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Inter-neural co-regulation before and after an interactive perturbation in mother-infant dyads

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Hyperscanning techniques allow inter-neural synchrony (INS) estimation and represent an innovative approach to investigate neural processes underlying social exchanges. Electroencephalographic (EEG) studies showed that collaborative parent-infant social interactions might be characterized by varying levels of INS. Nonetheless, despite social mismatches are common occurrences in early interactions, limited research has focused on INS variations elicited by interactive perturbations. (1) to assess variations in INS before and after an experimentally-induced interactive perturbation; (2) to investigate the relationship between INS and behavioral coupling. Sixty-six dyads of 9-month-old infants and their mothers participated in the well-validated Face-to-Face Still-Face (FFSF) procedure combined with dual-EEG recordings. Infants and maternal behaviors were microanalytically coded and phase locking values (PLV) over frontal, central and posterior regions in the *theta* and *alpha* bands were computed from the dual-EEG recordings. A significant increase in alpha coupling emerged before and after perturbation. Conversely, a reduction in *theta* coupling was highlighted in the subgroup of female infants. Behavioral and EEG-based dyadic co-regulation in the theta band was evident only prior to the perturbation. This study highlights the role of *alpha* and *theta* wave coupling in response to parental unresponsiveness, with differential patterns emerging when the dyadic system is perturbated.

Keywords EEG, Gaze, Hyperscanning, Infant, Mother, Still-face, Regulation, Synchrony, Stress

The first years of life are key for child development and infant interactions with the caregiving environment—occurring in the early months of life—are especially critical for later child social, emotional and cognitive development[1,2]. It should be highlighted that, contrary to naïve expectations, such early adult-infant interactions in the context of parental nurturing and caregiving are not characterized by continuous synchrony or attunement[3,4]. Rather, interactive ruptures and perturbations are inherent features of parent-infant interactions and they are meant to be naturally occurring occasions for child development of emotional regulation skills and stress resilience[5]. The Face-to-Face Still-Face (FFSF)[6] paradigm serves as a well-established method to investigate these interactions[7–9]. It presents an experimentally induced interactive perturbation by initially engaging mothers and infants in a period of face-to-face play, followed by a brief period where the mother adopts a still-faced expression, withholding contingent social responses[10].

This experimentally induced disruption in the social interaction flow allows researchers to study how mothers and infants co-regulate their emotional states in response to interactive perturbations. The FFSF has provided important information on a broad range of early interactive and developmental phenomena, such as infant physiological responses to social stress[11,12], temperament[13], gender differences[14] and responses to maternal behavior[8,15]. The FFSF may also offer precious insights into later trajectories of child development and health[16,17].

In the last decade, hyperscanning has rapidly emerged as a powerful tool to investigate the neurophysiological underpinnings of social interactions[18,19]. Hyperscanning involves measuring the neural activity simultaneously from multiple individuals that can be engaged in a joint task[20–22]. This allows researchers to assess inter-

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neural regulation, which is meant to reflect the level or extent to which neural activity synchronizes among individuals[23,24]. For this aim, different studies have used dual-electroencephalographic (EEG) recordings during parent-infant interaction (for reviews on the topic see[25–28], looking at different indexes of synchronization (e.g., using phase or amplitude-based estimates)[19] both with non-directional and directional approaches[29]. Phase coupling offers a powerful approach to investigate brain co-regulation by capturing the ways in which neural activity aligns across interacting individuals. This alignment may arise from common entrainment to shared environmental features[28], as well as from actor–observer dynamics, in which one participant's neural activity can be reset[30] or shifted—up or down from their intrinsic frequency band[31]—to follow the attentional or cognitive state of the other, reflecting moment-to-moment coordination of neural processes. These investigations often focus on *theta* (4–8 Hz) and *alpha* (8–12 Hz) oscillations that are prominent EEG rhythms implicated in various social, attentional and emotional processes across lifespan. In adults, *theta* activity has been linked to attention control, information processing[32,33] and emotional regulation[34,35] while *alpha* oscillations are associated with inhibitory processing and attention allocation[36,37]. Similarly, in infants, *theta* activity is associated with sustained attention, socially relevant information processing[38–40] and emotional arousal[41]. *Alpha* band activity in infants has been linked to social processing and self-regulation mechanisms[42,43].

Inter-Neural Synchrony (INS) is thought to be a potential underlying mechanism supporting social coordination and emotional reciprocity. For example, recent evidence seems to point to higher interbrain synchrony in parent-child interaction during cooperative tasks compared to individual activities[44–46]. Moreover, higher levels of interbrain synchrony have been reported during children's interaction with their parents as compared to strangers[46,47]. Furthermore, interaction promoting social cues such as positive emotion displays[48] and direct gaze[49,50] seem to elicit increases in interbrain synchrony during parent-child interactions. However, evidence on associations between INS and specific social cues is still debated, with studies failing to report significant associations with previously observed behaviors, both for mutual gaze[51] and positive emotionality[47]. Furthermore, brain-behavior coupling appears to be more particularly complex and probably not univocally detected by linear associations[52,53].

Despite the growing interest in social development and the utility of both FFSF and EEG hyperscanning, there is a paucity of research investigating parent-infant EEG coupling or co-regulation across interactive perturbations like the FFSF paradigm. In a recent pilot study, Gartstein and colleagues[54] explored individual frontal *alpha* asymmetry in parents and infants across the FFSF paradigm and documented a rightward shift in frontal EEG activity in mothers and infants. While these authors piloted a promising application of the EEG hyperscanning technique coupled with the FFSF task, there is no evidence to date of proper dyadic co-regulation of inter-neural activity across interactive perturbations adopting frequency-specific dyadic indexes of brain-to-brain coupling as recently suggested by Turk and colleagues[19]. Furthermore, our understanding of the EEG-behavioral associations, particularly in terms of dyadic co-regulation during these perturbations, remains limited.

The present study aims to address this gap in knowledge by investigating the dynamics of inter-neural coherence between mothers and their 9-month-old infants during the FFSF paradigm using EEG hyperscanning. We focus on changes in *theta* and *alpha* band using a phase-based non-directional index i.e., Phase-Locking Value (PLV), across the play and reunion episodes of the FFSF paradigm. We also explore the relationship between PLV and periods of mutual gaze and positive emotion matching during the two episodes. Analyses will be controlled for sex. This investigation will shed light on the neurophysiological mechanisms underlying social interaction, emotional regulation, and dyadic co-regulation during early infancy.

