Research report

Between-brain coherence during joint n-back task performance: A two-person functional near-infrared spectroscopy study

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HIGHLIGHTS

- N-back task performance assessed in paired and single players using fNIRS.
- Paired players revealed significant increase in cortical between-brain connectivity.
- Paired players revealed significant larger cortical hemodynamic responses.
- Paired players showed no increase in behavioral performance.
- Findings designate fNIRS as suitable tool for monitoring between-brain connectivity.

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ABSTRACT

The present study aimed to step into two-person neuroscience by investigating the hemodynamic correlates of between-brain connectivity during joint task performance. To test this approach, wireless functional near-infrared spectroscopy (fNIRS) was used to record brain signals during performance of a dual n-back task simultaneously in paired players as compared to a single player.

Evaluating functional connectivity between the paired players’ brains using wavelet transform coherence (WTC) analysis revealed (1) a significant increase in between-brain coherence during joint task performance as compared to baseline condition. These patterns were observed in two frequency bands, i.e. in the heart rate (HR) frequency and in low-frequency oscillations (LFOs). (2) Averaged hemodynamic responses revealed larger responses in total hemoglobin concentration changes [\(\Delta[Hb]\)] for the paired players as compared to the single players; in addition, within the paired players groups joint task performance revealed larger changes in [\(\Delta[Hb]\)] compared to a rest period and to a baseline condition. (3) No increase in behavioral performance was found in the paired players as compared to the single players.

Our findings designate fNIRS as suitable tool for monitoring interpersonal performances between two subjects. The results show that two-person performance leads to relevant and significant effects, which are detectable using between-brain connectivity analysis. Using this approach can provide additional insight into interpersonal activation patterns not detectable using typical one-person experiments.

Our study demonstrates the potential of simultaneously assessing cerebral hemodynamic responses for various two-person experimental paradigms and research areas where interpersonal performances are involved.

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1. Introduction

Research in the field of interpersonal performance focuses on the exploration of the coordinative structures that substantiate joint task performance. Joint task performance refers to the ability to perform tasks which provide a benefit to another individual or are beneficial to two players and may therefore be advantageous as compared to single performance [1–3]. As recently summarized by Ramenzoni et al. [1] joint tasks (e.g., joint attention, communication, task sharing, and team sports) require two or more subjects to coordinate, intentionally or spontaneously, to attain a common goal, e.g. [2,4,5]. The ability to engage in and sustain reciprocal relations is regulated by cognitive [6] and perceptual-motor processes [7,8], which work in concert to enable coordination during joint action [2].

Further, as recently summarized by Atmaca et al. [9] cognitive and social scientists have started to distribute tasks between two co-players to investigate whether individuals form similar
task representations when task performance is shared and when the whole task is performed alone [10–20]. Here, the terms ‘task co-representation’ and ‘shared task representations’ refer to the idea that during joint task performance, each co-player represents and follows not only his/her own part, but also the part to be performed by the co-player. In the following study we aimed to understand the term joint task performance according to this definition. These previous studies did provide inconsistent results concerning the question whether this type of joint task performance can lead to improved or rather decreased performance. Both positive and negative results were found, depending both on the task presentation and the type of task. It therefore remains a general question whether a representation of a task under the control of one’s co-player is only affecting the co-player’s behavioral performance and/or brain response or do co-players reflect each other’s actions in behavioral performance and/or brain response in such a way that changes can be observed in both co-players’ responses?

However, the attempt to elucidate the between-individual neural mechanisms of human joint task performance has only recently attracted researchers’ attention and an objective of moving toward two-person neuroscience. As recently emphasized by Babiloni et al. [21], a major limitation in most existing neuroimaging studies characterizing brain responses during interpersonal performance is that only one of the participating brains is measured each time. The interplay between jointly acting, cooperating, competing or communicating brains is thus not measured directly, but inferred from independent observations aggregated by cognitive models and assumptions that link behavior and neural activation. Studying real joint task performance is mainly limited due to practical limitations of traditional neuroimaging methods, such as functional magnetic resonance imaging (fMRI), to simultaneously record in two subjects. Hence, to conduct brain imaging in joint task performance, neuroimaging methods are required that allow for simultaneous investigations of two brains.

Recently, a few studies have attempted to overcome these challenges. Babiloni et al. [21] found consistent activation in medial prefrontal cortex in two players during the so-called Prisoner’s Dilemma using a dual-electroencephalography (EEG) setup. In another study using the same dual-EEG setup, these authors reported functional connectivity patterns in prefrontal and anterior cingulate cortex (ACC) during a card game, particularly in two players belonging to the same team [22,23]. De Vico Fallani et al. [24] were able to predict non-cooperative interactions during the Prisoner’s Dilemma using graph-theory of dual-EEG data, i.e. the decision to defect vs to cooperate could be estimated in advance by evaluating the changes of connectivity pattern between brains. Cui et al. [25] reported increased between-brain coherence over superior frontal cortices during cooperative game play in two players, but not during competition as assessed by functional near-infrared spectroscopy (fNIRS). Funane et al. [26] observed increased between-brain spatiotemporal covariance over prefrontal cortices as assessed using fNIRS data during a cognitive-motor cooperation task when subjects successfully synchronized their tasks.

