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# The influence of laughter on interbrain synchrony, cooperation and their respective correlation

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### German Abstract

Welche Beziehung besteht zwischen zwischenmenschlicher Anpassung und Kooperation, wo finden wir ihre jeweiligen neuronalen Korrelate, und welche Rolle spielt das soziale Schmiermittel Lachen in dieser Beziehung? In dieser Arbeit wird der Einfluss des Lachens auf die Zwischenhirnsynchronität im Gyrus frontalis inferior, in der Temporoparietalen Verbindung und im Gyrus temporalis superior untersucht. Darüber hinaus wird die Beziehung zwischen der Zwischenhirnsynchronität in diesen Regionen und der Kooperation untersucht, und ob Lachen die Kooperation unabhängig von der Zwischenhirnsynchronität beeinflusst. Die Arbeit basiert auf funktioneller Nahinfrarotspektroskopie und Verhaltensdaten aus der Mutterstudie "Laughing Together", einem Projekt der Abteilung für Entwicklungspsychologie der Universität Wien. Das Lachen wird durch ein Wortspiel, Tierclips und die allgemeine experimentelle Atmosphäre zwischen den Bedingungen manipuliert. Der Kooperationserfolg wird anhand der Leistung in einem kooperativen Brettspiel gemessen. Es werden gleichgeschlechtliche Dyaden mit Teilnehmern im Alter zwischen 18 und 25 Jahren gebildet.

Die Versuchsergebnisse konnten nicht zeigen, dass Lachen die Zwischenhirnsynchronität oder die Kooperation beeinflusst. Eine höhere Zwischenhirnsynchronität führte nicht zu einer besseren Kooperation.

Mögliche Gründe, warum die Nullhypothese nicht verworfen wurde, sind die geringe Stichprobengröße, das Scheitern der Lachmanipulation oder der theoretische Charakter des Konzepts der Zwischenhirnsynchronität, dem es an kausalen Belegen für höhere kognitive Funktionen fehlt.

#### **Summary**

What is the relationship between interpersonal alignment and cooperation, where do we locate their respective neural correlates, and what role does the social lubricant laughter play in this relationship? This thesis investigates the influence of laughter on interbrain synchrony in the Inferior frontal gyrus, the Temporoparietal junction and the Superior temporal gyrus. It further explores the relationship between interbrain synchrony in those regions and cooperation, and whether laughter influences cooperation independently of interbrain synchrony. The thesis is based on functional Near-Infrared Spectroscopy and behavioural data from the mother study "Laughing together", a project of the Department of Developmental Psychology at the University of Vienna. Laughter is manipulated through a word game, animal clips and the general experimental atmosphere between the conditions. Cooperation success is measured through the performance in a cooperative board game. Same-sex dyads

are formed with participants aged between 18 and 25.

The experimental results could not show that laughter influences interbrain synchrony nor cooperation. A higher interbrain synchrony did not lead to a better cooperation. Possible reasons why the null hypothesis was not rejected include the small sample size, failure of the laughter manipulation, or the theoretical nature of the concept of interbrain synchrony, which lacks causal evidence for higher cognitive functions.

#### Introduction

In everyday interactions, whether professional or private, there is often a discrepancy in how efficiently we can interact with one another. While some people seem to connect instantly in terms of values and capabilities, facilitating success in achieving a common goal, with others it can sometimes feel like an endless road of defining how to interact before even beginning to work together. Interbrain synchrony may explain this observation on a neuronal level. It refers to a similar rhythm of brain activity in two or more individuals during social interactions. This aligned rhythm relies on the reciprocal exchange of signals emitted by the respective brain and body (Hasson et al., 2012). Hyperscanning, which involves the simultaneous scanning of two or more people's neuronal activation, is an approach to observe interbrain synchrony in social interactions.

The 'Laughing Together' study, conducted by the Developmental Psychology Unit of the University of Vienna and supervised by Dr. Carolina Pletti and Verena Schäfer, B.A. M.Sc., aims to provide new insights into the relationship between laughter and interpersonal synchrony. The study uses a hyperscanning functional Near-Infrared Spectroscopy (fNIRS) approach to understand the role of interbrain synchrony in this topic. FNIRS is an efficient measurement tool that is often used in hyperscanning studies because of its resistance to motion artifacts (Czeszumski et al., 2020). This is particularly important when measuring neural activation during ecologically valid social interactions. FNIRS measures the hemodynamic response of the cerebral cortex and is therefore a correlational method.

This master's thesis uses fNIRS and behavioural data from 'Laughing together.'

# Cooperation

The capacity to collaborate with others allows individuals to achieve goals that would otherwise be unattainable. This behaviour is driven by cognitive mechanisms that facilitate the coordination of actions with partners and the distribution of acquired resources to incentivise continued cooperation (Melis & Tomasello, 2013). As demonstrated by the authors, this behaviour appears to be a recipe for success across species.

As it is not possible to make direct cross-comparisons between social behaviour in humans and other species due to the differing levels of complexity in human social interactions, there exist various theories on cooperation. From a game theory perspective, which provides mathematical techniques for analysing situations of conflict and cooperation between rational decision makers (Myerson, 2013), it may seem advantageous to free-ride on the cooperation of others to maximise one's own payoffs from a micro perspective (Rand & Nowak, 2013). However, cooperative behaviour at a societal level is often the most beneficial approach. The link between the two viewpoints may be direct or indirect reciprocity, which is the expectation of receiving something in return from a collaborator or someone in relation, at some point in time, for one's own willingness to collaborate (Rand & Nowak, 2013). This explanation encompasses short- and long-term decisions on cooperation. Game theory provides a logical explanation for cooperation. However, it views the individual as Homo economicus, an agent of perfect rationality, always acting on its own maximum benefit and utility (Rittenberg & Tregarthen, 2012). This perspective lacks the complexity required to fully describe human behaviour.