Methods

Participants

Sixty-six mother-infant dyads were included in the present study (41% female). Dyads were recruited from local prenatal classes at participating hospitals. Families were contacted when the infants were in the 8–10 months of age range. All infants were born at-term from healthy pregnancies, did not experience pre/perinatal severe health problems or neurological symptoms. All mothers had no neurological and/or psychiatric disorder diagnoses, no clinical history of epilepsy, traumatic brain injuries or significant head traumas. Parents provided written informed consent prior to the inclusion in the study. The study was approved by the Ethics Committee of Policlinico San Matteo, Pavia, Italy (protocol 0008588/23, date 16.02.2023). All methods were carried out in accordance with guidelines and regulations from the Declaration of Helsinki for studies involving human subjects.

Procedures

FFSF procedure

Mother-infant dyads participated to a video-recorded FFSF procedure. EEG data acquisition during the procedure was carried out with a 32-channel Smarting Pro (mBrainTrain) system with wireless connection, at 250 Hz sampling rate. After a familiarization phase for the infant and debriefing presentation for the parent, mothers first and subsequently infants were fitted with an appropriately size EEG cap and conductive gel was applied to optimize signal conductivity. The FFSF procedure included three episodes: (a) Play episode (2 min) – mothers were seated face-to-face with their infants (placed in a highchair) and asked to interact freely with them, possibly avoiding the use of toys and/or pacifiers (b) Still-Face episode (1 min) – mothers were asked to interrupt any interaction and communication toward the infant and to maintain a still (neutral and unresponsive) face expression while maintaining eye-contact (c) Reunion episode (2 min) – the unconstrained playful interaction was resumed. This procedure has been widely used to assess behavioral[17,55] and neurophysiological[56,57] measures of socio-emotional stress regulation after an interaction disruption. Previous research suggested that it is common occurrence to have infants who are unable to complete the paradigm due to their stress levels in behavior-only FFSF studies[58]. As such, compared to the classic FFSF procedure, we only reduced the length of the Still-Face episode from 2 min to 1 min in order to assure that the greatest proportion of infants could finish the task and provide appropriate EEG and behavioral data for the Reunion episode. Two external cameras were used to record the session. The separate videotapes of parent and infant were later synchronized and off-line coding was preformed focusing on specific maternal and infant interactive behaviors (see Behavioral Coding below for details).

EEG data processing and phase locking values estimation

Dyadic EEG data were pre-processed with a fully automated pipeline in MATLAB. The pipeline performance with automatic settings was previously assessed and compared to manual selection with comparable results on PLV values[59]. Parent and infant data were bandpass filtered at 1–30 Hz with the default EEGLAB Hamming windowed sinc FIR bandpass filter[60]; flat and outlier channels were detected – with the Neonatal EEG Artifact Removal NEAR plugin[61] and retained (participant included if < 15% bad channels); noise correction through Artifact Subspace Reconstruction ASR[62] was applied (k = 10); Independent Component Analysis ICA was conducted and components flagged – by ICLabel plugin[63] – as having > 50% probability of being ocular artifacts were rejected; bad channels previously identified were interpolated (with spherical interpolation) and re-referencing to the average signal was applied. The number of interpolated channels was kept very low (mean = 0.41; max = 3/18). The signal was then epoched in 1000 ms long non-overlapping epochs and epochs with voltage exceeding ± 150 μV[64] were rejected to exclude epochs contaminated by residual muscle artifacts or noise. Epochs were rejected in both participants of the dyad to maintain signal temporal alignment (dyads included with min = 30 epochs per episode). Connectivity was assessed using phase-locking values (PLV), an index measuring frequency-specific transients of phase locking[48,65] in 18 channels of interest (excluding external channels). The exclusion of external channels ensured better signal quality based on cap application (better impedance) and lower muscle noise based on location, assuring lower channel interpolation and higher epoch retention. PLV was selected as the index of INS to assess neural entrainment and its relationships with coordinated behavior[20]. PLV as other non-directed phase connectivity measures has limitations[66] but has commonly been used in previous studies focused on early parent-infant interaction[44,48,51] thus optimizing the current study comparability with the available literature[67]. The value ranges from 0 to 1, values closer to 0 indicate random signals with unsynchronized phases whereas values closer to 1 indicate strong phase coupling between the two signals. A custom MATLAB script was used to compute PLVs following previous work with mother-infant EEG data[68]. Mean phase coherence (MPC) between the time series of the mothers' and the infants' signals (after filtering for the frequency bands of choice) was computed through a custom MATLAB function that extracted the instantaneous phase using the Hilbert transform and then computed the average phase difference for each epoch. Two signals x(t) and y(t) with instantaneous phases ϕx(t) and ϕy(t) were considered phase synchronized if their instantaneous phase difference was constant. The computation was conducted considering homologous channels for the sake of interpretability. PLV was estimated considering *theta* (3–7 Hz) and *alpha* (6–12 Hz) bands that include both parent and infant typical ranges. PLV values were finally aggregated, computing frontal (Fz, F3, F4, FC1, FC2, FC5, FC6),central (Cz, C3, C4, CP1, CP2, CP5, CP6) and posterior (Pz, P3, P4, POz) region means.

Behavioral coding

Interaction videos were micro-analytically coded, by using the Noldus The Observer TX software for infant's and caregivers' target behaviors according to an adaptation of the Parent-Infant Coding Scheme (PICS, Version 4.0, *unpublished manual*)[69] as reported in Table 1. Measures were transformed in percentages of time for each FFSF episode, to accord for small variations in episodes duration within and between dyads. Furthermore, from the software interface, percentages of time were extracted for specific behaviors co-occurrences: *positive emotionality match* (both participants displaying a positive emotional state) and *gaze match* (both participants displaying a face-directed gaze direction).