The aim of the present study was to monitor behavioral performance and hemodynamic responses during a dual n-back task in paired players as compared to single players and to subsequently compare the behavioral results with cortical between-brain connectivity. Our experiment was based on the questions whether performing the n-back task in a joint manner may result in (1) better behavioral performance as compared to single task performance, which in turn may be reflected in (2) increased between-brain connectivity and/or (3) larger hemodynamic responses over prefrontal cortices?

2. Materials and methods

2.1. Subjects

15 subjects participated in the study. All subjects (mean age ±SD 22.9 ± 1.3) were right-handed (mean laterality quotient (LQ) ±SD = 93.9 ± 26.5) according to the Edinburgh Handedness Inventory [27]. Subjects were assigned to two groups, with group 1 consisting of paired players (N = 4 pairs) and group 2 consisting of single players (N = 7 subjects). There were no significant differences between groups in terms of sex, age or handedness.

Non-verbal fluid intelligence (GI), i.e. the ability to reason and to solve new problems independently of previously acquired knowledge, were assessed by an online version [28] of the Raven’s Advanced Progressive Matrices [29] with no difference between groups (total = 119.3 ± 9.5 (mean IQ ±SD); group1 = 118.5 ± 9.3; group2 = 119.6 ± 10.0).

Exclusion criteria were any history of visual, neurological or psychiatric disorder or any current medication; all subjects had normal or corrected-to-normal vision. All subjects gave written informed consent. The study was approved by the ethic committee of the Canton Zurich and in accordance with the latest version of the Declaration of Helsinki.

2.2. Experimental protocol

Group 1: In each session, the set-up consisted of a pair of players sitting in front of a PC (Dell Inspiron 6400) on a table next to each other (Fig. 1, Top). During the task phase, the two players were asked to perform an n-back task. N-back tasks have been studied extensively in single performance and offer easy and suitable integration to allow for joint action performance. In general, an n-back task is a continuous performance task that is carried out in neuroimaging to stimulate brain activity in test subjects and was introduced by Krichef [30]. In particular, we used a dual-task i.e. a variation of an n-back task in which two independent sequences are presented simultaneously, which as was first proposed by Jæger et al. [31,32]. To generate the dual n-back task in this study we used the software called Brain Workshop [33] (http://brainworkshop.sourceforge.net) which is available online.

The dual n-back task consisted of memorizing the location of a briefly shown visual input (colored squares in our case) in one of nine zones arranged in a 3 × 3 manner (Fig. 1, Bottom). For example, in a 2-back task, the player had to confirm whether the newly shown visual input was in the same zone as it was two visual inputs ago. During our paired experiment, the two players were asked to perform the n-back task in a joint manner. The Brain Workshop settings were set as follows: (1) ‘Use position’ was activated, i.e. visual stimuli at different positions in a square were used (no auditory stimuli), ‘Simultaneous visual stimuli’ was set to 2, i.e. two visual inputs, and ‘Interference’ to 12.5%, i.e. a certain percentage of the time Brain Workshop generates trials designed to be particularly tricky, such as by making the current stimulus match the stimulus (n − 1), (n+1), or (2n) trials ago. All other task settings were set to: No. This resulted in a purely visual n-back task in which each of the two players was asked to concentrate on and memorize one of two colored squares (player 1: blue squares, player 2: green squares). Players were asked to give their responses by pressing on a keyboard.

The following task pattern was chosen to ensure a consistent challenging level: each session was set to start with a task trial at the 2-back level. Further, each session was set to increase the level (i.e. 3-back, 4-back or 5-back) if the players achieved more than 80% correct answers per trial and to decrease one level if the players three times did not achieve 90%. At the 50% and 25% level in the next trials. Accordingly, the number of trials was automatically calculated after each task round depending on the n-back level (N-trials × N-trials-factor × N-trials-exponent: where n is the current n-back level and the default values consisting of: N-trials (number of trials = 20), N-trials-factor (multiplication factor for number of trials = 1), N-trials-exponent (exponent factor for number of trials = 2). This resulted in 24 trials for 2-back level, 29 trials for 3-back level, 36 trials for 4-back level and 45 trials for 5-back level. All participants underwent 15 task rounds, alternated with 20 s rest periods resulting in approx. 30 min for the whole session. Both players were given the instruction to contribute to the overall joint task performance, i.e. to achieve together as many correct answers as possible, while avoiding incorrect or missed answers. Each player was asked to concentrate on his/her part of the task, i.e. focusing on his/her input category (blue/green square), and to follow the responses of the other player in a timely close manner. During the rest periods subjects were instructed to remain motionless, focus on the black screen and engage in no mental effort.