Additional insight may be gained from affective and social neuroscience. Vanutelli and colleagues (2017) discovered that emotional mechanisms can provide a satisfactory reward during the production of common behavioural effects. Successful cooperation boosts the self-perception of social position and hierarchy by the cooperators and strengthens their performance in future common endeavours (Balconi & Vanutelli, 2017). Cooperation appears to be intuitively rewarding through emotional mechanisms and, in the case of success, even reinforcing.

In general, emotions and emotional signalling can play an important role in promoting cooperation. Individuals are more likely to gain trust for a joint cooperation when they base their signal on emotion rather than reason. This is based on the finding that the addressed cooperators trust in emotion is more often justified than if they trusted a signal based solely on reason (Levine et al., 2018). Given the potential risk of exploitation in collaborative contexts, it is important to have a reliable mechanism for determining whether or not to trust another individual. This reciprocal process involves physiological and behavioural signals displayed by the sender's face, and the synchronisation of the receiver's expression and emotions (Behrens et al., 2020). The purpose of this adaptation is to empathise with each other and

understand each other's (Behrens et al., 2020; Mills et al., 2019). Numerous studies from various disciplines have demonstrated that synchronisation on physiological, emotional, behavioural, and neural levels enhances engagement in and the success of cooperation (Behrens et al., 2020; Dávid-Barrett & Dunbar, 2012; Levine et al., 2018; Vico Fallani et al., 2010).

### Interbrain Synchrony

When individuals share the same environmental input, engage in the same behavioural tasks, or communicate in a human-to-human interaction, their neural activation appears to align. The stronger this alignment, the better the collaborative effort works (Kawasaki et al., 2013; Vico Fallani et al., 2010). This process can also be described as brain-to-brain coupling or interbrain synchrony.

Interpersonal physiological synchrony refers to the spontaneous temporal coordination of physiological processes (Mayo et al., 2021). Brain-to-brain coupling operates similarly but requires further explanation.

According to Hasson et al. (2012), for two brains to couple, one individual's perceptual system must establish a coupling with another individual's motor system. This binding mechanism stems from the brain's ability to couple with the physical environment through stimulus-to-brain coupling. The physical world provides an array of stimuli in the form of mechanical, chemical, and electromagnetic energies emitted by various objects. Organisms convert different types of signals, such as chemical or mechanical, into electrical impulses through receptors. This allows the brain to gather information about the external environment and generate appropriate behavioural responses. In addition, organisms actively move and use sensory receptor surfaces, such as hands, eyes, and tongues, to sample information from their surroundings.

This concept involves signals generated by another brain and body that bear resemblance to one's own. Brain-to-brain coupling is a process where two brains establish a connection through the transmission of a physical signal, such as light, sound, pressure, or a chemical compound, across a shared physical environment. According to Hasson et al. (2012), this process is comparable to a wireless communication system. Observing the actions, sensations, or emotions of another individual can trigger cortical representations in the perceiver, known as vicarious activations (Keysers & Gazzola, 2009). These activations may align neural

responses between individuals (Hasson et al., 2012). Various mechanisms can couple neural responses across two brains, with vicarious activation being just one example.

Various studies have highlighted that the successful establishment of interbrain synchrony leads to more efficient and effective social interaction.

In a study using the hyperscanning paradigm, Fishburn et al. (2018) found that cooperative tasks resulted in more synchronous neural activation between participants compared to individual tasks, observing others doing the task, or sharing an experience such as watching a movie together. This expands on the concept of shared intentionality, which posits that in a cooperative interaction, two or more individuals share a goal and must coordinate their efforts by sharing representations, predicting actions and actions of others, as well as integrating those facets (Searle, 1983; Sebanz et al., 2006). Additionally, beyond cooperative tasks, neuronal coupling predicts the success of an interaction. Stephens et al. (2010) discovered that the level of interbrain synchrony between a speaker and a listener is a predictor of the comprehension of the story being told. According to Vodrahalli et al. (2018), the neuronal activation of an experience is similar to the brain activity of someone who is experiencing it. Therefore, it appears that the more we can empathize with others, the more effectively we can interact with them. This hints to the concept of "Theory of mind" (or "Mentalizing" which commonly is used synonymously (Abu-Akel & Shamay-Tsoory, 2011; Bull et al., 2008; Dolan & Fullam, 2004)), which refers to the ability of representing and attributing affective and cognitive mental states to self and other (Abu-Akel & Shamay-Tsoory, 2011). In a broader sense, it means that we have a theory of someone else's mind, hence we reason about another person's representational mental states (Saxe & Kanwisher, 2003). However, there are mixed results on the effect of Theory of Mind on cooperative behaviour and cooperative intentions with some studies finding none or even a negative correlation (DeAngelo & McCannon, 2017; Sylwester et al., 2012), while a new study with a larger sample showed that there is a positive direct relationship between Theory of Mind and cooperative success (Markiewicz et al., 2023).

#### Laughter

Are there any more concrete processes than the abstract concept of interbrain synchrony that can explain successful social interaction? One possible explanation is the social behaviour of 'laughter', which facilitates social bonding through the shared release of endorphins (Dunbar, 2022) and acts as a booster for social performance.

Laughter is a cross-cultural behaviour and one of the first social vocalizations made by human infants (Gervais & Wilson, 2005). Laughter is generally considered a social behaviour, occurring thirty times more frequently in social contexts than when alone (Provine & Fischer, 1989). As laughter in a conversation is predominantly produced by the speaker, it appears that one of its functions is to facilitate interactions and promote positive feelings in the listener. In cases of nervous laughter, it may express anxiety (Gervais & Wilson, 2005). There are two types of laughter: voluntarily generated non-Duchenne laughter and spontaneous Duchenne laughter.

A non-Duchenne smile is identified when only the corners of the mouth are raised (Ekman et al., 1990). This type of laughter is self-generated and emotionless (Gervais & Wilson, 2005). On the other hand, when laughing with the stimulus-driven and emotionally valenced Duchenne smile, the cheeks and eyes are also raised (Ekman et al., 1990; Gervais & Wilson, 2005).

