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# Affiliative bonding between teachers and students through interpersonal synchronisation in brain activity

Lifen Zheng,<sup>1</sup> Wenda Liu,<sup>2</sup> Yuhang Long,<sup>2</sup> Yu Zhai,<sup>2</sup> Hui Zhao,<sup>2</sup> Xialu Bai,<sup>2</sup> Siyuan Zhou,<sup>2</sup> Kanyu Li,<sup>2</sup> Huan Zhang,<sup>3,4,5</sup> Li Liu,<sup>2</sup> Taomei Guo,<sup>2</sup> Guosheng Ding,<sup>2</sup> and Chunming Lu<sup>2,6</sup>

<sup>1</sup>Center for Teacher Education Research, Faculty of Education, Beijing Normal University, Beijing 100875, China, <sup>2</sup>State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, Beijing 100875, China, <sup>3</sup>Key Research Base of Humanities and Social Sciences of the Ministry of Education, Academy of Psychology and Behavior, Tianjin Normal University, Tianjin 300074, China, <sup>4</sup>Faculty of Psychology, Tianjin Normal University, Tianjin 300387, China, <sup>5</sup>Center of Collaborative Innovation for Assessment and Promotion of Mental Health, Tianjin 300074, China, and <sup>6</sup>IDG/McGovern Institute for Brain Research, Beijing Normal University, Beijing 100875, China

Correspondence should be addressed to Chunming Lu, State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, No. 19 Xinjiekouwai Street, Beijing 100875, China. E-mail: luchunming@bnu.edu.cn.

 $^{\dagger}\mbox{Lifen}$  Zheng and Wenda Liu contributed equally to this study.

### Abstract

Human beings organise socially. Theories have posited that interpersonal neural synchronisation might underlie the creation of affiliative bonds. Previous studies tested this hypothesis mainly during a social interaction, making it difficult to determine whether the identified synchronisation is associated with affiliative bonding or with social interaction. This study addressed this issue by focusing on the teacher–student relationship in the resting state both before and after a teaching period. Brain activity was simultaneously measured in both individuals using functional near-infrared spectroscopy. The results showed a significant increase in brain synchronisation at the right sensorimotor cortex between the teacher and student in the resting state after, but not before, the teaching period. Moreover, the synchronisation increased only after a turn-taking mode of teaching but not after a lecturing or video mode of teaching. A chain mediation analysis showed that brain synchronisation during teaching partially mediated the relationship between the brain synchronisation increase in the resting state and strength of the affiliative bond. Finally, both role assignment and social interaction were found to be required for affiliative bonding. Together, these results support the hypothesis that interpersonal synchronisation in brain activity underlies affiliative bonding and that social interaction mechanically mediates the bonding process.

Key words: affiliative bond; resting state; teaching; teacher; student; fNIRS

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### Introduction

As a survival response to evolutionary pressures, humans tend to form interpersonal bonds. Theoretical accounts posit that the process of affiliative bonding is potentially associated with a general neurophysiological mechanism in mammals, which is reflected as interpersonal bio-behavioural synchrony (Feldman, 2017). In support of this account, previous evidence has revealed physiological and behavioural synchronisation between individuals in various social relationships during interactions (Schellekens et al., 2009; Ulmer-Yaniv et al., 2016; Vicary et al., 2017; Wilson et al., 2018; Pauly et al., 2020; Schoenherr et al., 2019). At the neural level, evidence also shows interpersonal neural synchronisation (INS), i.e. phase and/or amplitude alignment of the neuronal or haemodynamic signals across time, in leader-follower pairs when leaders emerge (Sanger et al., 2012, 2013; Yun et al., 2012; Jiang et al., 2015; Zheng et al., 2018; Vanzella et al., 2019), in teacher-student pairs during teaching (Holper et al., 2013; Dikker et al., 2017; Takeuchi et al., 2017; Pan et al., 2018; Zheng et al., 2018; Bevilacqua et al., 2019; Nozawa et al., 2019) or between individuals when a work alliance is created (Zhang et al., 2018; Fachner et al., 2019). Moreover, while role assignment before a direct social interaction induced a specific pattern of INS between the leader and the follower (Sanger et al., 2012, 2013; Konvalinka et al., 2014), other studies on leader-follower, student-student or teacherstudent relationships showed that self-reported social bonds were associated with a distinct pattern of INS only during or after a direct social interaction (Jiang et al., 2015; Dikker et al., 2017; Bevilacqua et al., 2019). These findings seem to support the hypothesis that INS is associated with affiliative bonding. The unresolved issue, however, is that the identified patterns of INS in these studies could be associated with affiliative bonding, role assignment or social interaction, as these processes were intermingled when INS was detected.

The increased INS identified in previous functional nearinfrared spectroscopy (fNIRS) studies was roughly located in the inferior frontal cortex and superior temporal cortex (STC) of the mirror neuron network and temporo-parietal junction (TPJ) of the mentalising network (Jiang et al., 2012, 2015; Nozawa et al., 2016; Zhang et al., 2017, 2018; Dai et al., 2018; Pan et al., 2018; Liu et al., 2019; Mayseless et al., 2019; Vanzella et al., 2019). The INS in these networks seems to characterise the features of social interaction (Jiang et al., 2012). For instance, the INS is higher during face-to-face dialogue compared to back-to-back dialogue or face-to-face monologue (Jiang et al., 2012). Additionally, positive feedback increases INS to a greater extent than negative feedback does (Zhang et al., 2017; Lu et al., 2019; Lu and Hao, 2019). In addition, the INS is also associated with high-level cognitive processing such as problem-solving and shared representations of syntax (Liu et al., 2019; Lu and Hao, 2019; Mayseless et al., 2019). Thus, we hypothesised that INS within the mentalising and/or mirror neuron networks should also be able to characterise affiliative bonding that was created during a social interaction.