Variable	Levels	Description
A. Both interactive partners		
Emotional state	Negative	Clear display of negative emotionality (e.g., eyes, mouth, general movements of the face or the body, and other vocal or non-vocal signals) including fussing and crying.
	Positive	Clear display of positive emotionality (e.g., eyes, mouth, general movements of the face or the body, and other vocal or non-vocal signals) including smiles and laughs.
Gaze direction	Face-directed	Attention focus is on the interactive partner face
	Avoiding	The subject is actively avoiding eye-contact as displayed by head and body movements/posture.
B. Parental specific codes		
Vocal inputs	Nurturing	Vocal comments that convey playful and social engagement such as singing, laughing, playing nursery; express appreciation or acceptance of infants' behaviors or state or are finalized to sooth infants' stress. These also include mind-related comments (e.g., "you think", "you want") and mirroring of infants' communicative bids.
Tactile inputs	Nurturing	Tactile stimulations that convey playful and social engagement such as tickling, squeezing; finalized to sooth or regulate the behavioral state of the interactive partners and conveying a sense of affective closeness such as stroking, kissing, massaging.

Table 1. Selection of codes from the Parent-Infant coding scheme (PICS, version 4.0; Brambilla et al., 2023).

Plan of statistical analysis
All analyses were conducted with R version 4.4.3 for Windows 11, setting statistical significance cut-off at $p < 0.05$.

Preliminary analyses Preliminary to our primary analyses, behavioral measures changes were investigated among the different phases of the FFSF procedure. To describe changes in infants' behavior in response to the FFSF procedure, repeated-measure analyses of variance (ANOVAs) were performed assessing episode effects (3 levels: play, still-face, reunion). Similarly, changes in maternal voice (2 levels: pragmatic, nurturing) and touch (2 levels: pragmatic, nurturing) as well as dyadic behavioral measures (positive emotionality and gaze match) were tested with repeated-measure ANOVAs assessing episode effects. Analyses were also repeated controlling for sex, and episode*sex effects.

Principal analyses For aim 1 of the current study, inter-neural synchrony changes (i.e., mean PLV values obtained respectively in the *theta* and *alpha* bands and in frontal and central electrodes) in response to the interaction disruption were investigated. Repeated-measures ANOVA (Model theta 1; Model alpha 1) was used to examine theta and alpha PLV values across episodes (play, reunion) and regions (frontal, central, posterior). To examine whether infant sex accounted for additional variability in PLV beyond the within-subject factors, the same repeated-measures ANOVA was re-estimated with sex included as a between-subjects factor (Model theta 2; Model alpha 2). Nested models with and without sex were then compared to assess whether the inclusion of sex and its interactions explained additional variance in the multivariate outcome.

For aim 2, exploratory correlation analyses were performed investigating potential associations between inter-neural synchrony and dyadic behavioral measures. Bivariate Pearson's *r* correlation indexes were implied to test the co-variation of PLVs with dyadic positive emotionality and gaze match separately for the FFSF play and reunion episodes.

Results

The current sample consisted of 66 caregiver–infant dyads. In total, 104 dyads were tested, of these, 24 dyads were excluded prior to EEG preprocessing: 13 due to procedure interruption due to infant fussiness and 11 due to non-adherence to task instructions (i.e., the still-face episode was not performed as instructed, or the infant was seated on the caregiver's lap during parts of the procedure). Four additional dyads were excluded because of technical recording failures resulting in unavailable EEG data. After EEG data pre-processing, 66 dyads were retained for the final analyses, while 10 were excluded due to insufficient EEG data quality, based on interpolated channels and artifact-free epochs (overall retention rate = 64%). Descriptive statistics for infants (i.e., age at testing, gestational weeks and birth weight) and parents' (i.e., maternal and paternal age, maternal and paternal education) socio-demographical information for the included dyads are presented in Table 2. Included and excluded dyads were comparable for socio-demographical measures, descriptives for the excluded dyads and comparisons between included and excluded dyads can be found in Supplementary Materials S1.

Mothers and infants' individual behavioral responses to the interactive perturbation

Infant behavior
Looking at infants' negative emotionality displays throughout the experimental procedure (see Fig. 1), the repeated measures ANOVA highlighted a significant effect of episode $F(2,130) = 28.60$; $p < 0.001$; $\eta p^2 = 0.31$. In particular, post-hoc pairwise comparisons showed a significant increase in negative emotionality display emerged between play and still-face episodes $t(65) = -8.47$; $p < 0.001$, and a significant decrease between still-face and reunion $t(65) = 2.20$; $p = 0.031$. However, negative emotionality during the reunion episode was still higher compared to play levels $t(65) = -5.14$; $p < 0.001$. Coherently, repeated measures ANOVA on infants' positive emotionality display highlighted a significant effect of episode $F(2,130) = 29.90$; $p < 0.001$; $\eta p^2 = 0.32$. Post-hoc pairwise comparisons showed opposite patterns compared to negative emotionality (play > still-face; reu > still-face; play > reu; all ps < 0.001).

Regarding infants' gaze avoidance behaviors throughout the experimental procedure (see Fig. 1) the repeated measures ANOVA highlighted a significant effect of episode $F(2,130) = 24.10$; $p < 0.001$; $\eta p^2 = 0.27$;. Post-hoc pairwise comparisons showed a significant increase in gaze avoidance between play and still-face episodes $t(65)$

	Mean	SD	Min	Max
Infant measures				
Infant age (months)	9.47	0.698	8.13	11.4
Gestational weeks	39.34	1.176	37	42
Birth weight (grams)	3305.92	371.390	2250	4050
Socio-demographic measures				
Maternal age (years)	34.51	3.910	27	44
Paternal age (years)	36.23	4.753	26	50
Maternal education (years)	16.08	2.852	10	21
Paternal education (years)	15.06	3.655	8	21

Table 2. Descriptive statistics (mean, SD, and range) for infants and parents' socio-demographic information.

