When research does not synchronize: Comprehensive analyses show no mother–child physiological coupling

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Abstract

Biobehavioral synchrony refers to covariation in physiological signals between interacting partners and is widely hypothesized to support co-regulation, bonding, and early socio-emotional development. This assumption is particularly salient for children born preterm, who often experience reduced early physical contact and prolonged neonatal separation, potentially shaping later caregiver–child attunement. Here we present a large-scale, systematic investigation of mother–child physiological synchrony in 102 dyads (70 preterm 5-year-olds and 32 matched full-term controls). Heart rate was recorded across eight interaction paradigms spanning passive viewing to face-to-face play. We applied the major

commonly used analytic approaches, combined with rigorous surrogate-data controls and multiverse analyses, to assess the robustness of synchrony estimates. Across all paradigms, metrics, and analytic variants, synchrony in real dyads never exceeded that observed in pair-shuffled or segment-shuffled surrogate data. Patterns were virtually identical in full-term and preterm groups, and no reliable variability in synchrony remained that could be related to indices of prematurity. Together, these findings challenge the view that autonomic synchrony is a robust or ubiquitous feature of early social interaction and reveal substantial methodological fragility in current synchrony research. Our results underscore the need for more rigorous analytical frameworks and raise critical questions about when - and whether - physiological synchrony genuinely emerges in caregiver–child relationships.

Keywords: Physiological synchrony, Prematurity, Mother-child interactions, Multiverse analyses

1 Introduction

Humans are profoundly social beings, and development unfolds within relational contexts from the very beginning of life. Early caregiver–child interactions shape emotional regulation, stress responsivity, and socio-cognitive development [1]. A prominent line of research suggests that these interpersonal processes extend beyond shared emotions, thoughts, and behaviors to also encompass neural and physiological patterns, a phenomenon commonly termed biobehavioral synchrony [2–5]. Physiological synchrony, referring to the alignment of heart rates, respiratory rhythms, and hormonal activity, is posited as a central mechanism supporting co-regulation, social bonding, and the formation of secure attachment relationships [6–9].

Theoretical accounts posit the origins of physiological synchrony in early life [10, 11]. During gestation, the fetus is continuously exposed to maternal rhythms (e.g. heartbeat, respiratory, hormonal, and sensory), providing a pervasive scaffold for biobehavioral alignment. After birth, caregiver–infant interactions are characterized by sustained physical closeness, mutual responsiveness, and frequent opportunities for co-regulation, conditions thought to strengthen bodily alignment [12]. In stark contrast, infants born preterm experience an abrupt interruption of this prenatal embodied environment and often undergo prolonged separation in incubators and Neonatal Intensive Care Unit (NICU) settings, where medical procedures, limited contact, and elevated stress are pervasive [13]. Parent–infant interactions in the NICU are disrupted by separation, infant medical fragility, and heightened parental distress, all of which can hinder the emergence of synchrony [13, 14]. Preterm infants' reduced self-regulatory capacity and attenuated social cues may make interactions less rewarding [13, 14] while parental mental health difficulties—including anxiety, depression, and post-traumatic stress can impair sensitive responding [15–17], and are disproportionately common in NICU parents [18]. Together, these early perturbations have long led researchers to hypothesize that preterm children and their caregivers may develop atypical patterns

of physiological synchrony [12, 14], positioning this population as an ideal test case for evaluating both the robustness and developmental origins of physiological coupling.

Over the past decade, research on caregiver–child physiological synchrony has expanded rapidly, reporting co-fluctuation in measures such as heart rate, respiratory sinus arrhythmia, and skin conductance across a wide variety of tasks and emotional contexts [7, 10**?**]. Recent work indicates that the emergence and magnitude of physiological synchrony depend on multiple factors, including social context (e.g., cooperative versus competitive interactions), emotional intensity, communicative cues (e.g., gaze, speech and touch), and attachment-related characteristics [5, 17, 19, 20]. Despite this growing literature, findings remain highly heterogeneous, reflecting substantial variability in both experimental paradigms and analytic approaches [21, 22]. A widely used class of time-domain methods relies on (windowed) cross-correlation techniques [21, 22]. These methods, however, entail considerable flexibility in parameter choices such as window size, window overlap, lag range, and lag resolution, as well as in the approaches used to assess statistical significance [23–25]. Such methodological diversity introduces substantial researcher degrees of freedom, complicating comparisons across studies and raising concerns about reproducibility [26, 27]. Notably, if physiological synchrony were a robust and generalizable phenomenon, it should be detectable across standardized analytic frameworks. The frequent reliance on study-specific parameter tuning therefore calls into question the generalizability of reported synchrony effects.

To address these challenges, we conducted a large-scale, systematic investigation of mother–child physiological synchrony in 102 dyads, including 70 five-year-old children born preterm and 32 age-matched full-term peers. Heart rate was recorded across eight interaction paradigms encompassing a broad range of social contexts, from non-interaction control conditions (e.g., eyes-closed rest) to direct face-to-face interactions involving eye contact, conversation, and collaborative play, in which physiological synchrony would be theoretically most likely emerge. We implemented (windowed) cross correlation analyses -one of the most frequently used approaches in the synchrony literature- combined with rigorous surrogate-data controls and a comprehensive multiverse framework to evaluate the robustness of synchrony estimates. This design enabled a stringent test of a longstanding assumption: that physiological synchrony is a reliable feature of early social interaction, and that early disruptions to caregiver–infant contact, such as those associated with preterm birth, might meaningfully shape its developmental trajectory.

Contrary to these expectations, we found no consistent evidence that mother–child autonomic signals synchronized beyond what could be explained by surrogate data, in either full-term or preterm dyads, across any paradigm or any analytic variant. These findings challenge the notion that physiological synchrony is a pervasive or easily detectable feature of caregiver–child interaction and highlight the importance of systematic replication, standardized analytic frameworks, and cautious interpretation of results. By providing a large-scale, multi-paradigm, multi-method evaluation, our study offers a critical empirical foundation for reassessing the conceptual and analytic frameworks underlying physiological synchrony research.

