Journal of Visualized Experiments

Measurement of the Directional Information Flow in fNIRS-Hyperscanning Data Using the Partial Wavelet Transform Coherence Method

--Manuscript Draft--

Article Type:	Invited Methods Collection - JoVE Produced Video
Manuscript Number:	JoVE62927R2
Full Title:	Measurement of the Directional Information Flow in fNIRS-Hyperscanning Data Using the Partial Wavelet Transform Coherence Method
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TITLE:

Measurement of the Directional Information Flow in fNIRS-Hyperscanning Data Using the Partial Wavelet Transform Coherence Method

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SUMMARY:

This protocol describes partial wavelet transform coherence (pWTC) for calculating the time-lagged pattern of interpersonal neural synchronization (INS) to infer the direction and temporal pattern of information flow during social interaction. The effectiveness of pWTC in removing the confounds of signal autocorrelation on INS was proved by two experiments.

ABSTRACT:

Social interaction is of vital importance for human beings. While the hyperscanning approach has been extensively used to study interpersonal neural synchronization (INS) during social interactions, functional near-infrared spectroscopy (fNIRS) is one of the most popular techniques for hyperscanning naturalistic social interactions because of its relatively high spatial resolution, sound anatomical localization, and exceptionally high tolerance of motion artifacts. Previous fNIRS-based hyperscanning studies usually calculate a time-lagged INS using wavelet transform coherence (WTC) to describe the direction and temporal pattern of information flow between individuals. However, the results of this method might be confounded by the autocorrelation effect of the fNIRS signal of each individual. For addressing this issue, a method termed partial wavelet transform coherence (pWTC) was introduced, which aimed to remove the autocorrelation effect and maintain the high temporal-spectrum resolution of the fNIRS signal. In this study, a simulation experiment was performed first to show the effectiveness of the pWTC in removing the impact of autocorrelation on INS. Then, step-by-step guidance was offered on the operation of the pWTC based on the fNIRS dataset from a social interaction experiment. Additionally, a comparison between the pWTC method and the traditional WTC method and that

between the pWTC method and the Granger causality (GC) method was drawn. The results showed that pWTC could be used to determine the INS difference between different experimental conditions and INS's directional and temporal pattern between individuals during naturalistic social interactions. Moreover, it provides better temporal and frequency resolution than the traditional WTC and better flexibility than the GC method. Thus, pWTC is a strong candidate for inferring the direction and temporal pattern of information flow between individuals during naturalistic social interactions.

INTRODUCTION:

Social interaction is of vital importance for human beings[1,2]. For understanding the dual-brain neurocognitive mechanism of social interaction, the hyperscanning approach has recently been extensively used, showing that the patterns of interpersonal neural synchronization (INS) can well characterize the social interaction process[3–14]. Among recent studies, an interesting finding is that the role difference of individuals in a dyad may lead to a time-lagged pattern of INS, i.e., INS occurs when the brain activity of one individual lags behind that of another individual by seconds, such as that from listeners to speakers[5,9], from leaders to followers[4], from teachers to students[8], from mothers to children[13,15], and from women to men in a romantic couple[6]. Most importantly, there is a good correspondence between the interval of the time-lagged INS and that of social interaction behaviors, such as between teachers questioning and students answering[8] or between parenting behaviors of mothers and compliance behaviors of children[15]. Thus, time-lagged INS may reflect a directional information flow from one individual to another, as proposed in a recent hierarchical model for interpersonal verbal communication[16].

Previously, the time-lagged INS was mainly calculated on the functional near-infrared spectroscopy (fNIRS) signal because of its relatively high spatial resolution, sound anatomical localization, and exceptionally high tolerance of motion artifacts[17] when studying naturalistic social interactions. Moreover, to precisely characterize the correspondence between the neural time lag and the behavioral time lag during social interaction, it is essential to obtain the INS strength for each time lag (e.g., from no time lag to a time lag of 10 s). For this purpose, previously, the wavelet transform coherence (WTC) procedure was extensively applied after shifting the brain signal of one individual forward or backward relative to that of another individual[5,6,18]. When using this traditional WTC procedure for fNIRS signals, there is a potential challenge because the observed time-lagged INS may be confounded by the autocorrelation effect of the fNIRS signal for an individual[19–21]. For example, during a dyadic social interaction process, the signal of participant A at time point *t* may be synchronized with that of participant B at the same time point. Meanwhile, the signal of participant A at time point *t* may be synchronized with that of participant A at a later time point *t*+1 because of the autocorrelation effect. Therefore, a spurious time-lagged INS may occur between the signal of participant A at time point *t* and that of participant B at time point *t*+1.

Mihanović and his colleagues[22] first introduced a method termed partial wavelet transform coherence (pWTC), and then applied it in marine science[23,24]. The original purpose of this method was to control the exogenous confounding noise when estimating the coherence of two signals. Here, to address the autocorrelation issue in the fNIRS hyperscanning data, the pWTC method was extended to calculate time-lagged INS on the fNIRS signal. Precisely, a time-lagged INS (and a directional information flow) from participant A to participant B can be calculated using the equation below (**Equation 1**)[23].

$$\mathrm{pWTC}_{\mathrm{BtoA}} = \frac{\left|\sqrt{\mathrm{WTC}(\mathrm{B_t},\mathrm{A_{t+n}})} - \sqrt{\mathrm{WTC}(\mathrm{A_t},\mathrm{A_{t+n}})} \cdot \sqrt{\mathrm{WTC}(\mathrm{A_t},\mathrm{B_t})}^{*}\right|^2}{[1\text{-}\mathrm{WTC}(\mathrm{A_t},\mathrm{A_{t+n}})] \cdot [1\text{-}\mathrm{WTC}(\mathrm{A_t},\mathrm{B_t})]}$$

Here, it is assumed that there are two signals, *A* and *B,* from participants A and B, respectively. The occurrence of signal *B* always precedes that of signal *A* with a time lag of *n*, where $WTC(A_t, B_{t+n})$ is the traditional time-lagged WTC. $WTC(A_t, A_{t+n})$ is the autocorrelated WTC in participant A. $WTC(A_t, B_t)$ is the time-aligned WTC at time point *t* between participant A and B. * is the complex conjugate operator (**Figure 1A**).

[Place **Figure 1** here]

This protocol first introduced a simulation experiment to demonstrate how well the pWTC resolves the autocorrelation challenge. Then, it explained how to conduct pWTC in a step-by-step way based on an empirical experiment of naturalistic social interactions. Here, a communication context was used to introduce the method. This is because, previously, the time-lagged INS was usually calculated in a naturalistic communication context[3,4,6,8,13,15,18]. Additionally, a comparison between the pWTC and the traditional WTC and validation with the Granger causality (GC) test were also conducted.

PROTOCOL:

The human experiment protocol was approved by the Institutional Review Board and Ethics Committee of the State Key Laboratory of Cognitive Neuroscience and Learning at Beijing Normal University. All participants gave written informed consent before the experiment began.

1. The simulation experiment

1.1. Generate two time series of signals that correlate with each other, with one signal having autocorrelation at a 4 s time lag. Set the correlation coefficient of r between the two signals to 0.4.

1.2. Furthermore, generate two time series of signals without any correlation but with autocorrelation in one signal.

1.3. Calculate values of traditional 4 s time-lagged INS with **Equation 2** based on the generated signals with or without correlation, which can be named time-lagged $\mathrm{INS_{WTC}}$ with autocorrelation and time-lagged baseline $\mathrm{INS_{WTC}}$ with autocorrelation.

NOTE: Here, the traditional time-lagged WTC is expressed by the following equation (**Equation 2**)[25]:

$$\mathrm{WTC}(W_t, M_{t+n}) = \frac{\left|S\left(C^{*}_{W_t}(i,t) \cdot C_{M_{t+n}}(i,t)\right)\right|^2}{S\left(\left|C_{W_t}(i,t)\right|^2\right) \cdot S\left(\left|C_{M_{t+n}}(i,t)\right|^2\right)}$$

where, *C* denotes the continuous wavelet transform operator at different scales *i* and time points *t*. *S* denotes the smoothing operator. * denotes the complex conjugate operator. W and M indicate two individual time series of signals.

1.4. Remove autocorrelation from the generated signals. Then, calculate the values of traditional 4 s time-lagged INS_{WTC} with **Equation 2** based on the generated signals with or without correlation, which can be named time-lagged INS_{WTC} without autocorrelation and time-lagged baseline INSWTC without autocorrelation.

1.5. Calculate the values of 4 s time-lagged pWTC with **Equation 3** based on the generated signals with or without correlation, named time-lagged INS_{pWTC} and time-lagged baseline INS_{pWTC}.

NOTE: The pWTC can be calculated based on the following equation (**Equation 3**)[23]:

$$\mathrm{pWTC}_{\mathrm{WtoM}} = \frac{\left|\sqrt{\mathrm{WTC}(W_t, M_{t+n})} - \sqrt{\mathrm{WTC}(M_t, M_{t+n})} \cdot \sqrt{\mathrm{WTC}(W_t, M_t)}^{*}\right|^2}{\left[1 - \mathrm{WTC}(M_t, M_{t+n})\right] \cdot \left[1 - \mathrm{WTC}(W_t, M_t)\right]}$$

where, $WTC(W_t, M_{t+n})$ is the traditional time-lagged WTC. $WTC(M_t, M_{t+n})$ is the autocorrelated WTC of one individual. $WTC(W_t, M_t)$ is the time-aligned WTC. * is the complex conjugate operator.