Additionally, some studies have examined INS between the same brain regions of two individuals (Jiang et al., 2012, 2015; Liu et al., 2017, 2019; Zhang et al., 2017), whereas others did so across different brain regions (Dai et al., 2018; Zheng et al., 2018; Lu and Hao, 2019; Mayseless et al., 2019). While some of these latter studies reported INS across different brain regions (Algoe et al., 2017; Zheng et al., 2018; Lu and Hao, 2019; Mayseless et al., 2019), other studies detected a significant increase in INS in the same brain regions even though cross-region INS was investigated (Dai et al., 2018; Zheng et al., 2018; Zheng et al., 2018). Importantly,

evidence indicates that social interaction has a hierarchical structure such that different levels of processes correspond to different spatial and temporal patterns of INS (Zheng et al., 2018). Specifically, prediction-related INS seems more closely associated with cross-region INS and a time-lag effect (Zheng et al., 2018), whereas shared representation-related INS seems more closely associated with same-region INS and a time-alignment effect between the time courses of partners (Stolk et al., 2014; Liu et al., 2019). Because prediction is usually involved in social interaction (Vouloumanos and Waxman, 2014) but probably not in a state when social interaction is absent, we hypothesised that when affiliative bonding was disentangled from social interaction (i.e. when social interaction did not occur), an affiliative bond might be commonly represented between partners. The shared representation of an affiliative bond between partners might be associated with the same-region INS and a timealignment effect within the mentalising and/or mirror neuron networks.

A second line of work on the bio-behavioural synchrony account investigates the role of a specific pattern of social interaction behaviours in affiliative bonding (Feldman, 2017). According to previous studies, while social affiliation is created when group members act jointly or contingently with each other (Marsh et al., 2009), turn-taking is the core process of social interaction (Pickering and Garrod, 2013) and enables us to better predict and infer the mental states of others (Vouloumanos and Waxman, 2014). Online turn-based social interaction is suggested to be a pre-requirement for (Diamond, 2003) and effective in triggering the neurobiological signatures of affiliative bonding (Winterheld et al., 2013; Algoe et al., 2017). Recent evidence shows that turn-based interaction induces stronger INS than other modes of interaction during conversation (Jiang et al., 2012; Ahn et al., 2018; Liu et al., 2019; Mayseless et al., 2019; Perez et al., 2019). However, how social interaction is associated with affiliative bonding remains to be tested, because as mentioned above, affiliative bonding and social interaction were intermingled in previous studies. Based on previous theoretical accounts and empirical evidence, here we hypothesised that a turn-taking mode of interaction might be more effective in affiliative bonding and thus result in a stronger social bondrelated INS than other modes of interaction did.

To address these issues and test the hypotheses, this study investigated the brain synchronisation patterns of affiliative bonding in a resting state both before and after a social interaction. The resting state refers to the state when external stimuli are absent and no task performance is required. While the brain activity associated with a specific external stimulus or task performance is context-dependent, the spontaneous fluctuations in brain activity in the resting state are considered to be independent of a specific context (Fox and Raichle, 2007) and are capable of characterising the intrinsic functional architecture of the human brain (Honey et al., 2009). Most importantly, a recent study demonstrated that the parent-child similarity in their restingstate intrinsic network (RSN) connectome was associated with their day-to-day emotional synchrony and the child's emotional competence (Lee et al., 2017), though whether the RSN similarity is associated with affiliative bonding or other factors remains unknown. Based on evidence of both brain synchrony and the resting state, we predicted that when an affiliative bond was created, it would persist into the resting state immediately after role assignment and social interaction. Thus, a distinct pattern of INS might be detected in the resting-state post-interaction. We expected to detect same-region INS and a time-alignment effect within the mentalising and/or mirror neuron networks as we hypothesised above.

To test how social interaction was associated with affiliative bonding, the present study focused on the teacher-student relationship because (i) it is one of the most important social relationships, (ii) previous research has tested INS in various modes of teacher-student interaction but not in affiliative bonding in the resting state (Holper et al., 2013; Dikker et al., 2017; Takeuchi et al., 2017; Pan et al., 2018; Zheng et al., 2018; Bevilacqua et al., 2019) and (iii) it is possible to disentangle the processes of role assignment, social interaction and affiliative bonding in this relationship. Three different modes of teaching were used in this study, i.e. a turn-taking mode, a lecturing mode and a video mode (Zheng et al., 2018). We predicted that a turn-taking mode of teaching would lead to a stronger INS in a subsequent resting state and a stronger affiliative bond than other modes of teaching. Moreover, INS associated with teaching might play a mediating role in the relationship between the INS associated with affiliative bonding in the resting state and the strength of the affiliative bond created during a social interaction.

fNIRS was used to measure haemoglobin concentrations simultaneously from teacher-student pairs in the resting state both before and after teaching to reflect brain activity and calculate brain synchronisation. Previous studies have demonstrated covariation of the resting-state fNIRS signals across time between brain regions of the visual, auditory, sensorimotor or language systems within a single brain (White et al., 2009; Lu et al., 2010; Zhang et al., 2010). The evidence indicates a possibility to investigate brain synchronisation interpersonally in the resting state, though as far as we know, no such studies have been conducted using fNIRS. In this study, fNIRS has relative advantages compared to other techniques, such as fMRI, in studying affiliative bonding in the resting state. Specifically, while previous studies have shown that spatial closeness promotes social cooperation (Greiner et al., 2014), fNIRS allows examination of pairs of partners in the same space. In addition, fNIRS is suitable for studying the teacherstudent interaction, which allows us to further clarify the role of social interaction in affiliative bonding. Because of the limitation of fNIRS in spatial resolution, only the outer cortex of the brain could be measured, and nearby measurement channels (CH) might collect brain activity from the same brain regions (Boas et al., 2004). Thus, it was possible that although different measurement CHs were reported, the same brain regions were measured. The correspondence between measurement CHs and brain regions was estimated based on international 10-20 systems and confirmed using MRI images (see Methods).

### **Materials and methods**

#### Participants

The participants were the same as those described in Zheng *et al.* (2018). Sixty adults were recruited from universities in Beijing through advertisements (male vs female=1:1, mean age=23±2.3 years) and were assigned as students. In addition, four adults (two females, mean age=25±2.4 years) were also recruited from Beijing Normal University through advertisements and assigned as teachers. They had received training as a teacher for 6–7 years. Before the experiment, the teachers and students were not acquainted. The 60 students were pseudorandomly (equal numbers of males and females in each group) split into three groups for three teaching modes (see below). The 20 students in each group were randomly assigned to

the four teachers. All participants were right-handed (Oldfield, 1971) and had normal or corrected-to-normal vision. The average age of the students did not differ significantly among the three teaching modes ( $F_{(2, 57)} = 0.01$ , P = 0.99,  $\eta_p^2 = 0.0003$ , observed power = 0.051).