2 Results

Fig. 1 summarizes the experimental design and analytical approach. The eight interaction paradigms were grouped into three categories: no interaction (eyes closed, replay), box interaction (eyes open, positive conversation, negative conversation), and table play (building blocks, tidying blocks, 3D puzzle). Two experimental configurations were employed: the box set-up, adapted from Hessels et al. [28], and a classical table set-up. Physiological synchrony was quantified using (windowed) time-lagged cross-correlations [23], with dyad shuffling and segment shuffling applied as surrogate data generation methods to establish statistical thresholds.

2.1 Actual synchrony does not exceed synchrony in surrogate data

Fig. 2 summarizes the main findings for the full participant sample. The leftmost column contrasts average synchrony in real mother–child dyads with synchrony from randomly shuffled participant pairs (Fig. 2A,C,E; pair-shuffling surrogate data). The rightmost column displays effect sizes comparing real synchrony with synchrony obtained from shuffled data segments within the same dyad (Fig. 2B,D,F; segment-shuffling surrogate data). Rows 1-3 show windowed cross-correlation (WCC) results (10-s window, ±5-s time lag), using raw correlations, absolute correlations, or peak-picking values, while the bottom row (Fig. 2G) shows simple cross-correlations across the full condition period.

Most importantly, after correction for multiple comparisons, real dyadic synchrony did not exceed synchrony observed in surrogate data across any of the eight interaction conditions or analytic approaches Table 3. At the uncorrected level, a single condition (i.e. the eyes-open task) showed higher synchrony in the real than in the surrogate data when using the mean raw correlation metric, for both dyadic and segment shuffling ($p = 0.0141$ and $p = 0.025$, respectively). In addition, the negative conversation and tower-building conditions (mean raw correlations) yielded effect sizes that differed significantly from zero ($p = 0.0324$ and $p = 0.0276$, respectively); however, in these cases real dyads did not differ from shuffled dyads. Crucially, none of these effects survived correction for multiple comparisons and therefore do not provide evidence for genuine synchrony.

More fundamentally, statistical significance based solely on differences in mean correlations between real and surrogate data - although commonly reported in the literature [23, 29–32] - is insufficient to establish true synchrony. Demonstrating synchrony requires rejection of the null hypothesis that real data are drawn from the surrogate data distribution [24, 32, 33]. At an α level of 0.05, this corresponds to real-data values exceeding the 95th percentile of the surrogate distribution in the case of dyadic shuffling, or effect sizes exceeding a significancy threshold of 2 in the case of segment shuffling [34]. None of these criteria were met, or even approached, in the present analyses.

2.2 Higher co-fluctuation during interactive conditions does not indicate true synchrony

The WCC pair-shuffling analysis using absolute correlations and peak-picking values (Fig. 1A,E) revealed significant condition differences: interactive tasks (eyes-open, conversation, puzzle, tower building, clean-up) showed higher co-fluctuation than non-interactive conditions (eyes-closed, replay) (all $p < 10^{-5}$ for eyes closed and all $p < 10^{-4}$ with one exception for replay, see Suppl. Tables 1,2). Although such condition differences are often interpreted as evidence of synchrony, identical patterns were observed in the shuffled-pair surrogate data, and synchrony levels did not differ between real and shuffled dyads. This demonstrates that the observed condition effects reflect task-driven signal structure rather than genuine interpersonal synchrony.

2.3 Time-lagged synchrony remains far below statistical thresholds

Synchrony may manifest as concurrent co-fluctuation or as leader-follower dynamics, in which one partner's arousal changes precede arousal changes in the other. To examine these temporal patterns, Fig 3 visualizes synchrony across the ± 5s lag range for each condition. Although some plots appear to show increased synchrony at certain lags, considering the surrogate-based 95th percentile threshold makes clear that these relative peaks fall well within noise. Thus, we find no evidence for meaningful time-lagged coupling.

2.4 A multiverse analysis across analytic parameters

Because synchrony research lacks standardized methods, we systematically varied analysis choices and key parameters. The WCC analyses in Fig. 1 are based on 10-s windows and ± 5-s lags – choices that are defensible given the temporal resolution of heart rate data but nonetheless arbitrary. Suppl. Figure 1 shows WCC results using 5-s windows/±2.5-s lags and 30-s windows/±15-s lags. Across these alternatives, patterns were nearly identical to the main analysis. Real synchrony never exceeded surrogate synchrony after correction for multiple comparisons, with only a few exceptions in the real vs. dyad-shuffling contrasts: negative conversation (30s-window, raw correlations, p=0.0041), tower building (30s-window, raw correlations, p=0.0011), and eyes-open task (5s-window, raw correlations, p=0.0001); see Suppl. Table 3,4. However, none of these effects were consistent across surrogate methods as no significant findings emerged with the segment shuffling approach, and crucially, none of these contrasts exceeded the 95th percentile of the surrogate distribution.

To comprehensively examine the full parameter space, raw cross-correlations were computed for all combinations of window sizes (4–12 s) and maximum lags (4–12 s). Fig. 4 illustrates these results for a non-interacting condition (eyes-closed) and for an interacting condition characterized by eye contact, vocal turn taking, and shared positive engagement (positive conversation). Both deviations of raw cross-correlations from zero and mean differences between real and dyad-shuffled data are shown, following an approach analogous to [23]).

Despite the crucial difference between these conditions, both exhibited broadly similar patterns across the parameter space, with only a limited number of window–lag combinations yielding elevated synchrony indices. Although a small subset of parameter combinations produced t-values exceeding the conventional threshold ($t > 2$), none survived correction for multiple comparisons. Notably, selective parameter choices could spuriously suggest synchrony with comparable frequency in both interacting (positive conversation) and non-interacting (eyes-closed) conditions.

This conclusion is reinforced by Supplementary Fig. 2, which summarizes all conditions across the full parameter space. Synchrony patterns appeared largely random and unsystematic, and even the strongest effects remained well below the corrected significance threshold ($t > 4$).

