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Hemodynamic and Electrophysiological Biomarkers of Interpersonal Tuning during Interoceptive Synchronization

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Abstract: This research explored the influence of interoception and social frame on the coherence of inter-brain electrophysiological (EEG) and hemodynamic (collected by functional Near Infrared Spectroscopy, fNIRS) functional connectivity during a motor synchronization task. Fourteen dyads executed a motor synchronization task with the presence and absence of interoceptive focus. Moreover, the motor task was socially or not-socially framed by enhancing the shared intentionality. During the experiment, delta, theta, alpha, and beta frequency bands, and oxygenated and de-oxygenated hemoglobin (O2Hb and HHb) were collected through an EEG-fNIRS hyperscanning paradigm. Inter-brain coherence indices were computed for the two neurophysiological signals and then they were correlated to explore the reciprocal coherence of the functional connectivity EEG-fNIRS in the dyads. Findings showed significant higher correlational values between delta and O2Hb, theta and O2Hb, and alpha and O2Hb for the left hemisphere in the focus compared to the no focus condition and to the right hemisphere (both during focus and no focus condition). Additionally, greater correlational values between delta and O2Hb, and theta and O2Hb were observed in the left hemisphere for the focus condition when the task was socially compared to non-socially framed. This study showed that the focus on the breath and shared intentionality activate coherently the same left frontal areas in dyads performing a joint motor task.

Keywords: interoceptive attentiveness; hyperscanning; fNIRS; lateralization; inter-brain coherence; interpersonal synchronization; social frame

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1. Introduction

The perception of inner body signals, namely interoception, has mostly been investigated as a mechanism related to the inner world of the individual, while only recently has novel research focused on how interoception has an influence on social processes [1–3]. At this regard, the body of studies that investigate the impact of interoception on social dynamics and processes has been defined "social interoception" [4]. Within this research line, little is known about the impact of interoception on interpersonal synchronization processes.

Thus, with the aim to expand this field of research, we have recently conducted a study to explore the effect of a peculiar dimension of interoception on inter-brain hemodynamic coherence [5]. In particular, we have observed how the manipulation of Interoceptive Attentiveness (IA), that is, the capability to intentionally focus the attention on one's body signal for a determined span of time [6,7], combined with social synchronization, has an impact on the interpersonal neural synchronization (INS).

To do so, the functional Near Infrared Spectroscopy (fNIRS) was exploited as an optical imaging technique to measure the concomitant hemodynamic variations in the brain of the dyads performing the two basic synchronization tasks. At the methodological level, although fNIRS measurements present several advantages and reliability, this technique does not appear to completely capture and explain in-depth the nature of dynamic social processes when employed alone. In particular, the manipulation of IA and the shared intentionality promoted higher left compared to right prefrontal cortex (PFC) inter-brain coherence between the two participants performing synchronization tasks [5]. This effect was interpreted considering the twofold role of the left PFC as a marker of positive emotions derived from the synchronization and as a key neuroanatomical region of the "mutual attention system", whose main characteristic is the mutual and synchronized activation aspect [8]. This work showed the usefulness of coherence indices as a marker of the attention on the breath as an interoceptive condition and of the adjustment of the social frame in synchronization tasks, but also presented various caveats.

Beyond the fNIRS, the electroencephalogram (EEG) can also be used for sensing brain activity linked to social and emotional processes [9]. EEG frequency band analysis in particular sheds light on the function of various brain regions in emotional processes. The activity of low-frequency bands, such as delta and theta bands, was considered a marker of emotional processes, social skills, and empathic responses [10,11], whereas the activity of high-frequency bands, such as the alpha and beta bands, has been linked to cognitive processes [12,13]. A multimethod approach exploiting the concurrent collection of the hemodynamic signal and EEG frequency bands was used before to explore the impact of interoceptive manipulation on the social process of empathy for pain [14]. Moreover, EEG frequency bands were previously explored in relation to the effect of interoceptive manipulation on synchronization tasks [11,12]; however, it has never been combined with the application of fNIRS to explore this topic.

Therefore, the combination of fNIRS with EEG techniques allows the complementary analysis of the neuronal and hemodynamic components of brain activity during social processes [9,15].

Despite some limitations related to spatial and temporal resolution that may be taken into account in the use of fNIRS to explore ecological social interactions, this technique exhibits quite good ecological validity, signal resolution and usability when compared with other neuroimaging techniques (such as the functional Magnetic Resonance Imaging) [16]. Besides fNIRS, the EEG allows us to observe individuals' cortical activity in various conditions, enabling researchers to study the mechanisms behind INS in a variety of real-world settings. Moreover, it proves to be a useful method for recording participants' brain signal with a millisecond time window, offering a satisfactory temporal resolution [17].

In addition to the use of an integrated approach, the adoption of the hyperscanning paradigm, which embraces "a two-person neuroscience" approach, enables a better investigation of the mechanisms underlying social joint dynamics through the recording of the brain activity of the two interagents [18–20]. The hyperscanning paradigm enables simultaneous recording of the activity of two or more people engaged in interaction or performing a joint task, obtaining information on intrabrain connectivity mechanisms, which shows that the same brain areas are activated in single people, and interbrain connectivity, which reveals the intercerebral connections of dyads [21]. EEG may be applied in hyperscanning to record the brain activity of interagents moment-by-moment [17], whereas fNIRS can be adopted to explore the activation of shared cerebral areas during the social interaction [5,16,22].