Group 2: In contrast to the paired players, the single players only received one visual stimulus (blue square), but otherwise performed the same task as that described for group 1 (Fig. 1).

2.3. NIRS instrumentation

All players (paired and single players) were recorded using functional near-infrared spectroscopy (fNIRS) on the non-dominant hemisphere. The fNIRS is based on neurovascular coupling, which exploits the relationship between metabolic activity due to neural processing and the oxygenation and concentration of hemoglobin in blood vessels. Utilizing this tight coupling between neuronal
activity and regional cerebral blood flow, fNIRS measures regional hemodynamic changes associated with cortical activation [34]. We used a miniaturized, wireless and portable fNIRS technology [35] (Fig. 2) that does not require a subject’s body or head to be restrained, and can therefore overcome some of the limitations inherent to traditional neuroimaging methods in monitoring two-person simultaneously. The sensor components are mounted onto a four-layer rigid-flexible printed circuit board (PCB) which, in combination with a highly flexible casing made of medical grade silicone, enables the sensor to be aligned to curved body surfaces such as the head. The size of the device is $92 \times 40 \times 22$ mm and it weighs 40 g. The optical system comprises four light sources at two different wavelengths (760 nm and 870 nm) and four light detectors (PIN silicon photodiodes). The power is provided by a rechargeable battery, which allows continuous data acquisition for 180 min at full light emission power. The light intensity is sampled at 100 Hz and the resulting data are transmitted wirelessly to a host computer by Bluetooth within an operating range of about 5 m.

For fNIRS recording, two (one) sensors were placed over the players’ left hemispheres, covering Fp1 according to the international 10–20 system [36]. With each compact sensor measuring an area of 37.5 mm length and 25 mm width, we cover areas of prefrontal cortices. Hair under the sensors were carefully brushed away to ensure good skin contact; the sensors were fixed on a subject’s head using self-adhesive bandages which allow for a homogeneous contact pressure over the whole sensor surface (Derma Plast CoFix 40 mm).

3. Data analysis

3.1. Behavioral data

Behavioral task data of the paired and single players were computed according to the definitions given in Section 2.2 for the mean number of trials per session, the mean time per trial in 0.1 s, the mean n-back task performance, the mean score over all trials (from 0 to 100%) and the mean score for each of the input category (blue/green square) (from 0 to 100%). Statistical significance between the paired and the single players were assessed using independent samples t-test (confidence interval 95%, $p \leq 0.05$).

3.2. fNIRS data pre-processing

A program was written in MATLAB® (Version 2008a, Mathworks, Natick, Massachusetts, USA) to pre-process the raw light intensity values. By applying the modified Beer–Lambert law (MBLL), from the measured attenuation changes of NIR light after its transmission through tissue the concentration over time for oxy-hemoglobin ($O_2$Hb) and deoxy-hemoglobin (Hb) ($\Delta (O_2$Hb), [Hb]) were computed, which represent the dominant light absorber for living tissue in the NIR spectral band [37]. First, the fNIRS signals were sampled at 100 Hz for all four source-detector pairs of light-sources and detectors, resulting in four channels considered for analysis (Fig. 2). The ambient light intensities, i.e. from the environment, were subtracted from the fNIRS measurement values before low-pass filtering (7th order Chebyshev with 20 dB attenuation at 5 Hz) and the signals were then decimated to a sampling rate of 10 Hz. Then, the MBLL was used to compute the changes of $O_2$Hb and [Hb] applying differential path length factors (DPF) of 6.75 for the 760 nm and 6.50 for the 870 nm light sources [38]. The linear signal drift was then subtracted from the resulting $O_2$Hb and [Hb] signals. Source-detector pairs (channels) that showed severe artifacts or had a very low signal-to-noise ratio were excluded from analysis for each individual subject.

Since head and body movement can cause movement artifacts (MAs) in the [$O_2$Hb] and [Hb] signals, the method presented by Scholkmann et al. [39] was used to reduce MAs from these signals.
prior to analysis. Basically two types of MAs were present in the processed data: short spike-like artifacts and baseline shifts. The MA-reduction method, which was successfully used in previous studies [40–47], comprises an algorithm that is based on the calculation of the moving standard deviation (to semi-automatically detect MAs), the application of a spline interpolation to the MA-affected parts of the time series and subtraction of the spline interpolation function from the raw data (to reduce the MAs) and the reassembling of all not MA-affected parts with the MA-affected parts (to reconstruct the whole time series). The MAs removal was applied to the data of two subjects. For each data set the amount of MAs was approximately 3% or less.