2.5 Full-term and preterm toddler-mother dyads show no evidence of physiological synchrony

Given the early disruptions in maternal contact experienced by preterm infants, we hypothesized reduced physiological synchrony in preterm compared with full-term dyads. However, separate analyses conducted in the full-term and preterm groups (Suppl. Figure 3, Suppl. Tables 5,6) revealed no significant synchrony in either group, nor any evidence of higher synchrony in full-term relative to preterm dyads (Suppl. Table 7). Group-specific patterns closely mirrored those observed in the full sample, confirming a general absence of detectable physiological synchrony across both groups. Because no synchrony was observed, and no group differences emerged, we did not pursue the planned analyses examining associations between synchrony indices and neonatal prematurity parameters (gestational age, birth weight, and hours of kangaroo care).

3 Discussion

3.1 Summary of Findings

Synchrony research has expanded rapidly across developmental, clinical, and social neuroscience. The core hypothesis -that interacting partners exhibit coordinated physiological dynamics- is intuitively appealing and theoretically plausible. Yet, how synchrony should be operationalized, and the extent to which it constitutes a dominant feature of ongoing physiological activity, remain unclear. The field also lacks standardized analytic criteria and is characterized by substantial methodological flexibility. Against this background, the current study provides one of the most comprehensive tests to date of physiological synchrony in early development. Across a large sample of mother–child dyads, eight interaction paradigms, and an extensive multiverse of widely used analytic approaches, we found no evidence that real dyads exhibited stronger physiological synchrony than surrogate data. Synchrony estimates from actual mother–child pairs were statistically indistinguishable from both pair-shuffled and segment-shuffled controls, across all paradigms, including interactive and non-interactive contexts alike.

Although interactive tasks occasionally produced higher levels of co-fluctuation than passive tasks, these differences were fully mirrored in surrogate data, indicating that task driven signal structure -rather than interpersonal coupling- accounts for these effects. Multiverse analyses varying window lengths, time lags, and correlation metrics yielded highly convergent null results. Notably, both full-term and preterm dyads showed patterns nearly identical to those of the full sample.

Taken together, these findings challenge the premise that physiological synchrony is a reliable or readily detectable characteristic of mother–child interaction and underscore the substantial methodological fragility underlying current approaches to autonomic synchrony.

3.2 Why our study was well-designed to detect synchrony

If physiological synchrony were a robust feature of mother–child interaction, the present study was well-positioned to detect it. First, the study was well-powered, comprising among the largest sample of mother–child dyads examined with autonomic synchrony methods. Many prior reports rely on modest samples that are highly vulnerable to sampling variability and analytic flexibility [8, 35].

Second, we implemented an exceptionally broad and carefully designed set of interaction paradigms, representing one of the largest collections administered within a single study. All tasks involved direct one-on-one interaction, providing ample opportunity for dyadic attunement. The inclusion of both video-based "tunnel" interactions and face-to-face table play offered complementary contexts: tunnel paradigms minimized environmental distraction and heightened attentional focus, whereas table play ensured naturalistic physical co-presence. The absence of synchrony in either interaction context suggests that neither sustained visual focus nor physical proximity alone is sufficient to elicit autonomic coupling.

Third, the paradigms incorporated multiple features previously reported to enhance synchrony. Prior work suggests that direct eye contact can increase autonomic coupling [36–38], that vocal turn-taking can align moment-to-moment cardiorespiratory activity [39, 40], and that emotionally engaging interactions such as positive play [41, 42], collaborative tasks [43], reminiscing about shared experiences [32], and mild stress discussions [44, 45] can increase physiological co-fluctuation between parents and children. Collaborative, joint attention–based tasks have also been shown to strengthen bidirectional co-regulation [4]. Our design explicitly included such conditions and examined them in mother-child dyads, arguably the closest and most biologically salient relational bond, thereby providing an optimal context for detecting interpersonal attunement.

Fourth, we employed high-fidelity ECG acquisition using cabled BIOSEMI hardware. Preprocessing combined automated artifact detection algorithms with manual inspection to ensure high data quality.

Finally, we applied (windowed) cross-correlation, one of the most widely used and intuitive approaches to asses physiological synchrony [21]. While autocorrelation removal was applied only to full-duration cross-correlations and not to windowed analyses, applying this correction to windowed data would be expected to further attenuate already negligible correlations [46]. Although more complex nonlinear methods (e.g.,

coherence, recurrence quantification) could be used, these approaches likewise rely on temporally aligned dyadic signal structure and are therefore unlikely to reveal coupling in the absence of effects detectable via cross-correlations [33].

Taken together, the study's large sample, methodological rigor, and breadth of paradigms created strong conditions for detecting genuine physiological synchrony, but none emerged.

3.3 Insights from studies reporting synchrony

Our findings do not imply that all previous evidence for physiological synchrony is spurious. Rather, synchrony may be highly context-dependent, emerging only under specific conditions that were not present in our study.

Longer recordings may be critical. Autonomic processes unfold slowly, and brief laboratory paradigms may not allow sufficient time for dyadic autonomic trajectories to meaningfully align. Although the duration of our paradigms (2–8 minutes) is comparable to many developmental studies, they are substantially shorter than day-long home recordings or extended clinical interactions. For example, Tschacher and colleagues reported therapist–patient synchrony across approximately one-hour therapeutic sessions, although even in this enriched context effects remained below the 95th percentile threshold. Therapists may also be particularly attuned to subtle emotional cues, enhancing opportunities for physiological coupling [25, 47].

Emotional intensity may be necessary. Heart-rate–based synchrony appears to be strongly emotion dependent. Despite the social relevance of our tasks, arousal levels were likely mild to moderate. Autonomic synchrony may preferentially emerge during moments of heightened emotional intensity, such as distress, excitement, or strong affective engagement, which are inherently constrained in controlled laboratory settings [21, 48]. Relatedly, continuous physiological attunement may not be necessary for adaptive parenting. Instead, synchrony has been hypothesized to emerge selectively during moments of heightened child distress that necessitate co-regulation [49–51]. Our paradigms were not designed to elicit such states.

Other forms of synchrony may be more robust. Physiological synchrony is tightly constrained by autonomic variability and susceptibility to motion artifacts. In contrast, behavioral synchrony (e.g., gaze coordination, conversational turn-taking, mimicry) and neural synchrony (reflecting shared attention, perception, and action) have been observed even during low-arousal interactions and in broader social contexts, including neutral conversations with strangers [52], simple joint attention tasks [53], and routine coordinated activities such as finger movements or eye blinking [54]. Thus, our findings do not suggest an absence of mother–child attunement, but rather that such attunement may primarily manifest at behavioral or neural, rather than autonomic, levels.