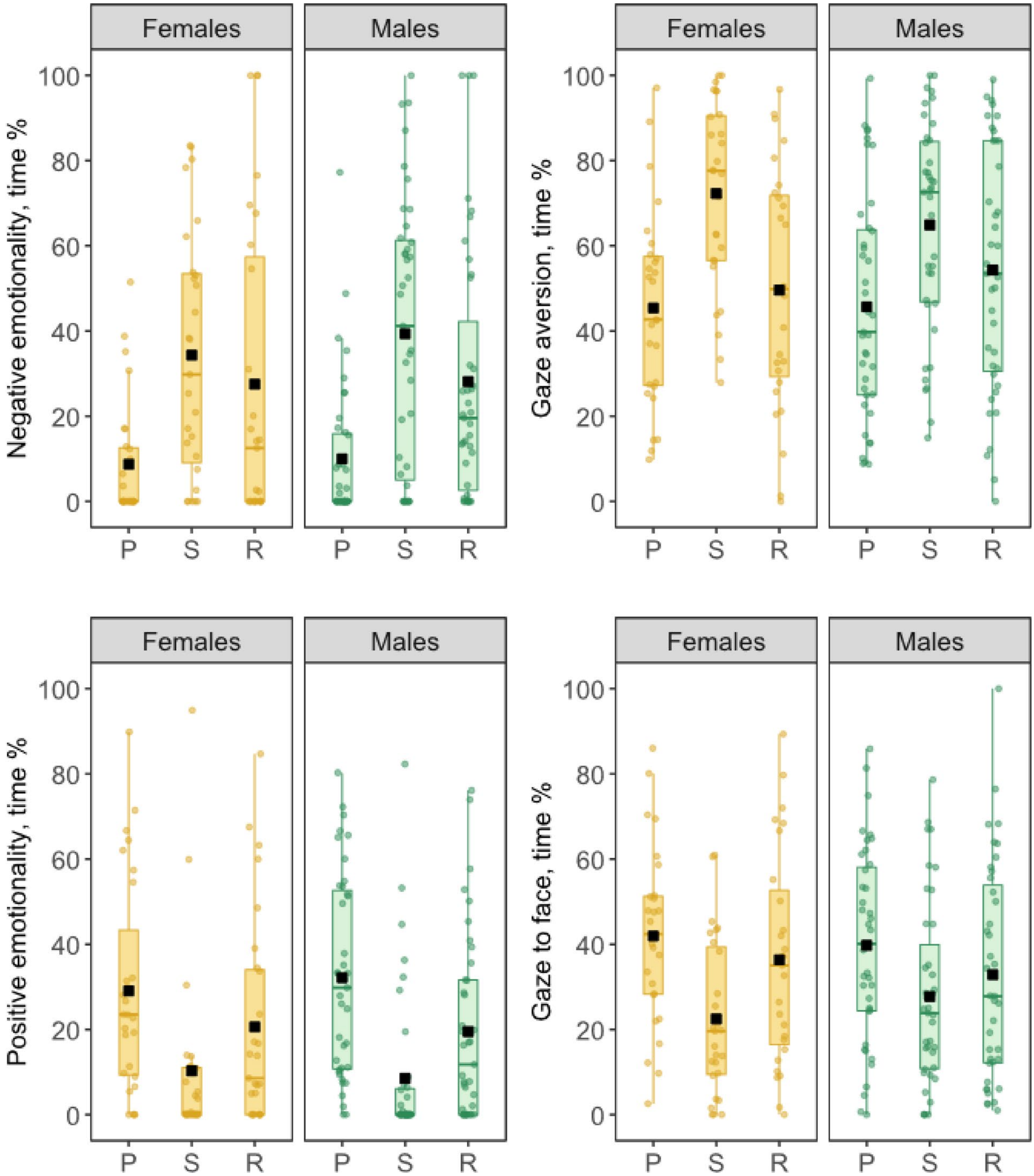

Fig. 1. Mean changes across the face-to-face still-face paradigm in infant behavioral measures displayed split by sex. P, Play; S, Still-Face; R, Reunion.

= − 7.00; $p<0.001$, a decrease between still-face and reunion $t(65)=4.51$; $p<0.001$ but with still higher levels in reunion compared to play $t(65) = -2.09$; $p=0.040$. Furthermore,. Coherently, looking at gaze orientation to the mother, the repeated measures ANOVA highlighted a significant effect of episode $F(2,130)=16.90$; $p<0.001$; $\eta p^2=0.21$. Post-hoc pairwise comparisons showed opposite patterns compared to gaze avoidance (play > still-face; still-face > reunion; reunion < play) All the analyses yielded similar results controlling for sex (see Supplementary Materials S2).

Maternal behavior

Maternal behavior changes were assessed between play and reunion episodes. Looking at changes in nurturing tactile input no significant main effects of episode $F(1,65)=0.64$; $p=0.427$; $\eta p^2=0.010$. Similarly, for nurturing vocal input no significant main effects of episode $F(1,64) = 0.87$; $p = .869$; $\eta p^2 = 0.013$ was highlighted. Finally, no significant changes emerged in maternal gaze to the infant between episodes $F(1,65) = 1.78$; $p = .187$; $\eta p^2 = 0.027$. Descriptive statistics of all maternal measures can be found in Table 3. All the analyses conducted controlling for sex yielded comparable results (see Supplementary Materials S2).

Dyadic behavioral indexes of response to the interactive perturbation
Repeated measure ANOVA focusing on positive emotionality match showed a significant effects of episode $F(1,56) = 5.57$; $p = .022$; $\eta p^2 = 0.091$. A significant effect of episode emerged in mutual gaze $F(1,56) = 11.80$; $p = .001$; $\eta p^2 = 0.17$. Significant decreases in positive emotionality match and mutual gaze were evident between play and reunion episodes. Descriptive statistics of all dyadic measures can be found in Table 3. All the analyses conducted controlling for sex yielded similar results (see Supplementary Materials S2).

Mother-infant INS changes in response to the interactive perturbation
In the repeated-measures ANOVA (Model theta 1 – Fig. 2A) examining theta PLV across episode (play, reunion) and region (frontal, central, posterior), no significant main effect of episode emerged, $F(1, 65) = 0.56$; $p = 0.459$; $\eta p^2 = 0.008$. In contrast, the main effect of region was statistically significant, $F(2, 130) = 3.28$; $p = 0.041$; $\eta p^2 = 0.048$. Post-hoc paired comparisons showed that central PLV was significantly lower than both frontal, $t(65) = 2.23$; $p = .029$, and posterior, $t(65) = -2.25$; $p = 0.028$ (please find complete post-hoc comparisons in Supplementary Materials S1). The episode × region interaction was not statistically significant, $F(2, 130) = 0.56$; $p = 0.572$; $\eta p^2 = 0.009$. Including sex as a covariate (Model theta 2 – Fig. 2B), the main effect of episode remained not significant, $F(1, 64) = 1.25$; $p = .268$; $\eta p^2 = 0.019$, while the main effect of region was again significant, $F(2, 128) = 3.37$; $p = .038$; $\eta p^2 = 0.50$. In addition, the between-subjects effect of sex was also significant, $F(1, 64) = 4.12$; $p = 0.047$; $\eta p^2 = 0.060$, as well as the episode × sex interaction, $F(1, 64) = 4.09$; $p = 0.047$; $\eta p^2 = 0.060$, indicating that changes in theta PLV across episodes differed by infant sex. No other interactions involving region or episode reached significance (all $ps > 0.50$). Follow-up paired comparisons showed that female infants exhibited a significant decrease in theta PLV from play to reunion, $t(64) = 2.04$; $p = 0.045$, whereas male infants did not show a significant change, $t(64) = -0.71$; $p = 0.482$ (please find complete post-hoc comparisons in Supplementary Materials S1). The model including only the within-subject factors did not significantly differ from the model additionally including sex as a between-subjects factor, (Pillai's trace = 0.17; $F(6, 59) = 2.03$; $p = 0.076$), suggesting that sex accounted for only limited additional variance in theta PLV beyond episode and region. Finally, theta PLV values during play and reunion didn't result to be significantly higher compared to surrogate dyads (see Supplementary Materials S3).