Therefore, by combining these two techniques, it would be feasible to overcome the distinct limitations of each tool, which include the low spatial resolution of EEG and the limited temporal resolution of fNIRS. In particular, even though the EEG provides data on a direct brain signal (electrical activity) and the fNIRS provides data on an indirect measure of neuronal activity (hemodynamic activity), their combination can enable the collection of various physiological signals and neurovascular coupling, revealing potentially useful for the practical applications of social research [23,24].

In the current work, the combination of EEG and fNIRS in hyperscanning allowed the recording of the hemodynamic and electrophysiological activity of the two interagents, providing details on the influence of interoception and social framing on mechanisms of functional interbrain connectivity between individuals while performing synchronization tasks.

Functional connectivity, which provides information on the synchronic and diachronic features underpinning people's interaction, is defined as the correlation between two temporal sequencies of activity related signals [25–27]. Indeed, a phenomenon of brain to brain coupling takes place during joint actions, as shown by various studies, which have noticed a correlation between cerebral activation in two people during joint movements, emotions, or feelings [26,28,29]. In particular, a number of hyperscanning experiments have found that participants in the exchange engage in implicit perceptual, cognitive, and motor linking mechanisms when performing joint actions. For instance, it has been noted that two individuals synchronize their motor behavior while they are both seated in rocking chairs [26,30], or how musicians partners synchronize their performance [31].

Using "two-person neuroscience", which simultaneously records the brain activity of two individuals, it is possible to learn more about the brain connectivity mechanisms of dyads during conditions implying social and emotional interactions [27,28]. This mechanism of similar activation cannot be recorded using conventional approaches used on an individual's single brain [32]. Moreover, intercerebral synchronization processes take place in a variety of social interaction contexts, including those in which the cooperative aim and the shared intentionality is stressed [5,33].

In the current study, two different interoceptive conditions were used to execute a motor synchronization task, and the coherence of inter-brain EEG-fNIRS patterns of functional connectivity was evaluated. The experimental setup made clear the two distinct interoceptive focus conditions: one in which participants' attention was focused on their breathing, and another in which it was not. Additionally, by emphasizing the shared intentionality, the motor task was socially or not-socially framed.

Given the former works, we expected to find higher EEG and fNIRS interbrain coherence for the focus compared to the no focus on the breath condition during the synchronization task [34,35].

Specifically, during the focus on the breath condition, we hypothesized to detect an EEG inter-brain coherence effect for specific frequency bands, namely low frequency bands, such as delta, theta, and alpha, given their twofold role in sustained attention and focus on the meditative state [12,36,37], and controlled motor synchronization [38].

Regarding the social framing manipulation, we expected to observe an increase in the inter-brain coherence effect for the socially framed compared to the non-socially framed motor synchronization, given the effect of the social frame we previously observed specifically for the motor task in our previous research [35].

Additionally, according to the literature cited above, we expected to observe a potential lateralization effect even in terms of inter-brain coherence, with a left compared to right hemispheric activation predominance for the positive emotions derived from the interoceptive focus on the breath and the social framed synchronization [5].

Finally, taking into account that coherence indices were used and correlated in previous EEG-fNIRS hyperscanning experiments to investigate the coherence of functional connectivity between the two neural signals [27], we also intend to check the direct relationship between these two orders of synchronization in the context of social interoception.

2. Materials and Methods

2.1. Participants

The research was conducted on a sample of 14 dyads of participants (28 participants in total, Mage = 26.87; SDage = 0.29), right-handed and normal or corrected to normal visual acuity. Specifically, the participants were university students and each dyad was made of two individuals of the same sex matched for age. They did not meet before the experiment. The following inclusion criteria were used for the recruitment of participants: they never met before the experiment, lack of psychiatric and neurological disorders. Exclusion criteria included: the presence of high levels of clinically relevant stress and stressful experiences during the last 6 months, pregnancy, past meditative experience, severe physical and chronic illnesses, convulsions and chronic pain. After being informed that they would not receive compensation for their participation, they voluntarily completed an informed consent form and consented to take part in the study. The research followed the principles and guidelines of the Helsinki Declaration. Moreover, the research was approved by the local ethics committee study (approval code 2020 TD-a.a.2020–2021) of the Department of Psychology of the Catholic University of Milan.

2.2. Procedure: Motor Synchronization Task and IA Manipulation

The dyad was located such that individuals could comfortably interact with each other face-to-face. The participants received full explanation of the procedural instructions. A 120-s baseline of each dyad member's EEG and hemodynamic resting state was collected before the experiment began. They were informed that they were required to execute a motor synchronization task. The task was presented in its basic form and also with a specific social frame. Also, the tasks were executed with and without the focus on the breath, A more detailed description of the experimental protocol can be found in our previous study [5,34,35].

To maintain the procedure's reliability, the same interoceptive manipulation was applied in prior studies, and it was demonstrated to have an effect on hemodynamic neural correlates [34,39]. To avoid any biases caused by sequence effects, the conditions were provided in a randomized and counterbalanced order.

Participants assessed their attention to their breathing, the other person, and the task on a scale of 0 to 10 during the debriefing phase that followed the activity. The entire experimental process was accomplished in one hour (Figure 1A).

