ABSTRACT

Both the putative Mirror Neuron System (pMNS) and the ventral medial prefrontal cortex (vmPFC) are deemed important for social interaction: the pMNS because it supposedly ‘resonates’ with the actions of others, the vmPFC because it is involved in mentalizing. Strictly speaking the resonance property of the pMNS has never been investigated. Classical fMRI experiments have only investigated whether pMNS regions augment their activity when an action is seen or executed. Resonance, however, entails more than only ‘going on and off together’: activity in the pMNS of an observer should continuously follow the more subtle changes over time in activity of the pMNS of the actor. Here we directly explore if such resonance indeed occurs during continuous streams of actions. We let participants play the game of charades, while we measure brain activity of both gesturer and guesser. We then apply a new method to localize directed influences between the brains of the participants: between brains Granger Causality Mapping. Results show that a guesser’s brain activity in regions involved in mentalizing and mirroring echo the temporal structure of a gesturer’s brain activity. This provides evidence for resonance theories and indicates a fine-grained temporal interplay between regions involved in motor planning and regions involved in thinking about the mental states of others. Furthermore, this new method enables experiments to be more ecologically valid by providing the opportunity to leave social interaction unconstrained. This, in turn, would allow us to tap into the neural substrates of social deficits, such as autism spectrum disorder.

4.1 INTRODUCTION

How do humans understand each other? In the last decade two parallel lines of research have investigated this question. On the one hand, the finding that some brain regions and neurons involved in performing an action are also active while viewing
the actions of others (jointly referred to as the Mirror Neuron System, MNS, (Aziz-Zadeh et al., 2006; Chong et al., 2008; Dinstein et al., 2007; Filimon et al., 2007; Gallese et al., 1996; Gazzola et al., 2006; Grèzes et al., 2003; Iacoboni et al., 1999; Keysers and Gazzola, 2009; Kilner et al., 2009; Ricciardi et al., 2009; Turella et al., 2009) has lead to the idea that we understand the actions of others in part by transforming them into the motor vocabulary of our own actions. On the other hand, reflecting on other people's thoughts and beliefs is mediated by another part of the brain, the ventromedial prefrontal cortex (vmPFC, including the anterior cingulate and paracingulate gyrus). This area is consistently activated when we think about other people's mental states (Amodio and Frith, 2006; Frith and Frith, 2006; Gallagher and Frith, 2003; Sommer et al., 2007). Given that we often deduce the beliefs and attitude of others through their actions, it is intuitively appealing to believe that these two networks would work together to achieve a coherent representation of the mental states of others (Keysers and Gazzola, 2007; Brass et al., 2007). However, a recent meta-analysis has shown that these two networks are often found to be dissociated (Overwalle and Baetens, 2009). In what follows, we will first briefly describe some key issues that have limited our understanding of how these two systems contribute to reading the mental states of other individuals during naturalistic situations, and then present a new experimental paradigm to explore their role and interaction in a naturalistic communicative situation.

In humans, the dorsal and ventral premotor, somatosensory cortex, anterior inferior parietal lobule and mid-temporal gyrus have the peculiar property of being active not only when we perform an action but also when we witness similar actions of others (Aziz-Zadeh et al., 2006; Chong et al., 2008; Dinstein et al., 2007; Filimon et al., 2007; Gazzola et al., 2006; Grèzes et al., 2003; Iacoboni et al., 1999; Keysers and Gazzola, 2009; Kilner et al., 2009; Ricciardi et al., 2009; Turella et al., 2009; Keysers, 2009). This set of brain regions has therefore jointly been referred to as the putative MNS (pMNS) (Keysers and Gazzola, 2009). It has been proposed that through this system, the brain of two interacting individuals 'resonate' with each other: "other people's mental states are represented by [...] tracking [...] their states with resonant states of one's own" (Gallese and Goldman, 1998). In this context, the term 'resonance' is used rather loosely and metaphorically; not in a strict physical sense but rather to suggest that the ups and downs in the activity of one person's motor system lead to sequences of actions and rest, which trigger similar ups and downs in the activity of the observer's (Gallese and Goldman, 1998; Gallese et al., 2004; Rizzolatti et al., 2001). This concept of resonance is very influential, however the only case in which this proposed temporal 'tracking through resonant states' has really been tested is for viewing repetitive cyclic ups and downs of the wrist (Borroni et al., 2005). Whether it applies to the natural streams of actions that typically lead us to read the minds of others, e.g. the sight of two gesticulating individuals on the side of the road, remains untested. This is
because experimental designs so far have merely tested whether the pMNS becomes active at the transition between a control condition and the sight of a single complex action. This shows that the pMNS of the observer is indeed triggered by the sight of an action. Single cell recordings (Keysers et al., 2003; Mukamel et al., 2010) and magnetoencephalography (Caetano et al., 2007) show that the temporal profile of this activity is indeed similar during action observation and execution, potentially providing a neural basis for resonance. The concept of resonance, however, entails more than only ‘going on and off together’ at the beginning and end of a single action: it involves a continuous tracking of the more subtle changes in activity during the execution and observation of entire streams of action. Natural social interactions are composed of complex sequences of actions where it is often difficult to know when one action ends and another starts. Here we will directly explore if such resonance indeed occurs within the pMNS during such continuous streams of actions.