1.6. Repeat the above procedures 1000 times.

1.7. After subtracting the baseline INS, compare the results of time-lagged INS_{WTC} with autocorrelation, time-lagged INSWTC without autocorrelation, and time-lagged INS_{pWTC} using the analyses of variance (ANOVA) method.

NOTE: Here, it is expected that the time-lagged INS_{WTC} with autocorrelation will be significantly higher than the time-lagged INS_{WTC} without autocorrelation and the time-lagged INS_{pWTC}, and no significant difference is expected between the time-lagged INS_{WTC} without autocorrelation and the time-lagged INS_{pWTC}.

2. The empirical experiment

2.1. Participants and procedure

2.1.1. Recruit appropriate participants.

NOTE: In this study, twenty-two pairs of close opposite-sex friends (mean age of women = 20.95, standard deviation (SD) = 1.86; mean age of men = 20.50, SD = 1.74) were recruited through advertising from undergraduates of universities in Beijing. All participants were right-handed and had normal or corrected-to-normal vision. Furthermore, no participants had any language, neurological, or psychiatric disorders.

2.1.2. Ask each pair of participants to sit face-to-face during the experiment. Ask them to communicate freely on a supportive topic in one session and on a conflict topic in the other session.

NOTE: The topics were used to induce the intended positive or negative emotional valence. Each communication session lasted 10 min, and the order of topics was counterbalanced.

2.1.3. Ask the participants to report about the supportive and the conflict topics as a standard set-up rule. Ask each partner to rate the positive or negative valence level that might have been induced on a definite point scale. Then, rank the reported topics according to the rating.

NOTE: In this work, the topics were selected with the following three steps. First, for the supportive topics, each participant was required to report 1–3 personal issues related to what she/he wanted to improve in her/his life. Each participant was required to report 1–3 cases that had induced or would induce conflict between them or that might endanger their relationship for the conflict topics. Second, each partner was required to rate the level of positive or negative valence each topic might induce on a 7-point scale (1 = not at all, and 7 = very much). Third, the reported topics were ranked according to the rating. The first two topics in the list of supportive topics and conflict topics were selected.

2.2. fNIRS data collection

2.2.1. Use 26-channel fNIRS topography system (see **Table of Materials**) to collect fNIRS data.

NOTE: Two customized optode probes set covered the bilateral frontal, temporal, and parietal cortices (**Figure 1B**).

2.2.2. Precisely, ask each participant to wear a cap with two customized probe sets (see **Table of Materials**).

2.2.3. Align the nasion, inion, and ear mastoids with Fpz, Opz, T7, and T8, which are typical landmarks of 10–20 international system[26].

2.2.4. Align channel (CH) 11 to T3 and CH25 to T4 following the international 10–20 system for the two probe sets[27,28].

2.2.5. Validate the anatomical locations of probe sets by scanning magnetic resonance imaging (MRI) data from a typical participant with a high-resolution T1-weighted magnetization-prepared rapid gradient-echo sequence (TR = 2530 ms; TE = 3.39 ms; flip angle = 7°; slice thickness = 1.3

mm; voxel size = 1.3 x 1 x 1.3 mm).

2.2.6. Use Statistical Parametric Mapping 12 (SPM12) to normalize the image to standard Montreal imaging institute coordinate (MNI coordinate) space[29]. Then, use the NIRS_SPM toolbox (see **Table of Materials**) to project the MNI coordinates of the probes to the automated anatomical labeling (AAL) template.

2.2.7. Collect the optical density data of near-infrared light at three wavelengths (780, 805, and 830 nm) at a sampling rate of 55.6 Hz (equipment default parameters).

2.2.8. Test the signal quality by using fNIRS topography system built-in equipment software (see **Table of Materials**).

2.2.9. Begin signal recording.

NOTE: Some published protocols have demonstrated how to collect fNIRS signals with various equipment and systems[30–32].

2.3. fNIRS data preprocessing

2.3.1. Export the data files from the equipment.

NOTE: In the current experiment, the built-in software automatically converted all-optical density data into oxyhemoglobin (HbO) concentration changes based on the modified Beer-Lambert law.

2.3.2. Remove the first and last 15 s of data for each session to avoid transient responses.

2.3.3. Use the MATLAB decimate built-in function to downsample the data from 55.6 Hz to 11.1 Hz.

NOTE: The power spectrum patterns between 55.6 Hz and 11.1 Hz are quite similar (**Supplementary Figure 1**).

2.3.4. Use the built-in MATLAB application function (Homer3, see **Table of Materials**) with appropriate filtering function to apply the discrete wavelet transform filter method to correct motion artifacts.

2.3.5. Use the MATLAB pca built-in function to remove global physiological noise. Remove the top 80% of the variance from the signals.

2.3.6. Remove physiological noise based on the previous studies[33]. Precisely, remove frequency bands of each signal above 0.7 Hz to avoid aliasing of high-frequency physiological noise (e.g., cardiac activity).

2.3.7. Then, remove frequency bands of each signal below 0.01 Hz to filter out very-low-frequency fluctuations.

2.3.8. Finally, remove frequency bands of each signal within 0.15–0.3 Hz to exclude the potential impact of respiratory activity.

2.4. First-level fNIRS Data Processing

2.4.1. First, calculate INS using traditional WTC (INS_{WTC}).

NOTE: Here, a women-led time-lagged pattern of INS_{WTC} was predicted to occur between the brain activity of women and that of men because previous studies have suggested different roles of women and men during a conversation[34,35]. The traditional WTC calculated this pattern of INS_{WTC} by shifting the brain activity of men backward relative to that of women (see **Equation 2**).

2.4.2. Calculate the women-led 2 s-lagged INS_{WTC} value after removing the initial 2 s of data from women and the last 2 s of data from men with **Equation 2**. Similarly, after removing the initial 2 s of data from men and the last 2 s of data from women, calculate the men-led 2 s-lagged INS_{WTC} value with **Equation 4**.

NOTE: Here, the wcoherence function, which is a built-in function of the wavelet toolbox of MATLAB, was used (see **Table of Materials**).

2.4.3. Repeat this procedure with different time lags n, i.e., n = 2 s, 4 s, 6 s, 8 s across all potential CH pairs (e.g., CH2 in women and CH10 in men, 676 pairs in total). Additionally, calculate the strength of men-led time-lagged INS_{WTC} the same way (**Equation 4**).

$$WTC(M_t,W_{t+n}) = \frac{|S(C^{*}_{M_t}(i,t)\cdot C_{W_{t+n}}(i,t))|^2}{S(|C_{M_t}(i,t)|^2)\cdot S(|C_{W_{t+n}}(i,t)|^2)}$$

2.4.4. Second, calculate INS using pWTC (INS_{pWTC}).

NOTE: pWTC was calculated based on **Equation 3**. The calculation of INS_{pWTC} was repeated with different time lags n, i.e., n = 2 s, 4 s, 6 s, 8 s across all potential channel pairs (e.g., CH2 in women and CH10 in men, 676 pairs in total). Additionally, the strength of the men-led time-lagged INS_{pWTC} was calculated the same way (**Equation 5**).

$$pWTC_{MtoW} = \frac{\left|\sqrt{WTC(M_t,W_{t+n})}-\sqrt{WTC(W_t,W_{t+n})}\cdot\sqrt{WTC(M_t,W_t)}^{*}\right|^2}{[1-WTC(W_t,W_{t+n})]\cdot[1-WTC(M_t,W_t)]}$$

2.4.5. Generate time-lagged time series of fNIRS signals at different time lags.

2.4.6. Calculate the values of the time-lagged WTC at different time lags.

2.4.7. Generate autocorrelated time series of fNIRS signals at different time lags. To calculate the 2 s-autocorrelated value for men, remove the first 2 s of data from the men and the last 2 s data from the men.

2.4.8. Calculate the autocorrelated WTC values at different time lags.

2.4.9. Generate time-aligned time series of fNIRS signals at different time lags. To calculate the 2 s time-aligned WTC, remove the first 2 s of data from the men and the women's first 2 s of data.

2.4.10. Calculate the time-aligned WTC values.

2.4.11. Enter time-aligned WTC, time-lagged WTC, and autocorrelated WTC values at different time lag into **Equation 3** and **Equation 5**—the equation of pWTC, generating INS_{pWTC}.

2.4.12. Finally, calculate INS using the GC method (INS_{GC}).

NOTE: To further validate the pWTC method and evaluate its advantages and disadvantages, GC-based INS was calculated using the GC method (INS_{GC}).

2.4.13. Based on the pWTC result, bandpass filters the HbO signal of each individual at the SMC (i.e., 0.4–0.6 Hz, see **Representative Results**).

2.4.14. Conduct a GC test (Econometric toolbox, MATLAB) within each dyad in the supportive and conflict topics separately.