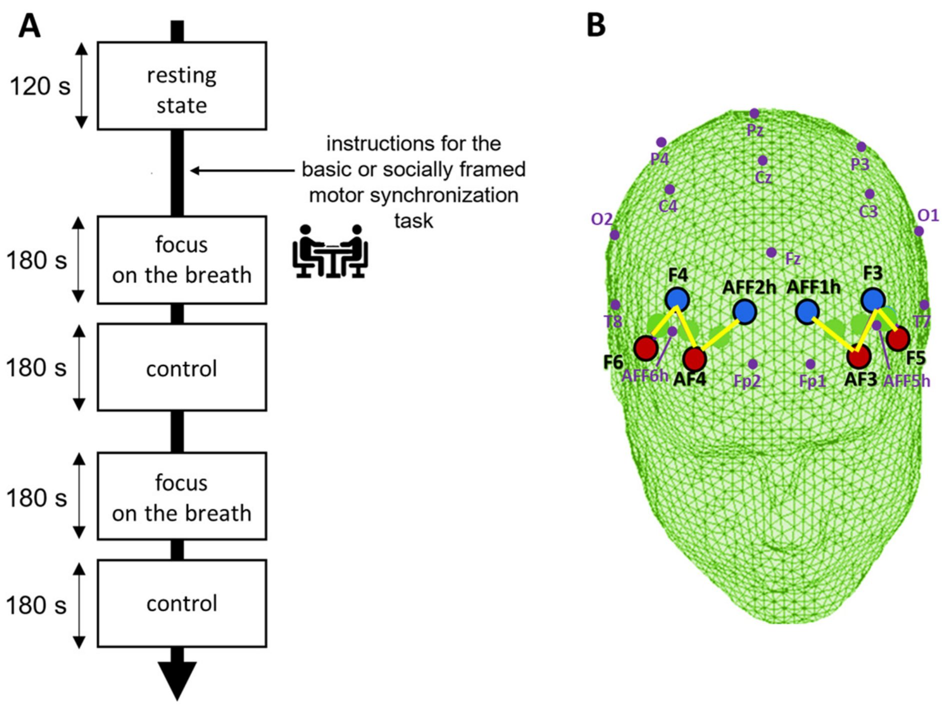

Figure 1. (**A**) Description of the experimental procedure for the EEG-fNIRS hyperscanning acquisition from the dyads. To avoid order effect, the task execution was randomized and counterbalanced for the interoceptive condition and the social frame. (**B**) Head rendering with the EEG-fNIRS montage layout. EEG electrodes placement is reported in purple colour. For the fNIRS montage, four emitters were installed at AF3, AF4, F5, F6, four detectors were at AFF1h, AFF2h, F3, and F4. Emitters and detectors are indicated in red and blue color respectively. Six channels (in yellow colour) were acquired using this optode configuration: Ch1 (AF3-F3), Ch2 (AF3-AFF1h), Ch3 (F5-F3), which correspond to the left PFC, and Ch4 (AF4-F4), Ch5 (AF4-AFF2h), Ch6 (F6-F4), which correspond to the right PFC [14,40].

2.3. EEG Recording and Signal Processing

Two 16-channel portable EEGs (V-AMP: Brain Products, München; LiveAmp: Brain Products, GmbH, Gliching, Germany) were used to acquire the EEG data. The 10/5 technique of electrode placement [41] was used to apply two ElectroCaps with Ag/AgCl electrodes grounded to the earlobes. For the dyad, electrodes were placed over the following positions Fp1, Fp2, AFF5h, Fz, AFF6h, T7, C3, Cz, C4, T8, P3, Pz, P4, O1, O2 for both participants (Figure 1B). By using the BrainVision Recorder software (Brain Products GmbH, Munich, Germany) data were collected using a bandpass filter of 0.01–250 Hz, a sampling rate of 1000 Hz and a 50 Hz notch input filter. Prior to data collection, the recording electrodes' impedance was examined and was consistently less than 5 kΩ. An off-line common average reference was used to prevent distortions caused by the signal-to-noise ratio [42]. An EOG electrode was also positioned on the canthi of the eye in order to record ocular movements.

Both resting-state and tasks-related data were filtered offline with a 0.5–45 Hz IIR filter (slope: 48 dB/octave), then segmented, and ocular inspection was applied for residual ocular, muscle, or movement artifacts (rejected epochs, 2%). To increase specificity, only artifact-free epochs were considered. EEG power spectra for artifact-free segments were finally computed via Fast Fourier Transform, averaged to calculate condition-specific power spectra, and the following frequency bands were then extracted: Delta (0.5–3.5 Hz), Theta (4–7.5 Hz), Alpha (8–12.5 Hz), and Beta (13–30 Hz). EEG data reduction was performed using BrainVision Analyzer 2.0 (Brain Products GmbH, Munich, Germany).

2.4. fNIRS Data Recording and Data Reduction

By using a six-channel optodes matrix of a NIRScout System (NIRx Medical Technologies, LLC, Los Angeles, CA, USA), we measured the variations in the concentrations of oxygenated hemoglobin (O2Hb) and deoxygenated hemoglobin (HHb). Four light sources/emitters and four detectors were placed over the scalp using a fNIRS cap in accordance with the 10/5 international standard [41].

The emitter-detector distance for consecutive optodes was kept at 30 mm, and it used two wavelengths of near-infrared light (760 and 850 nm). According to online atlases [43,44], the sources, detectors, and space between them were positioned in respect to the underlying functional region and the most adequate Brodmann area (Figure 1B).

The signals from the six channels were collected at a sample rate of 6.25 Hz with NIRStar Acquisition Software (NIRx Medical Technologies LLC, 15 Cherry Lane, Glen Head, NY, USA), then extracted and converted with nirsLAB software (v2014.05; NIRx Medical Technologies LLC, 15 Cherry Lane, Glen Head, NY, USA), based on their wavelength and position, producing mmol mm values that corresponded to the variations in the concentration of O2Hb and HHb per channel. Digital band-pass filtering at 0.01–0.3 Hz was applied to the obtained raw O2Hb and HHb data for each channel [28,45].