The repeated-measures ANOVA (Model alpha 1 – Fig. 2C) on alpha PLV indicated a significant difference between episodes, $F(1, 65) = 4.04$; $p = 0.048$; $\eta p^2 = 0.059$, with alpha PLV significantly increasing from play to reunion. No region effect emerged $F(2, 130) = 0.10$; $p = 0.909$; $\eta p^2 = 0.001$, and the episode × region interaction was likewise not significant, $F(2, 130) = 1.96$; $p = .146$; $\eta p^2 = 0.029$. A second model (Model alpha 2 – Fig. 2D) including infant sex as a covariate yielded a comparable pattern, although the episode effect no longer reached significance, $F(1, 64) = 3.20$; $p = .078$; $\eta p^2 = 0.048$. Neither the episode × sex interaction, $F(1, 64) = 1.10$; $p = .299$; $\eta p^2 = 0.017$, nor any effects involving region (all $ps > 0.16$) resulted significant. The between-subjects effect of sex was also not significant, $F(1, 64) = 2.15$; $p = .147$; $\eta p^2 = 0.033$. The model including only the within-subject factors episode and region did not significantly differ from the model additionally including sex as a between-subjects factor, and there was no evidence that sex accounted for additional variance in PLV across conditions (Pillai's trace = 0.08; $F(6, 59) = 0.91$; $p = 0.49$). Finally, alpha PLV values during reunion (and not play) resulted to be significantly higher compared to surrogate dyads in all regions (see Supplementary Materials S3).

Associations between INS and dyadic behavioral indexes across the interactive perturbation
Correlation analyses assessing associations between mutual gaze orientation (i.e., both participants in the dyad looking towards the interactive partner's face) with PLV yielded a significant result in the theta band in the posterior region during the play episode $r(55) = 0.396$; $p = .002$ (Fig. 3), and moderately sized but non-significant correlations in the frontal $r(55) = 0.182$; $p = .175$ and central $r(55) = 0.208$; $p = 0.120$ regions. Conversely, correlation tests between the same measures in the reunion episode did not highlight significant associations in the frontal $r(55) = 0.175$; $p = 0.194$, central $r(55) = -0.001$; $p = 0.995$, or posterior $r(55) = 0.104$; $p = 0.441$

	Play		Reunion	
	M (SD)	**range**	**M (SD)**	**range**
Maternal behavior				
Nurturing tactile input	29.40 (24.97)	0.00–91.44	31.81 (25.85)	0.00–86.57
Nurturing vocal input	60.47 (25.26)	0.00–99.24	58.07 (24.71)	0.00–98.82
Gaze to the infant	87.40 (15.56)	1.94–100.00	84.58 (15.89)	0.00–100.00
Dyadic behavior				
Mutual gaze	**37.50 (20.47)**	**0.00–83.90**	**28.70 (19.80)**	**0.83–73.00**
Positive emotionality match	**27.30 (21.94)**	**0.00–79.70**	**20.10 (22.40)**	**0.00–84.40**

Table 3. Descriptive statistics (mean, SD, and range) for maternal and dyadic behavior in the play and reunion episodes. Significant changes between episodes are highlighted in bold.