After pre-processing, for final analysis using MATLAB® and SPSS® (Version 17.0, SPSS Inc., Chicago, USA), the total hemoglobin concentration [THb] derived as the sum of the averaged [O2Hb] and [HHb] time series was calculated per trial and player. [THb] was chosen as prime parameter as it has been suggested that it is far less sensitive to vein contamination and therefore may provide better spatial specificity and might be used instead of [O2Hb] or [HHb] to map cerebral activity with fNIRS [48]. In addition, changes in [THb] represent changes in blood volume and are correlated with changes in blood flow [49].

3.3. Functional connectivity

To evaluate functional connectivity between the paired players’ time series wavelet coherence was computed as previously described by Grindest et al. [50]. Wavelet coherence, also known as wavelet transform coherence (WTC) is a method of measuring the cross-correlation between two time series as a function of frequency and time [51]. As such it can detect significant coherence between two time series of two subjects even though the common power might be low. For more thorough explanations of WTC, continuous wavelet transform (CWT) and cross wavelet transform (XWT) as well as illustrative examples, please see [50] and [52]. We used the wavelet coherence MATLAB® package presented in [50] which is available on the authors’ website (http://www.pol.ac.uk/home/research/waveletcoherence/).

Subsequent analysis was performed similar as previously described by Cui et al. [25]: for each channel from each player pair two time series were taken, e.g. [THb] in channel 1 from one player, and [THb] in channel 1 from the other player. WTC analysis on these two time series generated a color-coded 2-D coherence map (Fig. 4A). Two frequency bands were then selected for further analysis: 0.7–4 Hz (period length 0.25–1.5 s) and 0.06–0.2 Hz (period length 5–16 s). The first frequency band corresponds to the players’ heart rate (HR). The second frequency band corresponds to low-frequency oscillations (LFOs) with a centre frequency of approx. 0.1 Hz in humans [53,54]. Studies of the functional properties of LFOs have associated these oscillations with a network of connected brain regions that are active in the resting brain [55–58], which will be addressed in detail in the discussion. The average coherence value in these bands during the task phases and during the rest periods was then calculated. In addition, we assessed a ‘baseline’ value for between-brain coherence, i.e. an unrelated-paired-player WTC value as compared to the real-paired-player WTC values. This was calculated by using the time series of two unrelated players from the paired player group, i.e. two players from different sessions; this resulted in an equal number of baseline measures. Statistical significance was assessed using multivariate ANOVA with the fixed factors ‘Channel’ (channel 1–4) and ‘Condition’ (baseline vs rest vs 2-back vs 3-back vs 4-back). The factor ‘Condition’ differentiated the WTC values in the baseline control, the rest periods (time interval from 0 to 20 s) and the task phase (time interval from 20 to 100 s); within the task phase coherence values were further calculated separately for each trials of 2-back, 3-back and 4-back performance.

3.4. Averaged hemodynamic responses

Mean [THb] hemodynamic responses per task trial of the paired and single players were calculated for each channel. Statistical significance was assessed using multi- and univariate ANOVA with the fixed factors ‘Channel’ (channel 1–4), ‘Condition’ (rest vs 2-back vs 3-back vs 4-back) and ‘Player’ (player 1 vs player 2 vs single players).

4. Results

4.1. Behavioral data

Statistical significance of the behavioral task results assessed using independent samples t-test (confidence interval 95%, \( p \leq 0.05 \)) revealed significantly higher mean n-back performance for the single as compared to the paired players (\( t = 5.827, \ p \leq 0.001^{* * * } \) (Fig. 3). No other significant differences were observed (all other p-values with \( p \geq 0.3 \)).
4.2. Functional connectivity

Fig. 4 illustrates the WTC results of an exemplary pair of paired players for channel 1. The remaining channels did show a similar pattern. In Fig. 4(A) two frequency bands with high coherence (indicated in red colors) were found: first, high coherence values were found in the frequency band 0.7–4 Hz (period length 0.25–1.5 s) which is attributable to the players’ heartbeat. Second, high coherence values were found in the frequency band 0.06–0.2 Hz corresponding to LFOs (period length 5–16 s). In both frequency bands, coherence increased during the task phase as compared to the rest condition; the increase of coherence occurred consistently over all channels 1–4 in this player pair covering the prefrontal cortices.
Table 1
Multivariate ANOVA main effects and post hoc comparison: Wavelet transform coherence (WTC). Calculated for the means [tHb] WTC of the paired players (player 1 and 2) using the fixed factors ‘Channel’ (channel 1–4) and ‘Condition’ (baseline vs rest vs 2-back vs 3-back vs 4-back) for the heart rate (HR) frequency in the range of 0.7–4 Hz (corresponding to period from 0.25 s to 1.5 s) and the low-frequency oscillations (LFOs) defined from 0.06 to 0.2 Hz (corresponding to period from 5 s to 16 s).