An additional 56 adults (mean age = 20 years; s.d. = 1.6) were recruited to participate in a validation experiment and were randomly assigned into 28 two-person pairs. In each pair, the members were the same sex (to avoid a potential confound of mixed-sex interactions) (Daniel *et al.*, 2011; Baker *et al.*, 2016) and were strangers to one another (Aron *et al.*, 1992). All participants were right-handed (Oldfield, 1971), with normal hearing and normal or corrected-to-normal vision, and had no language, neurological or psychiatric disorders.

Written informed consent was received from all participants. The study protocol was approved by the Institutional Review Board of the State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University.

#### Experimental design

Figure 1a shows the overall arrangement of the experimental design. There were two 10-min resting-state sessions, one at the initial phase and the other at the ending phase of the experiment. During the resting-state sessions, the participants were required to keep still with their eyes closed, relax their mind and remain as motionless as possible (White *et al.*, 2009; Lu *et al.*, 2010; Zhang *et al.*, 2010). The interaction session immediately followed the first resting-state session and occurred before the second resting-state session.

#### Experimental procedures and materials

Task and materials. During the interaction session, a teaching task was performed, which has been described previously (Zheng et al., 2018). The teachers were required to teach the students about numerical reasoning, that is, looking for the hidden rule about a numerical relationship among a given sequence of numbers. This content was selected from a national standard guidebook [Chinese Civil Servants Administrative Professional Knowledge Level Tests (CCSAPAT), 2014] that aims to measure and improve various cognitive abilities including numerical reasoning in young adults (18-40-years old). Numerical reasoning involves finding hidden relations in a given number sequence. For instance, the number sequence of '2, 4, (), 8, 10, 12' follows the rule that all numbers are even numbers that differ by the constant of 2, so '6' is the correct answer. The CCSAPAT has been shown to have good validity and reliability (Wu, 2013). The students in our sample had not been exposed to the CCSAPAT.

*Experimental procedures.* All teaching was in the format of one teacher to one student. Each of the four teachers taught the same content to three groups of individual students in three different modes:

- (i) The turn-taking mode: The teacher and student sat side by side in front of a table in a silent room. A computer screen was placed on the table. The teacher first presented an example on the computer screen, and the student read and thought about the problem for  $\sim$ 20 s. Next, the teacher guided the student to solve the problem according to a Q&A approach described in a script for the teacher.
- (ii) The lecturing mode: The teacher and student sat side by side in front of a table in a silent room. A computer screen was

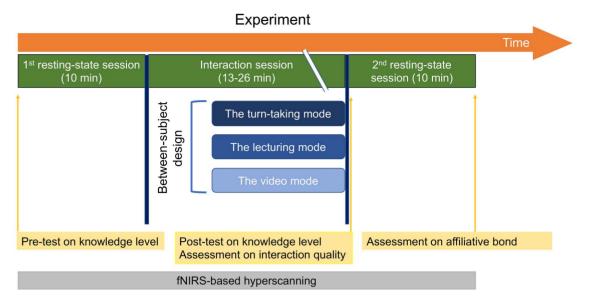


Fig. 1. Experimental procedures. (a) Set-up of the experiment. The teaching session occurred between the two resting-state sessions. Three groups of participants participated in three modes of teaching, i.e. turn-taking, lecturing and video. The participants' knowledge level was assessed immediately before the first resting-state session. The knowledge level, interaction quality and the strength of the affiliative bond were assessed immediately after the end of the second resting-state session. fNIRS data were simultaneously collected from the teachers and students during both the resting-state sessions and teaching session. (b) The positions of the optode probes. Note that the positions have been confirmed by MRI.

placed on the table. The teacher explained to the student the steps for solving each example using the computer screen. The teacher did not ask questions, and the student was not allowed to ask questions.

(iii) The video mode: Only the student sat in front of a table with a computer screen on the table. The videos in which the teacher simulated the lecturing mode alone (her/his brain activity was collected at this time) had been recorded previously. Then, the students learned by watching the video alone while brain activity was measured.

In the turn-taking and lecturing modes, the student sat next to the teacher because one-to-one teaching usually occurs this way in real life, and it is also convenient for the teacher and student to view and discuss the teaching contents presented on the computer. However, both the teacher and student were free to adjust their face direction so that both of them could see the face of their partners. In the video mode, the student could also see the face of the teacher as well as the teaching contents in the video. The experimental procedures were video recorded. The length of the teaching periods (i.e., from the beginning to the end of teaching behaviours) in each teaching style was flexible (13-26 min) and up to the teachers and students. Prior to the experiment, all teachers were trained in teaching the content.

### Behavioural assessment

Assessment of the affiliative bond. Immediately after the end of the second resting-state session, the students were required to assess how much they liked their teachers (item 1) and the teaching process (item 2) on 10-point scales. Inter-item consistency was computed, and reliability was high (Cronbach's alpha = 0.652). Then, the scores for the two items were summed to index the strength of the affiliative bond.

Assessment of the interaction quality. Immediately after the end of the teaching period, the students were also required to assess the quality of the teaching (item 1), their own learning (item 2) and the overall process of teaching (item 3) on 10-point scales. Inter-item consistency was computed, and reliability was high (Cronbach's alpha = 0.910). Then, the sum of the scores for the three items was used to index the interaction quality.

Assessment of the teaching outcome. Students' knowledge of numerical reasoning was tested immediately before the onset of the first resting-state session and at the end of the teaching period. A total of 20 4-choice items were selected from the CCSAPAT test bank, which were randomly split into twohalves: one for the pre-interaction test and the other for the post-interaction test. The difference between the pre- and postinteraction test scores was used as an index of the teaching outcome.