Parent–child bodily proximity may be key. Our sample comprised 5-year-old children, who remain strongly attached to caregivers but are no longer continuously physically connected to them. In infancy, extensive skin-to-skin contact, holding, rocking, and feeding create powerful channels for biobehavioral coupling. Stronger physiological synchrony reported in some parent–infant studies may thus reflect greater bodily proximity, which naturally diminishes as children become increasingly

mobile and autonomous. In the present laboratory setting, interactions were conducted without direct physical contact between parent and child.

Externally driven co-fluctuations may also contribute to apparent synchrony. Our surrogate analyses indicate that covariation between signals can, at least in part, arise from shared exposure to external events, including task transitions, stimulus onsets, or environmental changes, rather than from genuine interpersonal coupling. Without adequate control for these condition-driven influences, estimates of true dyadic synchrony may be inflated or difficult to quantify reliably.

3.4 Why we did not observe physiological synchrony

Our findings raise critical questions about the robustness and generalizability of physiological synchrony. Several, likely complementary, factors may account for the absence of detectable coupling in our study.

Physiological synchrony may be weaker and rarer than often theorized. Many prior reports are based on small samples, emotionally intense contexts, or flexible use of analytic methods. Moreover, our stringent surrogate-data criteria may have excluded effects that would previously have been interpreted as evidence for synchrony.

Autonomic signals are strongly driven by individual-level factors. Movement, respiration, baseline physiology, and task structure exert powerful influences on heart rate [55, 56]. These individual trajectories may overshadow any dyadic influences during moderately arousing tasks.

Most laboratory interactions may not elicit the conditions required for autonomic coupling. Play-based or conversational paradigms can sustain behavioral and neural attunement, yet may generate insufficient autonomic variability for physiological co-fluctuation to emerge. The novelty of the experimental setting and the cognitive demands of task performance may further shift children's focus toward individual self-regulation rather than interpersonal coordination. In addition, the presence of multiple adults in the laboratory, beyond the mother, likely increased social monitoring demands. Developmental factors may also play a role: at five years of age, children exhibit substantially greater physiological and behavioral autonomy than infants. Although maturation toward adult-like physiology could, in principle, support stronger coupling, increased self-regulatory capacity may instead reduce the dyadic interdependence required for physiological synchrony [7, 10, 57].

Synchrony may be highly state dependent rather than a stable interactional feature. Brief, intermittent moments of alignment may occur but remain too transient to be captured by global (and even windowed) cross-correlation–based metrics. Detecting such microstates may require novel analytic approaches with higher temporal specificity.

Analytic flexibility in the field may have inflated prior findings. Without rigorous surrogate controls, shared task timing or coincidental co-fluctuations can readily mimic interpersonal coupling. Our multiverse approach indicates that many commonly used methods are prone to overestimating synchrony [21].

In sum, physiological synchrony may emerge only under highly specific conditions, such as extended naturalistic recordings, strong emotional arousal, clinical interactions, or periods of close bodily contact. However, even when present, interpersonal

coupling may constitute only a minor source of variance in autonomic signals, easily overshadowed by individual-level physiological dynamics, task-driven fluctuations, and other non-dyadic factors. This may explain why synchrony effects, if they exist, are not consistently or robustly detected across studies.

4 Conclusion

Physiological synchrony has long been proposed as a core biobehavioral mechanism supporting co-regulation, emotional attunement, and the development of secure attachment. This line of reasoning is particularly compelling for populations whose early relational experiences may be disrupted. Preterm infants, in particular, frequently experience reduced early physical closeness with caregivers due to medical interventions and extended neonatal intensive care stays. Such early separation could plausibly alter the development of caregiver–child co-regulation, potentially resulting in atypical patterns of physiological synchrony later in childhood. Our study was therefore uniquely positioned to test whether prematurity is associated with altered mother–child autonomic coupling.

However, our results provide no evidence that physiological synchrony, as commonly defined and measured, is a reliable interpersonal phenomenon in mother–child interactions, let alone a mechanism capable of meaningfully differentiating full-term from preterm dyads. Across a large participant sample, a wide diversity of interaction paradigms, and an exhaustive multiverse of analytic decisions, synchrony estimates for real dyads did not exceed those obtained from rigorously constructed surrogate control data. These robust null results challenge the assumption that physiological synchrony is a pervasive or easily detectable feature of social exchanges. Instead, they suggest that autonomic co-fluctuation (captured here via heart rate during brief, moderately arousing laboratory paradigms) is far more limited, context-dependent, and method-sensitive than typically assumed. Furthermore, the absence of valid synchrony estimates precluded drawing any substantive conclusions about potential differences between full-term and preterm dyads.

Taken together, our findings highlight a critical need to re-evaluate the conceptual and methodological foundations of physiological synchrony research. Future work may benefit from longer-duration recordings, contexts characterized by stronger emotional arousal or distress, and paradigms that involve more sustained physical contact conditions under which autonomic attunement may be more likely to emerge. At the same time, our results underscore the importance of rigorous statistical control and multiverse approaches to avoid overinterpreting coincidental covariation as interpersonal coupling.

Ultimately, while the broader phenomenon of interpersonal attunement remains central to human social functioning, our findings suggest that autonomic synchrony may be only a narrow and highly context-bound facet of this process. As the field moves forward, a more cautious, critical, and empirically grounded understanding of when and how physiological synchrony genuinely occurs will be essential for advancing developmental, clinical, and social neuroscience.