Laughter can signal cooperative intent and affiliation, and laughing together can enhance self-efficacy and feelings of self-efficacy (Beckman et al., 2007).

This, in turn, can facilitate group performance in a working environment (Stajkovic & Luthans, 1998) and improve social investments (Cremer & van Vugt, 1998). Additionally, laughter can enhance cooperation among groups of strangers and their investment in the group (van Vugt et al., 2013). This seems to work better with a Duchenne rather than a non-Duchenne laughter (Deng et al., 2022; Reed et al., 2012).

Laughter can lead to interpersonal synchrony through emotional contagion (Banning & Nelson, 1987; Gervais & Wilson, 2005). The above-mentioned findings on behavioural alignment and its effect on interbrain synchrony suggest that laughter may enhance interbrain synchrony. This interpersonal coupling can further increase in-group cooperation and cohesiveness (Banning & Nelson, 1987; Gervais & Wilson, 2005; Vinton, 1989). Lackner et al. (2019) discovered that humour and laughter promote physiological synchronization between two male conversation partners who were previously unknown to each other, regardless of the amount of conversation between them. This finding supports the notion that shared experiences improve physiological synchrony (Palumbo et al., 2017). Additionally, humour can establish a common ground for interaction by exploring potential shared implicit constructs together (Clark, 2012; Flamson & Barrett, 2008).

# Regions of Interest (ROI)

#### Inferior frontal gyrus (IFG)

We find a neural correlate for this mental ability of interbrain synchronisation through shared intentionality in the Inferior frontal gyrus (Fishburn et al., 2018; Saxe & Kanwisher, 2003) as well as in the Temporoparietal junction when it comes to the representation of someone else's representations (TPJ; Saxe & Kanwisher, 2003). The IFG is part of the prefrontal cortex and involved in speech and language processing (Greenlee et al., 2007). It seems to be involved in phonologic, semantic, sentence- and discourse-level processing during conversations or language related tasks (Gernsbacher & Kaschak, 2003; Greenlee et al., 2007). The right IFG further shows activation when detecting the emotional content of speech, conveyed by the emotional tone (Buchanan et al., 2000; Gernsbacher & Kaschak, 2003).

The IFG further contains motor representations of actions in the operation-execution system of social alignment (Shamay-Tsoory et al., 2019). The concept of the operation-execution system of social alignment describes the functional route behavioural alignment takes in movement, emotion, or cognition: observing a behaviour may activate one's own representations for the behaviour, which proceeds to motor areas where responses are prepared and executed (Thornton & Knoblich, 2006). When interacting with a cooperative partner activation in the right IFG is increased while it is reduced when dealing with a competitor (Liu et al., 2015). The authors argue that this difference in activation emerges from increased motivation to achieve a goal with the help of others and decreased motivation due the disturbance of a competitor. Neural synchronisation of the inferior frontal cortex, which consists of the IFG, between conversation partners seems to be dependent on an equal face-toface dialogue, a back-to-back dialogue or face-to-face monologue could not deliver an interbrain synchronisation (Jiang et al., 2012). Neural alignment in this area therefore seems to result from multi-modal sensory information integration and turn-taking behaviour. The IFG shows to be an area that combines involvement in multiple speech related processes as well as emotion detection in speech and plays a role in the success of face-to-face conversations and cooperation scenarios through neural alignment.

#### Temporoparietal junction

The TPJ is a region situated at the posterior end of the Sylvian fissure and is responsible for integrating internal and external information to differentiate between self and others' mental states, such as beliefs, desires, and intentions (Abu-Akel & Shamay-Tsoory, 2011). Hemispherical functions appear to differ. The left TPJ (ITPJ) overlaps with the Wernicke area and the angular gyrus, regions involved in language comprehension (Binder, 2015; Seghier,

2013). It is also involved in inferring about someone else's mental states, such as beliefs (Samson et al., 2004).

The right TPJ (rTPJ) is involved in an individual's ability to orient their attention to a new stimulus (Corbetta et al., 2000). Activation of the rTPJ is linked to generating, testing and correcting internal predictions about external sensory events (Decety & Lamm, 2007). This creates the basis for empathy and theory of mind as it enables distinguishing between different perspectives on the same situation (Decety & Lamm, 2007).

The right TPJ mediates behavioural alignment through the mutual social attention system, which is responsible for mentalizing and theory of mind. This system consists of the rTPJ, which represents social connectedness and social stimulus-driven attention, and the prefrontal cortex, which processes attention (Gvirts & Perlmutter, 2020).

The TPJ is involved in cooperative activities and communication (Gvirts & Perlmutter, 2020; Stephens et al., 2010) and is related to social connectedness (Eddy, 2016) and different forms of attention. It is active during mutual attention (Gvirts and Perlmutter, 2020) and plays a role in selective social attention (Dai et al., 2018; Eddy, 2016). The TPJ is a central area for Theory of Mind as it enables the distinction between self and other mental states, as well as different perspectives on social situations. It also guides attention, shows activation during social connection, and plays a role in language comprehension.

#### Superior temporal gyrus (STG)

The STG is located ventrally of the Sylvian fissure (and therefore of the TPJ) and dorsally of the superior temporal sulcus. It is mainly associated with processing specific sounds and language comprehension (Bigler et al., 2007; Gernsbacher & Kaschak, 2003). Some studies have shown that the STG is involved in social emotions such as pride, guilt, embarrassment, shame, jealousy, and admiration when taking someone else's perspective (Ruby & Decety, 2004). It is also involved in processing emotions generated by visual, auditory, and olfactory stimuli (Royet et al., 2000). Bigler et al. (2007) propose a link between the amygdala and prefrontal cortex through the STG forming a regulatory system involved in social cognition. This system regulates cognitions influenced by social context.