#### fNIRS data acquisition

The imaging data were collected from the teacher and student simultaneously using an ETG-4000 optical topography system (Hitachi, Japan). Four sets of customised optode probes were used in each pair. Each set had four emitters and four detectors that consisted of 10 measurement channels (CH) with 30 mm optode separation. Following previous studies on social relationships (Jiang et al., 2015; Zheng et al., 2018), the probe sets covered the bilateral frontal, temporal and parietal cortices. The probe set on the left hemisphere was more anterior, whereas that on the right was more posterior, to better cover the left frontal cortex and right temporal-parietal cortex. CH2 was placed at FP1 on the left hemisphere, and CH19 was placed at F8 on the right hemisphere, according to the international 10-20 system (Figure 1b). The probe sets were checked and adjusted to ensure consistency within the teacher-student pair and across pairs.

To confirm the anatomical position of each optode, MRI was performed on one typical participant with a high-resolution T1-weighted magnetisation-prepared rapid gradient-echo sequence (TR=2530 ms; TE=3.30 ms; flip angle= $7^{\circ}$ ; slice thickness=1.3 mm; in-plane resolution= $1.3 \times 1.0$  m<sup>2</sup>; number

of interleaved sagittal slices = 128). SPM8 (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, London, UK) was used to normalise the MRI to the standard Montreal Neurological Institute (MNI) coordinate space (Ashburner and Friston, 2005). The anatomical positions below the optode were identified according to the automated anatomical labelling template (Tzourio-Mazoyer *et al.*, 2002). This information was used to provide neuro-functional explanations of the affiliative bonding based on the roughly corresponding brain regions of each CH combination.

The absorption of near-infrared light at two wavelengths (695 and 830 nm) was measured at a sampling rate of 10 Hz. Based on the modified Beer–Lambert Law, changes in the oxy-haemoglobin (HbO) and deoxyhaemoglobin (HbR) concentrations were obtained by measuring the changes in near-infrared light absorption after its transmission through the tissue. Previous studies have shown that HbO is a sensitive indicator of the change in regional cerebral blood flow and has a high signal-to-noise ratio (Hoshi, 2007). Thus, this study focused on the HbO concentrations only.

#### Behavioural data analyses

To examine whether an affiliative bond was formed between the teachers and students after the end of teaching, we tested the strength of the affiliative bond against a target value of five (medium strength level of the affiliative bond on a 10-point scale) using the Bootstrap method (1000 times). Here, a target value of five was used in place of pre-interaction assessment scores because the participants reported that it was strange to assess their affiliation when they did not know each other. Oneway ANOVA was performed to examine the potential differences among the three participant groups.

#### fNIRS data analysis

Individual-level analysis. Data from the first and last 10 s of each session were considered to be unstable and thus were deleted during pre-processing. Furthermore, during pre-processing, no filtering or detrending procedures were applied. These procedures were performed on the coherence value as described below. In addition, we did not perform any artefact corrections at the single-subject level, as wavelet transform coherence (WTC) normalises the amplitude of the signal according to each time window and thus is not vulnerable to the transient spikes induced by movements (Nozawa et al., 2016; Liu et al., 2019). Moreover, the movement artefacts were expected to be minimised because the participants were required to remain as motionless as possible in the resting state.

Additionally, we conducted analyses to investigate whether movement artefacts might contaminate the WTC results in the resting state as well as during teaching. First, raw data points that contained suspected artefacts were identified through a running-window procedure. Specifically, for a given data point N, mean and standard deviation (s.d.) were calculated based on its neighbours N - 2, N - 1, N + 1 and N + 2. If N > mean+/-3\*s.d., it was identified as being confounded by artefacts. In total, fewer than 1 and 3% data points during the resting-state session and the teaching session, respectively, were identified. Then, N was replaced with the mean. Statistical tests (see below) were conducted both before and after the replacement of artefacts. The results did not differ, which was consistent with previous report that low percent of artefacts (<5%) will not affect the results of brain synchronisation (Liu *et al.*, 2019).

Next, WTC was calculated using a MATLAB function 'wcoherence' (Grinsted et al., 2004) to assess the cross-correlation between the two fNIRS time series generated by each pair of participants as a function of frequency and time (Torrence and Compo, 1998). For example, for a specific teacher-student pair, two time series of HbO were obtained, one from CH1 of the teacher and the other from CH2 of the student. Then, WTC was applied to these two time series to find regions in the timefrequency space where the two time series covaried. These data generated a 2-D matrix of the coherence value. In the matrix, each line corresponded to a specific frequency point (the frequency resolution ranged from ~0.001 Hz in the low-frequency part to 0.1 Hz in the high-frequency part), while each column corresponded to a specific time point. All possible CH combinations between the teacher and student were examined. Because there were 20 measurement CHs for each participant, 400 pairs of time series were generated for each pair of participants, and WTC was thus carried out 400 times. Next, the coherence values were time-averaged across the entire resting-state session and converted into Fisher z-values.

Group-level analysis. Brain synchronisation difference among the three participant groups before social interaction. To test whether participants in the three groups differed significantly in brain synchronisation pre-interaction, one-way ANOVA was performed along the full frequency range (0.01-0.7 Hz) across all CH combinations (400 in total). Following previous studies (Guijt et al., 2007; Tong et al., 2011; Barrett et al., 2015), data above 0.7 Hz were not included to avoid aliasing of higher-frequency physiological noise, such as cardiac activity (~0.8-2.5 Hz); data below 0.01 Hz were also not used to remove very low-frequency fluctuations. Finally, data within the frequency range of respiratory activity ( $\sim$ 0.15–0.3 Hz) were not considered. As a general approach to the multiple comparisons problem, an FDR threshold was determined from the observed P-value distribution and hence was adaptive to the amount of signal in the data (Genovese et al., 2002; Nichols and Hayasaka, 2003).

Brain synchronisation before social interaction. To test whether there was significant brain synchronisation when the roles of the teacher and student were assigned but teaching had not occurred, a random-paired permutation test was conducted. That is, for each teaching mode, all participants were randomly assigned to form new two-member pairs (i.e. pairs of participants who had been in the same interaction mode but had not interacted with one another), and then synchronisation was recomputed. This permutation test was conducted 1000 times to yield a distribution of synchronisation for all CH combinations, which were then compared with the mean value of synchronisation in the original pair of participants in each participant group.