NOTE: Four groups of F-values are obtained for INS_{GC}: (1) from women to men on the supportive topic (W2M_supp); (2) from men to women on the supportive topic (M2W_supp); (3) from women to men on the conflict topic (W2M_conf); and (4) from men to women on the conflict topic (M2 W_conf). The F-values are used to index the INS_{GC}.

2.5. Second-level fNIRS data processing

2.5.1. Transform INS with Fisher-z transformation, and then average INS at the temporal dimension.

NOTE: Here, Fisher-z transformation was conducted using a custom MATLAB script with **Equation 6**[36]:

$$z = \frac{1}{2} \cdot \ln\left(\frac{1+r}{1-r}\right)$$

where, r is the value of the WTC or pWTC, and z is the Fisher-z transformed value of the WTC or pWTC.

2.5.2. For the averaged INS at each time lag, conduct a paired two-sample *t*-test (supportive *vs*. conflict) on each CH pair across the frequency range. Then, identify all significant frequency clusters ($P < 0.05$).

2.5.3. Conduct a cluster-based permutation test to establish a threshold for the results.

2.5.3.1. Reassign dyadic relationships by randomly assigning the participants to new two-member pairs, i.e., the participants of a dyad that had never communicated with one another. Recalculate the INS at each time lag, perform paired *t*-tests again in the new sample, and identify significant frequency clusters again.

2.5.3.2. Select the cluster with the largest summed t-value. Repeat the above procedures 1000 times to generate a null distribution of the maximum false-positive t-values.

NOTE: The distribution is served as the chance level. The familywise error rate (FWER) is controlled at $q = 0.05$, which means that only the top 5% of the null distribution of the false-positive t-values exceeds the threshold (R^*).

2.5.3.3. Compare the summed t-value of each identified frequency cluster in the original sample with the null distribution to obtain significant statistical results.

2.5.4. Conduct a context (supportive, conflict) x direction (women to men, men to women) analysis of variance (ANOVA) to test the difference in INS direction between different conditions (i.e., topics) ($p < 0.05$).

2.5.5. Conduct a paired two-sample two-tailed t-test between the results of WTC (Wt, Mt + n) and WTC (Mt, Mt + n) to test the potential impact of autocorrelation on INS.

NOTE: The INS of WTC (Mt, Mt + n) reflects autocorrelation.

REPRESENTATIVE RESULTS:

Simulation results

The results showed that the time-lagged INS_{WTC} with autocorrelation was significantly higher than the time-lagged INS_{WTC} without autocorrelation ($t(1998) = 4.696$, $p < 0.001$) and time-lagged INS_{pWTC} ($t(1998) = 5.098$, $p < 0.001$). Additionally, there was no significant difference between time-lagged INS_{WTC} without autocorrelation and INS_{pWTC} ($t(1998) = 1.573$, $p = 0.114$, **Figure 2A**). These results indicate that pWTC can effectively remove the impact of the autocorrelation effect on INS.

Additionally, when the WTC value was set to be close to 0 or 1, the time-lagged INS_{pWTC} still showed reliable results when the WTC value was away from 0 or 1 (**Supplementary Figure 2**).

Empirical experiment results

INS pattern using the traditional WTC method

The results showed that at 0.04–0.09 Hz, INS_{WTC} in the sensorimotor cortex (SMC, CH20) of both women and men was significantly higher in the supportive topic than in the conflict topic when the brain activity of men lagged behind that of women by 2 s, 4 s, and 6 s (2 s: $t(21) = 3.551$, $p = 0.0019$; lag 4 s: $t(21) = 3.837$, $p = 0.0009$; lag 6 s: $t(21) = 3.725$, $p = 0.0013$). Additionally, at 0.4–0.6 Hz, INS_{WTC} in the SMC was significantly higher in the conflict topic than in the supportive topic when men's brain activity lagged behind women's by 4 s ($t(21) = 2.828$, $p = 0.01$, **Figure 2B**).

Additionally, to compare the direction of INS_{WTC} in different topics, a topic (supportive, conflict) x direction (women to men, men to women) ANOVA was first conducted on INS_{WTC} of the SMC under a 2–6 s time lag. The 0.04–0.09 Hz results did not show any significant interaction effects at any time lag (*ps* > 0.05). For the 0.4–0.6 Hz frequency range, the results showed that the interaction effect was marginally significant ($F(1, 21) = 3.23$, $p = 0.086$). Pairwise comparisons showed that INS_{WTC} from women to men was significantly higher in the conflict topic than in the supportive topic (*M.D.* = 0.014, *S.E.* = 0.005, $p = 0.015$), whereas INS_{WTC} from men to women did not differ significantly between topics (*M.D.* = 0.002, *S.E.* = 0.006, $p = 0.695$).

Finally, to test the impact of autocorrelation on the results of traditional time-lagged INS_{WTC}, INS_{WTC} was compared between $WTC(W_t, M_{t+4})$ and $WTC(M_t, M_{t+4})$ at 0.04–0.09 Hz and 0.4–0.6 Hz, respectively. Note that the INS_{WTC} of $WTC(M_t, M_{t+4})$ reflects autocorrelation. The results showed that at the 0.4–0.6 Hz, there was no significant difference between the INS_{WTC} of $WTC(W_t, M_{t+4})$ and that of $WTC(M_t, M_{t+4})$ ($t(21) = 0.336$, $p = 0.740$). At 0.04–0.09 Hz, the INS_{WTC} of $WTC(Mt, Mt+4)$ was significantly higher than that of $WTC (W_t, M_{t+4})$ ($t(21) = 4.064$, $p < 0.001$). A comparison was also conducted between the frequency ranges of 0.04–0.09 Hz and 0.4–0.6 Hz regarding INS_{WTC} of $WTC(M_t, M_{t+4})$. The results showed that the INS_{WTC} of $WTC(M_t, M_{t+4})$ was significantly higher at 0.04–0.09 Hz than at the 0.4–0.6 Hz ($t(21) = 5.421$, $p < 0.001$). These results indicate that the time-lagged INS_{WTC} was affected by autocorrelation in both the low- and high-frequency ranges, but the impact was larger for the lower-frequency range than for the higher-frequency range.

INS pattern using the pWTC method

The results showed that the difference in INS_{pWTC} between the conflict and supportive topics reached significance at the SMC of both women and men at 0.4–0.6 Hz when male brain activity lagged behind that of women by 4 s ($t(21) = 4.224$, $p = 0.0003$). At 0.04–0.09 Hz; however, no significant results were found, nor were their effective results at other frequency ranges (*Ps* > 0.05, **Figure 2C**).

An additional ANOVA test was conducted on the INS_{pWTC} of the SMC at 0.4–0.6 Hz. The results showed that the interaction between topic and direction was marginally significant ($F(1,21) = 3.48$, $p = 0.076$). Further pairwise comparisons showed that INS_{pWTC} from women to men was significantly higher in the conflict topic than in the supportive topic (*M.D.* = 0.016, *S.E.* = 0.004, $p = 0.002$), whereas INS_{pWTC} from men to women did not differ significantly between topics (*M.D.* = 0.0007, *S.E.* = 0.006, $p = 0.907$, **Figure 2D**).

INS pattern using the GC method

An ANOVA test was conducted on the INS_{GC} at the SMC within the 0.4–0.6 Hz only. The results showed a significant interaction between topic and direction ($F(1,21) = 8.116$, $p = 0.010$). Pairwise analysis showed that INS_{GC} from women to men was significantly higher in the conflict topic than in the supportive topic ($MD = 5.50$, $SE = 2.61$, $p = 0.043$). In contrast, the INS_{GC} from men to women was not significantly different between topics ($MD = 1.42$, $SE = 2.61$, $p = 0.591$, **Figure 2E**).

[Place **Figure 2** here]

FIGURES LEGENDS:

Figure 1: Overview of pWTC. (**A**) The logic of the pWTC. There are two signals *A* and *B,* within a dyad. The occurrence of *A* always follows that of *B* with a lag *n*. A gray box is a wavelet window at a certain time point *t* or *t+n*. Based on the pWTC equation (represented in the figure), three WTCs need to be calculated: the time-lagged WTC of A_{t+n} and B_t; the autocorrelated WTC in participant A of A_t and A_{t+n}; and the time-aligned WTC at timepoint *t*, A_t and B_t. (**B**) The layout of optode probe sets. CH11 was placed at T3, and CH25 was placed at T4 following the international 10–20 system[27,28].

Figure 2: Results of the simulation and empirical experiment. (**A**) The simulation results of three simulated samples. The time-lagged INS_{WTC} with autocorrelation was significantly higher than time-lagged INS_{WTC} without autocorrelation and INS_{pWTC}. There was no significant difference between time-lagged INS_{WTC} without autocorrelation and pWTC. (**B**) The t-map of INS_{WTC} in the empirical experiment, showing significant context effects within 0.04–0.09 Hz when SMC activity of men lagged behind that of women by 2–6 s. There was also a marginally considerable context effect within 0.4–0.6 Hz when SMC activity of men lagged behind that of women by 4 s. (**C**) The t-map of INS_{pWTC}, showing a significant context effect within 0.4–0.6 Hz when SMC activity of men lagged behind that of women by 4 s. (**D**) Comparison of directional INS_{pWTC} at different topics by pWTC. Directional INS from women to men is significantly higher in conflict contexts than in supportive contexts. (**E**) Validation of directional INS by GC test (INS_{GC}). The resulting pattern of INS_{GC} is similar to INS_{pWTC}.