The literature on the role of the vmPFC in social interaction suffers from another problem. The vmPFC not only seems to be involved in reflecting on the mental states of others (Amodio and Frith, 2006), it is also one of the brain regions that systematically decreases its activity whenever participants process external stimuli (the ‘default network’) (Raichle and Snyder, 2007). Studies investigating mentalizing and the default network show strongly overlapping results while exploring seemingly very different functions (Spreng et al., 2009). The fact that the vmPFC is not typically found to be active while people observe the behaviors of others (Overwalle and Baetens, 2009) is therefore difficult to interpret: is activity due to interpreting another mind masked by the fact that its overall level of activity is reduced compared to baseline because of attention to external stimuli? A powerful way to examine this possibility would be to look at the activity of the vmPFC during the observation of longer streams of actions, as the overall level of activity in the default network might then be decreased but the subtle ups-and-downs could still reflect the mentalizing activity of this region in response to the sequence of actions.

Here, participants played the game of charades in the MR-scanner to allow us to examine brain activity during longer streams of gestures. The game of charades was chosen because its success as a commercial game shows how powerfully it triggers the naturalistic motivation to communicate a mental state to a partner through hand actions. It also has the advantage of making the participants generate and observe streams of actions that are naturalistic both in duration and complexity. Given that the type of gestures involved in this game are hand actions, charades can serve to examine the unresolved issue of whether the pMNS would make two individuals’ brains resonate during longer streams of actions. Furthermore, since the aim of charades is also to make one player guess a concept that is in the mind of the other player, it is also a powerful instrument to check if fluctuations in the activity of the vmPFC during longer streams of gestures could reflect mentalizing processes triggered
by the behavior of another individual. Indeed both the pMNS (Montgomery et al., 2007; Pazzaglia et al., 2008; Schippers et al., 2009) and the vmPFC (Montgomery et al., 2007) have been implicated in the observation of single gestures, maximizing our chances to examine the yet unexplored issue of whether the activity of these regions during longer streams of actions would indeed resonate with the activity of the brain of the gesturer.

We therefore asked couples to take turns in the fMRI scanner while we measured their brain activity. Each partner knew that on half of the trials, they would see a word on a screen and would have to gesture this word into a video camera for their partner later to guess; and on the other half, they would see a video of their partner’s gestures and have to guess what the word had been. Using this manipulation, a single fMRI scanner was enough to measure the brain activity both when one person generates gestures and (later) when another person decodes these very gestures. By aligning the time courses of the two brains’ activity, as measured using fMRI, relative to the video recording, we can then directly investigate the temporal coupling of the two brains activity during gestural communication. In order to do this quantitatively, we introduce a new analysis method: we extend Granger Causality Mapping originally used to track information flow within a brain (Roebroeck et al., 2005) to a between brain Granger Causality Mapping (bbGCM, Fig. 4.1 and Supplementary Information 2). BbGCM quantifies the influence from a selected seed region $Y$ in the gesturer’s brain to all voxels $X_i$ of the guesser’s brain by statistically comparing the G-causalities in both directions, i.e. $(Y \rightarrow X_i) - (X_i \rightarrow Y)$ (Roebroeck et al., 2005). A preliminary analysis of the same data using a traditional general linear model approach ignoring the temporal relationship between the brain activities of each couple has been published previously (Schippers et al., 2009) and shows involvement of pMNS areas, but not the vmPFC. Using bbGCM, however, we will show that even if one ignores the beginning and end of a gesture, activity in both the pMNS and the vmPFC of the observer does carry fine grained information about the time course of the activity in the brain of the gesturer, providing a powerful demonstration of resonance across brains during gestural communication.