Brain synchronisation increase after social interaction. To identify brain synchronisation that was specifically associated with affiliative bonding, paired two-sample t-tests were conducted between the resting-state fNIRS data (0.01-0.7 Hz) that were collected before and after teaching for each participant group. Based on these results, the frequency bands of interest were determined for each group based on a cluster-based permutation approach (Maris and Oostenveld, 2007). Specifically, first, for each teaching mode, all participants were randomly assigned to form new two-member pairs, and then synchronisation was recomputed. The synchronisation difference between pre- and post-interaction was examined using paired two-sample t-tests along the full frequency and across all CH combinations each time the permutation test was run. Second, a threshold of P < 0.05 was applied to each frequency point, resulting in a grid of all significant effects. Third, based on this grid, clusters that were composed of neighbouring significant frequency points were obtained. The clusters were formed along the frequency but not the CH combinations to avoid potential confounding of global physiological noise. The t-values within the largest cluster were then averaged. Fourth, this procedure was repeated 1000 times, generating a distribution of cluster-based t-values. Finally, the cluster-based t-values from original pairs were compared with the distribution of 1000 times permutation. The clusters whose averaged t-value was significantly larger than the mean of the distribution (cluster-based threshold, P < 0.05) were selected as frequency bands of interest for further analyses.

The coherence values within these selected frequency bands were averaged separately. Then, 2 (pre vs post)×3 (turn-taking, lecturing and video) mixed-model ANOVA was conducted on the time-averaged and frequency-averaged data with FDR correction across all CH combinations (P < 0.05). Finally, a series of planned paired two-sample t-tests was also conducted for each group.

To investigate whether the change in brain synchronisation was specific to pairs of participants interacting during the task, a validation approach was applied using the random-paired permutation procedure. That is, the mean value of the synchronisation changes in the original pair of participants was compared with the distribution generated by the 1000 times permutation. This procedure was applied to all CH combinations.

The post-interaction synchronisation of each participant group was also tested against a distribution generated by the random-paired permutation procedure (1000 times).

Time lag of the time courses of the teacher and student. To test whether a prediction or a representation sharing mechanism was involved in brain synchronisation, the coherence value was recalculated by shifting the time course of one participant forward or backward by 2–10 s (step=2 s), respectively. Then, a series of paired two-sample t-tests (i.e. pre- vs post-interaction) was applied to each time lag. It was hypothesised that the prediction mechanism should be associated with a time-lag effect, whereas the representation sharing mechanism should be associated with a time-alignment effect between the time courses of the teacher and student.

With which factors, i.e. affiliative bonding or social interaction, was the brain synchronisation increase associated? Two hierarchical linear regression model analyses were conducted with the change in brain synchronisation as the dependent variable (Bootstrap method, 1000 times, P < 0.05). In the first analysis, the strength of the affiliative bond was entered first (Model 1), and then teaching outcome and interaction quality were entered (Model 2). In the second analysis, teaching outcome and interaction quality were entered first (Mode 1), and the strength of the affiliative bond was entered next (Model 2).

A chain mediation role of social interaction in affiliative bonding. To test whether social interaction had a mediation role in affiliative bonding, a chain mediation analysis was conducted (Bootstrap method, 1000 times, P < 0.05), with the brain synchronisation increase in the second resting state as the independent variable (i.e. the difference between the second and first resting state in brain synchronisation, X, see the equations below), the strength of affiliative bond as the dependent variable (Y) and brain synchronisation in the teaching period as the mediation variable (M). The following equations show the chain mediation model (Judd and Kenny, 1981):

$$Y = cX + e1, M = aX + e2, Y = c'X + bM + e3.$$

where c, a, b and c' represent coefficients and e1-3 represent residual errors.

In addition, Zheng et al. (2018) previously reported that social interaction involves a hierarchical structure: (i) synchronisation at TPJ (teachers) – TPJ (students) is associated with a general interaction process that is not related to teaching outcome (0.06–0.07 Hz), (ii) synchronisation at anterior aSTC – TPJ is associated with actual knowledge transmission (0.5–0.7 Hz) and (iii) synchronisation at TPJ (teachers) – aSTC (students) is associated with the teachers' prior prediction of the knowledge and mental states of the students (0.5–0.7 Hz). We therefore calculated a brain synchronisation index by averaging the above three synchronisation (i.e. TPJ–TPJ, aSTC–TPJ and TPJ–aSTC) to characterise the overall pattern of teacher–student interaction (i.e. M in the above equation).

### Experimental procedures, data acquisition and analyses in the validation experiment

To test whether role assignment and social interaction were both required for affiliative bonding, an additional experiment was conducted. During the experiment, participants sat in a quiet room. There were two 8-min resting-state sessions, one at the initial phase and the other at the ending phase of the experiment. The interaction session was between the two resting-state sessions. During the interaction session, three modes of interaction were included to allow pairs of participants to interact in all possible ways and maximise the potential changes in brain synchronisation in the resting state (see Supplementary material). An ETG-4000 optical topography system (Hitachi Medical Company) was used to collect brain functional data from the two participants of each pair simultaneously (also see Supplementary material). Paired two-sample t-tests were conducted between the resting-state fNIRS data (0.01-0.7 Hz) that were collected before and after social interaction (FDR correction, P < 0.05).

### Results

#### The creation of an affiliative bond

Scores of affiliative bond in all three participant groups were significantly higher than 5 (Ps < 0.01, Figure 2). Oneway ANOVA showed significant differences among the three participant groups ( $F_{(2,59)} = 5.263$ , P = 0.008,  $\eta_p^2 = 0.155$ , observed power=0.813). Further post hoc analyses indicated that the affiliative bond score was significantly higher in the turn-taking mode participant group than in the video mode participant group (P=0.008, mean difference=2.450, 95% confidence interval: 0.541, 4.359) and marginally higher than in the lecturing mode participant group (P = 0.095, mean difference = -1.700, 95% confidence interval: -3.609, 0.209); however, no significant differences were found between the lecturing mode and video mode participant groups (P = 0.727, mean difference = 0.750, 95% confidence interval: -1.159, 2.659). These results confirmed the creation of an affiliative bond and showed that the turn-taking mode was more effective in creating a teacher-student bond than the other modes were.