Raw time-series were visually evaluated subject-by-subject both during the experimental phase and the signal processing to detect noisy channels brought on by motion artifacts or amplitude changes (criterion for rejection: amplitude of hemoglobin [Hb] signal above or below ±5 SD; visual inspection). A 3% loss of the data occurred, rejected due to artifacts. Channels with poor optical coupling and lack of heartbeat oscillations at 1 Hz were disregarded during this visual evaluation [43]. Additionally, a linear-phase Finite Impulse Response (FIR) filter on respiration was applied (0.3 Hz), which produces a symmetric impulse response [46,47].

Following the biosignal analysis, the mean concentration of each channel for the tasks was calculated. The effect size in each condition was determined using the mean concentrations in the time series for each channel and subject. The effect sizes (Cohen's d) were calculated by dividing the difference between the baseline and trial means by the baseline standard deviation (SD): $D = (m1 - m2)/s$, where m1 and m2 are the mean concentration levels for the baseline and trial, respectively, and s is the baseline SD. The effect sizes from the 6 channels were averaged in order to increase the signal-to-noise ratio. While raw fNIRS data were initially relative values that could not be directly averaged across people or channels, normalized effect sizes were averaged regardless of the unit since effect size is unaffected by the differential pathlength factor (DPF).

For the purposes of statistical analysis of the fNIRS data, the channels were averaged together to generate the Lateralization factor for the left (Ch1-Ch2-Ch3) and right (Ch4-Ch5-Ch6) hemispheres, which correspond to the left and right PFC.

3. Results

Below, distinct results' sets related to assessments of the EEG frequency range and hemodynamic dependent measurements will be discussed.

The first step of analysis (Step 1) encompassed the application of coherence analysis separately to the EEG and fNIRS data for each dyad.

To test the link between EEG and fNIRS coherence indices, asa second step (Step 2), a set of correlational analyses (bivariate Pearson correlational values) was applied to the coherence indices previously calculated for fNIRS and EEG.

Finally, the third step (Step 3) pertained to the use of ANOVA tests to these correlational values, which are regarded as dependent measures in ANOVAs.

3.1. Step 1: EEG and fNIRS Coherence Results

3.1.1. Step 1: EEG Coherence

A first analysis was conducted to obtain the EEG inter-brain coherence, by computing the partial correlation coefficient Πij for each dyad, applied to each frequency band. These indices were obtained by normalizing the covariance matrix's inverse

$$\Gamma = \Sigma^{-1}$$

$\Gamma = (\Gamma ij) = \Sigma^{-1}$ inverse of the covariance matrix.

This analysis permits evaluating the relationship between two signals (i, j) independently of one another [48], and has been previously applied often in earlier EEG hyperscanning research [32,49].

3.1.2. Step 1: fNIRS Coherence

Moreover, for this first step of analysis, we also computed the coherence indices for each fNIRS channel in each experimental condition, for both the O2Hb and HHb. These indices were obtained by following the formula reported for the EEG coherence results (Step 1).

The lateralization factor for the left and right hemispheres, which were derived as the average of the homologous channels for the O2Hb and HHb, respectively, underwent a sequential coherence analysis. Only the results for the O2Hb were taken into consideration and reported due to the small number of significant coherence values for the HHb.

3.2. Step 2: Correlational Analyses between EEG and fNIRS Coherence Indices

To test the relationship between EEG and fNIRS coherence indices, a successive set of correlational analyses (bivariate Pearson correlational values) was applied to the coherence indices. In the graphs below, we have reported the correlational values between EEG and fNIRS coherence indices for each dyad of participants (Figure 2A–E).

3.3. Step 3: ANOVAs Applied to the Correlational Values

These coherence indices were inserted as dependent measures for the subsequent ANOVAs with independent within variables Condition (2: focus on the breath, no focus on the breath) × Frame (2: no social and social) × Lateralization (2: left and right). The IBM SPSS Statistics (version 25) was utilized for this analysis. Any significant interactions between simple effects were explored using pairwise comparisons for all ANOVA tests, and the Bonferroni correction was used to reduce the potential bias of repeated comparisons. The degrees of freedom for all ANOVA tests were adjusted using the Greenhouse-Geisser epsilon where required. The size of statistically significant effects was determined using partial eta squared (η^2).