Supplementary Figure 1: The power spectrum plot for sample rate at 11.1 Hz (blue line) and 55.6 Hz (red line). The power spectrum pattern for the two is quite similar.

Supplementary Figure 2: The pWTC maps of floor and ceil WTC. (**A**) Left panel: the time-lagged WTC map generated by two same signals, the x-axis is time point, and the y-axis is frequency-band. The mean value of WTC at all points is ~1. Right panel: the pWTC map of two similar signals. The pWTC map is quite similar to the WTC map. (**B**) Left panel: the time-lagged WTC map generated by two random signals, the x-axis is the time point, and the y-axis is the frequency-band. The mean value of WTC at all points is ~0. Right panel: the pWTC map of two similar signals. The pWTC map is quite similar to the WTC map.

DISCUSSION:

In hyperscanning studies, it is usually essential to describe the directional and temporal patterns of information flow between individuals. Most previous fNIRS hyperscanning studies have used traditional WTC[25] to infer these characteristics by calculating the time-lagged INS. However, as one of the intrinsic features of the fNIRS signal[20,21], the autocorrelation effect might confound the time-lagged INS. To address this issue, in the protocol herein, a method termed pWTC was introduced[22]. This method estimates the time-lagged INS after partially out autocorrelation and maintains the advantages of the WTC method. This protocol offers step-by-step guidance on how to conduct pWTC and validates the results of pWTC by comparing its results with those of traditional WTC and GC tests.

The critical steps of applying pWTC in fNIRS-based hyperscanning data are demonstrated in this protocol. Specifically, first, to calculate the time-lagged WTC, the autocorrelated WTC, and time-aligned WTC must be calculated based on the time-lagged fNIRS time series. Next, the pWTC are computed at different time lags according to **Equation 1**. The results of the pWTC return a time x frequency matrix, and the values in the matrix ranges from 0 to 1. Thus, further statistical tests can be conducted on these values.

In the demonstration protocol, the representative results of the traditional WTC showed two significant effects at two frequency bands: 0.4–0.6 Hz. However, the impact within the 0.04–0.09 Hz did not survive the threshold in the pWTC results, suggesting that this effect might be confounded by the autocorrelation effect of the fNIRS signal. On the other hand, the results within the 0.4–0.6 Hz range were well replicated by the pWTC method. These results indicate that after removing the autocorrelation effect, pWTC provides more sensitive and specific developments in inferring INS's directional and temporal patterns between individuals. Another possibility, however, is that pWTC is not susceptible to INS's directional and temporal patterns in lower frequency ranges than in the higher frequency ranges, resulting in underestimation of the INS effect. Future studies are needed to clarify these possibilities further.

A comparison with the GC test further supports this conclusion. The results of the GC test were quite similar to those of the pWTC, showing important information flow from women to men but not from men to women. There was a slight difference between the results of the GC test and pWTC, i.e., the interaction effect between topic and direction was marginally significant in the results of the pWTC but reached significance in the GC test. This difference may be because the pWTC is calculated at a finer timescale than the GC test. Thus, although both the pWTC and GC tests can provide reliable results when controlling for the autocorrelation effect, the pWTC is advantageous because it is not necessary to make stationary assumptions and holds a high temporal-spectrum structure.

The pWTC method also has its limitations. Similar to the GC test, the causality inferred from pWTC is not a real causality[37,38]. Instead, it only indicates a temporal relationship between the signals of A and B. This issue should be kept in mind when applying the pWTC method. Second, pWTC only partials out the autocorrelation effect. Thus, other potential concurrent variables, such as shared environments or similar actions, may still impact the results. Consequently, conclusions about the direction and temporal pattern of information flow should be drawn after controlling these confounding factors.

Additionally, there were some complicated issues about fNIRS data preprocessing. Although fNIRS has a high tolerance of head movements, motion artifacts are still the most significant source of the noise[39]. Large head movements would still lead to a position shift of the optodes, generating motion artifacts such as sharp spike and baseline shifts. To address these issues, many artifacts correction approaches were developed such as spline interpolation[40], wavelet-based filtering[39], principle component analysis[41], and correlation-based signal improvement[42], etc. Cooper and his collegues[43] have compared these approaches based on real resting-state fNIRS data and found that wavelet-based filtering produced the highest increase in contrast-to-noise ratio. Further, Brigadoi and her collegues[44] have also compared these approaches in real linguistic task data and also found that wavelet-based filtering was the most effective approach in correcting motion artifacts. Thus, in this study, wavelet-based filtering was applied and also recommended for future fNIRS hyperscanning studies.

In general, pWTC is a valuable approach in estimating the directional and temporal patterns of information flow during social interaction. More importantly, it is believed that the pWTC method is also suitable for pseudo-hyperscanning studies (i.e., signals of two or multiple brains are not collected simultaneously[45,46]). In such experiments, although the direction of information flow is fixed, it is also of interest to examine the duration of the time lag between the input of the signal and the process of the signal. Therefore, autocorrelation can also confound the results of the time-lagged INS. In the future, this method can answer many questions in hyperscanning and other interbrain studies. For example, to determine the dominant role in various social relationships, such as teachers and students, doctors and patients, and performers and audiences. Additionally, as pWTC maintains the temporal structures of INS, it is also possible to test the dynamic pattern of INS, such as group attitude convergence.

ACKNOWLEDGMENTS:
This work was supported by the National Natural Science Foundation of China (61977008) and the Young Top Notch Talents of Ten Thousand Talent Program.

DISCLOSURES:
The authors declare no competing financial interests.

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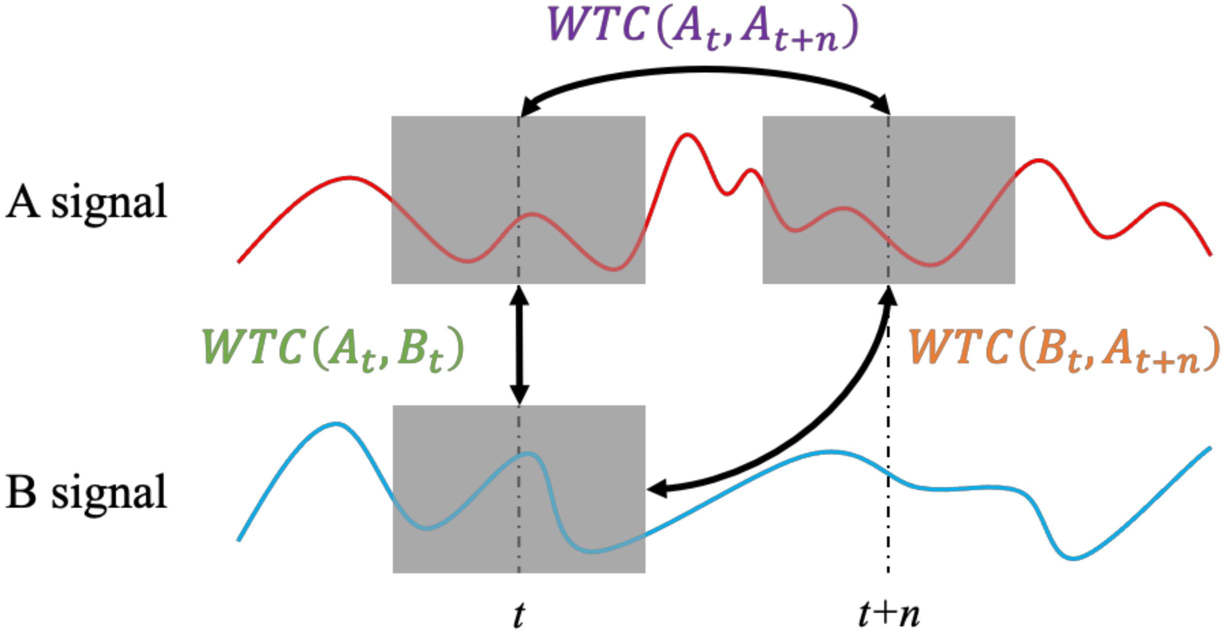
(A)

$$pWTC_{BtoA} = \frac{\left| \sqrt{WTC(B_t, A_{t+n})} - \sqrt{WTC(A_t, A_{t+n})} \cdot \sqrt{WTC(A_t, B_t)}^{*} \right|^2}{[1 - WTC(A_t, A_{t+n})] \cdot [1 - WTC(A_t, B_t)]}$$



(B)

