observations, many researchers have developed theories and conducted experimental studies to deepen our understanding of parent-infant interactions. John Bowlby developed the Attachment Theory (Bowlby and Solomon, 1989), with significant contributions from Mary Ainsworth, such as the Strange Situation procedure (Van Rosmalen et al., 2015). Thelen and Smith (2003) introduced the Dynamic Systems Approach. Ed Tronick (2007) developed the Mutual Regulation Model, and Louis Sander contributed the Systems Theory. In this chapter, models regarding the development of the sociocognitive development of the infant will be mentioned.

1.2. Dynamic System Theory

Ludwig von Bertalanffy, an Austrian biologist, is renowned for his pioneering work on General Systems Theory (GST). Von Bertalanffy proposed that complex systems, regardless of their type—biological, psychological, or social—share common organizational principles. His General Systems Theory posits that systems must be studied as wholes rather than merely as collections of parts. This holistic approach emphasizes the interconnectedness and interdependence of all components within a system, suggesting that the behavior of the system cannot be fully understood by analyzing its individual elements in isolation (von Bertalanffy, 1968).

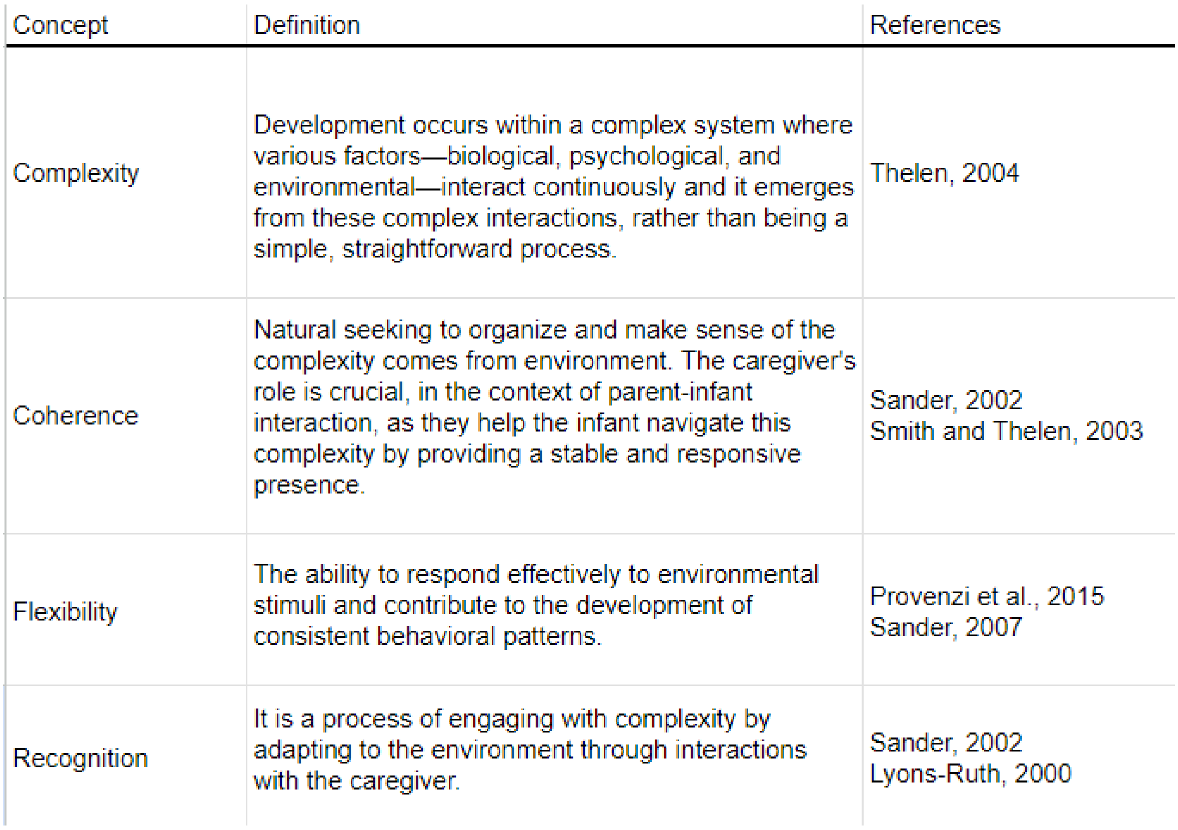
Von Bertalanffy's work introduced several key concepts fundamental to GST, including the idea of open systems, which continuously interact with their environment through the exchange of energy, matter, and information. He also highlighted the principle of equifinality, which asserts that a system can reach the same end state through different pathways. This perspective challenged the traditional linear and reductionist approaches, advocating instead for a more integrative and interdisciplinary framework for studying complex phenomena (von Bertalanffy, 1968).

General Systems Theory has had a profound influence on various fields, encouraging a shift towards understanding systems as dynamic, interactive, and evolving entities. This approach laid the groundwork for further theories and models that explore the complexity and adaptability of developmental processes.

Building on von Bertalanffy's foundational concepts, researchers like Esther Thelen extended these ideas into the realm of developmental psychology. Thelen, along with her colleague Linda B. Smith proposed a new theory (Table 1) called dynamic system (DS) which explains development as a dynamic process, involving continuous and complex interactions between an individual and their environment (Smith & Thelen, 2003). According to the DS, the parent-child dyad is a non-linear, dynamic and increasingly complex system rather than a linear and organized one (Grumi et al., 2022). Thelen and Smith explained dynamic approach with two assumptions: multicausality and nested timescales. By saying multicausality, they emphasized that development of living beings involves a myriad of interacting parts within the organism itself, which are continuously influenced by and influencing the complex external environment. This interaction affects behavioral changes and makes them develop over different timescales (Smith & Thelen, 2003). Esther Thelen applied dynamic system theory to explain how motor behaviors in infants emerge through the self-organization of neural, muscular, and perceptual systems. According to this view, behaviors are not pre-programmed but arise from the interactions of these systems with the environment (Thelen, 1994). This means that development is fluid and adaptable, rather than fixed and linear. Thelen's work has been influential in shifting the understanding of development from a static, stage-based perspective to one that sees development as a dynamic and context-dependent process.

Table 1.

Basic concepts to understand Dynamic Systems (DS) Theory.



Concept	Definition	References
Complexity	Development occurs within a complex system where various factors—biological, psychological, and environmental—interact continuously and it emerges from these complex interactions, rather than being a simple, straightforward process.	Thelen, 2004
Coherence	Natural seeking to organize and make sense of the complexity comes from environment. The caregiver's role is crucial, in the context of parent-infant interaction, as they help the infant navigate this complexity by providing a stable and responsive presence.	Sander, 2002 Smith and Thelen, 2003
Flexibility	The ability to respond effectively to environmental stimuli and contribute to the development of consistent behavioral patterns.	Provenzi et al., 2015 Sander, 2007
Recognition	It is a process of engaging with complexity by adapting to the environment through interactions with the caregiver.	Sander, 2002 Lyons-Ruth, 2000

1.3. Sander’s Contributions to the Dynamic Systems

Louis Sander made huge contributions to the DS theory and his work played a significant role in understanding the complexity of early human development and the dynamics of the caregiver-infant relationship. According to Sander, parent-child dyad can improve the flexibility to adequately respond to environmental stimulations and help to develop consistent behavioral patterns, as well as establish mutual responsive interactions that maintain stability and balance (Sander, 2007). To emphasize the importance of the particular characteristics of each interaction, rather than general patterns of behavior, Sander put forward a principle called “specificity”, stating that the way the caregiver responds to the infant’s current state and the context in which he/she gives the response will have a specific impact on the infant’s development (Sander, n.d.). Originally, specificity principle was introducted to Sander by Paul Weiss. A related concept which allows to understand how specificity works is: self-organization. The experience of specificity in development is deeply rooted in the "recognition process," which serves as a vital bridge connecting biological processes with developmental growth. This process suggests that during the early years of life, an infant's interactions with their mother and the complex environment around them gradually lead to adaptation. Over time, the infant begins to engage with this complexity by finding ways to fit and respond to it. This ongoing negotiation and connection in the interactions between the infant and the mother form the foundation for psychological organization, building a pathway from biological to psychological development (Sander, n.d.).

While Sander (2007) was explaining the concept of specificity, he came up with a paradox: how is stability (specificity of connection) achieved through instability (ongoing change and stresses)? It questions how such precise connections can form and remain stable through continuous and potentially disruptive changes. His concept of rhythmicity helps address this paradox by suggesting that regular and recurring patterns within the organism conribute to the stability and specificity connections.

1.4. Tronick’s Contributions

Tronick (1998) expanded Thelen's concept of self-organization beyond motor behaviors, suggesting that each person is a self-organizing system capable of managing their own mental states, encompassing various levels of consciousness and brain organization. These states are dynamic and evolve through interactions with other self-organizing systems, particularly in the context of close relationships. Building on this foundation, Tronick made significant contributions to our understanding of mother-infant interactions, especially through his development of the Mutual Regulation Model (MRM) and the Still-Face Paradigm (FFSF). These models emphasize how infants and caregivers co-regulate each other's emotional and psychological states, offering deeper insights into the complexities of early developmental processes.

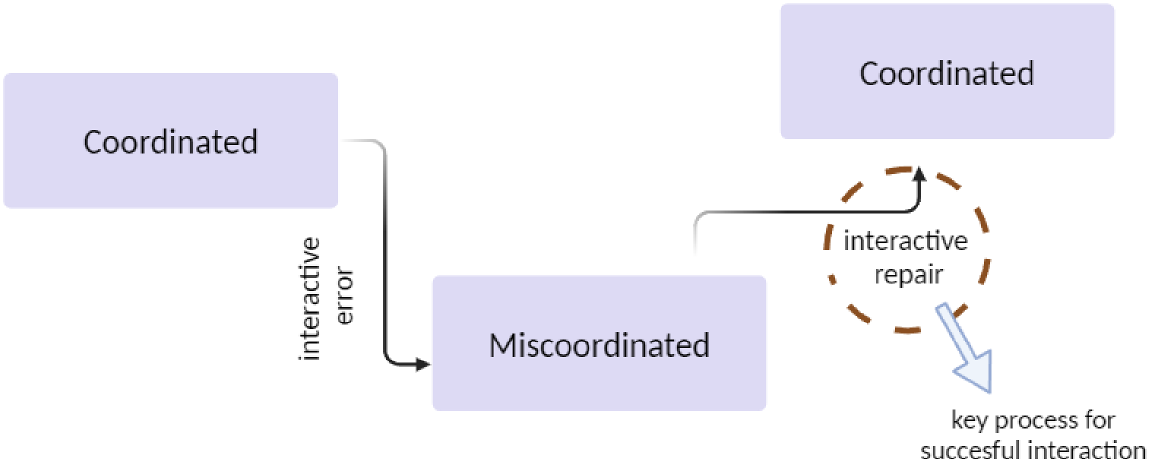
1.4.1. Mutual Regulation Model

After Sander’s invaluable contributions, one of the biggest and succesful approaches to the dynamic system studies was Mutual Regulation Model (MRM) by Ed Tronick (E. Z. Tronick, 1989). MRM explains how small scale social and emotional interactions during communication can create (or fail to create) a mutual understanding and shared awareness between two people (Tronick et al., 1998). Tronick sought to understand why some children develop traits of happiness and curiosity, while others exhibit anger and lack of focus. He discovered that the key lies in the presence of interactive errors during mother-infant interactions. Tronick observed that mismatched moments caused by these errors often transition to better-matched states through a process known as interactive repair. This repair process is crucial for healthy emotional and cognitive development (Tronick, 1989). According to Tronick, his studies highlight the critical role of experiencing both successful interactions and the resolution of interactive errors in the developmental trajectory of infants. Positive interactions foster a sense of accomplishment and provide positive reinforcement, essential for healthy emotional development. However, the inevitable occurrences of interactive errors and the resultant negative affect, such as frustration or sadness, are equally important. The successful repair of these errors and the subsequent transformation of negative affect into positive affect have several developmentally enhancing effects. These experiences are integral to the development of an infant's emotional communication and self-regulation skills. Through the process of navigating and repairing disruptions in interactions, infants enhance their capacity to communicate emotions effectively to others (other-directed affective communicative capacities) and to regulate their own emotional states (self-directed regulatory capacities). This experiential learning enables them to maintain engagement with their external environment, even in the face of stress or challenges (Gianino & Tronick, 2013).

The MRM posits that an infant's emotions are regulated by the mother's emotional expressions, and the infant's reactions are shaped by this implicit understanding of maternal emotional states. This process, known as mutual regulation, involves the ability to perceive and respond to each other's emotional states, allowing for appropriate and harmonious interactions. According to Tronick, mother-infant interactions fluctuate between coordinated (or synchronous) and miscoordinated states across a wide range of emotions (Figure 1). Miscoordinated states occur when one partner fails to accurately interpret the other's emotional cues, leading to inappropriate reactions. As previously mentioned, this miscoordination is termed interactive error. It can be resolved through a process known as interactive repair, where the interaction transitions from miscoordinated to coordinated states (Tronick et al., 1998). This mutually regulated process enhances the success of the mother-infant relationship.

Figure 1.

Illustrated to show mutual regulation model.



1.4.2. Face-to-face still-face paradigm

What happens if mutual interaction between a mother and her infant is disrupted? To investigate this, Tronick developed the face-to-face still-face (FFSF) paradigm (Tronick et al., 1978). This experimental setup is designed to observe the infant's response to a sudden change in maternal behavior and consists of three sessions lasting a total of six minutes: play, still-face, and reunion, each lasting two minutes (Figure 2).

In the first two-minute session, known as the *play episode*, the mother is instructed to engage with her infant in a normal, everyday manner. This interaction mimics the typical playful exchanges that occur at home, allowing the infant to experience a familiar and comforting environment. This session serves as a baseline for the infant's normal behavior and emotional state when engaged with a responsive caregiver.

The second session, the *still-face episode*, also lasts two minutes and introduces a significant change. The mother is instructed to cease all interaction with her infant, adopting a neutral and expressionless face. She must remain unresponsive, maintaining a still face without any emotional expression. This abrupt shift from a responsive to an unresponsive caregiver is designed to simulate maternal unavailability, providing a controlled environment to observe the infant's immediate emotional and behavioral reactions to this disruption in interaction.

The final session, the *reunion episode*, also lasts two minutes and involves the mother resuming her normal, playful interaction with her infant. This return to a responsive and engaging interaction allows researchers to observe how quickly and effectively the infant can recover from the distress experienced during the still-face episode.

During the still-face experiment, the infant attempts to maintain interaction with the mother. When these attempts fail, the infant's negative emotions become evident. This emotional change highlights the infant's self-regulatory capacity, which is observed during the still-face episode. The disruption of interactive regulation during this episode allows us to observe whether the infant exhibits adaptive behaviors, such as self-comforting or attempts to signal the mother (Beebe & Lachmann, 2015). According to the Tronick, adaptive infants demonstrate better self-regulatory behaviors by trying to stay engaged with the mother, whereas maladaptive infants show withdrawal, avert their gaze and generally disorganized behaviors (Gianino & Tronick, 2013).

When the still-face episode concludes and mothers are instructed to resume normal interaction as they did during the play episode, the infant's negative emotions may persist. The reunion session is designed to evaluate the infant's ability to re-establish a positive emotional state after the disruption. Tronick observed that while infants often return to a more positive and joyful state with their mothers during this session, residual feelings of anger and sadness can still be present (Weingberg & Tronick, 1996).

Figure 2.

FFSF paradigm.