4.2 MATERIALS AND METHODS

Participants

Twelve couples (total: 24 participants) were scanned while playing the game charades. Four participants had moved more than the voxel-size during the gesturing phase, which lead us to exclude 3 couples from the data analysis that contained these participants. All the analyses in this paper are performed on 18 participants. The mean age
Figure 4.1: Between brains Granger Causality during active guessing. (A) Time series X in the guesser’s brain is stimulus aligned with Y in the gesturer’s brain. The fixation periods between words (blue) are discarded. Guesser typically responded before the recorded 90s. Response (red) and post-response (grey) periods were thus removed. 15TR at onset and 5TR at offset of each guessing period were trimmed to remove transients. (B) Two regressions are compared at autoregressive order three (see Methods): one including only the past of X itself, and one additionally considering the past of Y. The residual error (variance) reduction from $\sigma^2(\epsilon)$ to $\sigma^2(\epsilon')$ quantifies how much Y G-causes X. (C) Reverse regressions are compared, and differential, directed influences exists if one variable helps more in predicting the other (see also Supplementary Information 2).
of the participants was 27.5 ± 3.8 years. Each couple consisted of a man and a woman involved in a romantic relationship for at least 6 months. More details are described in Supplementary Information 1.

**Task / Experimental Design**

The experiment consisted of two separate sessions on different days. In the first session, the couple was required to play the game of charades. In the second, detailed anatomical scans and a passive observation control condition were acquired. For the game of charades, participants took turns going into the scanner, alternating gesturing and guessing of words. Words were either objects (for example nutcracker, watch, pencil sharpener) or actions (for example painting, knitting, shaving, see Table 4.1 in Supplementary Information 1). Each participant performed two gesture and two guess runs in which they gestured 14 words and guessed 14 words in total (7 per run).

During a gesture run, the participant was presented with a word on the screen and was instructed to communicate this word to his or her partner by means of gestures. Every word had to be gestured for 90 seconds and was then followed by a 20 seconds fixation cross. During a guess run, the participant was shown the movies that were recorded in the gesture run of their partner. The task they had to perform was to guess what their partner was trying to gesture to them. Participants were asked to consider the gestures for at least 50 seconds before committing to a specific interpretation of the gestures. This was done to ensure at least 50 seconds of data in each trial to examine the time course of activity using between brains Granger causality. As a control condition for the guess run, the participants watched the movies they had seen during the guessing condition again. This time, they were instructed not to guess what was gestured, but only to passively view them. More details are described in Supplementary Information 1.

**Granger causality analyses**

Granger causality analyses were performed as described in Roebroeck et al. (2005) but applied here to data from different brains (see Supplementary Information 2). In short, given two time-series (for a seed and another point on the cortical surface), autoregressive models are estimated that quantify G-causality. Given a seed, maps are created that specify G-causal influence from the seed in the gesturer to all of the guesser’s brain, as well as influence in the reverse direction, i.e. from anywhere in the guesser’s brain to the seed in the gesturer’s brain. These two directions of G-causality are then subtracted from each other to generate differential G-causality maps, such
that positive values indicate more G-causality from the gesturer to the guesser than from the guesser to the gesturer. A separate differential G-map was calculated for each of the 8 seed regions (see below) for each participant. These differential G-causality maps were then taken, separately for each seen region map, to the second level (see below) and thresholded for multiple comparisons at $p < 0.05$ using a cluster threshold determined by a Monte Carlo simulation method (Forman et al., 1995; Hagler et al., 2006). The order of the estimated autoregressive models was 3, i.e. the 3 preceding time points are taken into account to predict the current activity, corresponding to $\sim 4$ seconds (3TR).