It is one of the central brain regions associated with spontaneous, authentic laughter (Lavan et al., 2017), the previously described Duchenne laughter. It becomes active when individuals perceive positive, arousing auditory stimuli (Lavan et al., 2017). The region of the auditory cortex involved in emotional perception of arousal and valence is also part of the Wernicke

Area and is responsible for predictive auditory language processing (Stephens et al., 2010). Additionally, the STG is involved in preprocessing predictable language representations (Dikker et al., 2014). This suggests that regions involved in predictive auditory networks are active prior to the onset of the auditory event. This proves to be important for a comprehensive conversation flow (Dikker et al., 2014, Stephens et al., 2010). The involvement of the STG in language processing and comprehension is well-established. Additionally, as part of the emotion regulation network, it plays a role in emotion processing. These two functions complement each other, contributing to the STG's involvement in spontaneous and authentic laughter.

#### Functional near-infrared spectroscopy (fNIRS)

Functional Near-Infrared Spectroscopy (fNIRS) is a non-invasive and non-ionizing method used for functional monitoring and imaging of brain hemodynamics (Scholkmann et al., 2014). It employs near-infrared light to penetrate several centimetres through tissue, including the scalp and skull, and assesses concentrations of oxygenated, deoxygenated, and total hemoglobin within the brain through spectroscopic interrogation.

The principles underlying fNIRS are based on the unique absorption spectra of oxyhemoglobin (HbO) and deoxyhemoglobin (HbR) in response to near-infrared light. fNIRS operates within the wavelength range of 650 to 950 nanometers, allowing for penetration through biological tissues and enabling the measurement of changes in cerebral blood flow associated with neural activity (Scholkmann et al., 2014). FNIRS measurements are designed with sources and detectors being positioned on selected portions of the scalp. The sources and detectors are represented by optodes, which are located above specific cortical regions (Morais et al., 2018).

Although fNIRS may not achieve the same spatial resolution as methods such as functional magnetic resonance imaging (fMRI), it has a distinct advantage in its high temporal resolution coupled with reasonable spatial resolution (Czeszumski et al., 2020). This unique feature allows for the examination of rapid changes in hemodynamic response with millisecond precision, presenting a valuable advantage in the study of dynamic cognitive processes (Czeszumski et al., 2020; Scholkmann et al., 2014).

The non-invasive nature, safety, cost-effectiveness, and portability of fNIRS make it a promising tool for use in both research and clinical settings (Czeszumski et al., 2020; Rahman et al., 2020). This imaging modality is particularly advantageous in populations and measurement procedures where other imaging techniques may be limited (Czeszumski et al.,

2020). The utility of this method extends to infants, children, and individuals with limited cooperation, providing a valuable alternative in scenarios that are challenging for traditional neuroimaging techniques (Scholkmann et al., 2014).

Since its first use twenty years ago, fNIRS has become an effective tool for studying normal brain physiology and deviations associated with disease (Rahman et al., 2020). The increasing interest in fNIRS is demonstrated by the exponential growth of publications, which has doubled approximately every 3.5 years over the past two decades (Scholkmann et al., 2014).

#### Hyperscanning

The study of social interactions in neuroscience has led to the development of hyperscanning, a technique that measures brain activity in multiple individuals simultaneously. This approach allows for the investigation of real-time dynamics between interacting brains, providing a significant advantage over traditional methods (Czeszumski et al., 2020). Unlike conventional paradigms that focus solely on the brain activity of individual participants during social interactions, hyperscanning allows for the concurrent examination of both intra- and interbrain neural relations (Schilbach et al., 2013). This methodology provides a unique avenue to understand the complexity of joint action, encompassing spontaneity, reciprocity, and multimodality. This poses a substantial challenge in neuroscientific examinations of social behavior.

FNIRS has been instrumental in advancing hyperscanning research due to its mobility and resilience to motion artifacts, making it particularly suitable for studying cognitive processes during social interactions.

Despite having a lower temporal resolution compared to EEG, which varies between 0.1 and 1 second (Quaresima & Ferrari, 2019), fNIRS has gained widespread use in cognitive neuroscience. The most commonly used method for calculating interpersonal neural synchrony in hyperscanning fNIRS experiments is Wavelet Transform Coherence (WTC; Nguyen et al., 2021). WTC assesses the relationship between the individual fNIRS time series in each dyad and each channel as a function of frequency and time. In this study, the WTC was estimated using the cross wavelet and wavelet coherence toolbox (Chang & Glover, 2010; Grinsted et al., 2004).

# Study design Procedure

This investigation takes place in a controlled laboratory environment, stipulated to endure a maximum duration of 120 minutes. Preceding the experimental procedures, same-sex dyads are purposefully formed, comprising participants unacquainted with one another and maintaining an age discrepancy within a range not exceeding five years. The participants are prepared with three ECG-electrodes, sixteen fNIRS-optodes and a microphone. The two upper ECG electrodes are placed beneath the clavicle left and right. The lower electrode is placed on the second rib from below on the left side of the upper body. The procedural sequence unfolds with participants initially completing a series of standardized questionnaires, thereby establishing a baseline assessment. The questionnaires include a scale about the felt sympathy towards the experimental partner, the Positive and Negative Affect Schedule (PANAS; Watson et al., 1988), and a handedness inventory. Subsequently, the manipulation phase commences.

Within the experimental group, laughter induction is pursued through the engagement of participants in a ludicrous word game and the viewing of humorous animal videos. Conversely, the control group engages in a neutral word game and is exposed to animal videos devoid of comedic content. The word game consists of a cloze, presented to one of the participants, while the other one chooses the words to fill in the gaps. After the exposition to the videos, participants in both groups rate the videos together. This ends the manipulation phase. The initial WTC within the dyad refers to this point in time, and represents the neural synchrony between both participants after they've gone through the manipulation phase together.

Following the manipulation phase, a 10-minute interval ensues wherein participants engage in uninhibited interaction without the presence of experimenters in the experimental chamber. The second WTC between the dyad refers to this point in time as the neural synchrony between participants after they freely interacted with each other.