Play (2 min)

Still-face (1 min)

Reunion (2 min)

1.4.3. Meaning-making

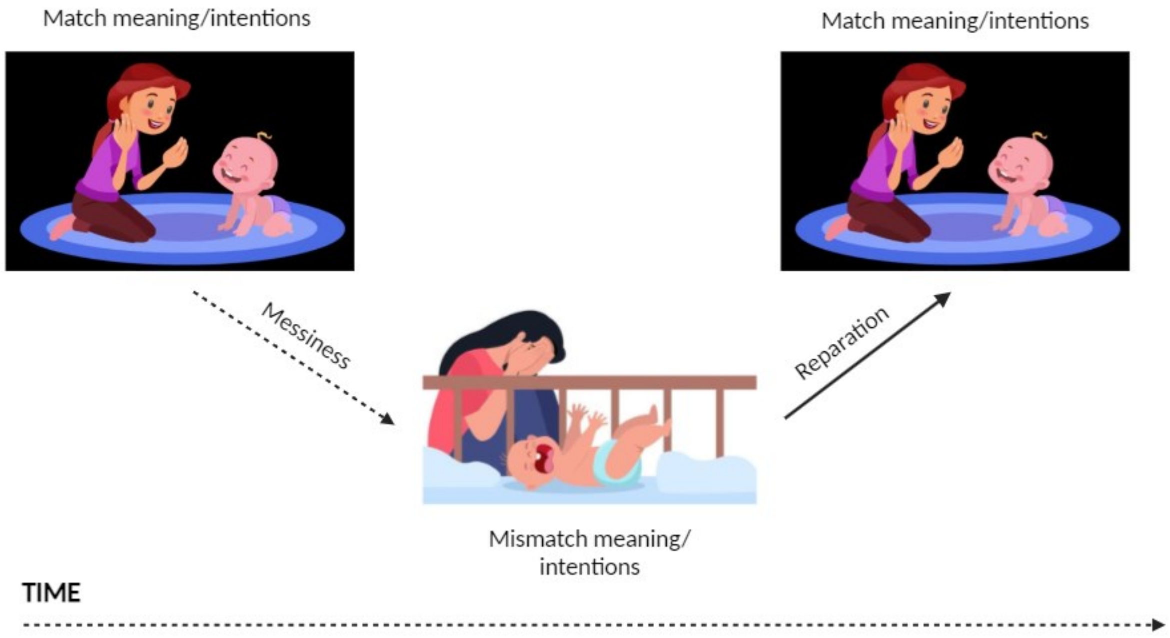
In our interactions with others, particularly with parents, the process is dynamic and non-linear, characterized by frequent mistakes, disconnections, and repairs. Understanding and interpreting the behaviors of others, and responding appropriately, requires making meaning out of these interactions. According to Tronick, these interactions are rarely perfect; they involve missteps, attempts, retries, synchronizations, and desynchronizations, creating what he describes as "messiness". This concept of messiness suggests that interactions between infants and caregivers oscillate between matched states (coordinated and synchronous) and mismatched states (uncoordinated and dyssynchronous), returning to matched states through intentional behaviors as showed in Figure 3 (Tronick, 2007). Matches occur when the infant's cues and the caregiver's response are in sync. For example, when a baby smiles and the caregiver responds with a smile or a positive gesture, it creates a matched interaction. Mismatches happen when there is a disconnection between the infant's cues and the caregiver's responses. For instance, if a baby plays with a toy happily and the caregiver takes the toy from the baby's hand, it results in a mismatched interaction. Repairs involve the caregiver recognizing a mismatch and taking steps into re-establishing connection and understanding. For example, if the caregiver initially misses the baby's cue but then notices and responds appropriately, it constitutes a repair. Tronick concepts of matches, mismatches, and repairs highlight the dynamic and ongoing process of meaning-making in infant-caregiver interactions. Through this process, infants learn that while perfect synchrony is not always possible, the ability to repair mismatches is key to developing a stable attachment and understanding the social world (Tronick, 2007).

We can easily observe this meaning-making process during still-face experiment. During the still-face episode, infants typically attempt to engage their unresponsive parents by smiling, gesturing, and vocalizing. These efforts reflect their natural inclination to seek interaction and connection. However, when their attempts to elicit a response fail, the infants become visibly distressed. This distress often leads to physiological arousal, manifesting in behaviors such as drooling, choking, and spitting up. To cope with the unresponsiveness, infants begin to disengage, averting their gaze, turning their heads away, or arching their backs. These actions communicate their state of dysregulation and represent their attempt to manage the relationship through disengagement. In the reunion phase of the protocol, when the parent resumes normal interaction, infants generally regain their positive affect and reengage with their parents. Despite this reengagement, they often display an initial hesitancy before fully resuming social interaction. This hesitancy underscores the impact of the brief period of unresponsiveness on the infant's emotional state and highlights their need to re-establish trust and connection before fully engaging again (Harrison & Tronick, 2022).

In summary, studying the dynamics of meaning-making is crucial as it provides insights into how infants develop either resilient or maladaptive outcomes. This understanding is invaluable for clinicians working with parent-infant interactions, enabling them to better support and guide healthy developmental processes (E. Tronick & Beeghly, 2011).

Figure 3.

Interactive process of matching, mismatching, and reparation of meanings and intentions.



1.4.4. Hypothesis of dyadic expansion of consciousness

The dyadic expansion of consciousness theory synthesizes the previous foundational concepts in infant-caregiver interactions, illustrating how these interactions contribute to the development of consciousness and social understanding. This theory posits that through mutual, dynamic exchanges, both the infant and the caregiver's awareness and cognitive capacities are expanded (Tronick, 2007).

As previously mentioned, the mutual regulation model describes how infants and caregivers engage in a continuous, reciprocal process of emotional and behavioral regulation. These interactions are dynamic, involving frequent mismatches and repairs, which help maintain emotional stability and social connection (Tronick & Cohn, 1989). Therefore, while mutual regulation model takes regulation of emotional states through interactions as a center, dyadic expansion of consciousness theory focuses on new developed capacities and expanded awareness and understanding.

The Still-Face Paradigm vividly demonstrates the importance of mutual regulation. When a caregiver suddenly becomes unresponsive, infants initially attempt to engage through smiles, gestures, and vocalizations. The lack of response leads to distress and physiological arousal, highlighting the essential role of caregiver responsiveness (Tronick et al., 1978). While the infant and caregiver make a new meaning during the disruption of the interaction because of the still-face episode, a different state of consciousness can be constituted. This new co-created meaning affects each individual's own state of consciousness, and it affects their own sense of self (Harrison & Tronick, 2022).

Meaning-Making explains how infants and caregivers co-create and interpret emotional and social experiences. Through these interactions, infants develop an understanding of their experiences and a sense of self, learning that disruptions can be resolved, which fosters resilience (Fogel, 1993; Tronick, 2007).

The dyadic expansion of consciousness theory integrates these concepts by emphasizing how in early life interactions, each individual as a self organizing system expands their consciousness with an interaction of another self-organizing system into a more complex but coherent state (Tronick et al., 1998).

In essence, the dyadic expansion of consciousness theory captures the profound impact of the early relational environment, showing how the dynamic, reciprocal nature of infant-caregiver interactions fosters the growth of consciousness and the capacity for complex understanding.

1.5. Current Studies

Recent research has expanded the use of the Face-to-Face Still-Face (FFSF) paradigm. For instance, Provenzi (2016) used this paradigm to investigate variations in cortisol secretion among two groups of 3-month corrected-age infants: very preterm infants exposed to low levels of pain during their stay in the Neonatal Intensive Care Unit (NICU) and those exposed to high levels of pain. The study found that infants exposed to low levels of pain exhibited greater variations in cortisol secretion during the FFSF paradigm compared to the high-pain group. These results can be interpreted using the concept of allostatic load, which suggests that prolonged stress can lead to a new baseline of hypothalamic-pituitary-adrenal (HPA) axis activity (McEwen, 1998). Additionaly, habituation to repetitive pain which contains maternal unavailability may have resulted in these infants perceiving the still-face scenario as a normative, rather than a novel, stressor. Consequently, their physiological responses were may blunted, reflecting an adaptive mechanism to conserve energy and maintain stability in a high-stress environment.

Another aspect of this research compared negative emotionality between very preterm infants and full-term infants. Montirosso (2016) observed that negative emotionality during the FFSF paradigm was higher in very preterm infants compared to full-term infants. This indicates that very preterm infants may have a heightened sensitivity to stress and less developed emotional regulation capabilities, likely due to their early and more stressful experiences in the NICU. These findings underscore the impact of early-life stress on emotional development and highlight the need for supportive interventions for preterm infants.

Extending the FFSF paradigm, neuroimaging studies have provided further insights. For example, research on the FFSF paradigm using frontal alpha asymmetry (FAA) as an indicator of emotional responses found that the paradigm led to increased negative emotions and a desire to withdraw in both infants and caregivers (Perone et al., 2020). This study, which employed the relatively new but increasingly popular hyperscanning technique, examined the interpersonal neural synchronization of parent-infant interactions. These findings emphasize the impact of stress on emotional regulation and the emotional states of both infants and their caregivers during the SFP. This topic will be explored further in Chapter 2.

Chapter 2

Hyperscanning Research

2.1.Theoretical foundations of hyperscanning

Social interaction is a vital aspect of social mammals, with communication skills being among the most significant traits, particularly in humans. Our actions, emotional regulation, stress responses, and decisions are profoundly influenced by interactions with others. Consequently, understanding the neural correlates of social interaction is a key objective for neuroscientists and psychologists.

Pioneers in this field, Duane and Behrendt (1965), were the first to record multi-brain activity simultaneously using electroencephalogram (EEG). After their initial work, the practice of recording multiple brain activities was largely ignored until it was rearise in this century under the term "hyperscanning" (Koike et al., 2015). Thus, hyperscanning is a relatively new technique aimed at recording the neural activity of two or more individuals simultaneously (Kayhan et al., 2022).

Hyperscanning has the potential to provide insights into various aspects of social neuroscience, such as joint attention (Bilek et al., 2015), emotional regulation and stress responses (Reindl et al., 2018), the roles of specific brain regions (Vanzella et al., 2019), decision-making tasks (Astolfi et al., 2010), neurodevelopmental disorders (Zhou & Wong, 2024) and hyperscanning can be used in ecological applications as well such as studying neural coupling in musicians playing together or brain synchronization between pilots and co-pilots during flight (Provenzi et al., 2023).

Interpersonal neural synchronization (INS) refers to the phenomenon where two individuals' brains show similar patterns of activity at the same time, often in a rhythmic manner. More specifically, INS is concerned with the temporal alignment of brain activities that occurs when people interact with each other, reflecting how their brains synchronize during social interactions (Koul et al., 2023).

Researchers have employed the hyperscanning technique to investigate various components of social interaction using different methods and the most common hyperscanning techniques are EEG (Wass et al., 2018), fNIRS (Reindl et al., 2018), and fMRI (Tanabe et al., 2012), each with distinct advantages and limitations.

EEG offers higher temporal resolution compared to both fNIRS and fMRI, making it ideal for capturing rapid changes in brain activity. While fNIRS provides better temporal resolution than fMRI, it still stays behind EEG in this regard. Both EEG and fNIRS are portable, allowing for the measurement of interactions in real-life settings, unlike fMRI, which requires a stable environment. However, EEG suffers from low spatial resolution, and while fNIRS improves on this, its spatial resolution remains limited. In contrast, fMRI excels with high spatial resolution, enabling precise determination of specific brain regions involved in interactions (Koike et al., 2015).

The selection of an appropriate hyperscanning technique depends on the specific requirements of the study as well as the research question. Researchers must consider whether participants need to move freely, if real-life interaction scenarios are necessary, the need for analyzing brain activity over short periods, and the importance of distinguishing between closely located brain activity points.

For this thesis, we selected EEG primarily for its excellent temporal resolution, which is crucial for capturing the dynamic and fast-paced interactions in our study. Additionally, EEG is portable and child-friendly, with wireless systems available that allow for freedom of movement, making it particularly suitable for working with infants in naturalistic settings. Before delving into EEG hyperscanning techniques and related topics, I will outline the basic principles of EEG to enhance the clarity of this study.

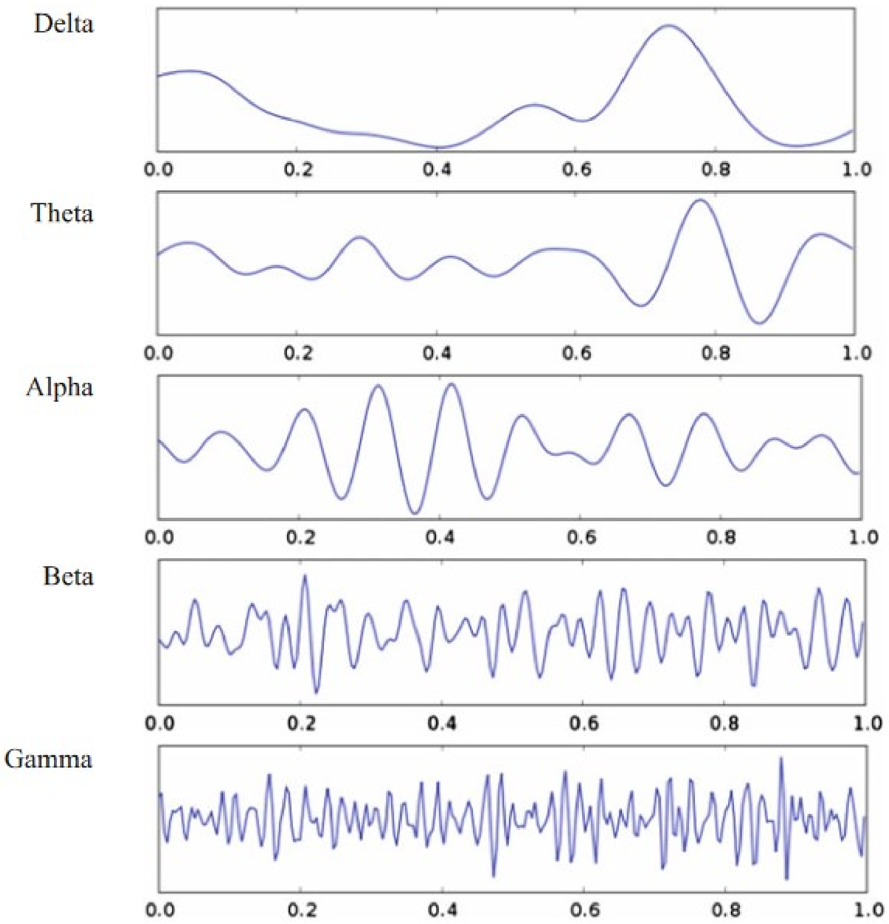
2.2. Basic principles of EEG and its application in hyperscanning

EEG is a neuroimaging technique used to measure and record the electrical activity of the human brain with a high temporal resolution. It is widely used by doctors, neuroscientists, and psychologists in research field to study brain waves and understand the brain-behavior relationship. The first use of EEG was pioneered by Hans Berger, a German neuropsychiatrist, in 1929. He used a German term called "elektrenkephalogramm" to describe the graphical representation of the electrical currents of the brain (Siuly et al., 2016).

An EEG signal, originating from brain activity, contains various frequency components that are categorized into distinct bands through frequency-domain analyses like the Fast Fourier Transform (FFT). These frequency bands, which represent different types of brain waves, include delta waves (0.5-4 Hz), theta waves (4.5-8 Hz), alpha waves (8.5-13 Hz), beta waves (13.5-30 Hz), and gamma waves (30.5-100 Hz). Each band is associated with different brain functions and states, providing insights into neural activity (Wright et al., 2020). Figure 4 presents different types of brain waves.

Figure 4.

Example of different types of normal EEG rhythms (Ref. Siuly et al., 2016)

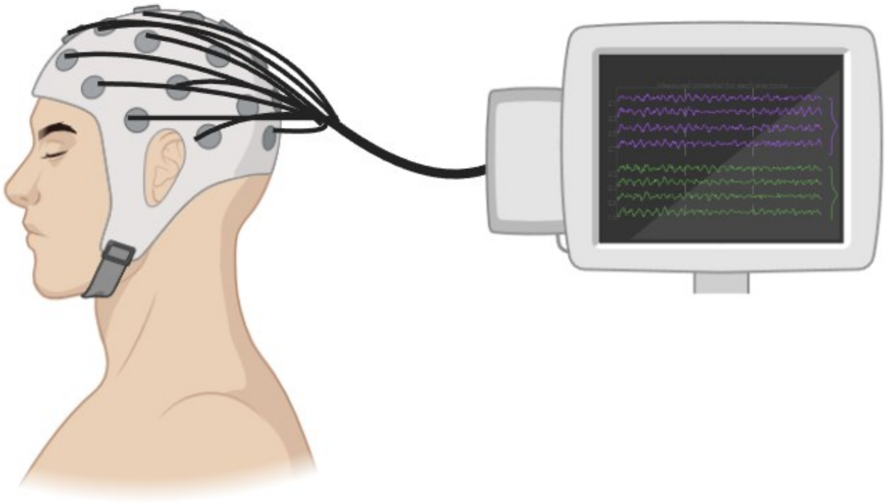


As in presented in Figure 5, the acquired EEG signals can be viewed on a computer connected to the electrode amplifiers. Electrodes are small sensors that are placed at various locations on the scalp, typically using a conductive gel or paste to ensure good contact and accurate signal transmission. In an EEG cap, electrode numbers can be up to 256, and higher electrode number improves the spatial resolution (Väisänen, 2008). The question that how we place EEG cap to the head can be explained by the international 10-20 electrode system. This system is characterized by the specific distances between adjacent electrodes, which are set at 10% or 20% of the total length measured either from the front to the back of the skull or from the right to the left. The precise locations for placing the electrodes are determined using two key anatomical landmarks: the nasion, located at the indentation between the forehead and nose at the eye level, and the inion, which is the noticeable bony bump at the midline on the lower part of the back of the head. These points serve as reference marks to ensure consistent and reproducible electrode placement, crucial for obtaining accurate and comparable neurophysiological data across different individuals and studies (Siuly et al., 2016).