### No significant difference in brain synchronisation among the three participant groups before social interaction

One-way ANOVA of the three participant groups did not reveal any significant difference in brain synchronisation after FDR correction (P < 0.05). These results indicated a good match in

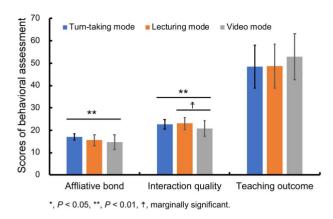


Fig. 2. Behavioural assessment of the strength of the affiliative bond, interaction quality and teaching outcome.

brain synchronisation level among the three participant groups before social interaction.

### No significant brain synchronisation before social interaction

The pre-interaction brain synchronisation of each participant group was tested against a distribution generated by the random-paired permutation procedure. No CH combinations survived the permutation test in any participant groups (Ps > 0.05). This result suggested that no significant brain synchronisation could be detected before social interaction even when the roles of the teacher and student had been assigned.

### Significant increase in brain synchronisation after social interaction

We hypothesised that the turn-taking mode of teaching might be more effective in creating an affiliative bond than other modes of teaching; thus, we were interested in the interaction between teaching mode and pre–post testing. Although no significant differences were found in the ANOVA results after FDR correction (Ps > 0.05), we examined the pattern of the interaction between teaching mode and pre–post testing. The result pattern showed that the brain synchronisation in the right sensorimotor cortex (SMC) of the teacher (CH16) and the student (CH10) at a frequency band of 0.04–0.05 Hz had the largest F-value ( $F_{(2,57)} = 7.204$ , P = 0.002, $\eta_p^2 = 0.202$ , observed power = 0.922) across all CH combinations of all frequency bands.

Planned paired two-sample t-tests showed that the turntaking mode induced a significant increase in brain synchronisation at the SMC of the teacher (CH16) and student (CH10) (0.04-0.05 Hz, t(19)=6.708, P < 0.0001, mean difference=0.057, 95% confidence interval: 0.039, 0.075, FDR correction, P < 0.05). No other significant results were found at any other CH combinations of this frequency band, nor were there significant results at any CH combinations of any other frequency bands (Ps > 0.05, Figure 3a and b).

Examinations of the brain synchronisation changes in the lecturing and video modes did not produce any significant results at the right SMC in the frequency range of 0.04–0.05 Hz (P > 0.05), nor were there significant results at any other CH combination of any frequency bands (Ps > 0.05) (Figure 3c–f).

### Validation of the pre-post brain synchronisation changes through a permutation test

The change of brain synchronisation post-interaction was further tested against a distribution generated by the permutation procedure (1000 times). Only the change of brain synchronisation at the right SMC of the teacher and student (CH16-10) after the turn-taking mode of teaching passed this test (P < 0.05, Figure 4a–c).

### Significant brain synchronisation after social interaction

The post-interaction brain synchronisation of each participant group was also tested against a distribution generated by the random-paired permutation procedure. The results showed that the brain synchronisation at the right SMC of the teacher and student (CH16-10) reached significance after the turn-taking mode of teaching only (P < 0.05, Supplementary Figure S1a–c). No other CH combination showed significant results (P > 0.05).

### Time alignment of the time courses of teachers and students

When the time course of one participant was shifted forward or backward by 2–10 s (step=2 s), respectively, no significant increase in brain synchronisation was detected in any groups (Figure 5). That is, the change in brain synchronisation reached a peak value when the brain activity of the teacher and that of the student were temporally aligned, which supported the representation sharing hypothesis.

# With which factors, i.e. affiliative bonding or social interaction, was the brain synchronisation increase associated?

Hierarchical linear regression model analysis showed that when the affiliative bond was controlled, teaching outcome and interaction quality did not make significant additional contributions to the increase in brain synchronisation ( $R^2$  change = 0.048, F change<sub>(2,56)</sub> = 1.527, P = 0.226). However, the affiliative bond still contributed significantly to the increase in brain synchronisation even when the teaching outcome and interaction quality were controlled ( $R^2$  change = 0.098, F change<sub>(1,56)</sub> = 6.2, P = 0.016). Thus, it seemed that the increase in brain synchronisation was more closely associated with affiliative bonding than with social interaction.

### A chain mediation role of social interaction in affiliative bonding

The chain mediation analysis showed that all three paths were statistically significant in the model ( $a_{beta}$ ,  $b_{beta}$  and  $c_{beta}$  in Figure 6). First, the path from the brain synchronisation increase in the second resting state (i.e. the difference between the second and the first resting state) to the strength of the affiliative bond reached significance ( $c_{beta} = 0.268, P = 0.048$ ), suggesting that there was a significant total effect of the model and thus the prerequisite of the mediation analysis was satisfied.

Second, the paths from the brain synchronisation increase in the second resting state to the brain synchronisation in

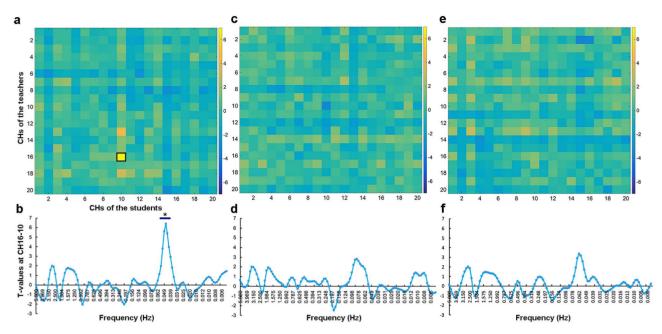


Fig. 3. Paired two-sample t-test of brain synchronisation. (a, c and e) Show increases in brain synchronisation in the turn-taking mode, lecturing mode and video mode, respectively. There were 20 CHs for each member of a pair and 400 CH combinations in total. The CH combination that showed a significant increase in brain synchronisation is highlighted by a black rectangle. (b, d and f) Show the distribution of brain synchronisation increases at the sensorimotor cortex (SMC, CH16-10) across the full frequency range in the turn-taking mode, lecturing mode and video mode, respectively. The short black line and star indicate the frequency range that passed the FDR correction (0.04–0.05 Hz).

social interaction and then to the strength of the affiliative bond reached significance ( $a_{beta} = -0.282$ , t = -2.177, P = 0.034;  $b_{beta} = 0.314$ , t = 2.417, P = 0.019). This result indicated a significant indirect effect, i.e. the overall pattern of teacherstudent interaction (i.e. brain synchronisation that was averaged across the brain synchronisation of TPJ-TPJ (0.06–0.07 Hz), aSTC-TPJ (0.5–0.7 Hz) and TPJ–aSTC (0.5–0.7 Hz)) mediated the relationship between the brain synchronisation increase of the SMC in the second resting state and the strength of the affiliative bond.