Subsequent to the interaction phase, participants proceed to complete additional questionnaires, providing a comprehensive post-manipulation evaluation. The questionnaires include a second set of the PANAS, a second scale about the felt sympathy towards the experimental partner, a survey about their liking of the other participant and how well they individually liked the watched animal videos as well as an Inclusion of Other in the Self Scale (IOS; Aron et al., 1992). The culmination of the experimental protocol involves the collaborative participation of dyad members in a cooperative game, serving as a concluding

activity within the designated timeframe. As for their cooperative game participants engage in the coordinative board game "Burgritter" (Tiggermann, 2006). Participants need to staple four blocks of different shape and size in a specific order with a rubber band, which they navigate through the four attached strings. The game consists of five rounds, one practice run and four 45 seconds long experimental trials. The cooperative performance gets quantified in a cooperation score, which gets calculated by dividing the cumulated time needed to finish all four rounds through the number of blocks participants were able to stack onto each other in total over four rounds. The lower the score, the better the two participants were able to cooperate.

#### FNIRS Optode Placement and Preprocessing of fNIRS Data

To correctly position the fNIRS optodes on the participant's scalp, we use optode-placementcaps that are specifically designed for this purpose. These caps come in a range of sizes, from 54 cm to 60 cm in diameter, to accommodate different head sizes.

The caps are based on the 10-20 system of electrode placement, which is the recognized standard for scalp electrode positioning in electroencephalography (Klem et al., 1999). The 10-20 system utilises external landmarks on the scalp to determine the relative cranial positions on the subject's head surface. The system assumes a consistent correlation between scalp locations and their underlying cerebral structures (Okamoto et al., 2004). It covers the area between the nasion and the inion.

The size of the head is measured from the frontal skull a few centimetres above the nasion to around the junction between the parietal and occipital bones. After the caps are placed on the participant's scalp, any hair blocking the light of the optodes is pushed aside. To prevent interfering light from the experimental chamber, a second cap is placed above the cap equipped with the optodes.

As for preprocessing the raw fNIRS data was converted from .fNIRX to .fNIRS data to make it compatible with the toolboxes on MATLAB (The MathWorks Inc., 2022). Then the data is segmented into the different parts: video phase – free interaction phase – game phase. The free interaction phase data is extracted between one minute after the start and one minute before the end of the phase. This accounts for distortions due to instructors leaving and later entering the experimental chamber. Afterwords the toolboxes homer2 (Huppert et al., 2009), Cross Wavelet and Wavelet Coherence Toolbox (Grinsted et al., 2004) are installed and used to calculate the wavelet transform coherence between the dyad. Then there is a visual data quality check, where the heart band of the channels shows the quality of a channel. Good channels have a clearly visible and continuous heart band without too many motion artifacts (Nguyen et al., 2021).

# Participants

The participants of "Laughing together" are between 18 and 25 years old and always matched with their same gender per dyad. Participants are only matched when the age difference is five years at maximum. In an online pre-screening survey, they have to not show any psychiatric dispositions or peculiarities.

Calculating the effect size of the mean difference between the comedy and the neutral condition in van Vugt et al. (2013) first experiment, we derive at a medium effect size of Cohen's d=0,51. With an  $\alpha$  error at 0,05% and the power at 0.80, a total sample size of 98 dyads for H<sup>1</sup> would be optimal, 49 per condition.

Following the findings of Duan et al. (2022), a synchronous pair shows better cooperation than a lesser synchronous pair with a medium effect size (Cohen's d=0,65). Using a medium effect size of Cohen's  $f^2=0,19$ , with an  $\alpha$  error at 0,05% and the power at 0.80, a sample size of 35 dyads for H<sup>2</sup> would be optimal.

Using the medium effect size (Cohen's d=0,51) of van Vugt et al. (2013) first experiment between comedy and neutral condition, we derive at Cohen's f=0,25 and hence with an  $\alpha$  error at 0,05% and the power at 0.80, a total sample size of 128 dyads for H<sup>3</sup> would be optimal.

# Hypotheses

As fNIRS studies have the limitation of a lower spatial resolution (Czeszumski et al., 2020) especially considering, that it is not possible to control for the individual's head shape as in functional Magnetic Resonance Imaging (fMRI) studies, not every channel will get looked at separately, but averaged per ROI.

As described above the WTC is calculated at two time points, after the manipulation and after the free interaction.

# Hypothesis one

As the right and left inferior frontal gyrus (IFG) play a central role in language processing, particularly in the detection of emotional content, they are areas to be considered when examining laughter. Theory of Mind and language comprehension are fundamental skills for understanding certain forms of humour (Vrticka et al., 2013). The right and left TPJ are areas

involved in humour and laughter, as they are considered central for Theory of Mind (Decety & Lamm, 2007) and language comprehension (Binder, 2015; Seghier, 2013). As previously mentioned, the STG plays a crucial role in various cognitive processes such as language comprehension (Bigler et al., 2007; Gernsbacher & Kaschak, 2003), emotional perspective-taking (Ruby & Decety, 2004), emotion processing (Royet et al., 2000), and spontaneous, authentic laughter (Lavan et al., 2017). Therefore, it is essential to consider the STG when examining laughter.

As laughter can lead to interpersonal synchrony through emotional contagion (Banning & Nelson, 1987), it is important to consider these regions when examining whether laughter leads to higher neural synchrony.

Therefore, the following hypothesis is derived:

# *H*<sup>1:</sup> *The participants in the laughter condition show a higher synchrony in the IFG, STG and TPJ than in the control condition.*

The interbrain synchrony in the IFG, STG and TPJ represents the dependent variable and the group (laughter and control) the independent variable.

Both WTC time points in the experiment are relevant to this hypothesis. The first time point represents the manipulation of laughter, as participants in the experimental group are expected to laugh by design. At the second time point, participants had the opportunity to laugh together in a free interaction influenced by the previously designed atmosphere of the experiment.