Figure 5.

An illustration to show a person wearing an EEG cap, which is used to measure the electrical activity of the brain. The cap is equipped with multiple electrodes that are connected to wires leading to a monitor. This monitor displays the recorded brain waves in the form of different wave patterns, which represent the brain's electrical signals.

Created in BioRender.com



Another important aspect of EEG is event-related potentials (ERPs), a measurement of brain responses towards a specific sensory, motor, and cognitive events (Luck, 2012). ERPs measurement is quite useful for both clinical and research settings to study neural mechanisms and see the movements of brain waves in response to a specific stimuli.

EEG can be used to detect brain diseases such as brain edema, Parkinson's disease, epilepsy (Li et al., 2021), sleep problems (Kazemi et al., 2022), developmental disorders such as Attention Deficit/Hyperactivity Disorder (Slater et al., 2022), Autism Spectrum Disorder and Fragile X Syndrome (Liang et al., 2022).

In conclusion, the main principles of EEG include measuring electrical brain activity, processing signals, offering high temporal resolution, analyzing event-related potentials (ERPs), and being a non-invasive technique.

While EEG is often used individually, the novel approach of hyperscanning has been introduced to study social interactions and entrainment between two or more people. EEG application in hyperscanning provides a fine temporal resolution as previously mentioned. It helps to assess how synchronization between brains changes at different brain wave frequencies according to the activity the participants are performing, and it helps to detect exact moment-to-moment alignment of neural activity across different brains. Briefly, thanks to fine temporal resolution, researchers can assess frequency dependency between brains and real-time synchronization (Koike et al., 2015).

2.3.EEG Hyperscanning Technical considerations

EEG hyperscanning, which involves the simultaneous recording of brain activity from multiple participants during interaction, necessitates careful planning to ensure high-quality data and precise data synchronization. Typically, each participant wears an EEG cap connected to an amplifier, and modern systems can accommodate high-density electrode caps (up to 128 or 256 channels) for detailed brain activity monitoring (Siuly et al., 2016).

In the paper of Barraza (2019), the setup of EEG hyperscanning is explained in detail. They provided a protocol which allows the measurements of synchronization between two brains. While the protocol can be provided to all groups of dual-EEG studies, for the aim of this thesis, I will focus on parent-infant dual-EEG studies. We can translate that protocol to parent-infant studies by connecting the EEG box/amplifier of the infant to the adult box/amplifier to allow savings of both recordings into one file (Turk, Endevelt-Shapira, et al., 2022).

In studies focusing on parent-infant interactions, the laboratory setup must support two interconnected EEG systems to record simultaneous measurements. It is crucial to facilitate direct eye contact between the parent and infant, as this interaction is significant for the behavioral outcomes. This can be achieved by employing an adjustable, age-specific infant chair that aligns their eye levels. Setting up EEG equipment for a parent and infant involves several important steps. The process begins with placing an EEG cap on the parent's head, making sure it fits comfortably and securely. Conductive gel is then applied to the electrodes on the cap to ensure good contact with the scalp. After this, an EEG cap is gently placed on the infant's head, and gel is similarly applied to the electrodes. Throughout these steps, maintaining a calm and gentle demeanor is crucial. This helps to create a relaxed environment, which is especially important for keeping the infant comfortable and ensuring the accuracy of the EEG readings. The laboratory environment must prioritize safety and comfort for the all kind of groups, but it is extremely important in studies involving infants. This includes maintaining a clean and organized space, securing cables, and removing any potential hazards (Turk et al., 2022).

Noreika (2020) identified 14 challenges associated with EEG research in infants, and their solutions were explained. One of the challenge, which is extremely important for the setup, is arousal variations in younger infants. Such variations can significantly impact EEG data, as infants' behavioral states can fluctuate rapidly from hyperarousal to calm or even sleep. To address this, it is advisable to design the experiment with brief, sessions with breaks, typically lasting 1-3 minutes. Therefore, we need to take infant’s age consideration for the measurement of parent-infant dynamics.

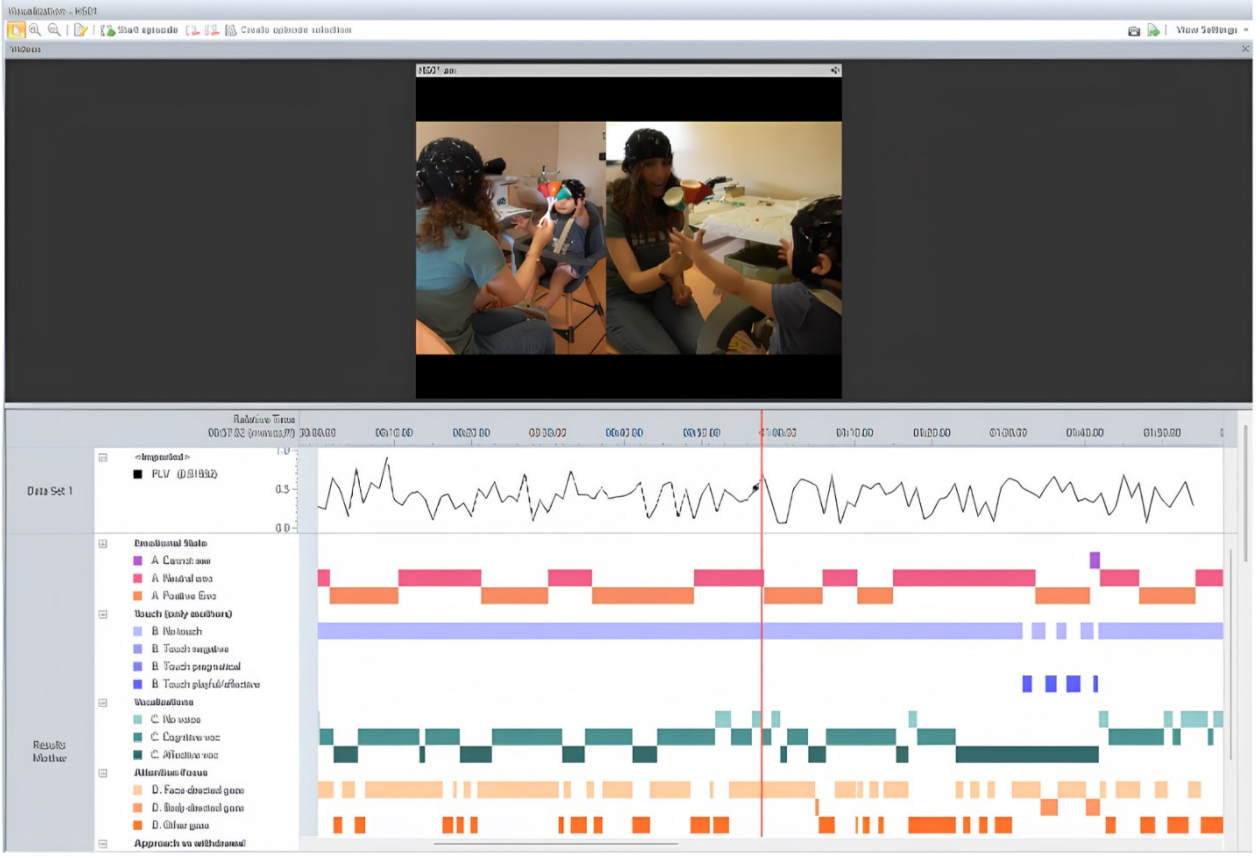
Another challenge is data loss caused by motion-related artifacts. This is because infants cannot be instructed to stay still, and even if parents are advised to minimize movement, natural interaction -including head movements and gaze shifts- is necessary for the study. These artifacts cannot be entirely prevented without sacrificing the natural behavior we are aiming to observe. Consequently, a significant amount of data may be contaminated and unusable. To mitigate this issue, it is advisable to double the number of participants in the study compared to what standard power calculations would typically require, ensuring sufficient usable data despite the expected loss (Noreika et al., 2020).

The selection of tasks within the experimental design must carefully consider the developmental, cognitive, and motor abilities of both the parent and the infant. Any disruption or mismatch in these abilities can significantly affect the integrity and interpretability of the data collected throughout the experiment. Therefore, it is crucial to establish well-defined baseline and control conditions tailored to the specific experimental questions being investigated. A "good baseline" refers to periods during which there is no interaction between the parent and infant, which can be evaluated to determine the neural baseline activity for both participants (Turk et al., 2022). This baseline serves as a critical reference point, allowing researchers to accurately interpret changes in brain activity in response to the experimental stimuli or tasks.

To take the full benefit of EEG hyperscanning studies, researchers often integrate EEG data with other modalities, such as video recordings. These additional data sources provide valuable behavioral insights that can be correlated with neural data obtained from EEG hyperscanning, thus enriching the interpretation of the observed phenomena (Feldman, 2007; Markova et al., 2019; Wass et al., 2018). It is crucial to ensure precise temporal synchronization across all data streams. This synchronization is essential for accurately mapping behavioral events to corresponding neural activity. Several tools and methodologies are available for this purpose, including Lab Streaming Layer (Kothe et al., 2024), Noldus Observer XT (Zimmerman et al., 2009), E-prime (Schneider et al., 2012) and OpenSesame (Mathôt et al., 2012). These tools provide the alignment of EEG signals with other recorded modalities, ensuring that data from different sources can be seamlessly integrated and analyzed. An example of Noldus Observer was illustrated in the Figure 6. The following sections will provide a detailed discussion of data synchronization techniques and their application in EEG hyperscanning studies.

Figure 6.

Retrived by NoldusObserver. It allows us to align neural and behavioral data temporally. This ensures that the timing of neural activity and related behaviors are matched, allowing for a precise comparison or analysis of how the brain's activity correlates with specific actions or stimuli over time.



Another critical consideration in hyperscanning studies is ensuring that EEG signals from multiple participants are synchronized, which is essential for accurate analysis. This can be achieved through hardware-based solutions, such as synchronization modules that send a common timing signal to all EEG systems, or software-based solutions, such as aligning data post-recording using event markers (Barraza et al., 2019). Companies like Brain Products, ANT, EGI, and BioSemi offer both hardware and software solutions to achieve synchronization between individual EEG measures and between signals from different participants (Turk et al., 2022).

For this thesis, we opted for software-based solutions to perform synchronization after data collection. This choice allowed us to integrate and visualize various modalities, such as video recordings (behavioral data) and EEG data (neural data), with greater flexibility and sensitivity during the experiment.

2.4. Metrics for measuring interpersonal neural synchronization and coupling

Brain-to-brain synchronization refers to the study of how neural activities in different individuals align or interact during social interactions or cooperative tasks. Several measures can be used to assess this synchronization, each capturing different aspects of interbrain coupling. I will explain them under two different categories; directional and non-directional measures.

Directional Measures

- Coherence analysis

Coherence measures the consistency of phase relationships between two time series within a specific frequency band, examining both time and frequency domains. It assesses how well brain signals from two individuals are synchronized at particular frequencies, indicating the degree of brain coupling. One of the indexes can be used in coherence analysis is Partial

Directed Coherence (PDC) which allows us to estimate INS while detecting the leader (parent or infant) and the follower (parent or infant) of the interaction (Turk et al., 2022). High coherence (close to 1) reflects a stable and consistent phase difference between the signals over time, suggesting strong synchronization. Conversely, low coherence (close to 0) indicates varying phase differences and weak or no synchronization (Thatcher, 2012).

- Granger Causality

Techniques such as Granger causality can investigate the causal influence between brain signals. Granger causality is a practical method used to determine whether one time series can predict or cause changes in another time series, indicating a directional influence between the two variables (Zhong et al., 2024). For instance, this method can examine whether neural activity from a mother's EEG predicts her infant's EEG, providing insights into the directionality of influence and dynamic interactions between their brains (Turk, Endevelt-Shapira, et al., 2022).

While analyzing hyperscanning data, coherence analysis focuses on the stability and consistency of coupling between two brains, whereas Granger causality is more concerned with identifying dominant or significant directional influences within this interaction.

Non-directional measures

These measures can be separated in two categorization as same frequency bands between adult and child (correlations, circular correlations, imaginary coherence and phase-locking values) and different frequency bands between adult and child (cross-frequency coupling).

- Correlations

When examining the relationship between specific behaviors or neural activities, correlation analysis can be employed (Wass et al., 2018). This approach assumes that both parent and infant exhibit same frequency bands related to the activity in question. Pearson’s correlation can be used to quantify this interaction, as it measures the instantaneous similarity between two signals within a given time-window and is effective for evaluating the similarity of power traces in a specific frequency band (Turk, Vroomen, et al., 2022). This method is valuable for understanding the similarity and strength of relationships between the brains of interacting individuals within a specific frequency band.

- Phase synchronization

Phase synchronization refers to the phenomenon where the phases of oscillatory signals become aligned, indicating that the signals are temporally coordinated. This is often observed when phase-lags between signals remain consistent over time, as illustrated in Figure 7. In the context of interbrain interaction, phase synchronization occurs when specific brain waves, such as delta waves, in one brain align with the corresponding brain waves in another brain, suggesting a shared neural process or communication (Turk et al., 2022).

Figure 7.

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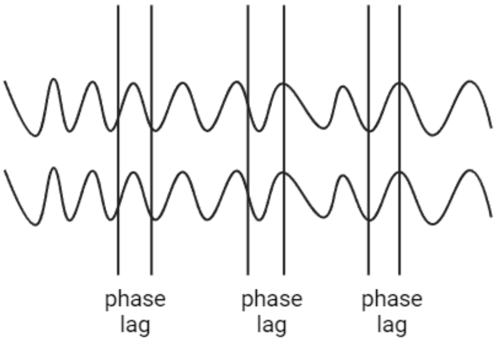


The Phase-Locking Value (PLV) is a widely used metric to quantify phase synchronization. It is calculated by determining the phase differences between pairs of electrodes, either within a single brain or across multiple brains, over a given time window. The consistency of these phase differences across various trials or events is then evaluated (Zhong et al., 2024). PLV values range from 0 to 1, where 0 indicates no synchronization (completely random phase differences), and 1 indicates perfect synchronization (consistent phase differences) between the signals (Dumas et al., 2010).

PLV is particularly valuable in hyperscanning studies, where it helps researchers to capture dynamic changes in neural activity between individuals engaged in social interactions or collaborative tasks. By comparing the PLV across different conditions or groups, researchers can infer the extent to which synchronized neural activity is associated with shared experiences, or coordinated behaviors (Dumas et al., 2010). For example, a study by Santamaria (2020) used Phase-Locking Value (PLV) to compare brain synchronization between mothers and infants during positive and negative interactions. The study found that positive interactions led to increased neural connectivity between the mother and infant. PLV measure provides insights into the neural mechanisms underlying social cognition and interbrain communication, making it a crucial tool in the study of interactive brain dynamics.

- Cross-frequency coupling

Cross-frequency coupling (Cohen, 2014) is a highly promising method for measuring interpersonal neural synchronization across different frequency bands. It is important to have cross-frequency methods in parent-infant research because adults and infants function on different frequency bands. This technique can be broken down into two main types: phase-phase cross-frequency coupling, phase-amplitude cross-frequency coupling and amplitude-amplitude cross-frequency coupling.

Phase-phase cross frequency coupling examines how the phases of oscillations in different frequency bands are aligned between two brains. For example, this method can reveal how often phase-locking occurs between the EEG signals of a parent and an infant across distinct frequency bands (Turk et al., 2022).

Phase-amplitude cross-frequency coupling, on the other hand, investigates how the amplitude of higher frequency oscillations in one brain is related to the phase of lower frequency oscillations in another brain (Noreika et al., 2020). For instance, it can be used to analyze whether the amplitude of high-frequency brain waves in an infant's EEG is modulated by the phase of slower, low-frequency oscillations in the mother's EEG. This coupling indicates that certain brain rhythms in one person might influence the timing of more rapid brain activities in another person, shedding light on the intricate dynamics of interbrain communication (Turk et al., 2022).

Amplitude-amplitude cross-frequency coupling is a relatively novel technique used to correlate the power of different frequency bins between two time series (Noreika et al., 2020).

- Circular correlations

Circular correlations are another non-directed measurement used to assess phase changes or similarities, without assuming that parent and infant have activity related to different frequency bands (Turk et al., 2022).

- Imaginary coherence

Imaginary coherence is another non-directed measurement used to assess changes or similarities in power or amplitude, assuming that parent and infant have activity within the same frequency band (Turk et al., 2022).