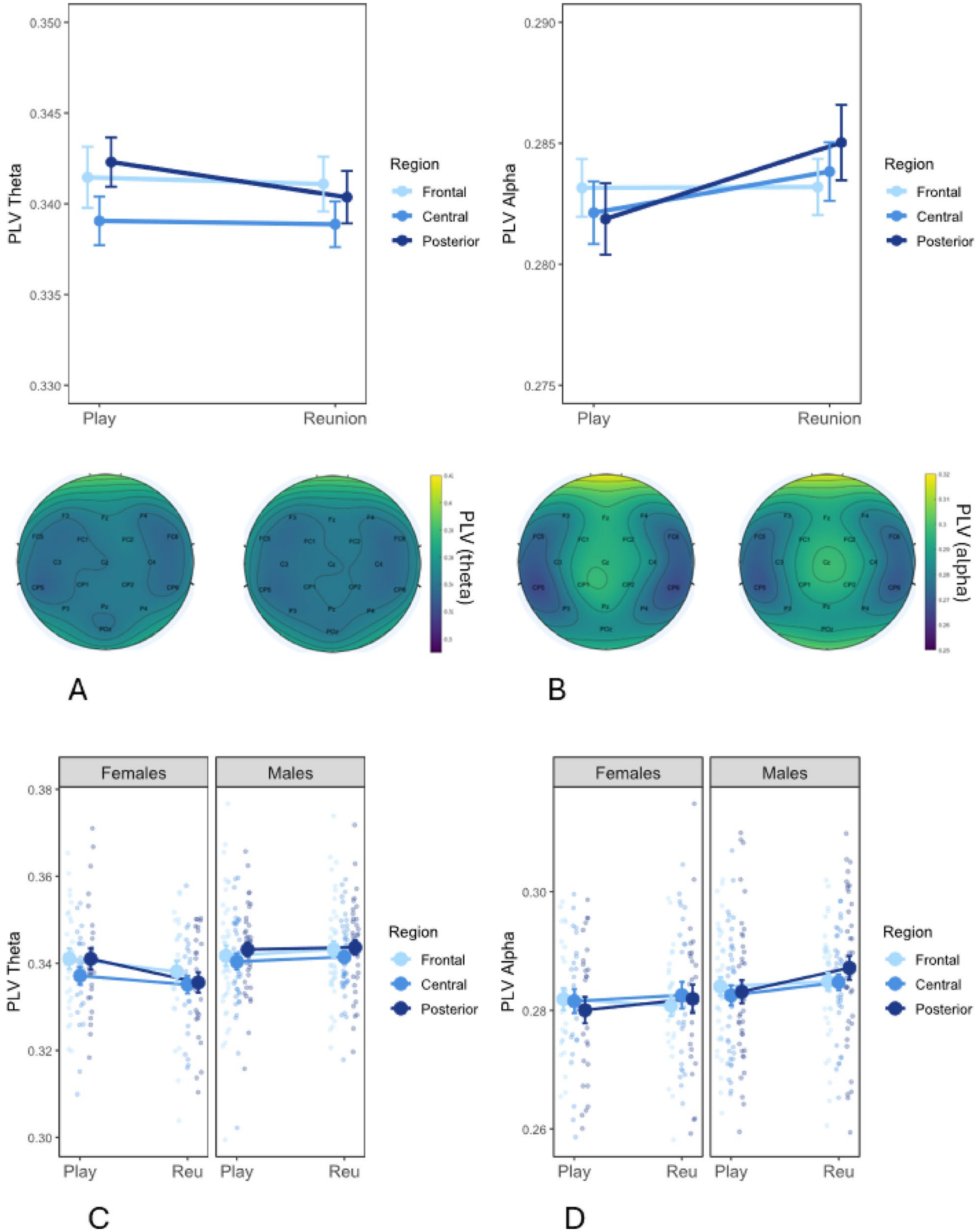

Fig. 2. Model theta 1 (**A**) and alpha 1 (**C**) region × episode PLV values; Model theta 2 (**B**) and alpha 2 (**D**) including infant sex.

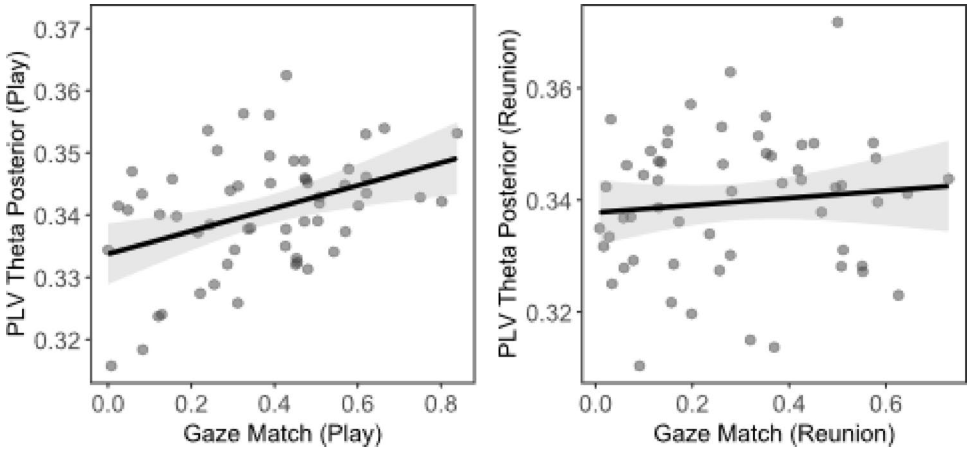

Fig. 3. Linear correlation scatter plots (with standard error bands) for behavioral mutual gaze (proportion of time) and theta PLV values associations in the play and reunion episodes, for posterior regions

regions (see Fig. 3). No significant correlations emerged between mutual gaze and PLV values in the alpha band (play rs(55) < |0.223|, $p > .085$; reunion rs(55) < |0.189|, $p > .159$). Similarly, no significant correlations emerged between measures of behavioral match in positive emotionality and PLV values across bands, episodes, and regions (theta: play rs(55) < |0.236|, $p > .077$; reunion rs(55) < |0.185|, $p > .168$; alpha: play rs(55) < |0.230|, $p > .085$; reunion rs(55) < |0.222|, $p > .098$) (for complete correlation matrixes see Supplementary Materials S1).

Discussion

The current study aimed at investigating dynamics of inter-neural synchrony between mothers and their 9-month-old infants during the FFSF paradigm using EEG hyperscanning. We assessed PLV changes across the play and reunion episodes of the FFSF paradigm and explored links between PLV and periods of mutual gaze and positive emotion matching during the two episodes.

Maternal, infant, and dyadic behavior

Preliminary data analyses on the behavioral measures collected confirm that the FFSF procedure is a robust and reliable paradigm to measure dyadic responses to interaction perturbation. Infants in the current study exhibited a typical pattern of responses, characterized by heightened negative emotionality and increased gaze aversion during the still-face episode, as previously reported[8]. However, it is noteworthy that approximately 25% of infants did not display negative emotionality during the still-face phase. Such a reduced emotional response may be attributed to the shorter duration of the still-face episode[9,70]. The analysis controlled for the infant sex showed similar results with no effect of sex on the behavioral measures collected. Although the literature on sex-based differences in responses to the FFSF procedure is limited[71], some studies have identified variations in both behavioral and physiological responses to this procedure[14,72]. Additionally, infant sex has been found to interact with other relevant variables in predicting responses[73,74], thus further analyses have been repeated controlling for this potentially confounding variable. Looking at maternal behavior no significant change was observed between play and reunion in tactile and vocal input and in gaze direction towards the infant. Maternal responsiveness, sensibility and soothing behaviors have been extensively studied as strategies to support infant emotion regulation[13] and evidence shows that sensitive mothers tend to be more responsive in both the play and reunion episode[70]. Conversely, in dyadic measures, a significant decrease in mutual gaze and in positive emotionality match was observed between the play and reunion phases. This finding is consistent with the significant increase in infants' gaze avoidance and negative emotionality during reunion episodes compared to play.