5 Methods

5.1 Participants

A total of 102 mother-child dyads participated in the study. Children were five years old and native Dutch speakers with at least one native Dutch-speaking parent. The sample comprised 70 preterm (PT) children (43 males; mean age = 5.60 years, SD = 0.14) recruited from the Resilience Cohort [58–60], born with a gestational age (GA) below 37 weeks and birth weight (BW) below 2500g. Of these, 37 were born moderate-to-late preterm (GA 33–36 weeks), 21 very preterm (GA 29–32 weeks), and 12 extremely preterm (GA $\leq$28 weeks). Exclusion criteria included severe cerebral lesions (grade III–IV intraventricular hemorrhage and periventricular leukomalacia) and neurological disorders (cerebral palsy or epilepsy). The control group consisted of 32 full-term (FT) children (16 males; mean age= 5.68 years, SD = 0.18), recruited via local schools and matched for age and sex. Inclusion criteria for FT children were GA between 37–42 weeks and BW above 2500g. Exclusion criteria for the FT group included a formal diagnosis of autism or ADHD, or a first-degree relative with either condition. Groups were comparable for age and sex distribution. Mean GA was 31.01 weeks (SD = 3.01) for PT and 39.35 weeks (SD = 1.31) for FT children. Mean BW was 1572.56g (SD = 492.59) and 3646.38g (SD = 512.18), respectively. PT children showed significantly higher scores on measures of behavioral problems (CBCL), autistic traits (SRS-2), and autism symptom severity (ADOS-2) compared to FT controls (1). For a full phenotypic description of the child sample, see Van den Broeck et al. [60].

Maternal characteristics did not differ between groups. Mothers of PT children (mean age = 37.7 years, SD = 5.21) and FT children (mean age = 37.5 years, SD = 3.26) showed comparable levels of state anxiety (STAI-Y1; p = 0.08), trait anxiety (STAI-Y2; p = 0.30), and depressive symptoms (BDI; p = 0.68; Table 1).

Families received compensation for their participation. Ethical approval was obtained from the Medical Ethical Committee of UZ/KU Leuven (S58807). Parents and teachers provided written informed consent, in accordance with the Declaration of Helsinki.

5.2 Design

5.2.1 Set-ups

Two experimental configurations were employed. In the box set-up, we adapted the dual camera video-call paradigm developed by [28] to facilitate naturalistic face-to-face interaction. Participants were positioned opposite each other in a shared room, separated by wooden enclosures fitted with half-silvered mirrors. This arrangement permitted direct visual and verbal exchange while concealed webcams, mounted at eye height behind each mirror, recorded gaze patterns without obstructing natural eye contact. The setup allowed for concurrent acquisition of ECG, EEG, electrodermal activity, audio/video, and eye-tracking data.

In the table set-up, each dyad was positioned at a table with mother and child seated directly across from one another. This setup enabled concurrent collection of EEG, ECG, electrodermal activity, and video data from both participants. In the

present study, we report exclusively on cardiac measures obtained from mother-child dyads.

5.2.2 Conditions

In the box set-up, each child engaged in a series of structured interactions with their mother. To ensure protocol fidelity while preserving child naivety, mothers received comprehensive briefings beforehand. Mother-child sessions followed a fixed sequence of five tasks: resting with eyes closed (2 min), silent mutual gaze (2 min), a covert replay phase in which children observed a pre-recorded video of their mother under the impression of a live interaction (2 min), reminiscing about a positive shared memory (3 min), and discussing a recent conflict (3 min). In the table set-up, dyads completed three joint tasks in a fixed order: collaborative construction of a block tower (8 min), followed by dismantling and tidying the blocks together (3–5 min), and joint assembly a 3D puzzle (panda or tiger) as a pair (5–15 min). Importantly, both the eyes-closed and replay condition involved no genuine interaction, whereas all other conditions comprised real-time mother-child interactions.

5.3 ECG pre-processing

Firstly, ECG signals were high-pass filtered using a zero phase, second order Butterworth filter and power line interference was removed with a 50Hz notch filter. R-peaks were automatically detected and subsequently inspected and corrected manually. All pre-processing was performed using R-DECO, an open-source MATLAB-based graphical user interface (GUI) that implements an envelope-based detection algorithm [61]. RR intervals were then computed as the distances between consecutive R-peaks and converted into heart-rate timeseries. The timeseries were cubic-interpolated to obtain evenly-sampled data. Because childrens' heart rates are substantially higher that adults', a sampling frequency of 8Hz, higher than typically used, was chosen[62].

5.4 Synchrony analyses

Synchrony between the two signals was quantified using four cross-correlation-based approaches. The first approach computed the plain cross-correlation over the full duration of each condition [32, 63]. Because this method incorporates the entire time series, signals were first detrended and corrected for autocorrelation using Autoregressive Integrated Moving Average (ARIMA) models [64]. Specifically, slow trends were removed by fitting a third-degree polynomial to each timeseries. ARIMA models were then fitted to the resulting residuals, with the optimal model selected for each timeseries using the Akaike Information Criterion (AIC). The final residuals served as input for the cross-correlation analysis.

The remaining three approaches employed windowed time-lagged cross-correlation [25, 65]. In these methods, signals were segmented into overlapping windows, and cross-correlations were computed between corresponding segments. To allow dynamic changes in leader-follower dynamics, for each segment pair, one segment was shifted across a range of positive and negative lags at a 0.125s resolution, yielding $2 \times \text{maxlag} + 1$ correlation values. In line with prior work, three alternative synchrony metrics were

derived: (i) the mean of the raw correlations, (ii) the mean of the absolute correlations [66–68], and (iii) the maximum correlation across lags (i.e. "peak-picking") [23, 38, 69]. Segment-level indices were averaged across windows to obtain a single synchrony value per dyad.

For each method and condition, statistical significance was assessed using surrogate data. Two surrogate-generation procedures were applied [24]. In dyadic shuffling, mother–child pairs were randomly reassigned such that signals from individuals who had not interacted were paired. In segment shuffling, temporal segments within each timeseries were randomly reordered, disrupting temporal alignment between paired segments. Both surrogate approaches were applied to the windowed analyses, whereas only dyadic shuffling was applied to the cross-correlation method. For dyadic shuffling, dyad-level synchrony indices from real and shuffled dyads were compared directly. For segment shuffling, effect sizes were computed for each dyad as the difference between the means of the real and shuffled segment-level indices, normalized by the standard deviation of the shuffled indices [25].

For the main analyses, a window length of 10 s, a maximum lag of 5 s, and a window overlap of 5 s were used. Two additional parameter configurations were examined; these results are reported in the Supplementary Material.