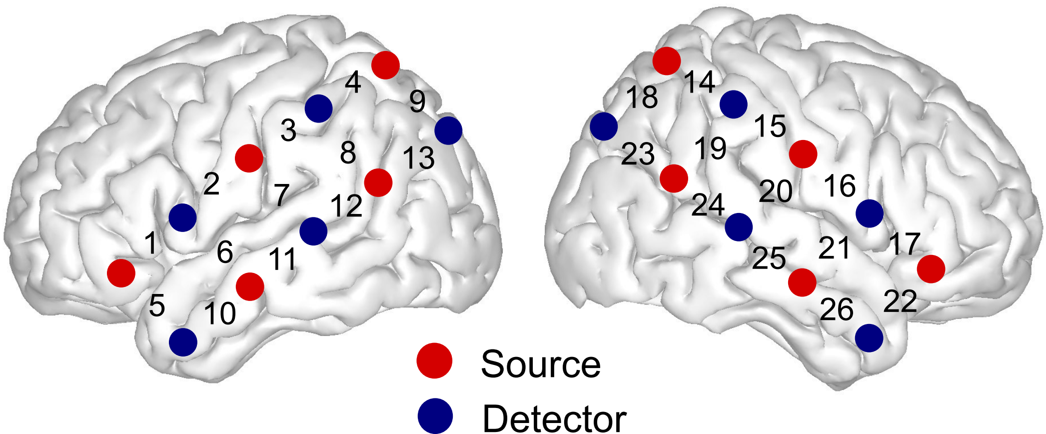
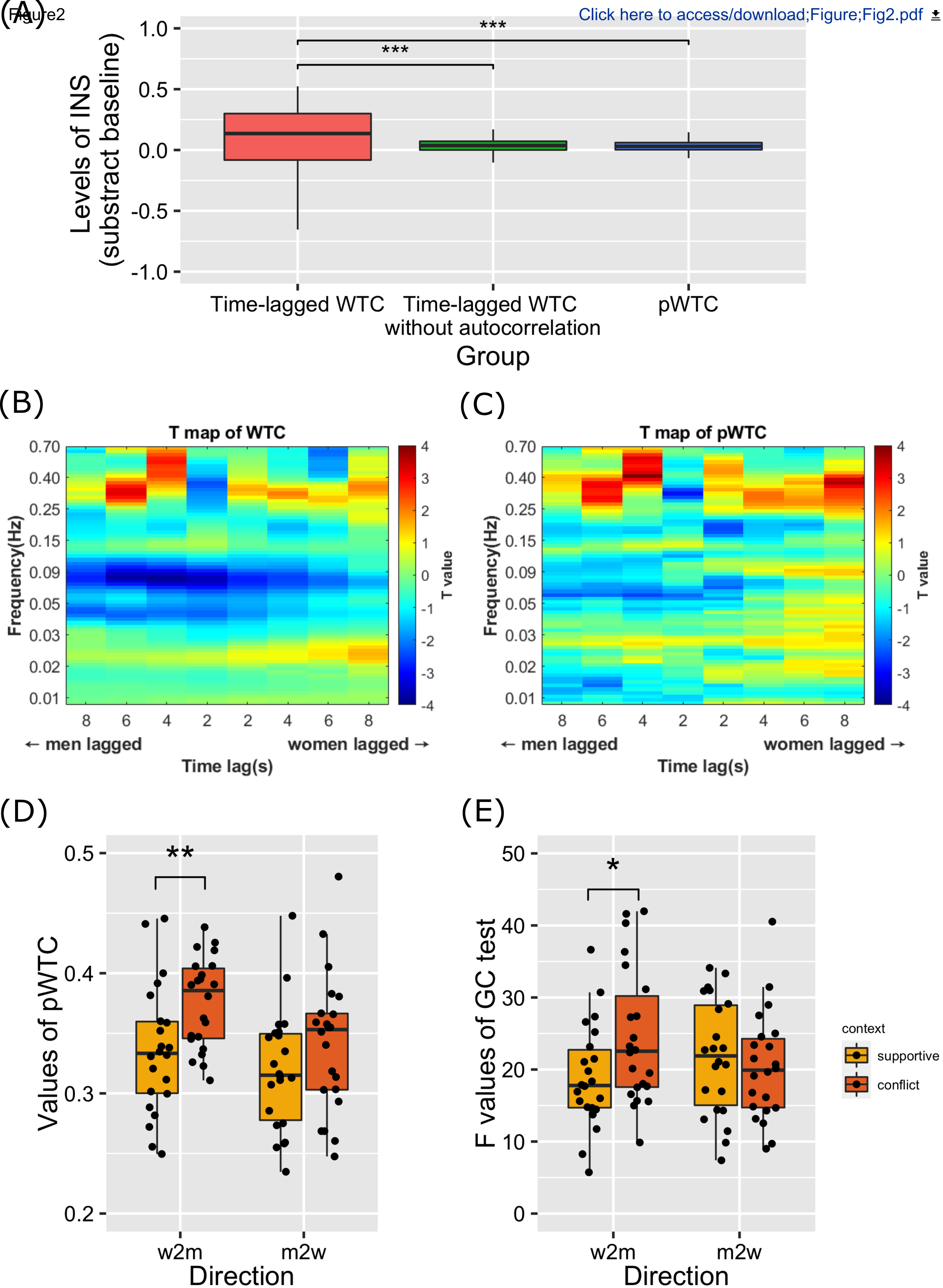


Figure2


(A)
***
***
Levels of INS (substract baseline)
1.0
0.5
0.0
-0.5
-1.0
Time-lagged WTC
Time-lagged WTC without autocorrelation
pWTC
Group
(B)
T map of WTC
Frequency(Hz)
T value
← men lagged
women lagged →
Time lag(s)
(C)
T map of pWTC
Frequency(Hz)
T value
← men lagged
women lagged →
Time lag(s)
(D)
**
Values of pWTC
w2m
m2w
Direction
(E)
*
F values of GC test
w2m
m2w
Direction
context
supportive
conflict

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Response to the reviewers' comments

We thank the reviewers and the editor for taking the time to review this manuscript. We have attempted to answer all questions raised by both reviewers and to substantially revise the previous version of the manuscript. We believe that the reviewers' helpful comments and constructive suggestions have helped us greatly improve the manuscript. Below, we have provided point-by-point responses to all concerns. In addition, we have uploaded a version of the manuscript with tracked changes.

Editorial comments:

Editorial Changes

Changes to be made by the Author(s):

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Answer(A)1: This revision has been edited and proofread by two professional editors from American Journal Experts (AJE, https://www.aje.com). We have also checked the whole manuscript thoroughly to avoid any errors.

2. Please provide an institutional email address for each author.

A2: Thank you. We have updated institutional email address for each author.

3. Please define the abbreviations before use (SPM, MNI, etc.)

A3: As requested, the abbreviations have been spelled out in the first use. Also see A3 in reviewer #2.

4. Please revise the following lines to avoid previously published work: 98-100, 143-146. Please refer to the iThenticate report attached.

A4: Thanks. The sentences have been modified.

5. Please revise the text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

A5: Changes have been made.

6. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials.

For example: LABNIRS, SIEMENS TRIO 3, etc.

A6: As suggested, "Table of Materials" has been added and the commercial languages in the main text have been removed.

7. Please include an ethics statement before the numbered protocol steps, indicating that the protocol follows the guidelines of your institution's human research ethics committee.

A7: The ethics statement has been provided before protocol steps.

8. Please ensure that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., "Do this," "Ensure that," etc.). The actions should be described in the imperative tense in complete sentences wherever

possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.

A8: Thanks. Changes have been made.

9. Please add more details to your protocol steps. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

A9: More details on "how" questions have been added. We hope that the revised protocol steps are sufficient for others to perform the actions.

10. Line 131: How is the absorption collected?

A10: We first obtained the optical density of the near-infrared light, which were then converted into concentration changes of oxyhemoglobin based on the Modified Beer-Lambert Law. This issue has been clarified.

11. Line 135-135: Please mention how the conversion of optical density into concentration changes performed. Is it done manually or automatically?

A11: Thank you. This operation was done automatically by the equipment. This information is now mentioned in the text.

12. Line 138: How is the data downsampled?

A12: A MATLAB built-in function *decimate* was used. This issue has been clarified.

13. Line 195-196: Please mention how the transformation is done? Is any software used?

A13: A MATLAB built-in function *wcoherence* was used to perform wavelet transform. Additionally, Fisher-z transformation was conducted based on the equation of the Fisher-z transformation. All information is added to the manuscript.

14. Please include a one-line space between each protocol step and then highlight up to 3 pages of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.

A14: Changes have been made. We highlighted the essential steps in yellow.

15. Please ensure that the discussion explicitly covers the following:

a) Critical steps within the protocol

b) Any modifications and troubleshooting of the technique

c) Any limitations of the technique

d) The significance with respect to existing methods

e) Any future applications of the technique

A15: Thanks. We have reorganized the discussion section carefully and tried our best to cover these five parts. Also see A1 in reviewer #2.

16. When giving a reference in the text, the corresponding number from the reference list must appear superscripted without a space after the word/group of words it applies to but before any punctuation (in the case of et al., place the superscripted number after et al. but before other punctuation). In the case of parentheses, decide based on the

context whether the reference applies to a specific part of the text within the parentheses (place the superscripted number within the parentheses) or to all text within the parentheses (place the superscripted number after the closing parenthesis). Ranges of reference numbers are separated by an en dash (no spaces), individual reference numbers are separated by commas (no spaces). Examples: Phang et al.5, (see previous publications)1–4,7,8

A16: Changes have been made.