Finally, however, because the path from the brain synchronisation increase in the second resting state to the strength of the affiliative bond was still significant when the brain synchronisation in the social interaction was controlled (i.e.  $c_{beta} = 0.340$ , t = 2.613, P = 0.012, Figure 6), the mediation was only partial, and other factors that were not considered in this study contributed to affiliative bonding.

## Role assignment and social interaction were both required for affiliative bonding

In this study, the roles of both the teacher and student were assigned prior to the resting-state session. However, as described above, the permutation test did not show any significant brain synchronisation in any groups pre-interaction (Ps > 0.05), suggesting that role assignment alone did not lead to an affiliative bond.

To test whether social interaction alone would lead to brain synchronisation changes in the subsequent resting state, an additional experiment was conducted (see Supplementary material) in which no social roles were assigned to any of the participants. They were required to perform an interaction task, and brain activity in the resting-state sessions was collected both pre- and post-interaction. For this experiment, no significant brain synchronisation changes were found for any CH combinations in any frequency ranges (paired twosample t-test, Ps > 0.05, FDR correction, Figure 7), suggesting that social interaction that was not designed to induce spontaneous emergence of an affiliative bond (Jiang *et al.*, 2015) did not lead to affiliative bonding. In sum, both role assignment and social interaction were required for affiliative bonding.

### Discussion

This study aimed to test the hypothesis that INS underlies affiliative bonding. The results showed a significant increase in brain synchronisation in the resting state when an affiliative bond was created. Moreover, the increase in brain synchronisation was selectively correlated with the strength of the affiliative bond but not with the quality of the social interaction. Finally, brain synchronisation in the social interaction seemed to have a chain mediation role between the relationship of brain synchronisation increase in the resting state and the strength of the affiliative bond. These results are discussed in sequence below.

First, our results showed a significant increase in brain synchronisation during a resting-state post-interaction compared to that pre-interaction. Previous theories have proposed a bio-behavioural synchrony account of affiliative bonding, that is, body movement and gaze behaviours, mental processes, biochemical processes and neural activity might synchronise between individuals who are bonded via social affiliation. Moreover, biochemical and neural synchrony are hypothesised to support behavioural and mental synchrony and affiliative bonding (Feldman, 2017). While previous studies have well demonstrated the increase in brain synchronisation in some social affiliations such as that of a leader–follower or a teacher–student relationship, they did not disentangle the process of affiliative bonding from role assignment and social interaction. This study addressed this issue by examining brain

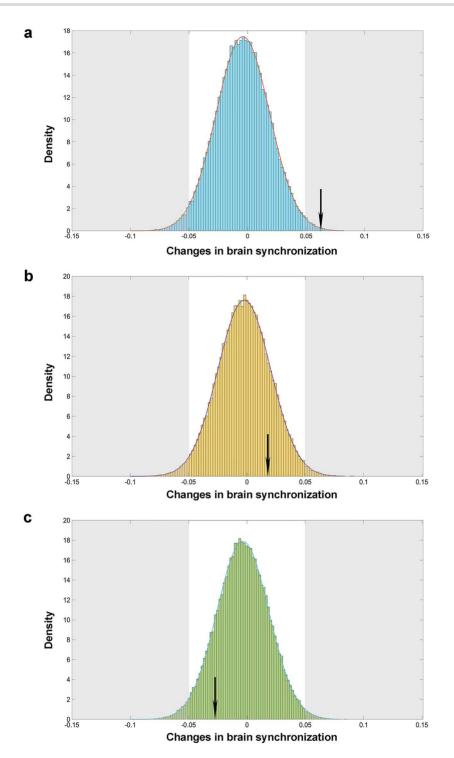
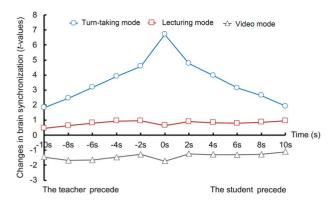


Fig. 4. Results of the permutation test on changes in brain synchronisation from pre- to post-interaction. For each panel, the x- and y-axes represent the brain synchronisation change and density of the distribution, respectively. The grey areas indicate 5% area (note that one side is 2.5%). The black arrows indicate the positions of brain synchronisation change post-interaction compared to that pre-interaction. Note that significant changes in brain synchronisation were found only after the turn-taking mode of teaching (a, P < 0.05) but not after the lecturing (b) or video modes (c) of teaching (P > 0.05).

synchronisation in the resting state when social interaction was absent. The findings suggest that a distinct pattern of brain synchronisation is associated with affiliative bonding between teachers and students, thus providing direct support for the bio-behavioural synchrony account. Second, the significant increase in brain synchronisation was found only after a turn-taking mode of social interaction. Moreover, the brain synchronisation in social interaction partially mediated the relationship between the brain synchronisation increase in the resting-state post-interaction and the



**Fig. 5**. Results of the time-lag analyses. The x-axis represents time lag when the teacher's brain activity preceded that of the student (left) or vice versa (right). The y-axis represents the changes in brain synchronisation in t-values. Note that the significant increase in brain synchronisation after the turn-taking mode of teaching reached a peak when the brain activities of the teacher and student were temporally aligned.

strength of the affiliative bond. An account of the social brain has posited that turn-based dynamic social interaction is a key constituent of grasping the minds of others and an action by an 'initiator' may lead to closer monitoring of the outcome of interaction (Schilbach *et al.*, 2013). The present findings provided initial support for this hypothesis by comparing the turn-based interaction mode with other modes of interaction and by testing brain synchronisation changes in the resting state before and after a social interaction.