To examine lag-specific synchrony and potential leader–follower dynamics, windowed time-lagged cross-correlations were additionally analyzed separately for each lag value. For each segment pair and lag, the segment-level index was defined as the raw cross-correlation obtained after shifting one segment by the specified lag. These indices were averaged across all segments to yield a single synchrony measure per dyad per lag. Statistical significance at each lag was assessed using dyadic shuffling.

Robustness to parameter choice was evaluated by systematically varying window size and maximum lag between 4 and 12 s [23]. For each parameter combination in this two-dimensional grid, windowed time-lagged cross-correlation (using mean raw correlations and dyadic shuffling surrogates) was performed. For each parameter combination, a set of shuffled dyads matched in number to the real dyads was randomly sampled. One-sample and two-sample t-statistics were computed to test, respectively, deviations of real dyad values from zero and differences between real and shuffled dyads.

Finally, in addition to analyses of the full sample, all primary analyses were conducted separately for the full-term and preterm groups, and synchrony indices were compared between groups using Mann-Whitney U-tests.

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Tables

Table 1 Sample characteristics of preterm and full-term mother-child dyads.

Children		**Preterm (N=70)**	**Full-term (N=32)**	
	Sex (M:F)	43:27	16:16	$\chi^2(3)=1.52$, p=0.68
	Age[1] (years)	5.60 ± 0.14	5.68 ± 0.18	W=1460, $p = 0.013^*$
Prematurity Indices[1]	GA (Weeks)	31.01±3.01	39.35±1.31	W=783.5, $p < 0.001^*$
	BW (g)	1572.56±492.59	3646.38±512.18	W=2176, $p < 0.001^*$
Characteristics[1]	CBCL T-scores	47.45±11.68	42.19±8.29	W=783.5, $p = 0.04^*$
	ADHD-Q	14.59±11.76	10.56±8.25	W=825, p=0.12
	SRS-2 T-scores 52.66±9.38	46.91±6.46	W=615, $p < 0.001^*$	
	DOS-2 T-scores 4.44±5.53	1.16±1.46	W=619, p=0.05	
Mothers		**Preterm Mother(N=70)**	**Full-term Mother(N=32)**	
	Age[1] (years)	37.7±5.21	37.5±3.26	W=1138, p=0.70
Characteristics[1]	Anxiety (STAI-Y1)	34.59±11.34	30.25±8.82	W=849, p=0.08
	Anxiety (STAI-Y2)	35.21±11.44	32.59±10.34	W=948, p=0.30
Depression (BDI)	7.18±6.45	6.09±5.08	W=1016, p=0.68	

Significant group differences are indicated with an asterisk (*). The statistically significant age difference between child groups corresponds to approximately one month and is considered negligible for the present research questions. ADOS-2 data were available for a subset of preterm children (n = 64). No significant group differences were observed for maternal characteristics (all $p > 0.05$). Abbreviations: M:F, male-to-female ratio; CBCL, Child Behavior Checklist; ADHD-Q, ADHD Questionnaire; SRS-2, Social Responsiveness Scale; ADOS-2, Autism Diagnostic Observation Schedule; GA, gestational age; BW, birth weight; STAI-Y1, State-Trait Anxiety Inventory – State subscale; STAI-Y2, State-Trait Anxiety Inventory – Trait subscale; BDI, Beck Depression Inventory; PT, preterm; FT, full-term.

[1] Expressed as Mean ± SD

Table 2 Number of dyads per condition.

Condition	No. of dyads
Eyes closed	86
Replay	89
Eyes open	92
Positive conv.	95
Negative conv.	88
Tower building	79
Clean up	76
3D puzzle	69

The full sample comprised 102 dyads. Following quality assessment of the ECG recordings, data from a subset of dyads were excluded from the analyses, and the number of exclusions varied across conditions.

Table 3 P-values of statistical surrogate tests.

	Dyadic shuffling				Segment shuffling		
Condition	CC	Raw corr.	Abs. corr.	PP	Raw corr.	Abs. corr.	PP
Eyes closed	0.4783	0.5297	0.4302	0.7429	0.4651	0.0595	0.2698
Replay	0.9142	0.7491	0.5747	0.1856	0.6748	0.7329	0.4816
Eyes open	0.5544	0.0141*	0.8267	0.2905	0.0250*	0.1244	0.0755
Positive conv.	0.4827	0.1353	0.5529	0.5374	0.0927	0.7689	0.6185
Negative conv.	0.7639	0.0809	0.8728	0.5992	0.0324*	0.1842	0.1812
Tower building	0.1303	0.1188	0.4636	0.0855	0.1188	0.5702	0.0276*
Clean up	0.3267	0.8916	0.9020	0.8610	0.3301	0.4442	0.080
3D puzzle	0.6875	0.2012	0.3736	0.5881	0.1654	0.1113	0.3086

Abbreviations: CC: Cross-correlation across full duration of condition; Raw corr: Raw correlation; Abs. Corr.: Absolute correlation; PP: Peak-picking correlation;

* Asterisks indicate conditions in which real data differed from surrogate data according to conventional, uncorrected significance criteria ($p < 0.05$).

Note that correction for multiple comparisons would require a threshold of $p < 0.00625$, and that comparison against the 95th percentile of the surrogate distribution would impose an even more stringent criterion.