2.5.Hyperscanning research on parent-infant interaction

Hyperscanning, the simultaneous recording of brain activity from multiple individuals, can be applied to various types of interactions. Specifically, theta and alpha bands are commonly examined in this context. Theta band activity is often linked to attention and cognitive processes, while alpha band activity can provide insights into emotional states. These frequency bands are vital for calculating interpersonal neural synchronization (INS) indexes.

For this thesis, the focus is on the parent-infant relationship, a key context for exploring how these frequency bands influence neural synchronization from a developmental perspective. This section will summarize findings from parent-infant hyperscanning studies, with a particular emphasis on EEG hyperscanning, to illustrate how these interactions affect neural synchronization and the role of theta and alpha frequency bands in these processes.

2.5.1. Interpersonal neural synchrony during naturalistic parent-infant interactions

During parent-infant interactions, infants develop a sense of self and others, which profoundly impacts their emotional expressions. Brain-to-brain synchronization, or interpersonal neural synchrony, provides insights into the outcomes of these interactions. Previous research has extensively employed fNIRS (functional Near-Infrared Spectroscopy) to investigate brain-to-brain synchronization during parent-infant interactions, focusing on a variety of developmental and psychological processes. These studies have explored how brain synchrony relates to emotion regulation processes (Reindl et al., 2018; Piazza et al., 2019), the effects of parental stress on neural coupling (Azhari et al., 2019; Alonso et al., 2023), and the role of brain synchrony in the development of language abilities (Zhai et al., 2023). Additionally, fNIRS has been used to examine brain-to-brain synchrony in the context of atypical developmental trajectories, such as those associated with Autism Spectrum Disorder (Minagawa et al., 2023; Wang et al., 2020). These studies collectively provide a comprehensive understanding of the factors influencing neural synchronization between parents and infants. Compared to fNIRS hyperscanning studies, EEG hyperscanning research on parent-infant relationships is relatively limited. However, for the purposes of this thesis, I will focus exclusively on EEG hyperscanning.

These studies collectively highlight the importance of parent-infant interactions in shaping neural synchronization and underscore the complex interplay between behavioral variables - such as emotional state, language development, and gaze- and neural synchronization.

Santamaria et al. (2020) found that parents and their infants exhibited stronger neural synchronization during positive maternal demonstrations compared to negative ones. This suggests that positive maternal expressions have a more substantial effect on the infant, fostering stronger neural alignment.

Additionally, Endevelt-Shapira et al. (2021) explored the effects of maternal chemosignals on neural synchronization in mother-infant and stranger-infant interactions. The study found that infant-mother interactions exhibited greater interpersonal neural synchrony in theta band compared to stranger-infant interactions. However, maternal chemosignals attenuated this difference, although they increased neural synchrony and positive arousal in infants even during stranger interactions.

Furthermore, Endevelt-Shapira and Feldman (2023) discovered that face-to-face interactions between mothers and infants, especially those involving positive facial expressions, lead to synchronized theta activity between their brains. Their findings build on previous research demonstrating that theta rhythms have several functions in the context of emotional processing. They highlighted that increased theta activity is associated with the processing of both positive and negative emotional stimuli in infants, underscoring the role of theta rhythms in emotional response. They suggest that because theta rhythms are involved in emotional, cognitive, attentional, and physiological processes, the underlying reasons for theta-band synchrony observed in infant-adult hyperscanning research need further investigation (Endevelt-Shapira et al., 2021).

In addition to the emotional aspects of brain synchronization, some studies have focused on the impact of parent-infant interactions on language development. It is essential to understand how our habits can influence the quality of these interactions, as certain behaviors may be detrimental. One relevant study examined the neurological correlates of screen exposure, comparing mother-infant dialogic reading with mobile-phone interrupted dialogic reading (Zivan et al., 2022). The results showed that mother-infant neural synchrony between the mother's language-related regions (left hemisphere) and the infant's comprehension-related regions (right hemisphere) decreased during mobile-phone interrupted dialogic reading compared to uninterrupted dialogic reading. This indicates that screen interruptions can negatively impact the neural connection crucial for language development.

One of the key aspects of behavioral synchronization is gaze. Gaze plays a crucial role in developing speech understanding during interactions. Previous studies have demonstrated that direct gaze, serving as a social cue, enhances interpersonal neural synchronization between adults and infants, particularly in the theta and alpha frequency bands (Leong et al., 2017; Wass et al., 2018). These frequency bands are known to be sensitive to attention, learning and changes in shared focus, concepts that are closely linked to eye gaze.

Research indicates that the characteristics of these frequency bands change with developmental age. For example, in infants, theta frequency is typically observed between 3 and 6 Hz and remains consistent until around 12 months of age. Eye gaze helps infants direct their attention to relevant information in their environment. Since the attention system starts to develop after 4 months, observing the interplay between theta and alpha activity in infants is most effective after this developmental milestone (Michel et al., 2015).

One study found a significant relationship between infant-adult theta cross-frequency phase-locking values (PLVs) and infant theta power specifically during free play, but not during the resting state. This suggests that higher infant theta power is positively associated with stronger synchronization of theta activity between the infant and the adult during interactive play (Kayhan et al., 2022). It seems that the dynamic and interactive nature of free play appears to enhance neural coupling, likely involving both mutual attention and eye gaze. Mutual attention helps synchronize focus on shared activities or objects, facilitating a shared experience. Eye gaze, on the other hand, acts as a powerful social cue, strengthening the connection and coordination between the adult and the infant. Therefore, mutual gaze should be considered a crucial factor in parent-infant studies, as it plays a significant role in fostering neural synchronization.

Until this part, I have explained parent-infant studies in a natural interaction context. Now, I will focus on parent-infant studies within the context of the Still-Face Paradigm (SFP). As mentioned before (see Chapter 1), the Still-Face Paradigm involves maternal unavailability and disruption of interaction. According to the mutual regulation model, infants express their internal state through emotional expressions, which are influenced by prior moments of interaction with their parent. Behavioral observations from the SFP have shown that infants may exhibit gaze aversion, withdrawal, crying, and fussing in response to maternal unavailability (Tronick et al., 1978). Researchers have used the SFP to induce short-term stress and reveal underlying physiological and neural mechanisms associated with these behavioral responses. I will try to focus on neural mechanisms more than others but studies are limited when SFP is included.

One study concluded by Perone (2020), investigated the relationship with maternal quality and infants' emotion regulation behaviors. They measured frontal alpha asymmetry (FAA) during SFP, and found that dyads with a more responsive mother exhibited higher and more left FAA, compared to dyads with less responsive mother. FAA is an important neural marker used to study emotional and motivational processes. It is measured by comparing the power of alpha brain waves in the left and right sides of the frontal cortex. Specifically, when there is more alpha activity on the left side (which means lower alpha power relative to the right side, resulting in higher FAA), it is associated with positive emotions and a tendency to approach situations. Conversely, when there is more alpha activity on the right side (lower alpha power relative to the left side, resulting in lower FAA), it is linked to negative emotions and a tendency to avoid or withdraw from situations (Perone et al., 2020).

Another study by Swider-Cios et al. (2024) examined FAA across the SFP in the context of maternal postnatal anxiety. They found that higher maternal postnatal anxiety was associated with lower FAA (more rightward) during the first still face episodes, suggesting a need for infants to withdraw from the situation. However, a seemingly contradictory finding was that infants exhibiting higher negative affect during the still face episode also showed higher FAA (more leftward). This indicates that, in the context of distress, infants' neural responses might differ from the typical patterns seen in adults, where higher FAA is usually associated with positive emotions and approach behaviors.

These results suggest that FAA in infants might have different implications compared to adults, especially during distressing situations like the still face episode. Therefore, it highlights the need for further research to fully understand FAA in infants.

In conclusion, this section has explored various studies on parent-infant interactions, particularly within the Still-Face Paradigm (SFP). The research highlights the significance of frontal alpha asymmetry (FAA) as a neural marker of infants' emotional and motivational states, showing that infants with more responsive mothers display more positive FAA patterns. Additionally, maternal postnatal anxiety impacts infant FAA, revealing complex emotional and neural responses. Overall, these findings underscore the importance of investigating neural mechanisms in understanding infant emotional regulation and development.

Chapter 3

Current Study

3.1. Introduction

From the first day of life, humans require a caregiver for the promotion of their cognitive and socio-emotional development, which is why researchers often focus on early interactions between caregivers and infants when studying human development. Research has shown that early-life interactions can impact an infant's cognitive flexibility (Tisborn et al., 2023), stress resilience (Averill et al., 2018), and long-term memory (Montirosso et al., 2013). Additionally, these interactions can be influenced by factors such as parental stress (Azhari et al., 2019), maternal interpretation of infant emotions (Laurent et al., 2024), parental sensitivity (Rattaz et al., 2023), and childbirth-related post-traumatic stress disorder (Pinto et al., 2023). In this developmental messiness, caregivers can help in developing regulatory capacity, and Face-to-Face Still-Face paradigm (E. Z. Tronick, 1989) is one of the procedure to assess this regulatory capacity which I will mention it in this current chapter. EEG hyperscanning is a novel approach for studying parent-infant interactions in dynamic and ecologically valid settings. This method allows for simultaneous recording of brain activity from both parent and infant, providing insights into how their neural systems synchronize during interactions. Regarding hyperscanning research, studies on INS are limited, with most focusing on natural interactions rather than controlled experimental paradigms. Previous studies have found a strong association between the theta band and gaze (Michel et al., 2015; Wass et al., 2018), and it has been demonstrated that theta oscillations are related to both emotional and attentional processes (Endevelt-Shapira & Feldman, 2023; Orekhova et al., 2006).

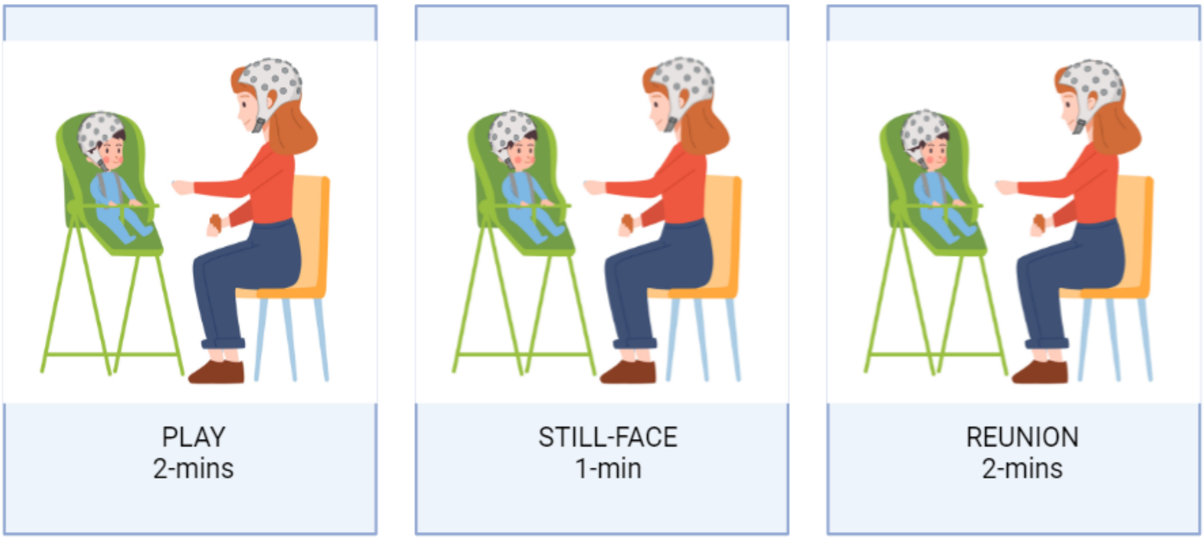
No previous study has investigated INS in the theta band while applying the SFP paradigm to parent-infant interactions. The specific aims of this study are to: 1) investigate infant' gaze (face-directed, object-directed and mutual gaze) during play and reunion episodes, 2) examine infant' emotional state (positive and negative) during play and reunion episodes, 3) explore INS in theta band between parent and infant during play and reunion episodes, 4) analyze the correlation between mutual gaze and INS in theta band during play and reunion episodes, and 5) examine the correlation between infant' emotional state and INS in theta band during these episodes. In line with previous behavioral findings (see Chapter 1), we anticipated that behavioral codings of positive and negative emotionality, as well as mutual gaze between parents and infants, would change significantly between the play and reunion episodes. Consistent with prior neural findings (see Chapter 2), we also expected to find a significant correlation between INS in the theta band and these behavioral codings during the play episode, compared to the reunion episode. Our research question aims to explore whether INS in theta band is correlated with these behavioral variables, and how this correlation varies between the play and reunion episodes.

Based on these research questions, we formulated the following hypotheses: Hypothesis 1 states that behavioral variables will show significant changes from the play episode to the reunion episode, influenced by the still-face episode (see Section 1.3.2). Hypothesis 2 predicts that significant correlations will be observed between INS in theta band and gaze, and between INS in theta band and emotional state behavioral variables.

To investigate above mentioned hypothesis, we used EEG hyperscanning to parents and infants during FFSF experiment. The total duration of the paradigm is decided as 5 minutes: 2 minutes for play, 1 minute for still-face, and 2 minutes for the reunion episode (figure 8). We kept the still-face episode 1 minute instead of 2 minutes as in the original study to prevent excessive stress, and preserve better signal for reunion episode.

Figure 8.

Our design for the experiment



Furthermore, we did not expect to observe INS during the still-face episode, given the well-established evidence that the still-face interaction disrupts social engagement (Abney et al., 2021; Montirosso et al., 2012; Provenzi et al., 2016). Therefore, our analysis focused on comparing INS between the play and reunion episodes.

3.2. **Method**:

3.2.1. Participants

Forty one parent-infant dyads were recruited between the June 2022 and March 2024. The mothers were contacted by either San Matteo Hospital database or prepartum courses which are given at location. Mothers who gave birth in San Matteo Hospital were called, and received all information related to our study. The ones who were interested in our study accepted our invitation. As another sampling strategy, during the prepartum courses (a course to prepare mothers for birth), we present our study to the mothers and if they were interested in it, they decided to join to our study. The dyads were recruited according to the following inclusion criteria: for the infants, age between 8 and 10 months, no presence of any developmental, neurological and/or medical conditions, normal birth weight and full-term birth; for the parents, age greater than 18 years, proficiency of Italian language, being the primary caregiver of the infant, absence of psychiatric or neurological conditions. The final sample was 20 parent-infant dyads because of the following reasons: fussiness of the infant (N=4), technical problems related to EEG equipment (N=5), inoperable EEG signal due to many interpolated channels or with less than 30 good epochs (N=11), outliear dyad with atypical familiar situation (infant born through in vitro fertilization; N=1).

3.2.2. Materials

EEG hyperscanning equipment: EEG data were collected using two wireless and portable EEG devices, each equipped with 32 channels, obtained through the mbraintrain (mbt) system (Smarting, mBrainTrain, Belgrade, Serbia). This setup allowed for unrestricted movement, ensuring that the infants' natural mobility was preserved without compromising the accurate acquisition of their brain signals, thereby enhancing the ecological validity of the study. Each EEG cap was paired with a portable amplifier, which sent the parent and infant signals to two different PCs via Bluetooth connection The two PCs were connected to each other through a LAN cable to allow for simultaneous EEG signal recording only from one station (namely the infant PC).