Inter-neural susceptibility to interactive ruptures in mother-infant dyads

We observed an increase in inter-brain alpha phase-locking values (PLV) from the play to the reunion episode across regions, indicating enhanced neural coupling during the post-perturbation interaction. Phase-based alpha synchrony has previously been reported in infant–mother dyads. Endevelt-Shapira and colleagues (2021)[47] identified a subset of significant alpha inter-subject connections, suggesting that this frequency band is sensitive to dyadic coordination rather than task-specific effects. Alpha-band inter-brain coupling has also been implicated in affective contexts: Santamaria and colleagues (2020)[48] reported higher alpha network density during positive relative to negative emotional conditions. Although this pattern contrasts with our finding of increased alpha synchrony during reunion—an episode characterized by heightened infant negative emotionality—these results are not directly comparable. In our paradigm, alpha synchrony was assessed following a perturbation, with emotionality indexed in the infant and likely accompanied by higher maternal engagement aimed at soothing and repairing interaction, rather than being experimentally elicited by maternal emotional displays. Within this framework, the observed increase in alpha synchrony may reflect processes related to dyadic co-regulation or interactive repair following disruption, although this interpretation remains tentative. An alternative, and not mutually exclusive, account relates to attentional and interactional asymmetries. Reunion episodes typically involve more mother-led regulation and scaffolding, which may preferentially engage alpha-band coupling. In line with this possibility, preliminary evidence suggests that alpha inter-brain synchrony is more prominent during mother-led interactions and is strongest over central, parietal, and occipital regions[75]. Converging findings further indicate that the dominant frequency of inter-brain coupling may shift as a function of interactional roles, with alpha synchrony emerging when mothers guide the interaction and children adopt a more receptive role, whereas theta synchrony predominates during child-led engagement and shows a more frontal distribution[44,76]. Importantly, because our analyses rely on non-directional connectivity measures, we cannot determine whether increased alpha synchrony reflects maternal-driven regulation, mutual adaptation, or shared responses to task structure. Future work incorporating directional or causal metrics, alongside fine-grained behavioral indices of repair and affect regulation, will be necessary to clarify the functional significance of alpha-band synchrony in post-perturbation parent–infant interaction.

Furthermore, changes in phase synchronization between play and reunion episodes emerged in the *theta* band in the dyads including female infants. These findings are partly consistent with previous studies reporting higher interbrain synchrony during parent-child interactions in cooperative tasks and uninterrupted play, as opposed to individual activities, assessed using various techniques[44–46,77]. For instance, inter-neural connectivity in the *theta* bands has been recently tested and results showed that the inter-neural frontal network was more densely connected during cooperative play, and particularly when the adult observed the (preschool-aged) child at play, compared to individual conditions[44]. During the first year of an infant's life, Endevelt-Shapira and colleagues reported higher non-direct neural connectivity in the right-to-right brain *theta* band between mother and infant compared to interactions with a stranger[47]. This finding was further corroborated by a more recent study from the same group[78] which demonstrated significantly higher inter-neural connections during mother-infant face-to-face interactions compared to surrogate data. In the current study, decreases in *theta* phase synchronization following interaction perturbation are parallel to an increase in phase alignment in the alpha bands potentially further supporting a shift towards parent led interaction from a more dyadic or infant led interaction during play. This pattern could be more evident in female infants. Although in the current sample no difference in the behavioral measures collected between male and female infants was highlighted more subtle interactive style differences could underly this pattern. For example, temperamental differences have been reported between male and female infants. A meta-analysis of children aged 3 months to 13 years found that girls scored higher on effortful control, including inhibitory control and perceptual sensitivity, while boys scored higher on surgency, including activity and high-intensity pleasure; whereas negative affectivity showed negligible gender differences[79]. Given that perturbations and mismatches are common in everyday interaction, dyadic reparation is expected to be a frequent occurrence[5,80]. Investigating the mechanisms underlying dyadic reparation is crucial, considering the significant impact of this interactive dynamic on behavioral and physiological indexes of emotion regulation, as well as on subsequent infant socio-emotional health[81,82].

Associations between dyadic behavior and inter-neural synchrony

In the current study, no significant correlations emerged between dyadic positive emotional match and mother-infant inter-neural synchrony. This finding is in contrast with previous studies reporting that inter-neural synchrony is increased by positive emotion displays[48] but in accordance with other studies finding no significant association with positive emotionality[47]. This finding may be explained by the relatively high and consistent levels of positive emotionality exhibited by both mothers and infants in the current study. Interaction before and after disruption was characterized by bouts of positive emotionality in both partners, emotional synchronization may thus not have been the primary driver of inter-neural phase synchronization. It is important to note, that effective dyadic coordination in mother-infant interactions has been reported as predictive of better self-regulation outcomes for children[83,84]. However, the "optimal" levels of synchrony and emotional matching are still under scrutiny[25]. Emerging research suggests that potentially moderate, rather than high or low, levels of emotional co-ordination in early infancy could be associated with more favorable outcomes[85]. Assessments of non-linear associations between these variables should be investigated in the future.

Furthermore, in the current sample, a significant association emerged between *theta* PLV values and mutual gaze. This association was significant and positive only in the play session whereas no association was found in the reunion episode. Previous literature has reported evidence of an association between higher levels of INS and direct gaze[49,50] during parent-child interactions. However, other studies did not report significant associations with this behavior[51,86]. Inconsistencies could be due to differences in the procedures and/or the techniques used to assess INS. For instance, Marriott Haresing and colleagues (2023)[51] failed to report significant associations between mutual gaze and a non-directed index of INS (i.e., PLV). However, it is important to note that the paradigm used during the free interaction in this case included an object (as the aims extended to joint-attention dynamics). The mothers were asked to engage in a playful triadic conversation with the infant including the toy and were instructed to spend a comparable amount of time gazing at the toy and the infant. This difference in paradigm may potentially influence mutual gaze dynamics during interaction. Significant associations have been observed in paradigms that excluded object use and focused solely on face-to-face interaction. For example, Endevelt-Shapira and colleagues (2021)[47] found that in a stranger-infant face-to-face interaction (with maternal chemo-signals present), increases in visual attention were positively correlated with enhanced infant-adult interbrain *theta* non-direct coupling. In contrast, using a different direct measure of INS (partial directed coherence), Leong and colleagues (2017)[49] examined adult-infant face-to-face interactions during nursery rhyme singing and reported bidirectional influences during direct compared to indirect gaze.