<table>
<thead>
<tr>
<th>Condition</th>
<th>HR</th>
<th>LFOs</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>$F_{1.478} = 10.106$</td>
<td>$F_{1.001} = 0.003$</td>
</tr>
<tr>
<td>baseline vs rest</td>
<td>$p = 0.001**$</td>
<td>$p = 0.001**$</td>
</tr>
<tr>
<td>baseline vs 2-back</td>
<td>$p = 0.003**$</td>
<td>$p = 0.001**$</td>
</tr>
<tr>
<td>baseline vs 3-back</td>
<td>$p = 0.001**$</td>
<td>$p = 0.001**$</td>
</tr>
<tr>
<td>baseline vs 4-back</td>
<td>$p = 0.001**$</td>
<td>$p = 0.001**$</td>
</tr>
<tr>
<td>rest vs 2-back</td>
<td>$p = 1.000$</td>
<td>$p = 0.003**$</td>
</tr>
<tr>
<td>rest vs 3-back</td>
<td>$p = 0.001**$</td>
<td>$p = 0.140$</td>
</tr>
<tr>
<td>rest vs 4-back</td>
<td>$p = 0.598$</td>
<td>$p = 0.598$</td>
</tr>
<tr>
<td>2-back vs 3-back</td>
<td>$p = 0.995$</td>
<td>$p = 0.995$</td>
</tr>
<tr>
<td>2-back vs 4-back</td>
<td>$p = 1.000$</td>
<td>$p = 1.000$</td>
</tr>
<tr>
<td>3-back vs 4-back</td>
<td>$p = 1.000$</td>
<td>$p = 1.000$</td>
</tr>
</tbody>
</table>

To further validate these findings, Fig. 4(B–D) illustrates the cross wavelet transform (XWT) and the continuous wavelet transform (CWT) of the time series from the same player pair. Fig. 4(B) shows that the common power between the two players is highest around time 40 s. Further, the two Fig. 4(C and D) show that there are also changes of power of the individual time series in the frequency domain during the task period relative to the rest period for either player. These changes are most prominent around time 40 s, 70 s and 90 s visible in both frequency bands. However no significant relation was found between these power changes and the coherence increase observed in Fig. 4(A) (paired t-test, all p-values $p \geq 0.2$). In other words, individual time series analysis did not reveal coherence related patterns of brain activity and are therefore not the source of the coherence increase observed between the two time series.

For quantification of the coherence increase over all player pairs data were parsed as described above for the fixed factor ‘Condition’ (baseline vs rest vs 2-back vs 3-back vs 4-back). Multivariate ANOVA (Table 1, Fig. 5A) of the heart rate frequency bands revealed a main effect of the fixed factor ‘Condition’ indicating a significantly larger coherence increase during the task phase as compared to baseline. A similar pattern was observed for the LFOs frequency band, i.e. again a main effect of the factor ‘Condition’ indicating a significantly larger coherence increase during the task phase as compared to baseline. Within the task phase, both frequency bands showed the largest increase in between-brain coherence for trials of 2-back performance as compared to the coherence values for 3-back and 4-back performance. The main difference between the two frequency bands was observed in the coherence values for the rest period; whereas the heart rate frequency showed coherence values for the rest period equal or even larger than those calculated for the task phase, LFOs exhibited significantly smaller coherence values for the rest period as compared to the task phase. None of the frequency bands revealed an effect of the fixed factor ‘Channel’.

4.3. Averaged hemodynamic responses

Last, we calculated the mean [tHb] hemodynamic responses for the paired and the single players and assessed significant differences using multi- and univariate ANOVA (Table 2, Fig. 5B and C). Results revealed that all players, both paired and single players, elicited larger [tHb] responses during the task phase as compared to the rest period; this finding was highly significant and consistent over all n-back conditions. Further, while neither the paired nor the single players revealed significant differences between 2-back, 3-back or 4-back performance, the single player group showed significant largest averaged hemodynamic changes in response to the 5-back performance. Last, an overall comparison of the mean [tHb] responses within the task phase between players revealed significantly smaller [tHb] responses in the single players as compared to the paired players. The latter finding indicates that playing the n-back task in a joint manner resulted in significant larger prefrontal responses over the whole task performance as compared to their single player comparisons.

No effects of the factor ‘channel’ were observed. Therefore, since no consistent patterns were found for the factor ‘channel’ neither for the coherence values nor for the averaged hemodynamic responses, we do not address topographical aspects in the following discussion.