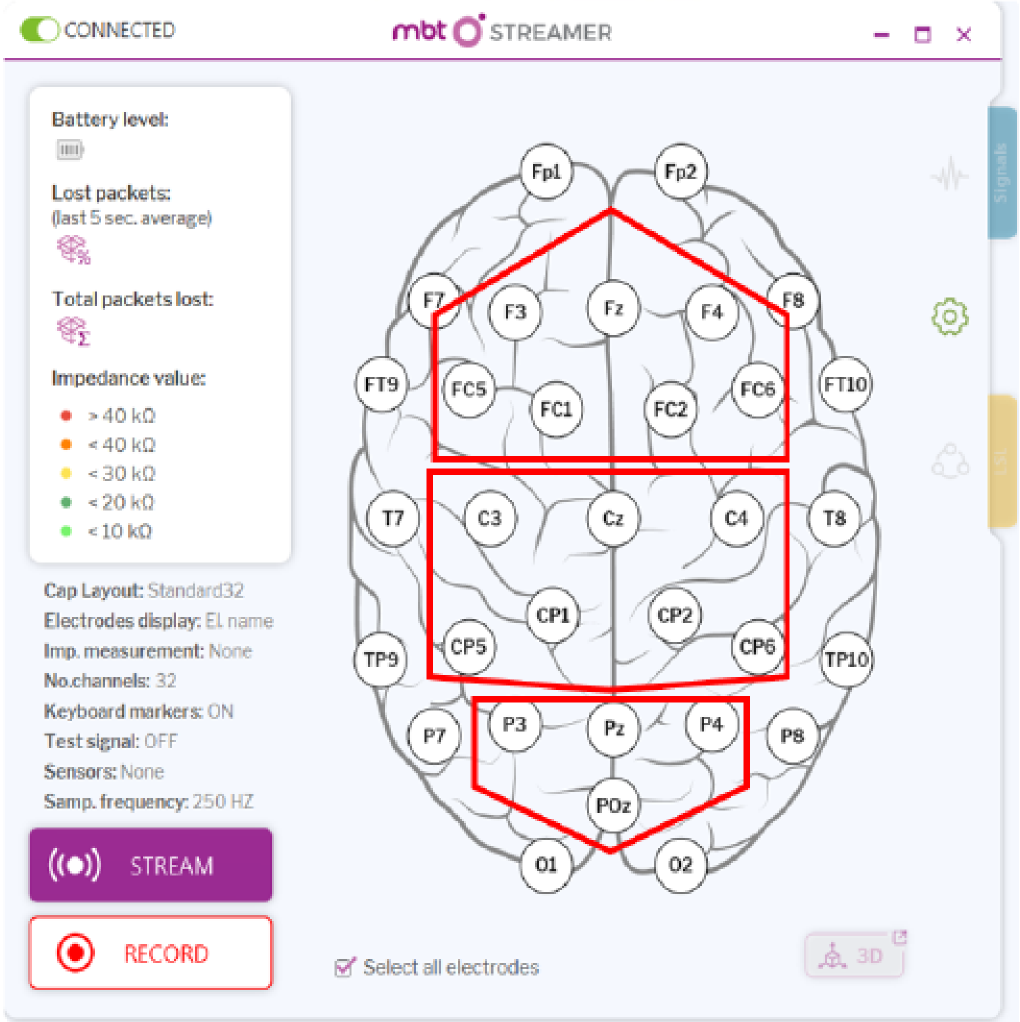
Video recordings: SFP was recorded using two video cameras to capture the movements of both parents and infants. One camera was positioned to focus on the parent's face and body, while the other was positioned to capture the infant's face and body. This setup was crucial for the behavioral coding procedure, as it involved coding across five domains focused on the facial expressions and bodily movements of both mothers and infants.

3.2.3. Setting and Procedure

Parent-infant dyads were invited to the our laboratory located in Fondazione Mondino and Istituto Neurologico Nazionale a Carattere Scientifico (IRCCS) hospital (Pavia, Italy). They were informed and asked to engage in a SFP while their brain activity was recorded through the EEG hyperscanning. Prior to commencing the session, the room was prepared. An infant seat and a chair for the mother were arranged, EEG equipment was set up. Upon the participants' arrival at the hospital, one experimenter greeted the mother. As they came to the room, three experimenters company to the mothers for the study and the infants was placed on a mat to play with toys which are provided from our laboratory to moderate their adjustment to this new environment. While the infants play with the toys, the procedure was explained to the mothers. After the explanation, we measured the mother's head circumference and choose EEG cap based on this measurement. Two experimenters were responsible for the preparation the EEG cap, while the other experimenter were responsible during the SFP. Once the cap was fitted onto the mother's head, the amplifier was connected, and two experimenters began applying conductive gel to the electrodes on the EEG cap. We intentionally began with the mothers to facilitate the infants' adjustment process. During the gel application, an experimenter responsible for computers and the SFP procedure checked the electrodes' channel impedances on the computer, aiming for values of around ~5 – 10 kΩ or lower. Especially the following electrodes should have good impedances (figure 9): frontal (Fz, F3, F4, FC1, FC2, FC5, FC6), central (Cz, C3, C4, CP1, CP2, CP5, CP6) and posterior (Pz, P3, P4, POz). In addition to reducing computational costs for analysis and targeting cortical areas with prominent theta responses, the electrode selection is supported by research highlighting the significance of the frontal, central and parietal regions in facilitating interbrain synchrony during direct communication. Moreover, previous studies on parent-infant dyads investigating interbrain synchronization have concentrated on similar electrode groups, reinforcing the rationale behind this choice (Bi et al., 2023; Orekhova et al., 2006; Santamaria et al., 2020). Once the process was completed successfully with the mother, the same procedure was applied to the infants. Subsequently, to ensure reciprocal design of the SFP, the infant was seated in front of the parent on the same height and at a distance of 70 cm (Mantis et al., 2014). Two cameras were placed in a way that both the mother's and the baby's faces could be seen clearly. Before starting to SFP, a neutral video, which is a one minute cartoon of a rocket flying to space, was watched to parents and infants, and a separator was put between them in order to prevent the infant to be focusing on the parent.

Figure 9.

The selected electrodes.



Cap Layout: Standard32
Electrodes display: El. name
Imp. measurement: None
No.channels: 32
Keyboard markers: ON
Test signal: OFF
Sensors: None
Samp. frequency: 250 HZ

☑ Select all electrodes

Once both parent and infant brain signals were checked, the procedure started with the '**start**' statement. The play episode lasted for 2 minutes, during which the mother was asked to interact with her infant in a typical manner within the given time. Parents were instructed to minimize the use of toys, although a few were provided to help the infant acclimate to the setting if they were particularly fussy or unengaged by parental interaction alone. Following the play episode, the still face episode began with the '**stop**' signal of the experimenter. The mother then needed to interrupt the interaction and assumed a neutral facial expression without any local or physical gestures. This episode lasted 1 minute. For the reunion, mothers and infants restarted interaction upon receiving the '**you may resume playing**' signal of the experimenter. The reunion episode also lasted 2 minutes, mirroring the duration of play episode. Throughout the SFP, the experimenter monitored the time ensuring that each phase adhered to the specified durations totaling 5 minutes. If the infant exhibited extreme frustration or stress during the still-face episode, the duration of this episode was abbreviated accordingly. During the entire SFP duration, only one experimenter stayed in the room, remaining concealed from view while still audible to the mother and infant. Each episode commenced with a simultaneous signal and key press, facilitating accurate timing for the coders to determine the start and end of each phase.

Once the recording session concluded, the amplifiers were detached from the cap, and the cap itself was removed. Assistance was provided to both mothers and infants to remove the EEG caps and wash their hair to eliminate any residual conductive gel. As a token of appreciation for their participation, each mother was presented with a printed picture taken while they were wearing the EEG caps.

3.3. Data Analysis

3.3.1. EEG hyperscanning data and pre-processing

In our study, we initially recruited forty-one parent-infant dyads; however, due to poor EEG signal quality, we excluded twenty-one dyads and proceeded with the analysis on the remaining twenty dyads. We developed an ad-hoc automated pipeline for EEG data pre-processing using EEGLAB (Delorme & Makeig, 2004) and MATLAB (The MathWorks, Inc.). Given the presence of multiple brains signals, our initial step involved splitting the signals into those belonging to the mothers and infants. Subsequently, we started with preprocessing the infant's EEG data, focusing on bandpass filtering at 1-30 Hz of the acquired signals. Our emphasis was on lower frequencies (1-30 Hz) because of the two primary reasons: higher frequencies are more prone to noise interference (Nottage & Horder, 2016), and we specifically targeted theta frequency as an indicator of social interaction (Cavanagh & Frank, 2014; Endevelt-Shapira et al., 2021; Endevelt-Shapira & Feldman, 2023; Michel et al., 2015). Flat and outlier electrodes were detected first in this process since it might indicate poor signal quality or loss of contact between electrode and the scalp. As following, we used the NEAR plugin (Kumaravel et al. 2022) to detect channels with flat or outlier signals: these channels were retained, i.e. stored in a different matrix, for later interpolation. Following this, we employed Artifact Subspace Reconstruction (ASR; Kothe and Jung, 2016), to correct noisy data, and then proceeded with performing Independent Component Analysis (ICA), another technique aimed at artifact mitigation. According to Delorme (n.d.), ICA can be understood as a signal processing technique used to separate independent sources that are linearly mixed across multiple sensors. During this step, the EEG signal is decomposed into a set of independent components which may correspond to artifacts rather than brain activity such as eye blinks, muscle activity, heart beat, eye movements. After identifying these components, each component displaying a probability of 50% or higher of being an ocular artifact was automatically rejected. This threshold was chosen because ocular artifacts are the only ones expected to appear in our target frequency (theta), while other artifacts such as line noise and muscular activity typically occur at higher frequencies and should not pose a problem. We further validated our manual component removal by cross-checking it with ICLabel, utilizing its labels to determine which components to retain and which to discard. After cleaning and modifying the data, we recalculated the new reference. To facilitate the examination and comparison of brain frequencies across the episodes of the SFP, we extracted trials for each episode. The play and reunion episodes were each 120 seconds long, while the still episode lasted 60 seconds. Therefore, the trials were segmented based on the these specified durations. We employed epoching to divide the continuous data stream into 1-second epochs for each SFP episode. Following this step, we performed automated rejection of the bad epochs, i.e. any epoch displaying a signal with amplitude higher or lower than 150µV (parameter used by Debnath et al. 2020). The epochs marked as rejected were saved into an Excel file. Once the data for the infant were cleaned, we applied the same process for the parents' data, ensuring that all epochs marked as rejected in the infant dataset were also marked as rejected in the parent dataset and vice versa. This was achieved by merging the Excel files containing the rejected epochs to determine the total number of rejected epochs across both datasets. Additionally, exclusion criteria were established for dyads at the preprocessing stage: if either the infant or parent data contained more than 10% of interpolated channels (equivalent to 6 or more channels) or if fewer than 30 epochs remained after epoch rejection, the signals were excluded from further analyses.

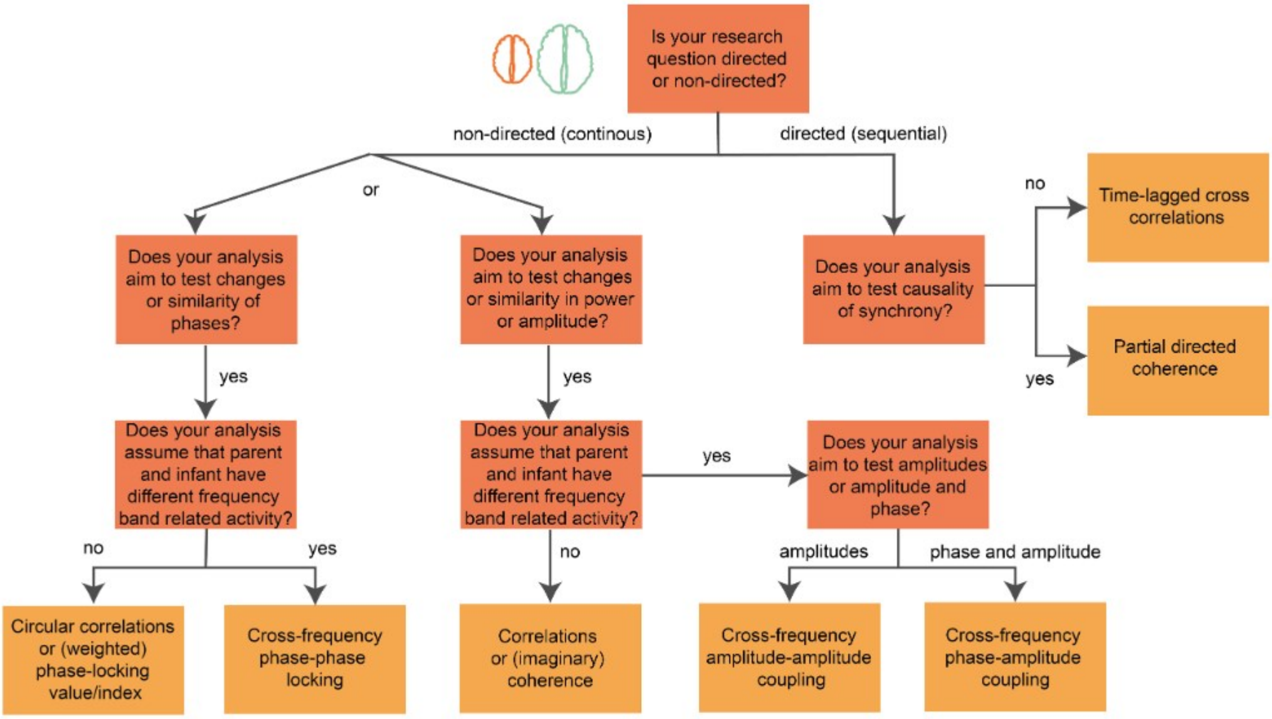
3.3.2. Interpersonal Neural Synchronization

For our analyses, we employed phase synchrony analyses, specifically focusing on the phase-locking value (PLV) method, which is a standard technique in EEG hyperscanning studies (Dumas et al., 2010; Kayhan et al., 2022). PLV is a non-directed measure of phase synchronization between two signals (Santamaria et al., 2020). While there are multiple options available for assessing interpersonal neural synchronization (INS), we opted for PLV due to our assumption that INS was non-directed, and because we hypothesized that mothers and infants might exhibit analogous frequency bands (Turk et al., 2022). Our decision is influenced by the decision-making pipeline designed by Turk (2022), and can be seen in Figure 10. To investigate these phenomena, we conducted interpersonal neural synchronization analyses focusing on theta frequency band, which has been repeatedly implicated in social interaction (Endevelt-Shapira et al., 2021; Endevelt-Shapira & Feldman, 2023; Michel et al., 2015; Orekhova et al., 2006). It's important to note developmental discrepancies in frequency band activity between infants and adults (Marshall et al., 2002). Accordingly, we investigated a blend of infant and parent frequencies. During our analysis of theta waves, we employed adult bands (4-7 Hz).

The selected electrodes for our PLV analyses were F3, Fz, F4, FC1, FC2, C3, Cz, C4, CP5, CP1, CP2, CP6, P3, Pz, P4, and POz.

Figure 9.

This flowchart assists in identifying a metric that aligns with the research question.



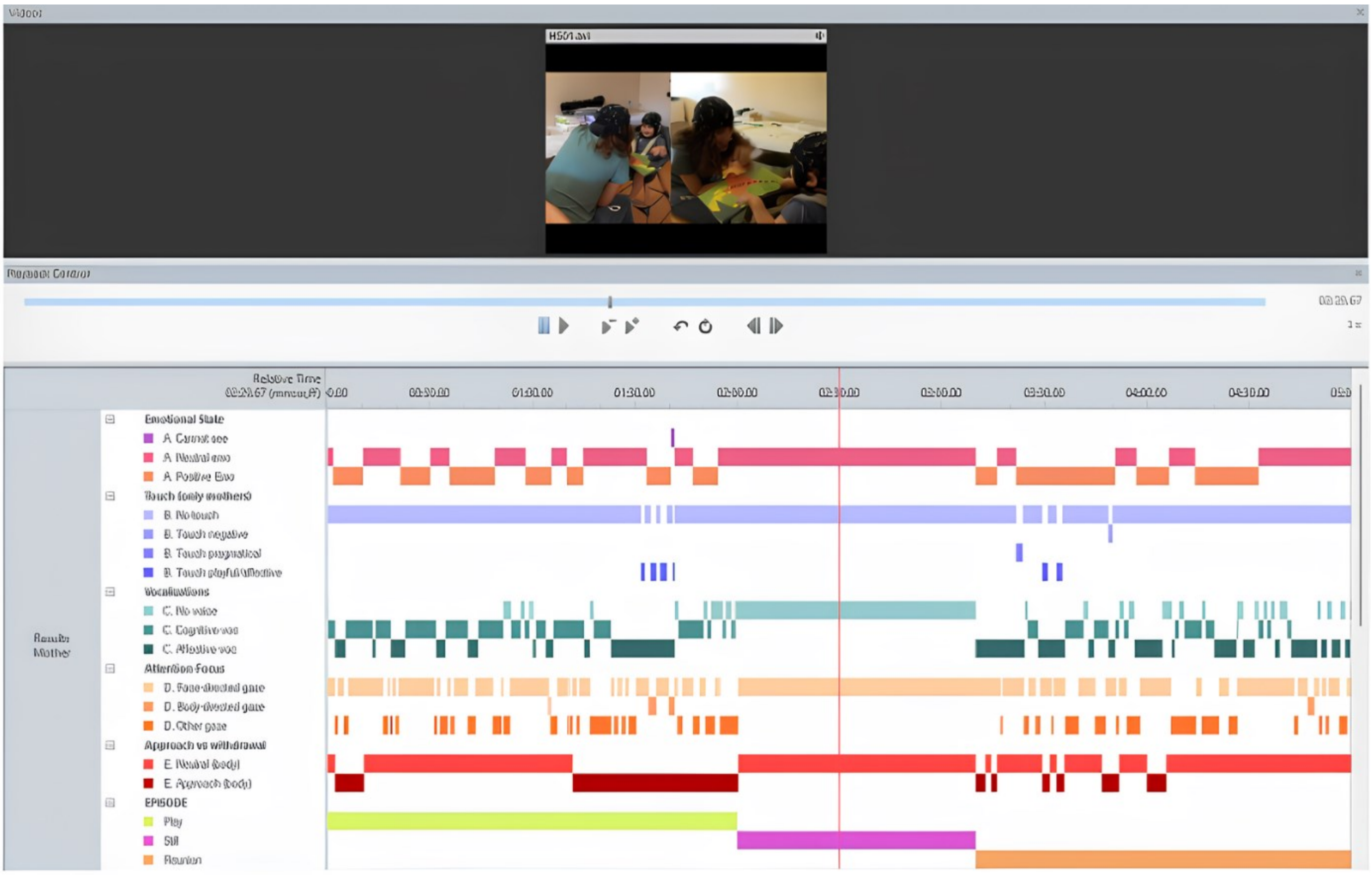
3.3.3. Behavioral Coding

We developed an ad-hoc behavioral coding system using Noldus for micro-analytic coding of videotaped parent-infant interaction. The focus is on specific infants' and parent's behaviors that can be discretely depicted in 1- or 2-seconds micro-analytic coding. It has 5 domains and each of them need to be coded twice, once for mother and once for infant (Figure 10). Domains are *emotional state, touch, vocalizations, attention, approach vs withdrawal*. Vocalizations domain is coded only for mother. For the purposes of this study, only emotional state and gaze domains are used in the analysis. A detailed explanation of the behavioral coding scheme for maternal and infant behavior can be found in the Appendix section.