#### Hypothesis two

As described above, interbrain synchrony, often represented by interpersonal synchrony, is positively correlated with cooperation and cooperative success (Fishburn et al., 2018; Vinton, 1989). To use Theory of Mind to picture someone else's affective and cognitive mental states and use this ability beneficially in social interactions, neural alignment is required (Kealoha, 2020). Markiewicz et al. (2023) demonstrated that the ability of Theory of Mind can lead to cooperative success. As previously mentioned, the left and right TPJ are believed to be involved in this process.

Since cooperation and success in cooperation can trigger intuitively rewarding emotional mechanisms (Balconi & Vanutelli, 2017; Vanutelli et al., 2017), the STG, which serves as a link between the amygdala and prefrontal cortex (Bigler et al., 2007), is also an area worth investigating in this process.

Shared intentionality is the foundation of cooperation, and the IFG plays a crucial role in the connection between interbrain synchrony and cooperation (Saxe & Kanwisher, 2003). IFG activity increases when interacting with cooperators (Liu et al., 2015) and during the integration of information from turn-taking social interactions (Jiang et al., 2012).

As cooperation reinforces interbrain synchrony and vice versa (Fishburn et al., 2018; Vico Fallani et al., 2010), the following hypothesis is proposed:

 $H^2$ : The extent of interbrain coupling in the IFG, STG and TPJ predict the performance in the cooperation game.

This hypothesis focuses on time point two after the free interaction phase, which is the final WTC measurement before the Burgritter game, representing the measurement of cooperation success.

#### Hypothesis three

To address the question of whether laughter facilitates cooperation, as predicted by van Vugt et al. (2013), and whether interbrain synchrony is linked to this process, we must take the degree of interbrain synchrony within the dyad into account. Stephens et al. (2010) and Gervais & Wilson (2005) suggest that interbrain synchrony and interpersonal synchrony enhance mutual understanding, increase cohesiveness, and promote cooperation. To determine if laughter finds its way in this process, it is necessary to control for interbrain synchrony.

As with hypothesis one, the IFG must be considered due to its role in detecting emotional content, which is essential for humour and laughter (Buchanan et al., 2000; Gernsbacher & Kaschak, 2003). Additionally, as described in hypothesis two, it is involved in cooperative interactions. The TPJ plays a fundamental role in Theory of Mind, contributing to the ability to understand certain forms of humour and share intentionality with a cooperator (Saxe & Kanwisher, 2003; Vrticka et al., 2013). Reed and colleagues (2012) found that Duchenne smiles predict cooperative behaviour more than non-Duchenne smiles. Therefore, the STG, which is one of the central areas for Duchenne smiles and part of a regulatory system for social cognition (Bigler et al., 2007), needs to be considered.

Based on these findings, H3 is derived as follows:

*H*<sup>3</sup>: Laughter facilitates the cooperative performance even after controlling for interbrain synchrony in the IFG, STG and TPJ.

Similar to hypothesis one, both time points must be considered. Time point one marks the end of the laughter manipulation, and time point two marks the end of the free interaction phase. This time period is characterized by the atmosphere dependent on the experimental condition. The second time period is important as it is measured shortly before the cooperation task begins.

# Data Analysis

#### **Descriptive Overview**

The data set comprises n = 42 dyads, with n = 22 dyads in the laughter condition and n = 20 dyads in the control condition. In the laughter condition two dyads were excluded, one because the questionnaire set, and manipulation was mixed with the control group and the other one because it had artificially high WTC values with maximal coherence of one over all channels. In the control group one dyad was excluded due to a fNIRS malfunction.

Table 1 provides a brief overview of the descriptive statistics of the ROI over both time points. The highest average WTC in the laughter group was in the right IFG at time point one with a mean of .388 (n = 19 dyads) and a standard deviation of .134. The highest average WTC in the control group was in the right TPJ at time point one with a mean of .391 (n =14) and a standard deviation of .090.

Figure 1 and Figure 2 provide a comparison between the WTCs of the ROI hemispheres and time points.

The mean cooperation score of the laughter group (n=20) was 16.922 with a standard deviation of 10.629. The control condition (n=19) had a mean of 16.811 with a standard deviation of 5.391. Overall, the mean cooperation score of all 39 dyads was 16.868 with a standard deviation of 8.382 (cf. Figure 3)

Because of the small sample size for the following hypotheses bootstrapping was used with 1000 samples and confidence intervals at 95%.

#### Table 1

WTC descriptives of the ROI over both time points

				Std.	
		Ν	Mean	Deviation	Std. Error
WTC IFG	Laughter	19	,3883	,13425	,03080
right time	Control	19	,3788	,13176	,03023
point 1	Total	38	,3835	,13128	,02130
WTC IFG	Laughter	20	,3475	,08708	,01947

right time	Control	19	,3494	,08455	,01940
point 2	Total	39	,3484	,08473	,01357
WTC IFG left	Laughter	20	,3501	,12885	,02881
time point 1	Control	19	,3889	,10248	,02351
	Total	39	,3690	,11689	,01872
WTC IFG left	Laughter	20	,3900	,10700	,02393
time point 2	Control	19	,3447	,08971	,02058
	Total	39	,3679	,10032	,01606
WTC TPJ	Laughter	16	,3179	,11454	,02864
right time	Control	14	,3911	,07841	,02096
point 1	Total	30	,3521	,10450	,01908
WTC TPJ	Laughter	16	,3685	,07770	,01942
right time	Control	15	,3771	,11908	,03075
point 2	Total	31	,3727	,09826	,01765
WTC TPJ left	Laughter	14	,3688	,15992	,04274
time point 1	Control	12	,3209	,06946	,02005
	Total	26	,3467	,12655	,02482
WTC TPJ left	Laughter	13	,3524	,10387	,02881
time point 2	Control	12	,3425	,10779	,03112





#### Figure 2

Boxplot of the Wavelet Transform Coherence in the IFG by Group, Hemisphere, and Interval