Third, the significant increase in brain synchronisation was found at the SMCs of both the teacher and student. This result confirmed our hypothesis that shared representations of the affiliative bond is associated with brain synchronisation between the same brain regions of a teacher-student pair. Previous studies have reported overlap between sensory and motor representations across a range of human actions such as grasping (Rizzolatti *et al.*, 2001) and musical instrument playing (Novembre and Keller, 2014), suggesting the potential involvement of the resonance-based simulation process in shared cognitive representations between individuals. The present findings additionally suggest that the simulation mechanism is also associated with representation sharing of social bonds between partners.

Fourth, time-lag analyses on brain synchronisation indicated that the fluctuations in brain activity in the teacher and student were temporally aligned in the resting state. Previous evidence has shown that social interaction is facilitated by a mutual ability to predict each other's subsequent action (Konvalinka et al., 2010; Stephens et al., 2010; Liu et al., 2017). Moreover, leaders are able to say the right things at the right time by predicting the turn-taking behaviours between her/him and the followers (Jiang et al., 2015). Most importantly, during successful teaching, the teacher's brain activity is associated with the subsequent brain activity of the student, and the length of the time lag roughly corresponds to the amount of time the teacher took to ask a question as well as the amount of time the student took to answer a question (Zheng et al., 2018). On the other hand, evidence also showed that a shared representation of the syntax (Liu et al., 2019) or concept (Stolk et al., 2014) between two individuals was associated with a temporal alignment of the two individuals' brain activity. The present findings confirmed the hypothesis that in the resting state when no social interaction occurred, the representation sharing mechanism and the timealignment effect might have a dominant role, whereas in the social interaction process, the prediction mechanism and the time-lag effect might have a dominant role.

Finally, our results showed that role assignment or social interaction alone was not sufficient for affiliative bonding. Previous studies on social relationships have conflicted with each other on this issue. For instance, some studies on leadership found that role assignment prior to the onset of social interaction induced leadership-specific brain synchronisation (Sanger et al., 2012, 2013; Konvalinka et al., 2014). Other studies on teacher-student relationships, however, found that role assignment alone did not lead to significant brain synchronisation (Holper et al., 2013; Takeuchi et al., 2017; Zheng et al., 2018). The present results tested the function of role assignment by conducting a permutation test and confirmed that role assignment did not produce a significant brain synchronisation change that was associated with the social bond between the teacher and student. The additional validation experiment further demonstrated that social interaction alone did not lead to significant brain synchronisation change in the resting state either. Thus, both role assignment and social interaction seem to be required for affiliative bonding. Another possibility, however, is that there are different mechanisms for the creation of different types of social affiliations. Future studies are required to test this hypothesis.

There are several limitations in this study. First, only four teachers were involved in this study. While this is better than using a single teacher, practice may have affected the interaction quality. However, we did not find a linear decrease in the interaction quality or brain synchronisation across interaction times (i.e. pairs of participants). Furthermore, even if the practice effect came into play, it was equal across the three interaction modes. Thus, this effect seemed unlikely to affect our results. Moreover, increasing the number of teachers also comes at the cost of increasing inter-dyad variability. Second, because of the limited spatial resolution and limited depth that fNIRS can detect, not all brain regions that are involved in affiliative bonding were captured in this study. Future studies using fMRI are needed to address this issue. Finally, the potential impact of motion artefacts on the true signal is a common issue in hyperscanning studies of free social interactions. Although the WTC normalises the amplitude of the signal according to each time window, which mitigates the transient spikes induced by movements, the normalised movement signal might still contribute to synchrony. In this study and in previous studies (Liu et al., 2019), the signal quality has been examined and compared between preand post-correction on motion artefacts. The results indicate that a percentage of motion artefacts that is lower than 5% might not significantly contribute to the statistical results. Thus, in future studies it is important to check the percentage of motion artefacts and to make appropriate corrections for the motion artefacts.

In summary, the findings of this study support the longstanding bio-behavioural synchrony account that interpersonal neural synchrony underlies affiliative bonding. The results further suggest that a brain synchronisation-based simulation mechanism in the sensorimotor system might have a key role in affiliative bonding. Moreover, our findings also support the perspective that turn-based interaction is more effective in creating social affiliation than other modes of interaction. Finally, the findings indicate that both role assignment and social interaction are required for affiliative bonding. These findings provide important insights into the neural mechanisms underlying social grouping and organising.

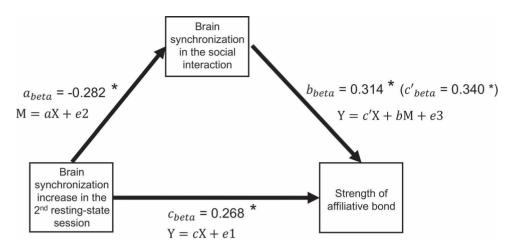


Fig. 6. The chain mediation model. The three rectangles represent the independent (X), dependent (Y) and mediation variables (M), respectively. The arrows indicate the chain relationship. Both the computing equations and coefficient values are shown beside the arrows. In the equations, X, Y and M represent the brain synchronisation increase in the second resting state, strength of the affiliative bond and brain synchronisation during the social interaction, respectively. c, *a*, *b*, and c' represent coefficients, and *e*1 – 3 represent residual errors.

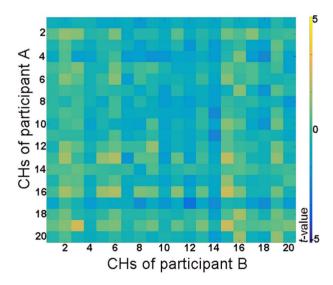


Fig. 7. Results of the validation experiment showing a t-map for the difference between pre- and post-interaction in brain synchronisation. Note that no significant changes in brain synchronisation were found after FDR correction (P < 0.05).

### Supplementary data

Supplementary data are available at SCAN online.

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### **Conflict of interest**

The authors declare no competing financial interests.

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