17. Please revise the table of the essential supplies, reagents, and equipment. The table should include the name, company, and catalog number of all relevant materials in separate columns in an xls/xlsx file. Please sort the Materials Table alphabetically by the name of the material.

A17: Changes have been made.

Reviewers' comments:

Reviewer #1:

Manuscript Summary:

This study proposed a new time-lag analysis method termed "partial wavelet transform coherence" (pWTC) for fNIRS-based hyperscanning study. The authors designed an opposite-sex communication task and measured participants' fNIRS signals simultaneously. By comparing results from pWTC, traditional WTC and Granger Causality (GC) analysis, they found a significant women-led time-lag pattern during communication and proved pWTC a useful approach in estimating the directional and temporal information flow during social interaction. Overall, the proposed method is new for fNIRS-based hyperscanning and could potentially be a new tool for revealing the inter-brain relationships.

Thank you.

Major Concerns:

1) While I understand the proposed method has been well developed and documented in other research fields, more explanations are needed to elaborate what specific challenges in the field of fNIRS hyperscanning could be solved, as compared to existing methods. Currently the authors talked about autocorrelation but did not mention the possible consequence of having autocorrelation for fNIRS.

One specific question following this point: The authors explained that the significant effect within the 0.04-0.09Hz from traditional WTC analysis might be confounded by the autocorrelation effect of fNIRS signal. Whether this autocorrelation effect checkable or verifiable, e.g. by directly comparing the level of WTC(Wt, Mt+n) and pWTC, or the level of WTC(Mt, Mt+n)?

A1: This is a nice suggestion. As requested, an example has been added to explain the potential confounding effect.

"For example, during a dyadic social interaction process, the signal of participant A at time point t may be synchronized with that of participant B at the same time point. Meanwhile, the signal of participant A at time point t may be synchronized with that of participant A at later time point t+1 because of the autocorrelation effect. Therefore, a spurious time-lagged INS may occur between the signal of participant A at time point t and that of participant B at time point t +1."

We have also performed additional analyses to directly test the difference between traditional WTC and pWTC. The methods and the results have been added.

"Conduct a paired two-sample two-tailed t-test between the results of *WTC*(W*t*, M*t*+*n*) and *WTC*(M*t*, M*t*+*n*) to test the potential impact of autocorrelation on INS. Note that the INS of *WTC*(M*t*, M*t*+*n*) reflects autocorrelation."

"Finally, to test the impact of autocorrelation on the results of traditional time-lagged INS_{WTC}, INS_{WTC} was compared between $WTC(W_t, M_{t+4})$ and $WTC(M_t, M_{t+4})$ at 0.04-0.09 Hz and 0.4-0.6 Hz, respectively. Note that the INS_{WTC} of $WTC(M_t, M_{t+4})$ reflects autocorrelation. The results showed that at the 0.4-0.6 Hz, there was no significant difference between the INS_{WTC} of $WTC(W_t, M_{t+4})$ and that of $WTC(M_t, M_{t+4})$ ($t(21) = 0.336$, $p = 0.740$). At 0.04-0.09 Hz, the INS_{WTC} of *WTC(Mt, Mt+4)* was significantly higher than that of $WTC\ (W_t, M_{t+4})$ ($t(21) = 4.064$, $p < 0.001$). A comparison was also conducted between the frequency ranges of 0.04-0.09 Hz and 0.4-0.6 Hz regarding INS_{WTC} of $WTC(M_t, M_{t+4})$. The results showed that the INS_{WTC} of $WTC(M_t, M_{t+4})$ was significantly higher at 0.04-0.09 Hz than at the 0.4-0.6 Hz ($t(21) = 5.421$, $p < 0.001$). These results indicate that the time-lagged INS_{WTC} was affected by autocorrelation in both the low- and high-frequency ranges, but the impact was larger for the lower-frequency range than for the higher-frequency range."

2) The method can be used not only for hyperscanning (I would interpret hyperscanning for simultaneous recordings from two or more persons), but also for inter-brain data

analysis when the data are not necessarily recorded simultaneously.

A2: Yes, other inter-brain data analyses would also benefit from this method. We have added statements to the discussion section. Thank you.

"In general, pWTC is a useful approach in estimating the directional and temporal patterns of information flow during social interaction. More importantly, we believe that the pWTC method is also suitable for pseudo-hyperscanning studies (i.e., signals of two or multiple brains are not collected simultaneously[34,35]). In such experiments, although the direction of information flow is fixed, it is also of interest to examine the duration of the time lag between the input of the signal and the process of the signal. Therefore, autocorrelation can also confound the results of the time-lagged INS. In the future, this method has the potential to answer many questions in hyperscanning and other interbrain studies. For example, to determine the dominant role in various social relationships, such as teachers and students, doctors and patients, and performers and audiences. Additionally, as pWTC maintains the temporal structures of INS, it is also possible to test the dynamic pattern of INS, such as group attitude convergence."

3) The rationale of using the data from a communication task also needs to be explained. Would the results be generalized to more hyperscanning or inter-brain scenarios? And what would be expected for the analysis results for the validation of the proposed method? For instance, the difference between the results of pWTC and GC might need to be discussed more.

A3: The reasons to introduce the pWTC method based on communication tasks are as follows. It is important to infer the direction of information flow in a naturalistic communication context (Jiang et al., 2021), because individuals involved in a communication often have different social roles that are either assigned a priori (e.g., Nguyen et al., 2020; Zheng et al., 2018) or emerged during a communication (e.g., Jiang et al., 2015). Meanwhile, it is particularly difficult to do so because of the lack of strict experimental control. Recently communication tasks have been more and more widely used to investigate various topics such as speech processing, conceptual alignment, turn-taking, etc. Therefore, we believe that a demonstration in a communication context would be more helpful propagating the use of the pWTC method. These statements have been added to the introduction section.

Additionally, more discussions about the difference between the results of pWTC and GC have been added.

"This conclusion is further supported by a comparison with the GC test. The results of the GC test were quite similar to those of the pWTC, showing significant information flow from women to men but not from men to women. There was a slight difference between the results of the GC test and pWTC, i.e., the interaction effect between topic and direction was marginally significant in the results of the pWTC but reached significance in the GC test. This difference may be because the pWTC is calculated at a finer timescale than the GC test. Thus, although both the pWTC and GC tests can provide reliable results when controlling for the autocorrelation effect, the pWTC is advantageous because it is not necessary to make stationary assumptions and holds a high temporal-spectrum structure."

4) Would it be necessary to conduct a simulation study before applying to real fNIRS data?

A4: We have added a simulation experiment to investigate whether pWTC is able to remove autocorrelation effect of the signal.

Methods:

"**NOTE:** To test the effect of the pWTC method, a simulation experiment was first conducted. Here, the traditional time-lagged WTC is expressed by the following equation [31]:

$$WTC(W_t, M_{t+n}) = \frac{|S(C^*_{W_t}(i,t) \cdot C_{M_{t+n}}(i,t))|^2}{S(|C_{W_t}(i,t)|^2) \cdot S(|C_{M_{t+n}}(i,t)|^2)}$$

Equation 2

where C denotes the continuous wavelet transform operator at different scales i and time points t. S denotes the smoothing operator. * denotes the complex conjugate operator. W and M indicate two individual time series.

Additionally, the pWTC is calculated based on the following equation:

$$pWTC_{WtoM} = \frac{\left|\sqrt{WTC(W_t, M_{t+n})} - \sqrt{WTC(M_t, M_{t+n})} \cdot \sqrt{WTC(W_t, M_t)}^*\right|^2}{[1 - WTC(M_t, M_{t+n})] \cdot [1 - WTC(W_t, M_t)]}$$

Equation 3

where $WTC(W_t, M_{t+n})$ is the traditional time-lagged WTC. $WTC(M_t, M_{t+n})$ is the autocorrelated WTC of one individual. $WTC(W_t, M_t)$ is the time-aligned WTC. * is the complex conjugate operator.

1. Generate two time series of signals that correlate with each other. Set the correlation coefficient of r to 0.4. Additionally, one signal has autocorrelation (r = 0.8) at a 4 s time lag. Furthermore, generate two time series of signals without any correlation but with autocorrelation in one signal.

2. Calculate values of traditional 4 s time-lagged INS based on the generated signals

with or without correlation, which can be named "time-lagged INS_{WTC} with autocorrelation" and "time-lagged baseline INS_{WTC} with autocorrelation".

3. Remove autocorrelation from the generated signals, and then calculate the values of traditional 4 s time-lagged INS_{WTC} based on the generated signals with or without correlation, which can be named "time-lagged INS_{WTC} without autocorrelation" and "time-lagged baseline INS_{WTC} without autocorrelation".

4. Calculate the values of 4 s time-lagged pWTC based on the generated signals with or without correlation, which can be named "time-lagged INS_{pWTC}" and "time-lagged baseline INS_{pWTC}".