We applied Noldus coding system to all the dyads with good EEG signal.

Figure 10.

Retrived by NoldusObserver. It allows us to code specific infants' and parent's behaviors that can be discretely depicted in 1- or 2-seconds micro-analytic coding.



3.4. Result

The data analysis was conducted using Jamovi version 2.3.28 (The Jamovi Project 2024 n.d.) whereas graphs were built using RStudio 2023.12.1 to test our research question, which explores whether INS in the theta band correlates with mutual gaze and emotional state behavioral variables, and how these correlations differ between play and reunion episodes. We performed repeated measures ANOVA and paired samples Student's t-tests, followed by post-hoc analyses and correlation matrices to further explore these relationships. The results are presented in the following sections through a combination of text, tables, and graphs.

Descriptive statistics - demographics

To begin, descriptive statistics were calculated for all variables to provide an overview of the data set. The sample consisted of 20 parent-infant pairs. The mean age of the parents was 36.3 years (SD = 4.17, range = 29-45 years), and the mean age of the infants was 9.1 months (SD = 0.91, range = 8-11 months). The sample was relatively homogeneous, with most infants being male (12 out of 20). This homogeneity in terms of maternal age, infant age, and infant sex may be relevant for interpreting the results.

3.4.1. Preliminary analyses 1 - Behavioral codings

Repeated measures ANOVA and Paired samples Student's t-tests were applied to detect significant changes in the selected variables (infant gaze and emotional state) throughout the episodes of FFSF.

Face-directed gaze (infant)

Descriptives

The descriptive statistics indicate that the mean duration of face-directed gaze was highest during the play episode, followed by the reunion episode, and lowest during the still episode.

Table 2.

Descriptive statistics for face-directed gaze during the play, still-face, and reunion episodes

Variable	**N**	**Mean**	**SD**	**Min**	**Max**
Face-directed gaze - Play	20	27	19.7	0.69	74.9
Face-directed gaze – Still	20	13	17.7	0	67
Face-directed gaze - Reunion	20	18.5	17.3	1	68.1

Repeated Measures Anova

A Repeated Measures ANOVA confirmed a significant effect of episode on face-directed gaze, F(2, 38) = 7.27, p = 0.002, with face-directed gaze being significantly higher during the play episode compared to both the still and reunion episodes.

Post-hoc analyses

Post-hoc analyses using paired samples t-tests were conducted to explore the differences in face-directed gaze across the three episodes: play, still, and reunion. The results indicated a significant reduction in face-directed gaze from the play to the still episode, with a mean difference of 14.00 percentage points (SE = 4.31), t(19) = 3.25, p = 0.004. This suggests that infants directed their gaze toward the parent's face significantly less during the still episode compared to the play episode.

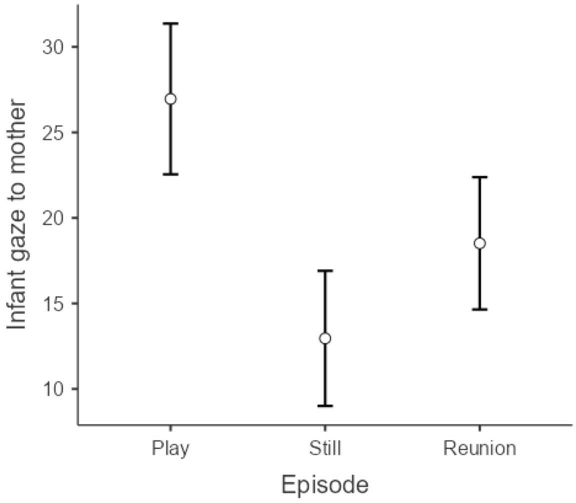
Similarly, a significant reduction in face-directed gaze was observed when comparing the play episode to the reunion episode, with a mean difference of 8.44 percentage points (SE = 3.71), t(19) = 2.28, p = 0.034. This indicates that even during the reunion episode, which follows the still episode, infants continued to direct their gaze toward the parent's face less frequently than during the initial play episode.

However, when comparing the still and reunion episodes directly, the mean difference in face-directed gaze was -5.56 percentage points (SE = 2.94), t(19) = -1.89, p = 0.074, which was not statistically significant. This suggests that there was no substantial change in face-directed gaze between the still and reunion episodes, indicating that the reduction in gaze observed during the still episode persisted into the reunion episode.

Table 3.

Post-hoc analysis for the differences in face-directed gaze across play, still-face, and reunion episodes.

Comparison	Mean diff.	SE	df	t	p
Play – Still	14.00	4.31	19	3.25	0.004
Play – Reunion	8.44	3.71	19	2.28	0.034
Still – Reunion	-5.56	2.94	19	-1.89	0.074


Infant gaze to mother
30
25
20
15
10
Play
Still
Reunion
Episode

Other object-directed gaze

Descriptives

The descriptive statistics show that the mean duration of other object-directed gaze was highest during the still episode, followed by the reunion episode, and lowest during the play episode.

Table 4.

Descriptive statistics for other object-directed gaze during the play, still-face, and reunion episodes

Variable	N	Mean	SD	Min	Max
Other object-directed gaze - Play	20	63.7	21.2	25.1	99.3
Other object directed gaze – Still	20	82.6	24.0	14.9	100
Other object directed gaze - Reunion	20	75.5	21.6	10.7	99

Repeated measures ANOVA

The analysis revealed a significant effect of episode, F(2,38)=9.49, p= <0.001, indicating that the amount of other object-directed gaze differed significantly across the play, still and reunion episodes.

Post-hoc analyses

Post-hoc analyses using paired samples t-tests were conducted to explore differences in other object-directed gaze across the play, still, and reunion episodes. The results revealed a significant increase in other object-directed gaze from the play episode to the still episode, with a mean difference of -18.96 percentage points (SE = 5.04), t(19) = -3.76, p = 0.001. This suggests that infants directed their gaze toward other objects significantly less during the play episode compared to the still episode.

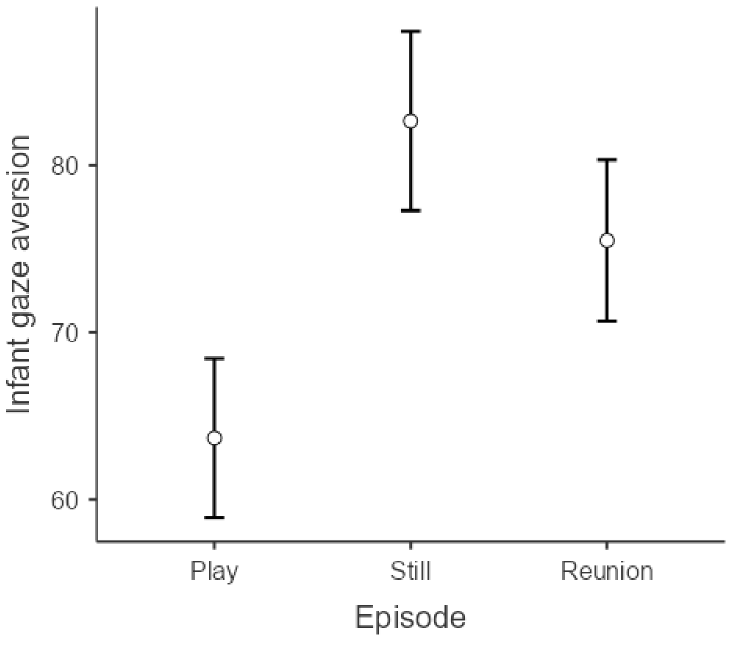
Similarly, a significant decrease in other object-directed gaze was observed during the play episode compared to reunion episode, with a mean difference of -11.81 percentage points (SE = 3.77), t(19) = -3.13, p = 0.005. This indicates that infants also spent more time directing their gaze toward other objects during the reunion episode compared to the play episode.

However, no significant difference was found between the still and reunion episodes, with a mean difference of 7.15 percentage points (SE = 4.34), t(19) = 1.65, p = 0.116. This suggests that the increase in other object-directed gaze observed during the still episode continued into the reunion episode, without significant change.

Table 5.

Post-hoc analysis for the differences in other object-directed gaze across the play, still-face, and reunion episodes.

Comparison	Mean diff.	SE	df	t	p
Play – Still	-18.96	5.04	19	-3.76	0.001
Play – Reunion	-11.81	3.77	19	-3.13	0.005
Still – Reunion	7.15	4.34	19	1.65	0.116



Mutual gaze

Descriptives

Descriptive statistics for mutual gaze across play and reunion episodes are presented in table. The descriptive statistics indicate that the mean duration of mutual gaze was higher during the play episode compared to the reunion episode.

Table 6.

Descriptive statistics for mutual gaze during the play and reunion episodes

Variable	N	Mean	SD	Min	Max
Mutual gaze - Play	20	26.3	19.8	0.79	75
Mutual gaze - Reunion	20	17.7	16.0	0.83	58.3

Paired-samples t-test

The results reveal a statistically significant difference in mutual gaze between the play and reunion episodes ($t(19)=2.451$, $p=0.024$).

Positive emotional state (infant)

Descriptives

The descriptive statistics suggest that infants displayed the highest mean duration of positive emotional states during the play episode, followed by the reunion episode, with the lowest duration observed during the still episode.

Table 7.

Descriptive statistics for positive emotional state (infant) during the play, still-face, and reunion episodes

Variable	N	Mean	SD	Min	Max
Positive emotionality - Play	20	22.3	20.5	0	71.4
Positive emotionality – Still	20	10.6	24.9	0	94.9
Positive emotionality - Reunion	20	20.7	26.9	0	84.7

Repeated measures ANOVA

The analysis revealed a significant effect of episode, F(2,38)=6.20, p= 0.005, indicating that the amount of positive emotional state (infant) differed significantly across the play, still and reunion episodes.

Post-hoc analyses

Post-hoc analyses using paired samples t-tests were conducted to examine differences in positive emotional state across the play, still, and reunion episodes. The results showed a significant decrease in positive emotional state during the still episode compared to the play episode, with a mean difference of 11.64 percentage points (SE = 3.12), t(19) = 3.73, p = 0.001.

This indicates that infants experienced a significantly lower positive emotional state during the still episode.

In contrast, there was no significant difference in positive emotional state between the play and reunion episodes, with a mean difference of 1.54 percentage points (SE = 4.03), t(19) = 0.38, p = 0.706, suggesting that positive emotional state did not change significantly from the play episode to the reunion episode.

However, a significant difference was observed between the still and reunion episodes, with a mean difference of -10.10 percentage points (SE = 3.57), t(19) = -2.83, p = 0.011. This indicates that positive emotional state significantly differed between the still and reunion episodes, with a notable change occurring from the still episode to the reunion episode.

Table 8.

Post-hoc analysis for the differences in positive emotional state (infant) across the play, still-face, and reunion episodes

Comparison	Mean diff.	SE	df	t	p
Play – Still	11.64	3.12	19	3.73	0.001
Play – Reunion	1.54	4.03	19	0.38	0.706
Still – Reunion	-10.10	3.57	19	-2.83	0.011

Negative emotional state (infant)

Descriptives

The descriptive statistics reveal that infants exhibited the highest mean duration of negative emotional states during the still episode, followed by the reunion episode, with the lowest duration observed during the play episode.

Table 9.

Descriptive statistics for negative emotional state (infant) during the play, still-face, and reunion episodes

Variable	**N**	**Mean**	**SD**	**Min**	**Max**
Negative emotionality - Play	20	7.30	12.5	0	38.8
Negative emotionality – Still	20	30.44	31.2	0	80.3
Negative emotionality - Reunion	20	16.97	22.1	0	66.8

Repeated measures ANOVA

The analysis revealed a significant effect of episode, F(2,38)=8.69, p=<0.001, indicating that the amount of negative emotional state differed significantly across the play, still and reunion episodes.

Post-hoc analyses

Post-hoc analyses using paired samples t-tests were conducted to investigate differences in negative emotional state across the play, still, and reunion episodes. The results indicated a significant increase in negative emotional state during the still episode compared to the play episode, with a mean difference of -23.15 percentage points (SE = 5.30), t(19) = -4.37, p <

0.001. This suggests that infants experienced a markedly higher negative emotional state during the still episode.

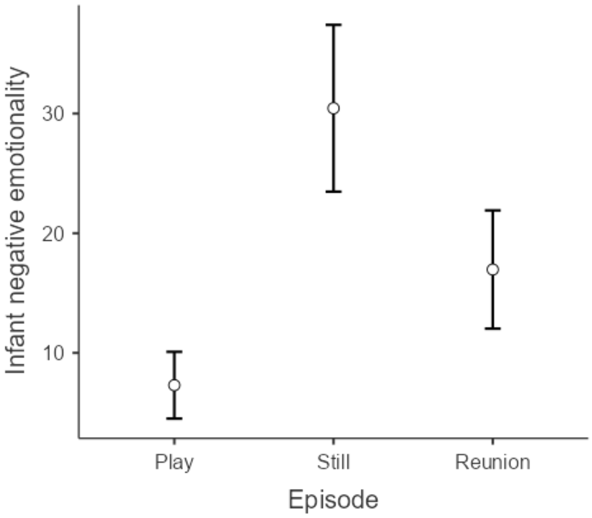
A significant difference in negative emotional state was also found between the play and reunion episodes, with a mean difference of -9.67 percentage points (SE = 4.14), t(19) = -2.34, p = 0.031. This indicates that negative emotional state was significantly different between these episodes.

However, there was no significant difference in negative emotional state between the still and reunion episodes, with a mean difference of 13.48 pergentage points (SE = 6.94), t(19) = -1.94, p = 0.067. This suggests that the negative emotional state observed during the still episode persisted into the reunion episode without a significant change.

Table 10.

Post-hoc analysis for the differences in negative emotional state (infant) across the play, still-face, and reunion episodes

Comparison	Mean diff.	SE	df	t	p
Play – Still	-23.15	5.30	19	-4.37	<0.001
Play – Reunion	-9.67	4.14	19	-2.34	0.031
Still – Reunion	13.48	6.94	19	-1.94	0.067


Infant negative emotionality
10
20
30
Play
Still
Reunion
Episode

Mutual positive emotionality

Descriptives

The descriptive statistics indicate that the mean duration of mutual positive emotionality was similar across the play and reunion episodes, with slightly higher values during the play episode.

Table 11.

Descriptive statistics for mutual positive emotionality during the play and reunion episodes.

Variable	N	Mean	SD	Min	Max
Mutual positive emotionality - Play	20	20.1	19.9	0	65.3
Mutual positive emotionality - Reunion	20	19.3	25.7	0	84.4

Paired samples t-test

The result reveal no significant difference in mutual positive emotionality between play and reunion episodes (t(19)=0.185, p=0.855).