5. Discussion

We present behavioral and hemodynamic data recorded in paired and single players simultaneously during performance of a dual n-back task. Our experiment was motivated by the hypotheses that performing the n-back task in a joint manner may result in (1) better behavioral performance as compared to single task performance, which in turn may be reflected in (2) increased between-brain connectivity and/or (3) larger hemodynamic responses over prefrontal cortices.

5.1. Behavioral data

To address our first question, i.e. that joint task performance would lead to better overall behavioral results as compared to single performance, n-back task data were compared between groups. Results revealed significant higher mean n-back performances for the single as compared to the paired players (Fig. 3). Hence, these findings gave a negative answer our first question. Two points might have contributed to this outcome. First, it could be suggested that behavioral results might have shown different values when including subjects previously trained in n-back task performance. Few previous studies [31,59,60] reported improved performance after training of n-back tasks, both for the n-back task performance itself as well as for its transfer of learning to other tasks involving non-verbal fluid intelligence (GF) (definition see Section 2.1). This assumption is based on the idea that working memory, such as assessed with an n-back task and GF correlate [61] although research still needs to entangle the relationship between the behavioral and neural level [62]. Second, accordingly it could be reasoned that a comparison between single and paired n-back task performances involving the same subjects, i.e. analyzed first during single task and then during paired player task, might have been more significant in terms of improved performance than with our two groups (which did not involve the same subjects). These two points therefore require further research.

5.2. Functional connectivity

Wavelet coherence analysis has been previously used in neuroscience to study functional connectivity between time series, e.g. [63–67] and has been recently applied to assess between-brain coherence derived from simultaneous recorded fNIRS time series in two interacting subjects [25].

Addressing our second question, the main finding of our study is that joint task performance revealed increased between-brain coherence in the paired players as compared to baseline as illustrated in an exemplary subject pair in Fig. 4(A) and plotted over all subjects in Fig. 5(A). Our results are validated by the fact that the
between-brain coherence increase was not reflected in the changes of power of the players. This finding answered our second question positively, i.e. playing the n-back task in a joint manner results in increased between-brain connectivity. This indicates that the simultaneous collection and analysis of brain activity from multiple interacting subjects can reveal an additional layer of information in the study of between-brain performance [25].

Two frequency bands were observed to contribute to this between-brain coherence pattern, i.e. the heart rate frequency and LFOs. First, concerning the heart rate frequency band, few previous

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**Table 2**

(Top) Univariate ANOVA main effects and post hoc comparison: Averaged [tHb] hemodynamic responses within players. Calculated for mean [tHb] responses of the paired players (player 1 and 2) and the single players using the fixed factor ‘Condition’ (rest vs 2-back vs 3-back vs 4-back). No effects were observed for the fixed factor ‘Channel’ (channel 1–4). (Bottom) Univariate ANOVA main effects and post hoc comparison: Averaged [tHb] hemodynamic responses between players. Calculated for mean [tHb] responses using the fixed factor ‘Player’ (player 1 vs player 2 vs single players). Shown are F-statistics with degrees of freedom (F(df)) and significant p-values are highlighted with (**).

<table>
<thead>
<tr>
<th></th>
<th>Condition</th>
<th>Post hoc</th>
<th>Player 1</th>
<th>F(df)</th>
<th>Sig.</th>
<th>Player 2</th>
<th>F(df)</th>
<th>Sig.</th>
<th>Single players</th>
<th>F(df)</th>
<th>Sig.</th>
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<tbody>
<tr>
<td></td>
<td>rest vs 2-back</td>
<td>p = 0.001**</td>
<td>F4 = 8.790</td>
<td>p ≤ 0.001**</td>
<td></td>
<td>rest vs 2-back</td>
<td>p = 0.001**</td>
<td>p ≤ 0.001**</td>
<td>rest vs 5-back</td>
<td>F5 = 37.195</td>
<td>p ≤ 0.001**</td>
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<tr>
<td></td>
<td>rest vs 3-back</td>
<td>p = 0.001**</td>
<td>p = 0.001**</td>
<td>p ≤ 0.001**</td>
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<td>rest vs 3-back</td>
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<td>p ≤ 0.001**</td>
<td>2-back vs 3-back</td>
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<td>rest vs 4-back</td>
<td>p = 0.001**</td>
<td>p = 0.001**</td>
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<td>rest vs 4-back</td>
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<td>2-back vs 4-back</td>
<td>p = 1.000</td>
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<td>2-back vs 4-back</td>
<td>p = 1.000</td>
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<td>3-back vs 4-back</td>
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<td>3-back vs 5-back</td>
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<td>3-back vs 5-back</td>
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<td>4-back vs 5-back</td>
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<td>4-back vs 5-back</td>
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<td>4-back vs 5-back</td>
<td>p = 1.000</td>
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<th>Player 1 vs player 2 vs single players</th>
<th>F(df)</th>
<th>Sig.</th>
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<td>Player</td>
<td>F4 = 13.492</td>
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<td>player 1 vs player 2</td>
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<td></td>
<td></td>
<td>player 1 vs single players</td>
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<td>player 2 vs single players</td>
<td>p = 0.025*</td>
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studies have shown that synchronization between two persons' heart rates can occur potentially reflecting a physiological basis for two-person action coordination [68,69]. Two different possible underlying processes could be suggested as explanation for heart rate synchronization between two persons. (1) The first process might be suggested in the case where two persons receive the same sensory input, such as watching same visual sceneries or performing a similar activity. Here, the heart rate changes in synchrony, however no direct coupling happens between the two persons. For example, Konvalinka et al. [68] investigated the correlation of heart rate dynamics in a Spanish fire-walking ritual between firewalkers and spectators. The authors reported that physiological effects of synchronized arousal were reflected in heart rate synchronization between active participants and related spectators, but not between participants and other non-related members of the audience. Müller et al. [69] reported between-person synchronization in heart rate oscillations among singers and one conductor engaged in choir singing. Both synchronization in respiration and heart rate variability increased significantly during singing relative to a rest condition. (2) The second process might be suggested in the case of a real physiological exchange between two persons, such as during direct communication or shared mental activity. For example, McCraty et al. [70] investigated heart–brain interaction effects across larger distances in subject pairs who were not in physical contact by combining EEG in one subject and electrocardiography (ECG) in another subject. Data showed that when two people are at a conversational distance, the electromagnetic signal generated by one person’s heart can synchronize with the other person’s brain rhythms, i.e. synchronization can occur between the alpha waves in one person’s EEG and the other’s ECG signal.