5. Repeat the above procedures 1000 times.

6. After subtracting the baseline INS, compare the results of time-lagged INS_{WTC} with autocorrelation, time-lagged INS_{WTC} without autocorrelation, and time-lagged INS_{pWTC} using the analyses of variance (ANOVA) method. Here, it is expected that the time-lagged INS_{WTC} with autocorrelation will be significantly higher than the time-lagged INS_{WTC} without autocorrelation and the time-lagged INS_{pWTC}, and no significant difference is expected between the time-lagged INS_{WTC} without autocorrelation and the time-lagged INS_{pWTC}."

Results:

The results showed that the time-lagged INS_{WTC} with autocorrelation was significantly higher than the time-lagged WTC INS_{WTC} without autocorrelation ($t(1998) = 4.696$, p

< 0.001) and time-lagged INS_{pWTC} ($t(1998) = 5.098$, $p < 0.001$). Additionally, there was no significant difference between time-lagged INS_{WTC} without autocorrelation and INS_{pWTC} ($t(1998) = 1.573$, $p = 0.114$). These results indicate that pWTC can effectively remove the impact of the autocorrelation effect on INS.

Minor Concerns:

1) Fig.2c-d. Asterisks in Panel C&D could be better labeled to indicate inter-group comparisons. Also, the font size could be a little bit larger for clear reading.

A5: Changes have been made to better indicate the meaning of the asterisks. Also, the front size has been increased.

2) Page 9, line 223. Mismatch between the "marginally higher" and "p = 0.01".

A6: We apologize for this mistake. This error has been corrected.

3) Equation 1. The value of pWTC was always positive and ranges from 0 to 1. I am wondering that, is there any possibility that comparable pWTC results could be obtained while the actual value WTC(Bt, At+n) was very small (close to zero) and close to 1?

A7: We actually tested these possibilities, and the results showed that when the actual value of WTC is about 1, the results of the traditional time-lagged WTC and pWTC methods are comparable.

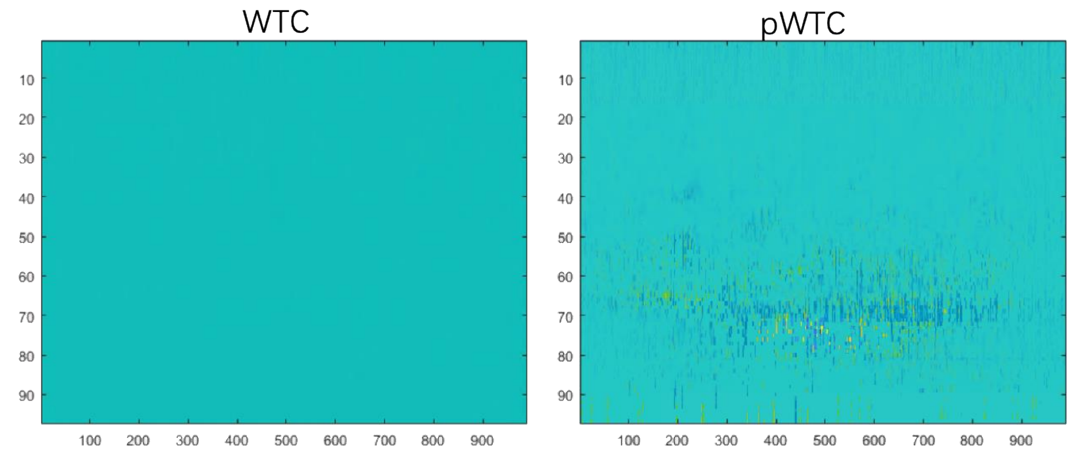

Fig. 1 Left panel: the time-lagged WTC map generated by two same signals, the x-axis is time-point, and the y-axis is frequency-band. The mean value of WTC at all point is about to 1. Right panel: the pWTC map of two similar signals. The pWTC map is quite similar to the WTC map.

Additionally, while the actual value of WTC is about 0, a similar pattern of results can also obtained from the traditional time-lagged WTC and pWTC methods.

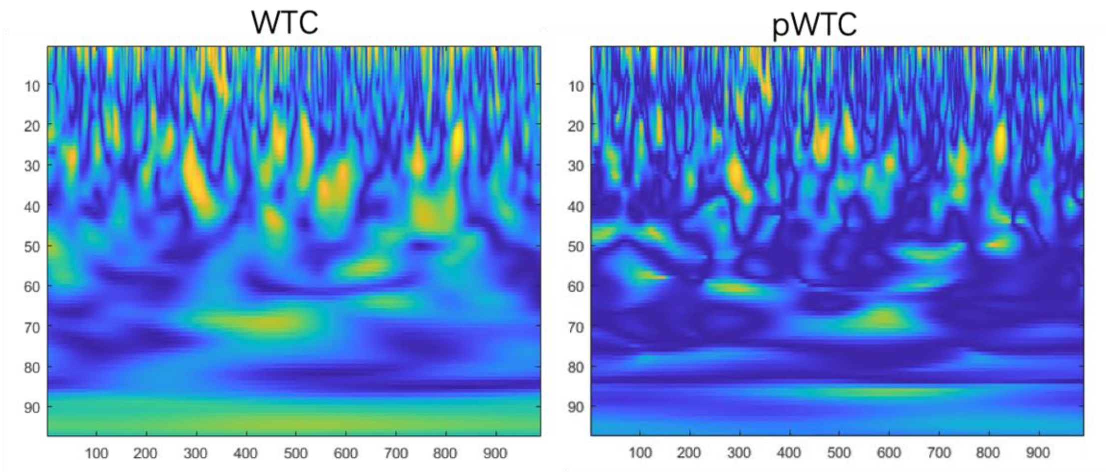

Fig. 2 Left panel: the time-lagged WTC map generated by two random signals, the x-axis is time-point, and the y-axis is frequency-band. The mean value of WTC at all point is about to 0. Right panel: the pWTC map of two similar signals. The pWTC map

is quite similar to the WTC map.

Reviewer #2:

Manuscript Summary:

Title: Describing the Direction of Information Flow in FNIRS-Hyperscanning Data Using the Partial Wavelet Transform Coherence Method (JoVE62927)

The manuscript looks attractive because it is well presented and it deals with a challenging topic, which is: describing the direction of information flow in fNIRS-hyperscanning data using a Wavelet method. Nevertheless, I have a few concerns for authors to address.

Thank you.

Major Concerns:

1. DISCUSSION part needs better organization. Indeed, it is the most hampering obstacle for me to support the paper being published at current stage. For example, what is the contribution of your work or your findings to the related researches? Are there any implications or implications or applications of the research you conducted in the paper?

A1: We apologize for the poor organization of the discussion section. As suggested, the discussion has been thoroughly re-written. We hope that the revision is acceptable.

Minor Concerns:

1. Line 70-71, the authors mentioned that "proposed a new method termed "partial wavelet transform coherence" (pWTC) for the calculation of time-lag INS on the fNIRS signal". pWTC was first introduced in 2009, so it is not new, but maybe new for

detecting time-lag pattern of INS. Please clarify this.

A2: Thank you for your correction. The statement has been modified as below.

"Mihanović and his colleagues[22] first introduced a method termed "partial wavelet transform coherence" (pWTC) and then applied it in marine science[23,24]. The original purpose of this method was to control the exogenous confounding noise when estimating the coherence of two signals. Here, to address the autocorrelation issue in the fNIRS hyperscanning data, we extended the pWTC method to the calculation of time-lagged INS on the fNIRS signal. "

2. Line 96-97. S.D. The authors should spell out full name of any abbreviation when first appear. Please also check the entire documents.

A3: The revised manuscript has been thoroughly checked to correct this problem.

3. Section 1.1. Participants were recruited through advertising in universities of Beijing, what is the background of their education?

A4: All the participants are undergraduates. We have added this information to the manuscript.

4. section 2 and 3 may be integrated as "fNIRS Data Collection and Processing". Here, "1.1 …", "3.1..." and other types of statements are not recommended. The authors might want to explain the experimental steps in order, however, "1.1" is more like a chapter heading rather than a statement of experimental steps, so that it should be adjusted.

A5: As suggested, the manuscript has been reorganized.

5. Section 3.3. downsample the data from 55.6 Hz to 11.1 Hz for what purpose?

A6: The purpose is to reduce computing cost. Additionally, as the figure below shows, the data of 11.1Hz is sufficient to record physiological components such as heart rate, breathing, etc. in the same way as 55.6 Hz.

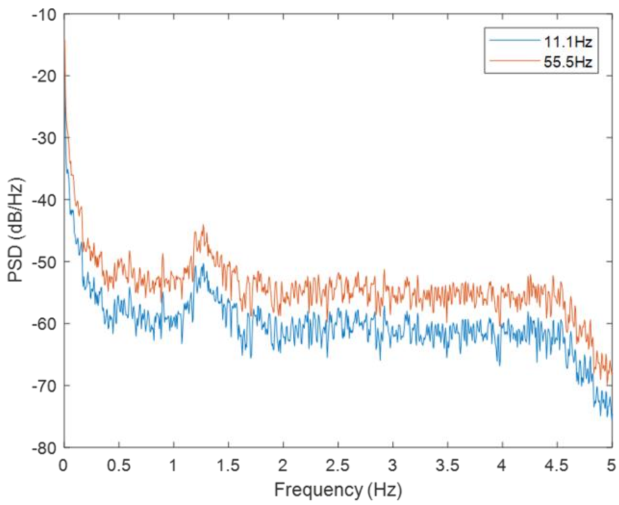

Fig. 3 The power spectrum plot for sample rate at 11.1 Hz (blue line) and 55.6 Hz (red line). The power spectrum pattern is quite similar.