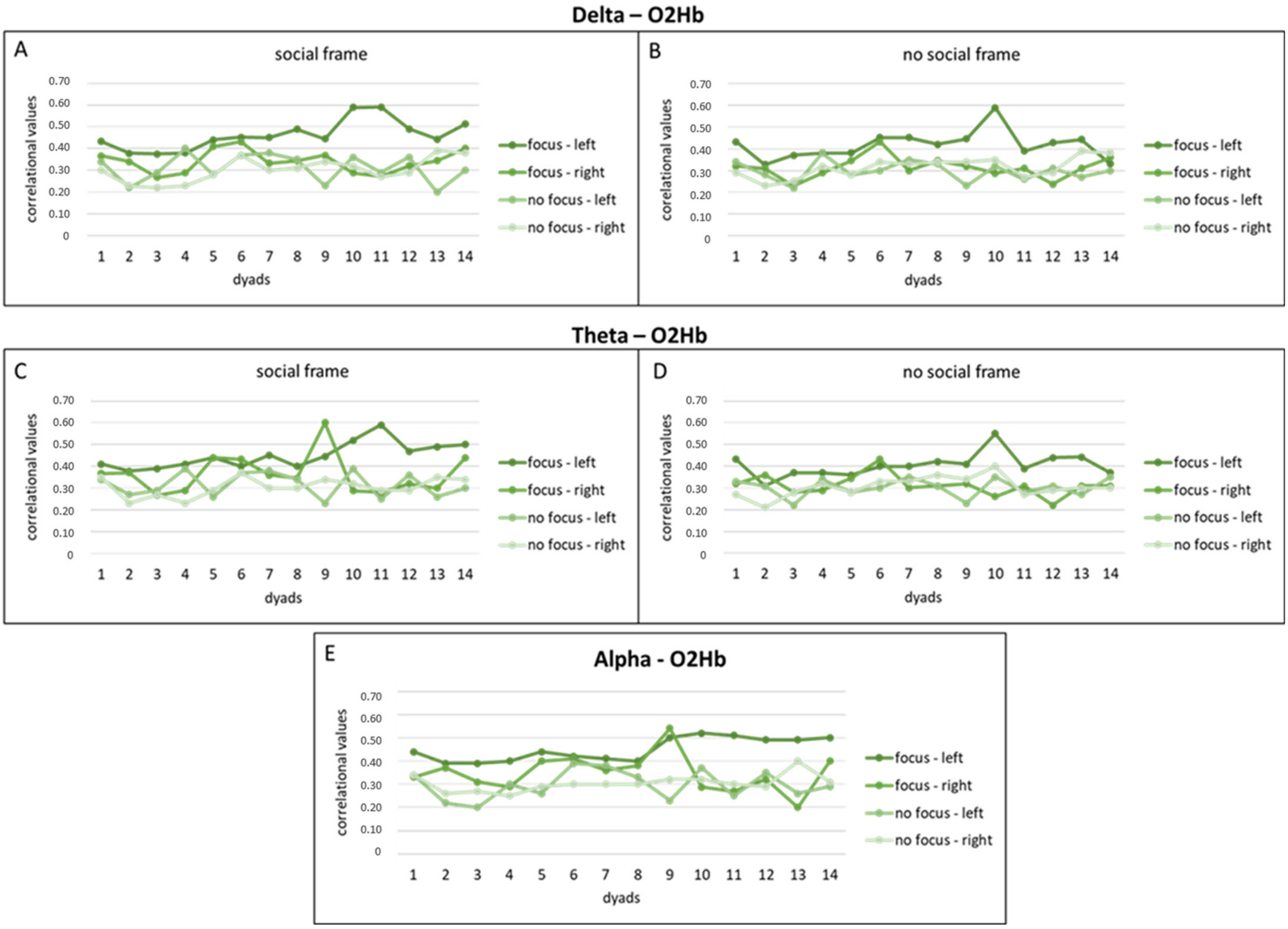

Figure 2. (**A**–**E**) Trends of the significant correlational values between EEG and fNIRS coherence indices. Trend of the correlational values between delta band and O2Hb values for the motor synchronization task in the socially framed (**A**) and no socially framed conditions (**B**). Trend of the correlational values between theta band and O2Hb values for the motor synchronization task in the socially framed (**C**) and no socially framed conditions (**D**). (**E**) Trend of the correlational values between alpha band and O2Hb values.

The ANOVA applied to the correlation coefficients as dependent variables for each dyad revealed significant effects. The significant outcomes of the ANOVAs are reported in the sections that follow.

3.3.1. Delta Band and O2Hb Correlation Values

A first significant interaction effect was found for the Condition × Lateralization (F [1,13] = 8.12, $p = 0.01$, $\eta^2 = 0.477$). Pairwise comparison revealed higher correlational values for the left hemisphere in the focus compared to no focus condition (F [1,55] = 8.90, $p = 0.01$, $\eta^2 = 0.490$), to the focus condition in the right hemisphere (F [1,55] = 7.91, $p = 0.01$, $\eta^2 = 0.390$), and to the no focus condition in the right hemisphere (F [1,55] = 7.09, $p = 0.01$, $\eta^2 = 0.376$) (Figure 3A).

Second, an interaction effect was observed for Condition × Frame × Lateralization (F [1,13] = 10.08, $p = 0.01$, $\eta^2 = 0.513$). In particular, pairwise comparisons showed greater correlational values in the left hemisphere for the focus condition when the task was socially compared to non-socially framed (F [1,84] = 8.90, $p = 0.01$, $\eta^2 = 0.490$). Moreover, pairwise comparisons showed greater correlational values in the left compared to right hemisphere for the socially framed task during the focus condition (F [1,84] = 8.16, $p = 0.01$, $\eta^2 = 0.451$) (Figure 3B). There were no additional statistically significant effects.