Based on these previous works, while we cannot determine from our data whether the second explanation mentioned above could be taken into account, we suggest that the heart rate synchronization observed between our players might reflect a process according to the first explanation. It could be assumed that the mental focus of joint concentration exhibited by our subjects during the joint task performance might have led to the increased heart rate coherence. An open question remains the interpretation of the coherence increase observed during the rest period. This finding might reflect processes according to the second explanation described above or might be merely a consequence of the heart rate relaxation after the joint task. What the coherence increase in the rest period tells us would require control conditions in further studies, such as by comparing different rest conditions involving variations in gaze point, eye position and/or visual fixation.

Second, the finding in the LFOs frequency band might be in line with previous studies that have discussed the origin of spontaneous slow oscillations in cerebral hemodynamic responses in single subjects. Two mechanisms might have contributed to our findings. (1) An increase in the activity of the sympathetic nervous system, i.e. induced by the mental effort during the task phase, can lead to an increase in the amplitude of the so-called Mayer waves (0.1 Hz) [71], which correspond to LFOs with a centre frequency of approx. 0.1 Hz in humans [53,54]. This in turn, might underlie the task related increase in coherence: increased amplitude of LFOs in both players might have resulted in an increased coherence between the players’ brains. (2) Another, second cerebral mechanism which has received much attention in the last decade could also be considered for the coherence found in LFOs. Studies of the functional properties of those oscillations have identified a network of connected brain regions that are active in the resting brain [55–58]. This network, also called the default mode network (DMN) or task-negative network (TN), is characterized by coherent neuronal oscillations at a rate lower than 0.1 Hz and includes medial prefrontal cortex (mPFC), posterior cingulate cortex (PCC) and medial, lateral and inferior parietal cortex [72–75]. In contrast, during goal-oriented activity, the TNN is deactivated and another network, the task-positive network (TPN), is activated including dorsolateral prefrontal cortex (dPFC), inferior parietal cortex (IPC) and supplementary motor area (SMA) [73,74], i.e. regions more commonly reported to be active following the transition to attention-demanding tasks [76]. The TNN deactivations are further related to the amount of focused attention a task requires, i.e. deactivation is greatest for the most difficult and attention-demanding task conditions [56,57], and a task must be suitably cognitively challenging for DMN deactivation to occur [72]. Hence, the interplay between TNN and TPN may be considered elements of a single default network with anti-correlated components [73,74,77].

Although the cognitive significance of the LFOs coherence patterns observed in the present study remains unclear, we suggest that the difference between the rest period and the task phase may reflect processes of the underlying TNN and TPN networks within the paired players. In particular, the TNN that is thought to correspond to task-independent introspectively focused thought might have been active during the rest condition, i.e. reflecting again the two players’ joint attention focus during that resting state, while the TPN that is thought to correspond to action and extrospective attentional orienting might have been active during the task phase, i.e. reflecting the task related mental focus of the paired players.