Figures

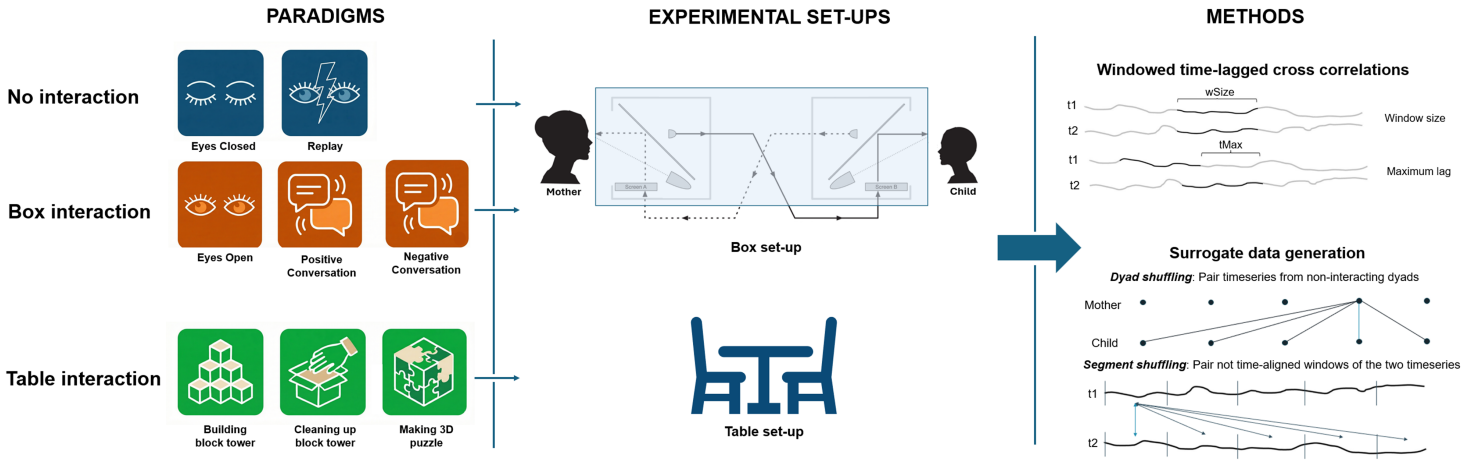

Fig. 1 Overview of the experimental design and analytical approach. Left: The eight interaction paradigms grouped into three categories: no interaction (eyes closed, replay), box interaction (eyes open, positive conversation, negative conversation), and table interaction (building blocks, tidying blocks, 3D puzzle). Middle: Schematic representations of the experimental set-ups, adapted from the box set-up [28] and the table-play configurations. Right: Illustration of the analytical pipeline, including windowed time-lagged cross-correlation analysis [23] and the two surrogate data control procedures applied: dyad shuffling and segment shuffling.

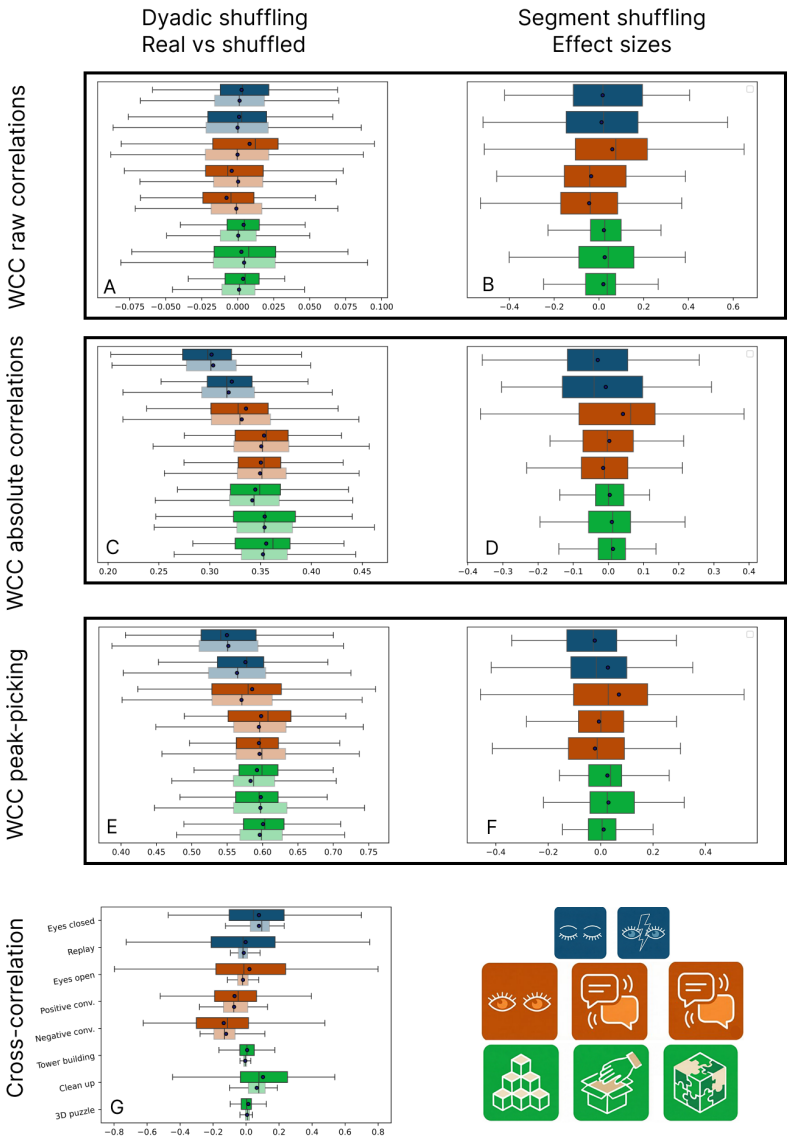

Fig. 2 Cross-correlation and windowed time-lagged cross-correlation (WCC) across interaction conditions. Results are shown for the full sample across eight interaction conditions, grouped by interaction level (blue: no interaction; brown: box interaction; green: table interaction). WCCs were computed using a 10 s sliding window with a 5 s time lag. The first column displays averaged raw correlations (A), absolute correlations (C), and peak-picking values (E) for real dyads (solid colors) and shuffled dyads (transparent color). The second column presents the corresponding effect sizes, quantifying differences between aligned and shuffled segments for each metric (B, D, F). The bottom row illustrates cross-correlations computed over the full duration of each condition for real and shuffled dyads (G).