One potential explanation for the association between INS and mutual gaze suggests that phase resetting around key communicative signals, such as mutual gaze, might be the mechanism through which INS is achieved. However, this hypothesis has been challenged by direct testing[51]. A different hypothesis relates to parental responsiveness and attentional monitoring of the infant. During joint parent-infant play, parental *theta* power has been shown to track changes in infants' attention, with greater maternal *theta* responsiveness positively correlated with longer periods of infant sustained attention[87]. Additionally, maternal communicative cues in joint attention have been found to enhance infants' neural responses to objects and modulate the mothers' own attentional processes[88]. Infants also appear sensitive to their caregivers' engagement with their attentional focus, exhibiting increased *alpha* suppression when their attention focus is joint, a neural pattern associated with predictive processing[86]. These findings may suggest that the ability of parents to follow and adapt to their infants' visual attention shifts, as well as infants' responses to this parental tracking, could underlie the connection between mutual gaze and INS. However, the potential association between mutual gaze and indexes of INS (and its putative underlying mechanisms) warrant further investigation. From this point of view, a reduced INS after an interactive rupture or perturbation might reflect an adaptive attempt to recalibrate previous inter-neural alignment to achieve a new dyadic state, something reminiscent of Tronick concept of dyadic expansion of consciousness[89].

Interestingly, in the current study, mutual gaze was not found to be significantly associated with PLV values following interaction perturbation. On one side, a significant decrease in mutual gaze was highlighted between play and reunion. This reduction, as well as a reduction in mutual gaze measures variability, could be at the base of this different pattern of associations. Another possible explanation for this observation could be linked to a disruption in dyadic attention tracking. Parents may be less engaged in following the infants' attention while re-engaging the infant in the interaction and other dyadic mechanisms may be at play supporting interaction reparation. For instance, maternal behavior (e, g., affectionate touch)[90], and caregiving profile[91] have also been linked to differences in INS. Future research should investigate the potential role and/or modulating effect of specific parental and dyadic behavior in supporting INS during interaction reparation.

Limitations

While our sample of 66 dyads provides moderate statistical power for detecting main effects, the study remains limited in its capacity to support well-powered analyses of smaller subgroups, potentially restricting inference on emerging dyadic clusters or further participant stratifications. Future research should aim to include a more extensive and diverse sample to validate and extend these findings across different populations, potentially including also at-risk/clinical populations.

A second potential limitation of the current study could be related to the use of EEG recordings, that are known to be more sensitive to motion artifacts compared to other techniques such as functional Near-Infrared Spectroscopy – fNIRS[92]. However, the use of EEG offers advantages, including greater temporal precision, higher portability, lower costs, and ease of application[93] making it a valuable tool despite this limitation. Future research could benefit from incorporating strategies to minimize motion artifacts or combining EEG with other modalities to enhance data accuracy. Further investigations should assess the impact of signal-to-noise ratio and the use of different artifact attenuation/rejection strategies on indexes on phase synchronization in the context of parent-infant live interaction.

A third limitation of the current investigation lies in the use of PLV itself, an index restricted to assessing phase-based, non-directional connectivity at the same frequency. This approach may not fully capture the complexities of interactive dynamics. Particular attention should be given to differences in main *alpha* band frequencies between adults and children, future research should explore cross-frequency interactions and expand to include associations in amplitude dynamics. Additionally, considering the complexity of interactive dynamics, that are typically characterized by both concurrent and sequential "action-response" sequences, future studies should incorporate analyses of directional connectivity to provide a more comprehensive understanding of these dynamics. Furthermore, in the current study PLV values were based on the same electrodes on mother and infant. This strategy may not fully capture different patterns of neural activity present in the dyad. Future research should consider employing methods that assess connectivity across multiple channels or within entire networks to provide a more nuanced understanding of neural interactions.

Furthermore, the current findings are focused on mean INS values; future literature should strive to also assess individual differences and granularity when investigating inter-brain coupling. In the present study, mean PLV values in the theta band were not significantly higher than those observed in surrogate dyads, whereas mean alpha-band PLV during reunion was significantly elevated relative to surrogate analysis. However, because mean values cannot determine whether specific dyads or brief interaction windows show coupling above chance, dyad-level specificity and fast temporal dynamics remain unresolved. This limits inference and emphasizes the need for future work examining individual differences and shorter time scales of neural coordination. Moreover, as different connectivity indexes capture distinct signal features, alternative analytic approaches may index partially dissociable components of co-regulation, enabling direct testing of multiple hypotheses rather than phase coupling dynamics. Future research should also strive to develop brain-to-brain specific metrics that incorporate additional signal properties to increase interpretability[94,95] and motivate new hypotheses on the mechanisms underlying neural attunement during social exchange hopefully embracing complexity[96,97]. Moreover, integration across complementary neuroimaging technologies provides a promising route to address current methodological constraints and overcome the limitations of single-modality connectivity estimates[98,99].

Finally, in the current study associations between inter-neural synchrony and dyadic behavior were investigated using linear correlation analysis only. Future research could benefit from applying more nuanced and individualized analytical techniques extending to non-linear associations (e.g., cross-recurrence quantification analysis) to reveal more intricate patterns of association between behavioral and neural synchronization.

Conclusions

The current study highlighted a significant increase in alpha and a (partial) reduction in *theta* band mother-infant neural coupling following an interaction perturbation, possibly indicating a disruption in neural attunement. Behavioral and EEG-based dyadic co-regulation was evident prior to the perturbation but diminished following it. This study contributes to our understanding of the dynamic interplay between neural and behavioral processes in response to interaction perturbations, highlighting the role of alpha and *theta* wave modulation in these regulatory mechanisms. Mother-child dyads experience frequent moments of disconnection during daily interactions. The current study's results could suggest that dyads may regulate these disconnections by changing inter-neural synchronization patterns and negotiating a phase of destabilization in the dyadic system to seek a new equilibrium. As such, differential synchrony patterns could represent a physiological mechanism contributing to the process of 'quotidian resilience' as a form of dyadic regulation that is crucial for the child's socio-emotional and socio-cognitive development.

Data availability

The raw data pertinent to the current study are available on the Zenodo repository at this doi link: https://doi.org/10.5281/zenodo.18300924

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Author contributions
E.C.: investigation, data analysis, data visualization, manuscript drafting; L.P.: conceptualization, funding acquisition, project administration, manuscript drafting, supervision; MPP: data investigation, data analysis; V.R.: conceptualization, supervision; L.B.: conceptualization, supervision; G.C., S.D., Y.G.: investigation, data curation; E.R.: conceptualization, data collection; R.B.: supervision.

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Declarations

Competing interests
The authors declare no competing interests

Ethical approval
The study was approved by the Ethics Committee of Policlinico San Matteo, Pavia, Italy (protocol 0008588/23, date 16.02.2023). All methods were carried out in accordance with guidelines and regulations from the Declaration of Helsinki for studies involving human subjects. Parents provided written informed consent prior to the inclusion in the study.

Additional information
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