3.4.2. Preliminary analyses 2 – INS measures (PLV) in the theta frequency band

Paired samples student’s t-tests were applied to detect significant changes in the selected variables (PLVs in the theta frequency band) throughout the episodes of the FFSF.

Descriptives

Descriptive statistics were calculated for all variables to provide an overview of the data set. Table summarizes the means, standard deviations, and ranges for the primary variables.

Table 12.

Descriptive statistics for an overview of the data set.

Variable	**N**	**Mean**	**SD**	**Min**	**Max**
PLV frontal theta - Play	20	0.372	0.016	0.340	0.402
PLV central theta - Play	20	0.371	0.015	0.335	0.395
PLV posterior theta - Play	20	0.365	0.014	0.331	0.381
PLV frontal theta - Reunion	20	0.369	0.015	0.338	0.397
PLV central theta - Reunion	20	0.366	0.012	0.348	0.389
PLV posterior theta - Reunion	20	0.367	0.011	0.350	0.384

Paired samples t-test

Comparison between play and reunion episodes across frontal, central and posterior regions in the that band made by paired samples t-test. None of the comparisons showed statistically significant differences.

Table 13.

Paired samples t-test statistics to provide comparison between play and reunion episodes across the brain regions.

Variables	**Student's t**	**df**	**p**	**D**	**C.I.**
PLV frontal theta (play, reunion)	1.831	19	0.083	0.409	-0.053; 0.861
PLV central theta (play, reunion)	1.569	19	0.133	0.351	-0.106; 0.798
PLV posterior theta (play, reunion)	-0.805	19	0.431	-0.180	-0.619; 0.264

Correlation matrices

Pearson's correlations were run to measure the relationship between the infant behavior during the FFSF and the PLVs measured in the theta frequency band.

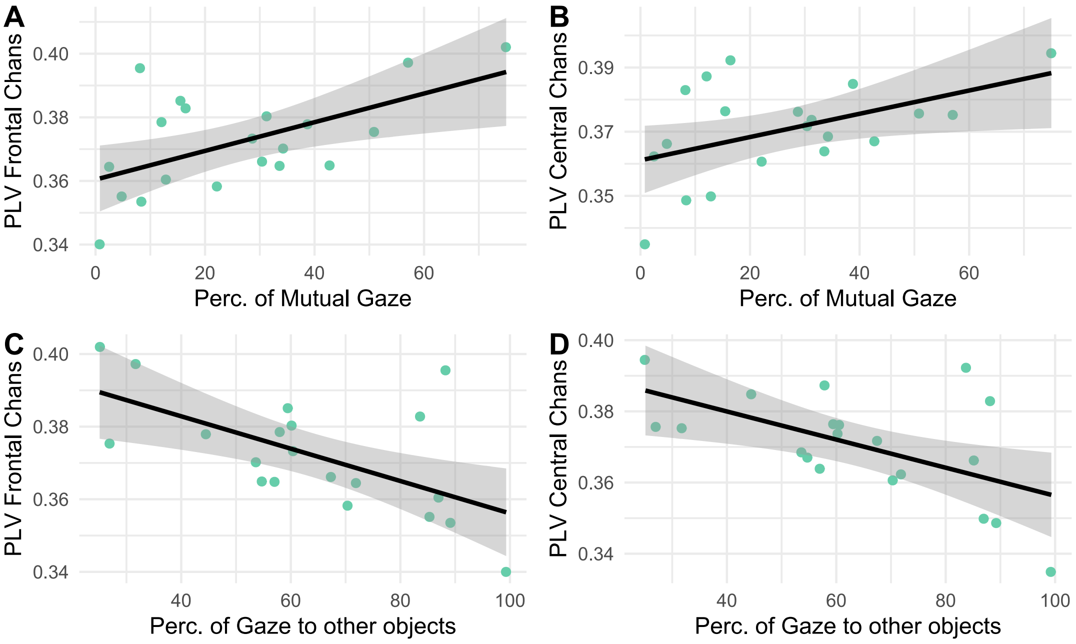
Hypothesis 1: Infant Gaze and PLV theta

Play and Reunion episodes

Correlation between the infant gaze and PLV theta band during the play episode were analyzed. The results indicate that the percentage of face-directed gaze is positively correlated with PLV theta activity in the frontal ($r = 0.576$, $p = 0.008$) and central regions ($r = 0.479$, $p = 0.033$) during play episodes. Similarly, the percentage of mutual gaze is positively associated with increased PLV theta activity in the frontal ($r = 0.568$, $p = 0.009$) and central regions ($r = 0.481$, $p = 0.032$). Conversely, the percentage of gaze directed towards other objects is negatively correlated with PLV theta activity across the frontal ($r = -0.602$, $p = 0.005$), central ($r = -0.562$, $p = 0.010$), and posterior regions ($r = -0.518$, $p = 0.019$). No significant relationships were found between body-directed gaze and PLV theta activity.

Correlation between the infant gaze and PLV theta band during the reunion episode were analyzed. The results revealed there were no significant relationships between the variables and PLV theta activity across any of the brain regions.

Episodes	Variables	PLV frontal theta	plv central theta	plv posterior theta
Play	Percentage of Face-directed gaze	0.576** (p = 0.008)	0.479* (p = 0.033)	0.376 (p = 0.103)
Reunion	Percentage of Face-directed gaze	0.040 (p = 0.866)	-0.016 (p = 0.948)	0.106 (p = 0.658)
Play	Percentage of body-directed gaze	0.149 (p = 0.531)	0.258 (p = 0.271)	0.372 (p = 0.106)
Reunion	Percentage of body-directed gaze	0.416 (p = 0.068)	0.3217 (p = 0.174)	0.250 (p = 0.287)
Play	Percentage of mutual gaze	0.568** (p = 0.009)	0.481* (p = 0.032)	0.360 (p = 0.119)
Reunion	Percentage of mutual gaze	0.027 (p = 0.911)	-0.020 (p = 0.932)	0.094 (p = 0.693)
Play	Percentage of gaze to other objects	-0.602** (p = 0.005)	-0.562** (p = 0.010)	-0.518* (p = 0.019)
Reunion	Percentage of gaze to other objects	-0.172 (p = 0.468)	-0.094 (p = 0.694)	-0.169 (p = 0.477)

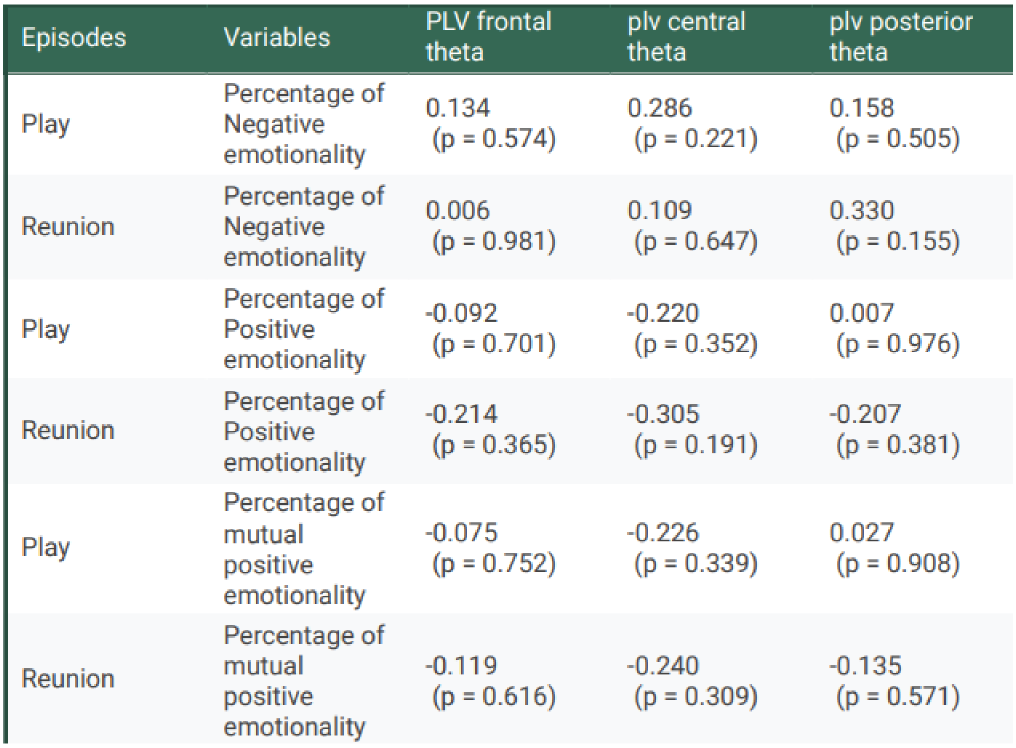


Hypothesis 2: Infant Emotional state and PLV theta

Play and Reunion episodes

Correlation between the infant emotional state and PLV theta band during the play episode were analyzed. The results revealed there were no significant relationships between the variables and PLV theta activity across any of the brain regions.

Correlation between the infant emotional state and PLV theta band during the reunion episode were analyzed. The results revealed there were no significant relationships between the variables and PLV theta activity across any of the brain regions.



Episodes	Variables	PLV frontal theta	plv central theta	plv posterior theta
Play	Percentage of Negative emotionality	0.134 (p = 0.574)	0.286 (p = 0.221)	0.158 (p = 0.505)
Reunion	Percentage of Negative emotionality	0.006 (p = 0.981)	0.109 (p = 0.647)	0.330 (p = 0.155)
Play	Percentage of Positive emotionality	-0.092 (p = 0.701)	-0.220 (p = 0.352)	0.007 (p = 0.976)
Reunion	Percentage of Positive emotionality	-0.214 (p = 0.365)	-0.305 (p = 0.191)	-0.207 (p = 0.381)
Play	Percentage of mutual positive emotionality	-0.075 (p = 0.752)	-0.226 (p = 0.339)	0.027 (p = 0.908)
Reunion	Percentage of mutual positive emotionality	-0.119 (p = 0.616)	-0.240 (p = 0.309)	-0.135 (p = 0.571)

Chapter 4

Discussion and Conclusion

Hyperscanning, as an innovative technique, marks a substantial advancement in the study of social interaction, particularly in the context of parent-infant relationships. This method, which allows for the simultaneous recording of brain activity from multiple individuals, provides new insights that traditional approaches could not capture. As a result, researchers have been able to move beyond previous limitations in parent-infant studies, gaining a deeper understanding of the complex dynamics that characterize these early interactions. This development has enabled the accumulation of more convincing evidence regarding the critical role that parent-infant interactions play in child development. Since the introduction of hyperscanning in this field, significant strides have been made in understanding various facets of these interactions.

4.1. Findings in infant gaze behavioral variable

One behavioral focus of our study was given on infant gaze behavior across different interaction episodes—specifically, the play, still-face, and reunion episodes. One of the key findings was that during the play episode, infants spent a significantly higher percentage of time directing their gaze towards their mothers' faces compared to the still and reunion episodes. The still episode, characterized by a lack of maternal responsiveness, elicited the lowest levels of face-directed gaze from infants. This finding aligns with Tronick's well-established research, which shows that infants tend to avert their gaze during stressful or uncomfortable situations, such as when their mothers suddenly become unresponsive, a phenomenon known as the "still-face" effect (Tronick et al., 1978). In contrast, during positive interactions, such as play, infants are more likely to maintain eye contact with their mothers, reflecting their engagement and comfort.

In this thesis, one of the particular attentions was given to the reunion episode, which follows the stressful still-face episode. Interestingly, we observed that there was no significant difference in the amount of face-directed gaze between the still and reunion episodes. However, there was a significant difference between the play and reunion episodes. This pattern suggests that the reduction in face-directed gaze seen during the still-face episode did not immediately resolve during the reunion episode, despite the reestablishment of maternal responsiveness. Although infants exhibited less gaze aversion during the reunion compared to the still episode, the difference was not statistically significant.

While infants' face-directed gaze decreased during the still-face episode, their object-directed gaze increased significantly. Our study found that object-directed gaze was highest during the still-face episode, with a significant increase observed from the play episode to the still-face and reunion episodes. This shift suggests that when deprived of social interaction, infants may redirect their attention to objects as a coping mechanism or alternative focus. The lack of significant differences in object-directed gaze between the still-face and reunion episodes indicates that the stress induced by the still-face episode persisted into the reunion, and the disruptions in interaction continued even after the mother's re-engagement.

In addition to examining face-directed and object-directed gaze, our study also assessed mutual gaze, but only during the play and reunion episodes. Since the still-face episode is designed to eliminate interaction cues—including gaze—we focused on these two episodes to understand how mutual engagement is affected by the still-face interruption. Our findings revealed a significant difference between the play and reunion episodes, with mutual gaze being considerably higher during the play episode.

Interpreting these results through the lens of the Mutual Regulation Model (Tronick et al., 1998), which posits that high-quality interactions are characterized by partners responding to each other's affective cues, we can infer that the still-face episode disrupted the dyadic interaction. According to Provenzi (2023), in a well-regulated interaction, parents and infants guide each other's behavior through mutual responsiveness. In our study, the reduced mutual gaze during the reunion episode suggests that the rupture caused by the still-face episode may have affected not only the infants but also the parents. This could indicate that parents, consciously or unconsciously, mirrored their infants' behavior, showing that both partners were affected by the prior disruption in their interaction.

4.2.Findings in emotional state behavioral variable

The second major focus of our study was on assessing infants' emotional states across different interaction episodes. Our findings aligned with our expectations, particularly in the context of positive emotional expressions. We observed a highly significant difference in positive emotional state between the play and still-face episodes, with a noticeable decrease in positive affect during the still-face episode. This result is consistent with the understanding that the still-face episode, characterized by a lack of maternal responsiveness, is stressful for infants, leading to a decline in positive emotional expressions.

Similarly, we found a significant increase in positive emotional state when comparing the still-face and reunion episodes. This indicates that when the mother re-engaged with the infant during the reunion episode, the infant's positive emotional state began to recover (recovery effect). Importantly, there was no significant difference in positive emotional state between the play and reunion episodes, suggesting that by the time of the reunion, infants were able to display positive emotions at levels comparable to those seen during the initial play interaction.

However, our analysis of negative emotional states revealed a different pattern. While the infants' positive emotionality during the reunion episode was similar to that observed during the play episode, their negative emotionality, which had increased during the still-face episode, persisted into the reunion episode. We found a significant increase in negative emotional state when comparing the play and reunion episodes. This suggests that even though infants showed positive emotions during the reunion, they also continued to experience and express the negative emotions that were triggered by the still-face episode. The stress induced by the still-face interaction did not completely disappear upon reunion, as evidenced by the lack of a significant difference in negative emotional state between the still-face and reunion episodes. This persistence of negative emotionality can be interpreted as a carry-over effect, where the stress and emotional disruption experienced during the still-face episode continued to influence the infant's emotional state even after the interaction resumed (Yaari et al., 2018). These findings are consistent with previous research, which has shown that infants exhibit emotional responses to disruptions in social interaction, indicating that they are capable of perceiving and interpreting such situations accurately (Montirosso et al., 2010; Yaari et al., 2018; Provenzi et al., 2015). The emotional responses observed in our study suggest that infants are not merely reacting reflexively; rather, they seem to understand the rupture in interaction as a meaningful change in their social environment. This ability to detect and respond to the breakdown in interaction demonstrates that even at a young age, infants are sensitive to the quality of social exchanges and can emotionally process the difference between responsive and unresponsive interactions.

In addition to analyzing positive and negative emotional states, our study also evaluated mutual positive emotionality during the play and reunion episodes. The results showed no significant difference between these two episodes, suggesting that the levels of positive emotionality exhibited by both infants and parents remained statistically similar during both interactions. This finding is particularly meaningful because it indicates that, despite the disruption caused by the still-face episode, infants and parents were able to maintain or quickly regain a positive emotional connection during the reunion.

During the reunion episode, when the parent resumes regular interactive play, infants typically exhibit what is known as the "reunion effect." This effect is characterized by a rebound of positive affect, reflecting the infant's ability to re-engage and express positive emotions once the interactive disruption has ended. However, this rebound of positive affect often occurs alongside a partial carry-over of negative affect from the still-face episode (Fuertes et al., 2024).

Our findings align with this pattern, as the lack of significant difference in mutual positive emotionality between the play and reunion episodes suggests that the infants successfully reestablished a positive emotional connection with their parents. This outcome indicates that the infants were resilient and able to recover their positive emotional state effectively. Despite this recovery, the partial carry-over of negative affect, which was observed in the increased negative emotionality during the reunion episode, further supports the notion of the reunion effect.