Figure 3





#### Hypothesis one

To assess if it is necessary to look at group level for each time point (called phases in calculation) two mixed ANOVA were calculated for the WTC for both hemispheres of the TPJ over both time points between the groups was calculated. The within-subject contrasts show that there was no significant main effect for phase, Greenhouse-Geisser F(1.00, 27.00) = .134, p = .717. There was also no significant interaction between phase and group, Greenhouse-Geisser F(1.00, 27.00) = 3.185, p = .086. There was no significant difference between groups estimated over both time points, F(1,27) = 1.47, p = .236. Therefore, all following analyses regarding H1 are explorative in nature. To assess if it is necessary to look at both hemispheres for the TPJ a two factor ANOVA was calculated. The interaction Group and Hemisphere was nearly significant, F (1,108) = 2.952, p = 0.089. Looking at the interaction between group and hemisphere again, this time for the IFG there is clearly no interaction present, F (1, 151) > 0.000, p = 0.999. This adds to the fact that looking at the hemispheres separately is only to be considered explorative.

Using a one-way ANOVA looking at the TPJ at both time points following results can be reported. Because the small sample size a Levene test of homogeneity of variances was conducted. For the TPJ at both time points and hemispheres variance homogeneity was achieved with F(1, 21) = 2.724, p = .114 for the right TPJ at time point one, F(1,21) = .858, p = .365 for the left TPJ at time point one, F(1, 21) = 3.797, p = .065 for the right TPJ at time

point two and F(1, 21) = .022, p = .884 for the left TPJ at time point two.

For the IFG variance homogeneity was achieved as well for both time points and hemispheres, with F(1, 36) = .378, p = .543 for the right IFG at time point one, F(1, 36) = .1.573, p = .218 for the left IFG at time point one, F(1, 37) = .025, p = .875 for the right IFG at time point two and F(1, 37) = .599, p = .444 for the left IFG at time point two. There was no significant difference to be found between groups in any hemisphere or time point of the TPJ WTC. The closest area to show a significant difference was the right TPJ at time point one, with F(1, 21) = 4.292, p = .051,  $\eta^2 = .170$ . At time point one the left TPJ showed no difference with F(1, 21) = .349, p = .561,  $\eta^2 = .016$ , at time point two both hemispheres also showed no significant difference, with F(1, 21) = 1.110, p = .304,  $\eta^2 = .050$ at the right TPJ and F(1, 21) = .011, p = .918,  $\eta^2 = .001$ .

No significant difference was to be found between groups in any hemisphere or time point of the IFG WTC as well. The right IFG showed no difference with F (1, 36) = .048, p = .828,  $\eta^2 = .001$  at time point one, at time point two with F (1, 37) = .005, p = .944,  $\eta^2 > .000$  and the left IFG as well no difference with F (1, 36) = .608, p = .440,  $\eta^2 = .017$  at time point one, and at time point two with F (1, 37) = 2.046, p = .161,  $\eta^2 = .052$ .

#### Hypothesis two

To check the conditions for multiple regression, partial regression plots between cooperation and the WTC of all ROI were output. Even though there is a clear spread, in all plots, except for the right TPJ at time point two, there is linearity visible (cf. Figure 4, Figure 5, Figure 6 and Figure 7). The scatter plot between standardised predicted values and residuals showed no pattern and therefore indicates homoscedasticity (cf. Figure 8). To check for normal distribution, a histogram and P-P diagram were generated. Both indicate a normal distribution (cf. Figure 9 and Figure 10). In addition, the standardised residuals at time point 2 lie between -1.697 and 2.080, which also indicates normally distributed data. To check whether outliers are significant for the calculation of the regression, the Cook distance was calculated, with a maximum of .460 at time point two, indicating no conspicuity towards biases towards outliers. A collinearity diagnosis was carried out to check for multicollinearity. No ROI showed any peculiarities to any time point (cf. Table 2).

The overall regression model proved to be non-significant with F (4, 18) = .497, p = .739. For no ROI there was a relation between WTC and Cooperation (cf. Table 3).







# Figure 5

Partial Regression Plot for the WTC of the left IFG at time point 2



Partial Regression Plot for the WTC of the right TPJ at time point 2



Dependent Variable: Cooperation

Figure 7

Partial Regression Plot for the WTC of the left TPJ at time point 2



Scatterplott between standardised predicted values and residuals for Cooperation



Dependent Variable: Cooperation

**Regression Standardized Residual** 

# Figure 9





P-P Plot of standardized residuals



# **Table 2**Collinearity Statistics at timepoint two

Model		VIF
1	(Constant)	
	WTC IFG right time point 2	1,094
	WTC IFG left time point 2	1,081
	WTC TPJ right time point 2	1,218
	WTC TPJ left time point 2	1,247

# Table 3

Respective Influence of RsOI WTC on Cooperation at timepoint two

		Unstandardized Coefficients		Standardized Coefficients		
Model		В	Std. Error	Beta	t	Sig.
1	(Constant)	17,064	5,721		2,983	,008
	WTC IFG r 2	,948	9,046	,025	,105	,918
	WTC IFG 12	2,949	6,841	,100	,431	,672
	WTC TPJ r 2	-8,886	7,819	-,281	-1,136	,271
	WTC TPJ 1 2	-,833	8,017	-,026	-,104	,918

#### Hypothesis three

The Levene's test of equality of Error variances proves to be non-significant, F(1, 21) = 1.751, p = .200 variance homogeneity seems to be evident for the ANCOVA. With controlling for interbrain synchrony there is no difference between the groups considering the cooperation score, F(1, 17) = 1.216, p = .285,  $\eta^2 = .067$ . No ROIs WTC influences the difference in cooperation between the groups (cf. Table 4)

#### Table 4

Mean differences in cooperation between the groups under control of the covariates