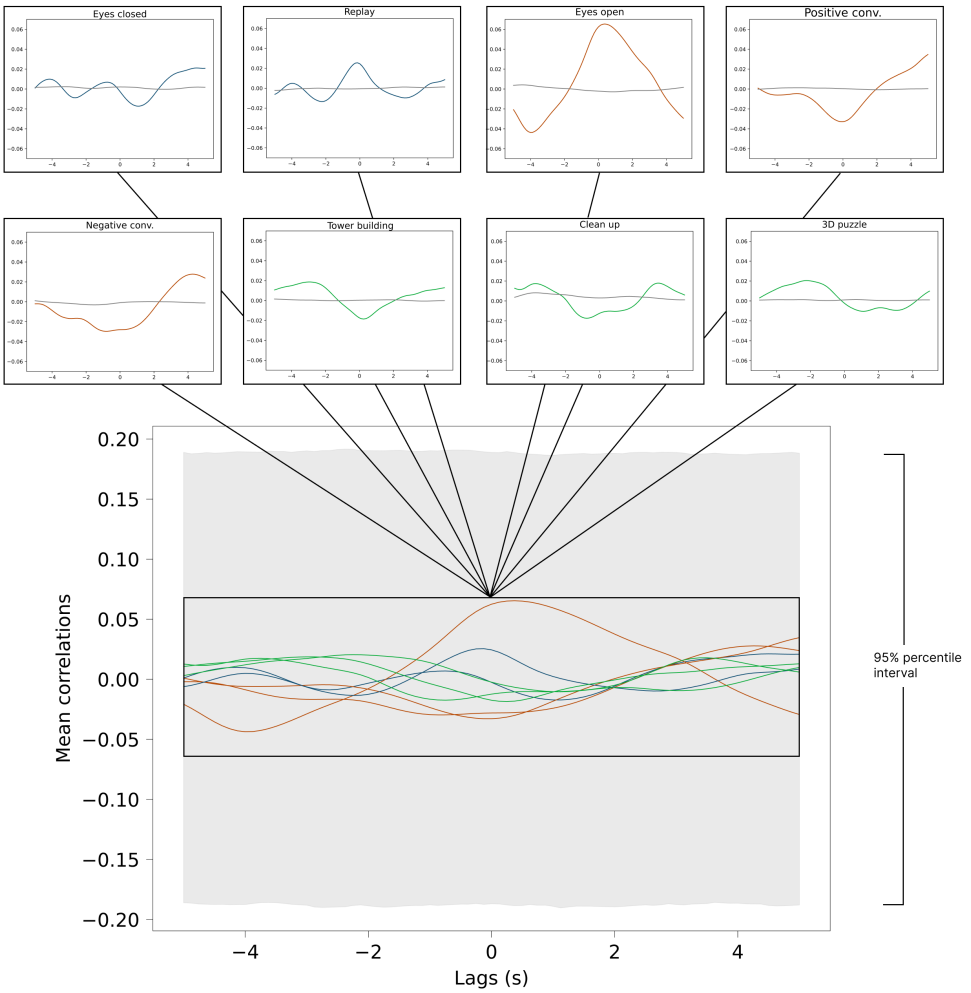

Fig. 3 Temporal patterns of physiological co-fluctuation. The top two rows show average correlation values across time lags ranging from -5s (child leading) to +5s (mother leading) for each of the eight interaction conditions. Colored lines represent synchrony indices for real dyads, whereas gray lines depict those obtained from shuffled dyads. The bottom panel shows the same synchrony indices (with corresponding colors), scaled relative to the 95th percentile interval of the surrogate distribution, which denotes the significance threshold (gray shaded area).

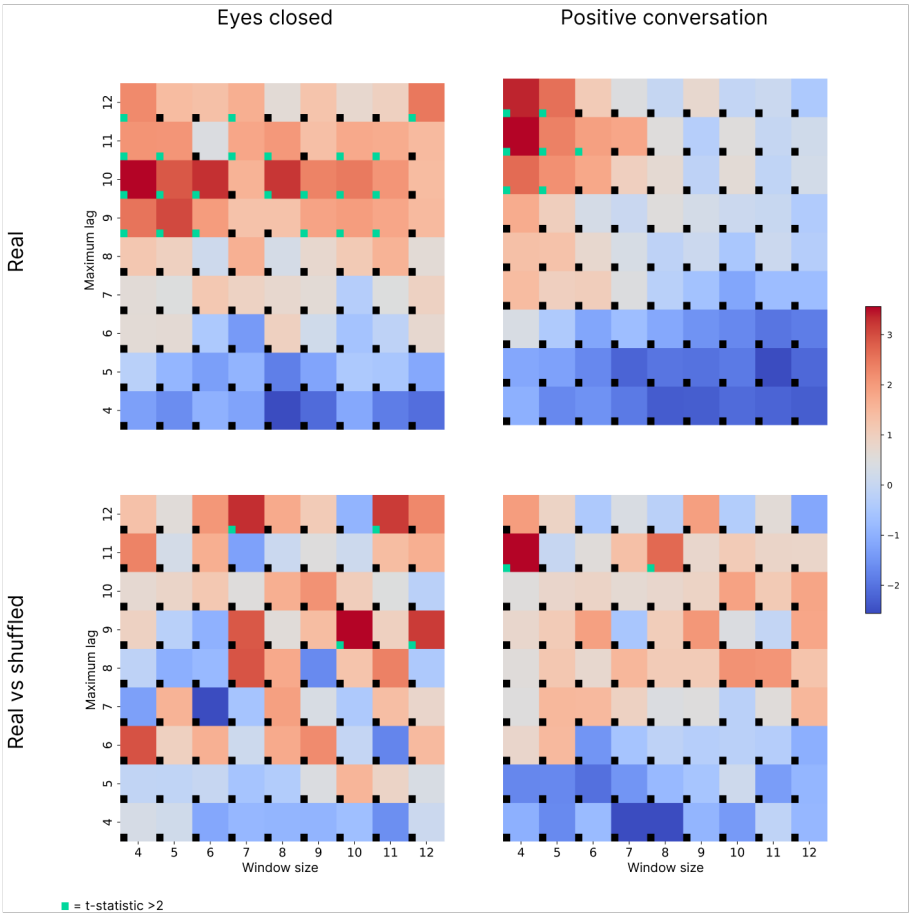

Fig. 4 WCC synchrony across varying window sizes and time lags The parameter space included window sizes and maximum time lags ranging from 4 to 12s. The top row shows one-sample t-statistics of raw correlation values for the eyes-closed and positive conversation conditions. The bottom row depicts the respective two-sample t-statistics comparing real and shuffled dyads. Green squares indicate $t > 2$.