4.3. Findings in PLV theta frequency band

Our analysis of the phase-locking value (PLV) in the theta frequency band suggests that, on average, theta activity remains relatively stable from the play episode to the reunion episode across all examined regions—frontal, central, and posterior. This stability indicates that theta band synchronization is not significantly affected by the transition from play to reunion, a finding that aligns with previous research showing elevated theta synchronization during interactive play and face-to-face interactions between mothers and infants (Kayhan et al., 2022; Orekhova et al., 2006; Santamaria et al., 2020; Wass et al., 2018). The absence of a significant difference between these episodes might imply that both maintain a high level of theta synchronization, consistent with the interactive engagement present in both contexts, which likely promotes similar synchronization levels.

However, while this stability could be interpreted positively—as evidence of the parent-infant dyad's resilience and ability to maintain engagement even after a disruption like the still-face episode—it also introduces some ambiguity. Specifically, the lack of difference could suggest that neither episode sufficiently engages the dyad to create high synchronization, or it might reflect a ceiling or floor effect, where differences are undetectable due to inherent limitations in the data. As such, the most cautious conclusion we can draw is that PLV theta synchronization remains stable between the episodes.

To fully understand the significance of these synchronization levels, it would be necessary to compare them against a baseline to determine whether they represent high, low, or moderate synchronization. A key limitation of our study is the absence of such a reference point, leaving us uncertain about the absolute level of synchronization observed. Future research should consider including a non-interactive baseline condition, such as a neutral or non-social episode, or analyzing the still-face episode as a non-social control, to provide a clearer context for interpreting theta synchronization levels.

4.4. Findings for correlation between PLV theta and infant gaze

We examined the correlation between PLV theta activity in the central, frontal, and posterior brain regions and infant gaze behavior during both play and reunion episodes. Our analysis revealed a significant positive correlation between PLV theta activity and face-directed gaze during the play episode in the frontal and central brain regions, but not in the posterior region. Similarly, a positive correlation was found for mutual gaze in these same regions. However, no correlation was observed between PLV theta activity and body-directed gaze. The only significant correlation across all regions was seen between PLV theta activity and other-directed gaze. Notably, these patterns were only present during the play episode, as no significant correlations were found during the reunion episode.

Surprisingly, no prior studies have analyzed PLV theta synchronization between parents and infants within the context of the Face-to-Face Still-Face (FFSF) paradigm. As a result, we cannot directly compare our findings across different episodes within the paradigm. However, our results are consistent with existing literature suggesting that face-directed gaze enhances interpersonal brain synchronization in the theta band, particularly during social interaction (Leong et al., 2017; Orekhova et al., 2006; Turk, Endevelt-Shapira, et al., 2022). Previous research has established that infant attention is associated with theta oscillations, with a pronounced theta effect localized to the frontal regions of the brain during attentional tasks (Xie et al., 2018).

The frontal region, especially the prefrontal cortex, plays a crucial role in higher-order cognitive functions such as attention, decision-making, and social cognition (Cavanagh & Frank, 2014). The observed positive correlation between PLV theta activity and both face-directed and mutual gaze in the frontal region suggests that this area is actively engaged during social interactions. This engagement likely facilitates the regulation and processing of social cues exchanged between the infant and parent. The frontal region's involvement is essential for understanding the intentions of others and maintaining engagement in social contexts, which could explain the increased theta synchronization observed during face-directed and mutual gaze.

The absence of significant theta synchronization in the posterior region during face-directed and mutual gaze could be attributed to the nature of posterior theta activation, which is predominantly observed during attention to social stimuli (Orekhova et al., 2006). This finding aligns with our observation of posterior theta synchronization during other-directed gaze behavior, suggesting that when the infant's sustained attention is focused on external stimuli rather than direct interaction with the parent, different neural mechanisms are at play.

Orekhova's (2006) study further suggests that theta activity is more prominent over the frontal region in infants and shifts towards the posterior region in older children, possibly due to the maturation of neural mechanisms. This developmental difference might explain the distinct patterns of theta synchronization observed in different scalp areas in our study. The lack of posterior theta synchronization during face-directed gaze in infants could reflect an immature neural system that is still developing the capacity for processing social information in a way that more mature brains do in older children.

In summary, our findings highlight the role of the frontal and central brain regions in supporting social interaction through theta synchronization, while the posterior region's involvement appears to be more context-dependent, possibly reflecting developmental differences in the neural mechanisms underlying social cognition in infants.

We did not observe any significant PLV theta synchronization in relation to infant gaze behaviors during the reunion episode. This finding aligns with our expectations, given the results of our behavioral analysis, which indicated that the interactive rupture caused by the still-face episode had lingering effects during the reunion. Specifically, the disruption in the infants' gaze behavior persisted into the reunion episode, preventing a full return to the patterns of interaction observed during the initial play episode. This finding underscores the lasting impact of the still-face episode on infant-mother interactions, highlighting how a momentary disruption can have prolonged effects on both behavior and brain activity.

4.5. Findings for correlation between PLV theta and emotional state

We investigated the correlation between PLV theta activity and infants' emotional states during the play and reunion episodes. Contrary to expectations based on previous literature (Orekhova et al., 2006; Endevelt-Shapira & Feldman, 2023; Endevelt-Shapira, 2021), our results revealed no significant correlation between emotional states and PLV theta activity across any brain regions. Research on the relationship between theta activity and emotional states is still limited, with most studies focusing on the alpha band (Perone et al., 2020; Perone & Gartstein, 2019;

Swider-Cios et al., 2024). It's possible that other unexamined variables influenced this correlation, or that an indirect relationship exists, which was not detected in our analysis. Additionally, confounding factors might have played a role. This potential correlation warrants further investigation in future studies to better understand the complexities of theta activity and its connection to emotional states.

4.6. Limitations and Conclusions

Investigating parent-infant social interactions and the corresponding brain activities is crucial for enhancing the quality of communication in their daily lives. Our study sought to understand how interpersonal neural synchronization is affected by disruptions in parent-infant interactions, specifically focusing on how behavioral variables—such as infant gaze patterns and emotional states—are reflected in theta brain rhythms.

Our findings highlight the significant impact of the Face-to-Face Still-Face (FFSF) paradigm on parent-infant interactions. The still-face episode effectively disrupted the natural flow of interaction, as seen in changes to infant gaze behaviors (including face-directed, other-directed, and mutual gaze). This disruption did not merely end with the still-face episode; rather, it carried over into the reunion episode, indicating a lasting effect on the infant's social engagement. In terms of emotional state, while infants showed a marked recovery in positive emotions following the still-face episode, they continued to exhibit elevated levels of negative emotions during the reunion episode. This persistence of negative emotionality reflects a "carry-over effect," where the emotional experience from the still-face episode continues to influence the infant's emotional state in subsequent interactions. This suggests that infants are sensitive to changes in social dynamics and can respond to these changes, but the emotional impact of the still-face episode has a lingering effect that challenges their ability to fully return to a positive emotional state.

In addition to these behavioral observations, we explored how these experiences corresponded with PLV theta synchronization across different brain regions during the play and reunion episodes. Most of our results aligned with our expectations, particularly in how gaze behaviors were linked to theta synchronization. However, the lack of significant correlations between theta synchronization and emotional state was surprising, given the existing literature on brain rhythms and emotional processing. This discrepancy suggests that the relationship between theta activity and emotional state might be more complex than initially thought, possibly influenced by factors not accounted for in our study.

Despite these insights, our study does have several limitations that should be considered. First, our sample size was relatively small, which may have limited the diversity and generalizability of our findings. A larger sample could provide a broader range of behaviors and brain activity patterns, offering more robust conclusions. Additionally, we focused solely on direct relationships between variables, without exploring the potential role of moderators—factors that could influence or change the strength or direction of these relationships. Future research should investigate potential moderators such as infant temperament, parent-infant attachment style, and parental stress. These factors are known to significantly impact parent-infant interactions and could provide a deeper understanding of the nuances in how brain synchronization and behavior are linked. Finally, it is important to acknowledge that the infant brain is still in a rapid state of development, and this must be considered when interpreting our results. In our study, we treated the parent and infant brains as homologous, using corresponding EEG channels for both. However, the infant brain undergoes significant changes during the first months and years of life, which means that brain rhythms and neural activity patterns can vary greatly depending on the age and developmental stage of the infant. For instance, previous research has shown that brain rhythms differ significantly from 2 months to 9 months of age (Michel et al., 2015), and from infancy to childhood (Orekhova et al., 2006). Future studies should carefully consider the developmental stage of infants when analyzing brain activity, as these developmental changes could influence the observed patterns of brain synchronization and their relationship to behavior.

Studying parent-infant interactions is vital not just for understanding behavior but for delving into the neurobiological processes that underpin these relationships. Our growing knowledge of the brain has been transformative for human development, particularly within the clinical field, where it has led to groundbreaking techniques and interventions that improve lives. This underscores the necessity of experimental research in this area. By exploring these interactions in-depth, we can generate insights that are crucial for clinicians, enabling them to integrate these findings into their practice and offer more informed, compassionate care. We are privileged to be in an era where we can conduct experimental research on humans, even at early developmental stages. This unique opportunity should be used to deepen our understanding of how we connect with one another and enhance the quality of our relationships. Our interactions and environment are integral to our well-being, and recognizing how even seemingly minor details, such as directed gaze, can impact interpersonal neural synchronization highlights the importance of these nuances.

This research reveals how small elements of our interactions can significantly influence our social connections and overall health. It reminds us that every facet of our relationships—however subtle—contributes to the richness of our human experience. By acknowledging and appreciating these details, we can strive to nurture our connections and foster healthier, more meaningful relationships.

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Appendix

Appendix 1 Behavioural coding scheme for maternal and infant behaviour during the SFP

DOMAIN	DESCRIPTION	Variable Type	Noldus CODING	Noldus Key
N.B. Subject	This always has to be coded twice (once per member of the dyad) as a first keypress together with all the other variables (e.g., Ie1, Me1, Ie2, Me2….), except for the "Touch" variable, where only mothers are coded (therefore, we only need to code M).	(*not a variable*)		I = Infant M = Mother
Episode	This also has to be coded twice, once for the mother and once for the infant (the mother coding for the still phase will not be extracted but it still needs to be coded as an episode).			Pl = Play (initial state) St = Still Re = Reunion
EMOTIONAL STATE	**Cannot see ->** The infant face is covered, or it is only partially evident (e.g., the infant look at the other side of the camera and the face is only half-visible) and is not possible to code the facial emotional expression. Use this code also for cases in which the emotional expression of the infant cannot be clearly coded due to technical issues. **Negative ->** The infant displays clear negative emotionality. Negative emotionality might be expressed through facial expression (eyes, mouth, general movement of the face muscle and/or of the body), and/or non-verbal communication (cry, to be fussy, stressed, shout). **Neutral ->** The infant does not display clear positive or negative emotionality through facial expression or other modalities (e.g., the infant is calm, relaxed). **Positive ->** The infant displays clear positive emotionality. Positive emotionality might be expressed through facial expression (e.g., eyes, mouth, and general movement of the face muscle and/or of the body) or vocalizations (e.g., laughing). To code 3, there should be clear expressions of positive emotionality	State event (duration)	A. Cannot see emo I A. Negative emo I A. Neutral emo I A. Positive emo I	e0 e1 e2 (initial state) e3
TOUCH (Only mother)	**No touch** -> The parent is moving and accidentally touches the infant with no intrinsic touch-related intentional purposes OR the infant starts the tactile contact with the parent OR there is no touch. **Negative** -> touch is somehow intrusive and provokes negative responses and stress (the negative response must be visible). The touch	State event (duration)	B. No touch B. Touch negative B. Touch pragmatical	t0 (inital state) t1 t2 t3

	may be intrusive, awkward, overwhelming, rough, etc. **Pragmatical/cognitive** -> touch used to accomplish a specific non-relational task (e.g., removing infants' hands from the mouth, adjusting infant's position on the infant-seat, adjusting infant's dress, cleaning the mouth of the child, etc.) OR touch used to get the infant's attention toward the parent (e.g., tapping, patting, squeezing, pinching, stroking, etc.). The infant should not be focused on the parent. This type of touch may (or not) be accompanied by attention getting vocal productions. **Affective/Playful** -> Parent touch characterized by a sense of closeness or by the goal of soothing and calm the infant. The parent may be kissing, stroking, or massaging the infant with affectionate intent or to regulate negative emotional states OR Parent touch characterized by high-paced, dynamic, repetitive, fast cinematic features. The parent may tickle, shake, squeeze, lift, move fingers on the infant's belly like little ants, move the hands or legs of the infant. Typically, the goal is provoking fun in the infant. Include through objects here.		B. Touch playful/affective	
VOCALIZATIONS	**Cannot hear/unspecified/no voice** -> The parent is silent OR It is not possible to be sure if the parent said something OR the vocal content is not clear. [use this variable for NO VOCALIZATIONS for the infants] **Negative OR Rejecting** -> Verbalizations that communicate rejection or negative comments on infants' behavior. **Cognitive** -> **Request OR Directiveness** (Verbalizations that are direct requests for specific behaviors (do this, take that, go there) OR **Attention getting** (Specific kind of request: the parent requests the child to look toward the parent herself. Usually, the child was not looking at the parent before this request) OR **Explain** (Verbalizations that provide explanations for time sequences (we did that, now we do this), object naming (this is a telephone), object characteristics (*"bello"*, this is done this way), object affordances (you can open it with this)). **Affective/Playful** (Verbalizations characterized by singing, laughing, nursery rhymes) OR **Mirroring** (Verbalizations that mirror or amplify using parent voice previous vocalizations or gestures produced by the infant. These verbalizations should occur immediately after an infant emotional display, gesture, surprise reaction, or verbalizations) OR **Affectionate OR**	State event (duration)	C. No voice C. Negative C. Cognitive C. Affective	v0 (initial state) v1 v2 v3

	Nurturing (Verbalizations that confirm the state of the infant, approve his/her behavior, or that are used to regulate infant distress. Compliments to the infant also are included e.g., *"bravissimo"*). **Mind-related comments** (Any comment that signals the interest of the parent for the mind of the infant. Keywords are "you think", "you want", "you like", "you prefer", "you wonder". Sometimes, these expressions are implicit in parent's verbalizations – for example, when after the Still-Face episode the parent asks, "Where was the mom?" or "What happened?" this can be coded as mind-related, as the verbalizations may be introduced, implicitly, by "You are wondering…?").[use this variable for all kinds of VOCALIZATIONS for the infants]			
ATTENTION FOCUS	Direction of gaze and head orientation: **Face-directed** -> The attentional focus is directed towards the interactive partner's face (N.B. Use this for mothers during the still face). **Body-directed** -> The attentional focus is directed towards the interactive partner's body (e.g., hands). **Other ->** every other gaze direction (including objects).	State event (duration)	D. Face-directed D. Body-directed D. Other	a1 (initial state) a2 a3
APPROACH WITHDRAWAL	Approach and withdrawal of bodies: **Neutral** -> normal posture **Withdrawal** -> evident leaning backwards or turning head away. **Approach ->** evident leaning forward or reaching forward with hands.	State event (duration)	E. Neutral E. Withdrawal E. Approach	b0 (initial state) b1 b2

NOTES:

Vocalizations:

(i) for the infant only no voice, vs all types of vocalizations (negative and positive) will be coded, using the variables "cannot hear" and "affective".

(ii) "prendo io?" = mother thinks baby wants to give her the toy/book/object → Mind related comments

(iii) if break is of 1 second or less, code together with previous vocalization. If more than 1 second, code as no voice

Touch:

(i) if the contact is passive, e.g., the parent leaves a hand resting on the body of the infant and no movement is detected do not code (only code the first instance in which the touch happens.

Attention focus:

(i) Object refers to physical objects (e.g., toys, clothes, and pacifier) but also to parts of the body other than the head of the interactive partner.

Approach/withdrawal:

Approach:

For the mother: we can usually observe a shift of the whole torso + head towards the infant. This is not the case when she leans for instance to get an object that the baby dropped.

For the baby: mind the intentionality. if the infant leans forward but to reach an object, this is not approach. Usually we can observe that the baby reaches with the arms while also looking at the mom's face.

Withdrawal:

backwards shift in the body (easy to observe for instance during still when infants turn away or lean backwards.

Acknowledgment

“Our souls
will not be soothed
by what we achieve
how we look
or all the hard work we do
even if we managed to
make all the money in the world
we’d be left feeling empty for something
our souls ache for community
our deepest being craves one another
we need to be connected
to feel alive”

-rupi kaur

Our achievements, joys, sorrows, and all of life's experiences are deeply shaped by the people we choose to surround ourselves with. Sharing these moments with our people can create the most unique and meaningful experiences of all. I always strive to build strong connections—whether with family, friends, mentors, or professors—because, as a song so beautifully says, "*you can't go in alone, everybody needs help, you gotta find your people, then you'll find yourself.*”

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