Last, we want to discuss the time course of the coherence increase as shown in Fig. 4 as well as the overall larger coherence increase during 2-back performance that occurred in both frequency bands. First, in the sample of the paired players shown in Fig. 4(A), the main coherence increase was observed in the middle of the task round, i.e. around 40–80 s, as compared to the start and the end of the task round. A similar pattern was found in the other paired players. In addition, we found that 2-back performance elicited a greater coherence increase as compared to 3- and 4-back performance. Two factors might have contributed to these findings. On the one hand, the start of all sessions was set at the 2-back level; at this point subjects might have been still more alert as compared to the end of a session, during which 3- and 4-back levels occurred more frequently and completed the sessions. Hence, the difference between alertness vs fatigue could have elicited a stronger vs weaker link between the paired players and therefore contributed to the larger coherence increase during the middle of the task as well as the 2-back as compared to 3- and 4-back levels. On the other hand, this finding could be a reflection of increasing difficulty. The enhanced difficulty levels at the end of a session, i.e. 3- and 4-back levels, could have disrupted proper joint task attention and thus decreased synchronization, while lower difficulty levels, i.e. 2-back levels, could have allowed for proper joint task performance and thus increased synchronization. This interpretation might hold true for both of the frequency bands, the heart rate and the LFOs.

All things considered, one major question remains: how do the heart rate and the LFOs synchronize even though there is no direct connection, and no common perceptible cue that would trigger synchronization? These effects may not only be relevant for neuroscience task configurations but also in related social experimental research. The results of our study require confirmation both thematically and methodically and might motivate additional investigations, of whether there is a relevant neural effect between jointly acting subjects, which has not been considered so far.

5.3. Averaged hemodynamic responses

Last, addressing our third question, we performed a comparison of the mean [Hb] responses using multivariate ANOVA (Table 2, Fig. 5B and C). Results revealed first, that both the paired and the
single players elicited larger [tHb] responses during the task phase as compared to the rest period and second, that paired players elicited significantly larger [tHb] responses as compared to the single players. This together answered our third question positively, i.e. that n-back task performance in a joint manner results in larger hemodynamic responses as compared to playing alone.

5.4. Experimental considerations and limitations

The following aspects will need to be validated and confirmed in a future study using a larger number of subjects to improve the power of the analysis.

First, concerning the experimental design, we are aware that the difference in the number of squares in the paired player as compared to the single player group could have contributed to differences in performance between the two groups. In particular, the additional second square in the paired player group, as compared to only one square in the single player group, might be one very simple explanation for differences between joint and individual task performance because there was less distracting information in the visual display in the single player group.

Second, a limitation of the presented study is that we did not measure the activity of the autonomous nervous system (ANS), for example using methods such as photocorticography (EDC). An assessment of the ANS might have supported an explanation of the increase in LFOs between-brain coherence. Moreover, frequency oscillations do not exist only in the cerebral tissue but have also been shown to be detectable in other human organs, such as the skin or the muscle, where the sympathetic nervous system has an influence [78,79]. Hence, it could have been investigated whether the activity of the ANS shows a comparable synchronization and whether this activity had a similar time course to the coherence increase during task performance. This would have further supported our discussion concerning the underlying processes observed in the frequency band of the LFOs.

Last, a general methodological fNIRS limitation is subject-to-subject variability in signal location that can occur due to sensor positioning. Although, external landmarks are used for sensor positioning using the international 10–20 system [80,81], these landmarks offer only probabilistic guidelines for individual differences in location. Hence, as with several other non-invasive brain imaging methods (e.g., EEG) anatomical information and variability between individuals are not directly obtained, making the localization of externally recorded signals difficult with respect to the underlying brain. These and the limitation of the usually restricted fNIRS sample volume [81] in our study may have led to differences in exact location of the interrogated tissue from subject to subject. Hence, by using Fp1 as landmark, we could only assume to cover prefrontal cortices in the individual subjects.

6. Conclusion

Our findings provide insight into two-person neuroscience by means of between-brain connectivity during joint task performance recorded using fNIRS. Evaluating functional connectivity using coherence analysis between the two players’ brains, revealed (1) significantly larger between-brain coherence in the heart rate frequency and in LFOs during joint task performance as compared to baseline. (2) Averaged hemodynamic responses revealed larger responses in total hemoglobin concentration changes [tHb] for the paired players as compared to the single players; in addition, within the paired players groups joint task performance revealed larger changes in [tHb] as compared to a rest period and to a baseline condition. (3) No increase in behavioral performance was found in the paired players as compared to the single players. Our findings designate fNIRS as suitable tool for monitoring between-brain connectivity between two subjects, which can provide additional insight into two-person activation patterns not detectable using typical one-person experiments. The results of the present study aim to show the potential of simultaneously assessing brain hemodynamic responses in jointly acting subjects for various other research areas where interpersonal personal performances are involved. The described phenomena pose an interesting question: How do two interacting subjects synchronize their oscillations without a direct connection or perceptible cue? This opens interesting new research approaches.

Conflicts of interest

The authors have no conflicts of interest.

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References