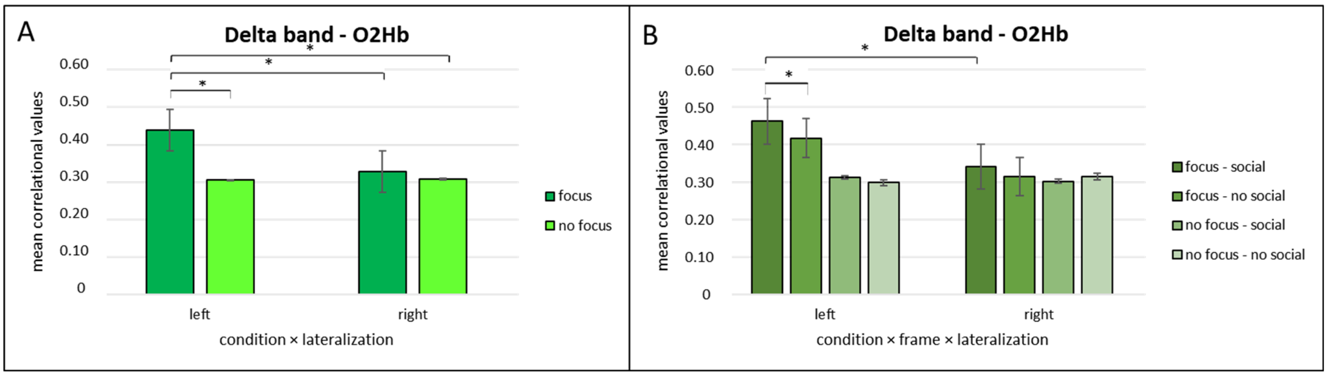

Figure 3. (**A**,**B**) Mean correlational values. (**A**) The bar graph shows the significant effect Condition × Lateralization. (**B**) The bar graph shows the significant effect Condition × Frame × Lateralization. All asterisks (*) mark statistically significant differences, with $p \leq 0.05$.

3.3.2. Theta Band and O2Hb Correlation Values

A significant interaction effect was found for the Condition × Lateralization (F [1,13] = 8.55, $p = 0.01$, $\eta^2 = 0.479$). Greater correlational values for the left hemisphere in the focus compared to no focus condition (F [1,55] = 7.16, $p = 0.01$, $\eta^2 = 0.410$), to the focus condition in the right hemisphere (F [1,55] = 7.44, $p = 0.01$, $\eta^2 = 0.420$) and to the no focus condition in the right hemisphere (F [1,55] = 7.67, $p = 0.01$, $\eta^2 = 0.392$) were revealed by pairwise comparisons (Figure 4A).

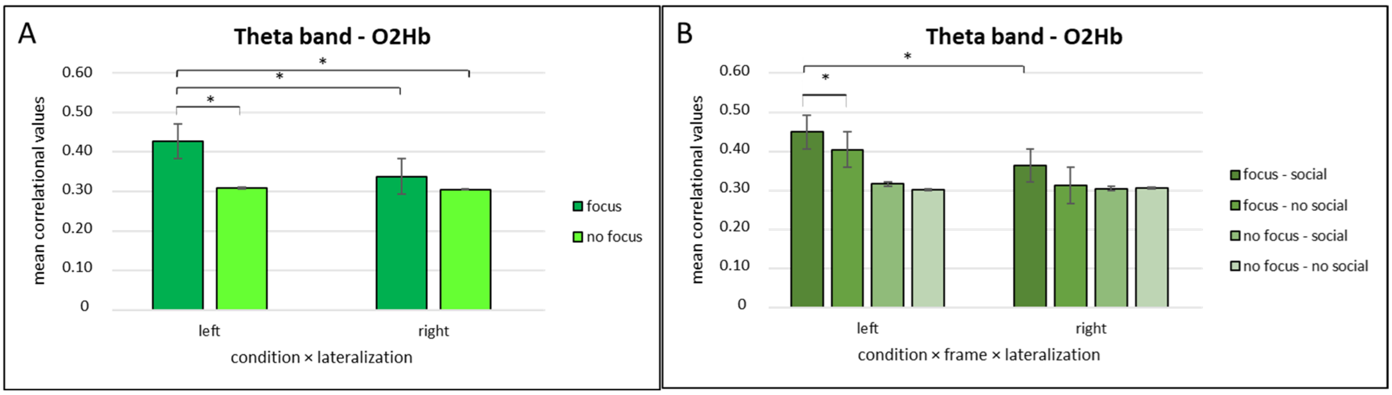

Figure 4. (**A**,**B**) Mean correlational values. (**A**) The bar graph shows the significant effect Condition × Lateralization. (**B**) The bar graph shows the significant effect Condition × Frame × Lateralization. All asterisks (*) mark statistically significant differences, with $p \leq 0.05$.

Moreover, an interaction effect was detected for Condition × Frame × Lateralization (F [1,13] = 9.42, $p = 0.01$, $\eta^2 = 0.543$). Pairwise comparisons showed greater correlational values in the left hemisphere for the focus condition when the task was socially compared to non-socially framed (F [1,84] = 8.20, $p = 0.01$, $\eta^2 = 0.476$). Furthermore, pairwise comparisons showed greater correlational values in the left compared to right hemisphere for the socially framed task during the focus condition (F [1,84] = 6.34, $p = 0.01$, $\eta^2 = 0.328$) (Figure 4B).

3.3.3. Alpha Band and O2Hb Correlation Values

For alpha band and O2Hb correlation values, only one significant interaction effect was observed for the Condition × Lateralization (F [1,13] = 8.55, $p = 0.01$, $\eta^2 = 0.470$). In particular, pairwise comparison revealed greater correlational values for the left hemisphere in the focus compared to no focus condition (F [1,55] = 7.76, $p = 0.01$, $\eta^2 = 0.443$), to the focus condition in the right hemisphere (F [1,55] = 7.21, $p = 0.01$, $\eta^2 = 0.403$), and to the no focus condition in the right hemisphere (F [1,55] = 7.98, $p = 0.01$, $\eta^2 = 0.409$) (Figure 5). No other statistically significant effects were found.