6. Section 3.4 hmrMotionCorrectWavelet, what is the benefit of using this algorithm?

A7: During fNIRS experiments, head movements will cause a shift in optical coupling between the fiber and the skin, which will further lead to rapid changes in the fNIRS signal such as sharp spikes. According to previous findings, the wavelet-based filtering method employed in this function can more effectively isolate the spikes from the real hemoglobin concentration changes than do other methods (Molavi and Dumont, 2012; Sato et al., 2006).

7. Section 3.5. Why you think 80% of variance from the signals are physiological noises?

A8: This is an interesting question. So far there is no consensus about how many percentages of variance from the signals are physiological noises and should be removed from the signals. In traditional block-designed experiment, more percent of variance (e.g., 97%) are expected than a naturalistic experiment because the rhymical change of task and baseline will modulate and amplify the physiological response such as the hemoglobin concentration changes in the head skin. Here we employed a lenient value to avoid removement of real signals in naturalistic communication tasks. In future we hope to have an experiment to specifically investigate this issue and to compare the effects of different percentages of variance on the results of WTC between different experimental contexts.

8. Line 184, I believe Granger Causality method deserves further explanation here.

A9: Thanks. We have added more details of Granger Causality method to the text and more discussions to the discussion section (also see A3 in response to reviewer #1).

"3. Finally, calculate INS using the GC test (INS_{GC}).

NOTE: To further validate the pWTC method and evaluate its advantages and disadvantages, GC-based INS was calculated using the GC method (INS_{GC}).

1. Based on the pWTC result, bandpass filter the HbO signal of each individual at the SMC (i.e., 0.4 – 0.6 Hz, see the results below).

2. Conduct a GC test (Econometric toolbox, MATALB) within each dyad in the supportive topic and the conflict topic separately. Then, four groups of *F*-values are obtained for INS_{GC}: 1) from women to men on the supportive topic (W2M_supp); 2) from men to women on the supportive topic (M2 W_supp); 3) from women to men on the conflict topic (W2M_conf); and 4) from men to women on the conflict topic (M2 W_conf). The *F*-values are used to index the INS_{GC}."

9. It's hard to understand this part in line 310-311, i.e., "It only indicates 'the direction of information flow' which cannot reveal whether A cause B or vice versa". Please clarify this. Also, references are needed here, for example:

Liu, H., et.al., (2020). "Inferring Subsurface Preferential Flow Features from a Wavelet Analysis of Hydrological Signals in the Shale Hills Catchment." Water Resources Research 56(11): e2019WR026668.

Rhif, M., et.al., (2019). "Wavelet Transform Application for/in Non-Stationary Time-Series Analysis: A Review." Applied Sciences 9(7): 1345.

A10: Thank you for the suggested references. This part of discussion has been revised mainly based on the aforementioned two papers.

" The pWTC method also has its limitations. First, similar to the GC test, the causality inferred from pWTC is not a real causality[32,33]. Rather, it only indicates a temporal relationship between the signals of A and B. This issue should be kept in mind when applying the pWTC method. Second, pWTC only partials out the autocorrelation effect. Thus, other potential concurrent variables, such as shared environments or similar actions, may still impact the results. Consequently, any conclusions about the direction and temporal pattern of information flow should be drawn after controlling for these confounding factors."

References

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Jiang, J., Zheng, L., & Lu, C. (2021). A hierarchical model for interpersonal verbal communication. *Social Cognitive and Affective Neuroscience*, *16*(1–2), 246–255. https://doi.org/10.1093/scan/nsaa151

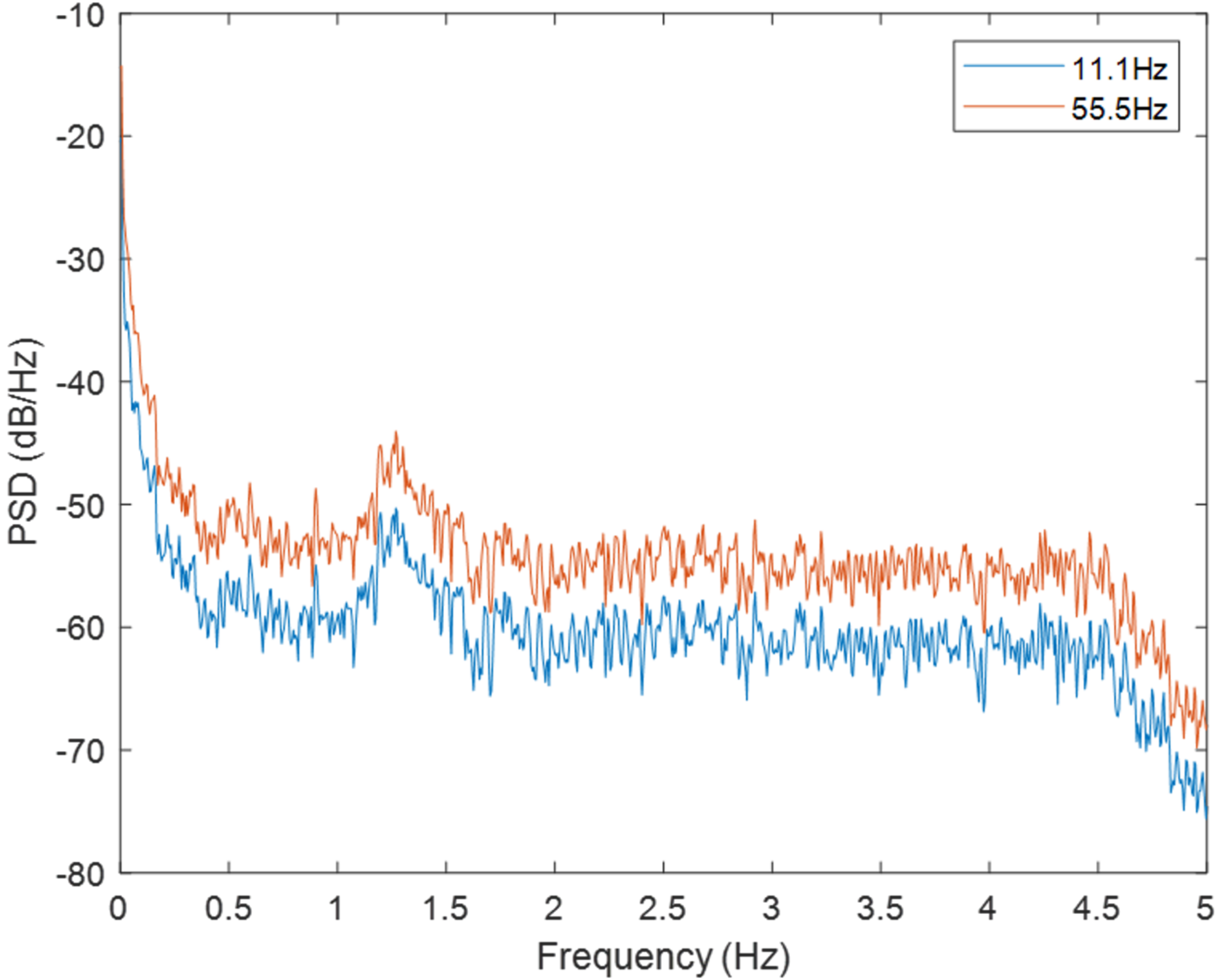
Li, Z., Li, J., Hong, B., Nolte, G., Engel, A. K., & Zhang, D. (2021). Speaker–Listener Neural Coupling Reveals an Adaptive Mechanism for Speech Comprehension in a Noisy Environment. *Cerebral Cortex*. https://doi.org/10.1093/cercor/bhab118

Liu, Y., Piazza, E. A., Simony, E., Shewokis, P. A., Onaral, B., Hasson, U., & Ayaz, H. (2017). Measuring speaker-listener neural coupling with functional near infrared spectroscopy. *Scientific Reports*, *7*(October 2016), 1–13. https://doi.org/10.1038/srep43293

Nguyen, T., Schleihauf, H., Kayhan, E., Matthes, D., Vrtička, P., & Hoehl, S. (2020). The effects of interaction quality on neural synchrony during mother-child problem solving. *Cortex*, *124*, 235–249. https://doi.org/10.1016/J.CORTEX.2019.11.020

Zheng, L., Chen, C., Liu, W., Long, Y., Zhao, H., Bai, X., Zhang, Z., Han, Z., Liu, L., Guo, T., Chen, B., Ding, G., & Lu, C. (2018). Enhancement of teaching outcome through neural prediction of the students' knowledge state. *Human Brain Mapping*, *39*(7), 3046–3057. https://doi.org/10.1002/hbm.24059

Supplemental Figure1



Supplemental Figure2