					95% Confidence Interval	
					Lower	Upper
Parameter	В	Std. Error	t	Sig.	Bound	Bound
Intercept	18,189	5,777	3,148	,006	6,000	30,378
WTC IFG r 2	1,216	8,995	,135	,894	-17,763	20,195
WTC IFG 12	3,625	6,828	,531	,602	-10,781	18,031
WTC TPJ r 2	-10,830	7,970	-1,359	,192	-27,645	5,985
WTC TPJ 12	-,285	7,985	-,036	,972	-17,132	16,561
[Group=1,00]	-1,656	1,501	-1,103	,285	-4,823	1,512
[Group=2,00]	$0^{a}$	•	•	•		•

# Conclusion and Discussion

In conclusion, none of the null hypotheses were rejected. The experiment did not demonstrate that laughter facilitates interbrain synchrony or that either interbrain synchrony or laughter has an impact on cooperation. Additionally, there was almost no difference in the cooperation score between the experimental and control groups, with means of 16.24 and 16.45 respectively (95%-CI[-5.53, 5.11]), t(39) = 0.0806, p = 0.9362. There are various reasons for these results, aside from the explanation that there are no effects, which was recently supported by a study. The study showed that Duchenne smiling does not predict cooperative behaviour. Instead, the personality trait of altruistic cooperativeness is the true predictor (Deng et al., 2023).

Besides that, the first reason is that the small sample size may have biased towards the null hypothesis. Statistical power is dependent on sample size, and this sample size is very low compared to effect sizes in the literature relating to the respective hypothesis. As described earlier, for H1 a total sample size of n = 98 dyads would be optimal, for H2 n = 35 dyads and for H3 n = 128 dyads. With barely around n = 20 dyads per condition, a type II error may occur for all hypotheses. This means that the null hypothesis may be incorrectly retained,

even though the alternative hypothesis is true.

Secondly, it is possible that the experiment did not work as intended. There are indications to support this explanation. Although the laughter between the two groups has not yet been fully coded, it appears that there is no significant difference in laughter between the experimental and control groups. Upon reviewing the recording of the free interaction phase, participants in the experimental group did not discuss the manipulation animal videos, whereas those in the control group talked about the animals in the video. Several participants reported experiencing headaches at the end of the experiment due to the pressure of the fNIRS cap and optodes. This may have influenced their behaviour during the free interaction phase, causing them to feel tired and resulting in a more distant behaviour towards their experimental partner.

Another reason for the non-significant findings is more fundamental in nature. The concept of interbrain synchrony is theoretical and targets higher cognitive functions, making it difficult to grasp both methodologically and conceptually. There are two perspectives on interbrain synchrony: mechanistic and epiphenomenal. According to the mechanistic perspective, interbrain synchrony enhances performance in socially interactive tasks (Novembre & Iannetti, 2021) and therefor acts as lubricant in social interactions. Despite the many benefits of hyperscanning, it is important to note that findings with this method are correlational and lack causality, which can lead to an epiphenomenal perspective on interbrain synchrony, which works similarly as the null hypothesis to this topic. Interbrain synchrony may simply be the result of two individuals sharing the same sensory environment, according to Novembre and Iannetti (2021). Hamilton (2021) suggests that more complex study designs are needed to challenge the epiphenomenal view on interbrain synchrony.

However, there are studies, such as Novembre et al. (2017), that have tackled this challenge. In that study they induced in-phase (simultaneous) beta band (20 Hz) oscillations in dyads using transcranial alternating current stimulation (tACS) on the left motor cortex. This improved interpersonal movement synchrony in a coordinative finger tapping task compared to an anti-phase (delayed stimulation of second participant) or sham condition (no stimulation). The study suggests that interbrain synchrony leads to better coordination, at least for movement. However, it does not really address the mechanism-epiphenomenon dilemma, as it only demonstrates that synchronized brain oscillations in the motor cortex result in synchronized behaviour, which describes interbrain synchrony as an epiphenomenon of simultaneously performing the same action. In contrast, Pan et al. (2021) have made progress in identifying the underlying mechanism of interbrain synchrony by inphase stimulating the inferior frontal brain regions of a song instructor and a learner with tACS. In particular, during 6 Hz stimulation, spontaneous and synchronous body movements were triggered. Additionally, intonation learning performance, measured by the accuracy of reproducing pitch, was enhanced compared to the anti-phase and sham conditions. The study conducted a correlational analysis and found a significant correlation between induced spontaneous and synchronized movement and learning performance. This suggests that stimulated synchronized movement leads to better learning performance. The study also conducted a mediation analysis, which confirmed the correlation and found that interpersonal movement synchrony acts as a mediator. This reduced the relationship between dual brain stimulation and learning performance, although it remained significant. This builds on their previous work (Pan et al., 2018), which found correlational evidence that the strength of interbrain synchrony between a song instructor and learner predicted the learner's intonation learning performance. The researchers had now discovered the first causal evidence for the enhancement of social interactive learning through interbrain synchrony. The mediator analysis results suggest that dual brain stimulation did not directly enhance learning, but rather indirectly through synchronized movement. This raises the question of whether neural alignment, represented through enhanced cortical excitability and neural oscillations, enhances information transfer between individuals. Biophysical computer models trained with real data support this conclusion (Dumas et al., 2012).

Despite the hype surrounding interbrain synchrony and its potential impact on social interactions (Hamilton, 2021), reliable results are still needed to fully comprehend the significance of aligning the neural activity of two individuals' brains. Multibrain stimulation may offer a promising solution to the hen-and-egg problem posed by the mere correlational results provided by hyperscanning. Human interaction is too complex to manipulate specific brain regions in order to achieve interbrain synchronized activation and expect clear results indicating a precise mechanism of this neural alignment. Studies in this area often have vague interpretations, as it remains unclear how manipulated interbrain synchrony affects social interaction (Pan et al., 2021). Interbrain synchrony may provide a new element to consider in human social interaction. However, until more details on the causal level of this complex matter are available, subjective interpretations must be avoided. The mechanism-epiphenomenon dilemma serves as an example.

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