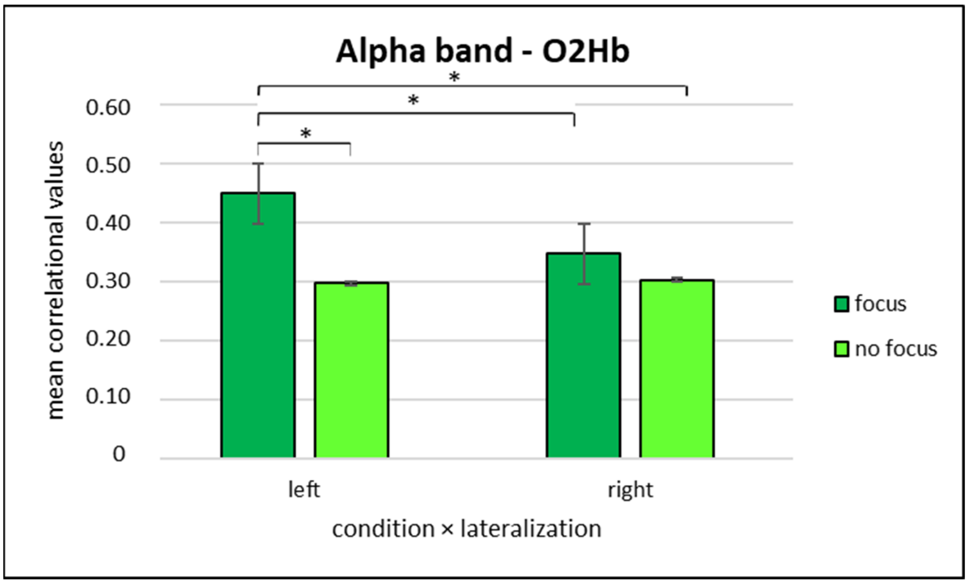

Figure 5. Mean correlational values. The bar graph shows the significant effect Condition × Lateralization. All asterisks (*) mark statistically significant differences, with $p \leq 0.05$.

4. Discussion

This study examined the coherence of functional inter-brain connection patterns during a motor synchronization task that was presented with and without a social frame and carried out under two different interoceptive conditions. Specifically, the interoceptive manipulation comprised of two different experimental conditions in which participants were required to focus their attention on the breath or just performing the task, without the explicit request to focus the attention on body correlates (i.e., focus vs. no focus on the breath condition). Moreover, to stress the shared intentionality, during the motor synchronization task participants were told that they needed to synchronize in order to develop greater teamwork skills. In this way, this instruction served as social frame to stress the shared intentionality and the social goal, whereas the lack of a social framing was caused by the lack of focus on the sharing of purpose [35].

Within this experimental design, the application of a multimethodological approach through EEG-fNIRS hyperscanning co-registration allowed to obtain the direct comparison between different levels of neurophysiological measures and analysis.

First, it allowed the collection and analysis of participants' multi-level neurophysiological responses (both electrophysiological and hemodynamic) related to the motor synchronization task performed in the distinct experimental conditions.

Secondly, it permitted the collection of the signal derived from the two interagents, providing details on the influence of interoception and social framing on mechanisms of functional interbrain connectivity between individuals while performing the synchronization task. In fact, an analysis of the coherence indices was done for both the EEG and the fNIRS signals. The inter-subject neural coherence between the dyads for the left and right hemispheres evaluated in each experimental condition was estimated using the coherence analysis. Thirdly, the correlation between electrophysiological and hemodynamic coherence indices allowed us to investigate the systematic relationship and coherence between these signals in terms of functional brain connectivity coherence. Indeed, what constitutes the added value of this study is the significant correlation between EEG-fNIRS coherence indices in the left hemisphere suggesting the coherence of functional connectivity between the two neural signals [27], during specific experimental conditions. Before our study, few studies adopted or solicited the multimodality hyperscanning EEG-fNIRS application [27,50]. To the best of our knowledge, this is, however, the first instance in which the direct link between these two orders of neuronal synchronization is examined and observed in the context of social interoception.

As to the specifics, in accordance with the first hypothesis, we observed a significant correlation between EEG-fNIRS interbrain coherence in the left hemisphere for the focus compared to the no focus on the breath condition and to the right hemisphere (both during focus and no focus on the breath condition) during the motor synchronization task.

Specifically, for the EEG data we observed higher inter-brain coherence in the left hemisphere for low frequency bands, namely delta, theta, and alpha during the focus on the breath condition while participants were performing the motor synchronization task.

Regarding the role of the theta and delta bands in motor synchronization tasks, previous research has revealed that guitarist pairings have highly synchronized theta and delta oscillations in frontal and central electrode locations when playing a song in duet, suggesting that frontal activation for these frequency bands depends on their role in social cognition [38].

Additionally, in relation to the functional significance of low frequency bands connected to the deliberate emphasis on breathing, Harmony et al. [51] claimed that the EEG delta oscillation, which reflects people's attention to their own internal processes, is connected to their performance at mental tasks. Additionally, delta and alpha oscillations in the PFC were suggested as a supporting inhibitory mechanism influencing people's motivation and attention and moderating their performance on mental tasks [52]. According to Harmony [53], the higher delta oscillation observed in mindfulness practitioners is the result of a PFC inhibitory mechanism and the reduced emotional and cognitive involvement.

The manifestation of frontal theta rhythm was also previously related to the parasympathetic component of the autonomic nervous system [54–56]. Additionally, theta power increase has previously been noted following 5 days of mind-and-body training [57].

Regarding the alpha band, Coomans and colleagues [58] evaluated the intersubject EEG bands' coherence of dyads executing mindful breathing exercises individually and in couples. The alpha band, which is interpreted as an increase in shared relaxation, and the theta band, which is related to the dyad's agreeableness and theory of mind, showed more EEG coherence during the joint practice session than during the individual session, according to the authors. Furthermore, it has been suggested [59] that increased alpha synchronization throughout frontal areas is a reflection of processes for "shutting off" external attention.

Considering the left lateralized effect highlighted in the results, Beauregard et al. [60] stated that higher alpha power detected over left frontal and temporal regions during meditation condition is an index of reduced cortical arousal associated with a relaxation response. About delta and theta manifestation in the left PFC, a previous study in the context of emotions showed that lateralized EEG activity (mainly low-frequency theta and delta bands) is intrinsically associated with the cortical hemodynamic responsiveness to the emotional patterns, for which specifically the right hemisphere activation was observed during negative emotions processing [9]. Further research is needed to confirm whether, on the other hand, this left lateralized impact for the delta and theta band in the context of social interoception is related to a pleasant emotional experience.

A possible partial explanation could be that this left PFC electrophysiological tuning is due to greater cooperation and relaxation induced by the focus on the breath during the motor synchronization task. The interoceptive focus had an impact on the low frequency bands, which are also associated with cooperation and social cognition in motor synchronization tasks and which promote a positive emotional experience for both the members of the dyad. It may be claimed that the interoceptive focus on breathing is directly connected to this impact on positive emotions, because the same effect is not seen in the control condition (without the attention on breathing) and in the right hemisphere during the motor synchronization task.

Nonetheless, a more exhaustive explanation can be provided if these EEG results are interpreted taking into account the concomitant increase of O2Hb in left PFC. In fact, the increase of EEG delta, theta and alpha band coherence indices was significantly correlated with the increase of O2Hb (measured through fNIRS) coherence indices in the left PFC for the focus on the breath condition during the motor synchronization task.

A significantly higher O2Hb coherence in the left PFC when the dyads performed the basic synchronization tasks in the focus on the breath condition was previously observed in a recent study [5]. This finding was interpreted by considering the twofold role of the left PFC, that is firstly a neuroanatomical region pertaining to the "mutual attention system", whose key feature is the element of reciprocal and synchronized activation [8]; and, secondly, it plays a more significant role in processing positive emotions than the right PFC. Indeed, approach motivation, the ability to regulate negative emotions, and general wellbeing are all associated with the frontal cortical asymmetry favoring the left hemisphere, as has been previously observed [61–64]. Moreover, this lateralization effect was even more evident when the task was socially framed, thus supporting the impact of shared intentionality during motor synchronization task on the PFC [35], and specifically on the left side. On the whole, this work suggests that even basic exercises of synchronization, if performed during the focus on the breath and explicitly socially framed, may increase an individual's inter-brain hemodynamic (O2Hb) coherence in the left PFC [5].

However, this previous fNIRS hyperscanning study aimed to investigate only the hemodynamic connectivity between the two members of the dyad during a social interoception experiment, while the current work verified if two signals (EEG and fNIRS) go in parallel during comparable experimental conditions related to interoception and social processes.

In fact, the current research adds further evidence in terms of correlation between the EEG and the fNIRS coherence indices. For low frequency bands (delta and theta) coherence indices, it was observed a significant correlation with O2Hb coherence indices in the left hemisphere during the focus on the breath condition for the socially framed compared to the non-socially framed motor synchronization. This lateralization effect observed in terms of inter-brain coherence for both EEG and fNIRS data could confirm initial evidence on the role of a left compared to right hemispheric activation predominance for the positive emotions derived from the interoceptive focus on the breath and the social framed synchronization.

5. Conclusions

In conclusion, the current study emphasizes how the focus on the breath and the social frame promotes individuals' EEG and fNIRS brain synchrony in the left hemisphere, and how these two neurophysiological signals are coherent in specific experimental conditions related to the manipulation of IA and social synchronization.

To the best of our knowledge, this is the first time that such multimethodological approach and functional connectivity analysis has been applied to the study of interoception and synchronization processes. We must, however, point out some limitations that might be taken into account in further research.

First, to increase the generalizability of the present findings, future studies must increase the sample size and consequently the number of homologous dyads taken into consideration in the functional connectivity analysis. Secondly, our fNIRS montage covered solely the PFC, which is an important cortical region for social-cognitive, emotional and interoceptive processing; however, future studies should consider the measurement of a larger portion of cerebral cortex. Thirdly, specific behavioral measures to verify the participants only focused on their breath and not on other body signals (e.g., skin temperature or heartbeat), which could be added in future studies. Another limitation which should be mentioned is that participants were mostly university students and that it would be advisable in the future to consider a more various sample in order to generalize the results.

In the current experiment, the breathing rate was not recorded during the interoceptive phase of the task because the focus of the study was on the neurophysiological effect (EEG and fNIRS coherence and their relationship) of the interoceptive attention to breath (which is a different top-down manipulation from the respiratory control), and not the autonomic correlates of the process. Additionally, in earlier studies on intersubject EEG coherence, such as the study of Coomans et al. (2021) [58], the same approach was adopted. Indeed, in this study, no collection or manipulation the respiratory rate or synchrony was reported, and the neurophysiological results showed interpersonal neural synchrony for the theta and alpha bands while healthy dyads were engaged in a mindful breathing exercise together. Despite this evidence, we suggest that future research could collect the breathing rate of each dyad to get a fuller picture of the phenomenon under investigation.

Finally, it should be noted that the question of how far these findings generalize across ecological tasks, but also across analysis methods and INS measures [21], remains an open avenue for future hyperscanning research.

To sum up, we were able to observe interbrain functional connectivity thanks to the use of the hyperscanning paradigm and multimethodological approach. Additionally, a parallel pattern of electrophysiological and hemodynamic data in the left hemisphere during the focus condition and when the motor task was socially framed has been identified thanks to correlation analysis.

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