### Seed ROIs

The ROIs that were used as seeds in the between brains Granger causality analysis were defined as those ‘mirror’ areas that were active both during gesturing and guessing using a traditional GLM analysis on the same data (Schippers et al., 2009).

### Instantaneous Motion Energy GLM

We extracted motion energy from the gesture movies using Matlab. For two consecutive frames of the recorded movies, motion energy was quantified in every pixel as the sum of the squared differences in the red, green and blue channels and then summed over all pixels. This time course was then mean corrected, convolved with the hemodynamic response function and sampled at the acquisition rate of the fMRI signal (TR = 1.33s).

### 4.3 RESULTS

#### 4.3.1 BbGCM: gesturer to guesser

The resulting bbGCMs are shown in Figure 4.2 separately for each ROI (top 4 rows), as well as summarized over all ROIs (bottom row). They show that activity in the pMNS of the gesturer indeed predicts brain activity in the brain of the guesser more than the other way around (warm colors). Given that we used only the very recent past (4 seconds) of the gesturer’s brain activity in the analysis, this provides the first evidence, to our knowledge, that the moment-to-moment activity in the guesser’s pMNS indeed mirrors the close past of the gesturer’s pMNS activity during gestural communication. Notably, regions of the vmPFC cortex (including the anterior cingulate and paracingulate gyrus) are also G-caused by activity in the pMNS. The opposite directionality (guesser to gesturer) is much rarer (only found in a small region on
Figure 4.2: Results of second-level bbGCM. Granger analyses executed separately for the left and right seed are shown together. The right side represents the guesser’s brain showing t-values of the paired t-test between gesturer → guesser versus guesser → gesturer G-causality (Random Effects, n=18). Upper four rows: differential G-causality originating from the seeds on the left. Bottom row: summary of all seeds (solid colors, left) and bbGCMs (right) with an outline of the pMNS according to a traditional GLM (Schippers et al., 2009) for visual orientation. BbGCM maps are statistically thresholded at $p < 0.05$ corrected for multiple comparisons by using a Monte Carlo simulation-based cluster-size threshold adjustment (Forman et al., 1995; Hagler et al., 2006)

the mesial wall, cold colors). This suggests that bbGCM is indeed able to track the prevalent direction of information flow between brains which has to be in the gesturer to guesser direction given that the guesser could see the gesturer in the video but not vice versa.
4.3.2 **BbGCM: gesturer to passive observation**

To examine whether these results were dependent on the observer actively trying to guess the meaning of the observed gestures, we had participants watch the same gesture movies again but with an instruction not to actively interpret the movies (see Methods). We computed between-brain influences between the gesturer’s brain activity while generating the gestures and their partner’s during this control condition (Fig. 4.3A and Fig. 4.4). We then directly contrasted these results with those during active guessing (Fig. 4.2 and Fig. 4.3B). Directed influence was significantly reduced within the ventral premotor and parietal regions associated with the pMNS when directly comparing the two situations (Fig. 4.3C and Fig. 4.5), and differential G-causality was more consistent during deliberate guessing than passive viewing (Fig. 4.3A vs. Fig. 4.3B). This suggests that a task to decode gestures does influence the consistency (and therefore statistical significance) with which an observer’s brain time locks onto the gestures, and thereby pMNS activity, of the gesturer. An instruction not to interpret the gestures however cannot ensure that participants indeed refrained entirely from interpretation and a traditional GLM analyses of the same data set (Schippers et al., 2009) showed that overall activity during active guessing and passive observation is indeed similar. Accordingly, contrasting bbGCM during active guessing and passive viewing is a very conservative approach to localizing the neural basis of gesture interpretation that would exclude all neural processes that are triggered automatically by the vision of gestures. However, at debriefing, participants reported having interpreted the gestures at least less consistently during passive viewing than active guessing, and we did find differences in bbGCM results. This shows that an instruction not to interpret the gestures of a partner does seem to partially decouple the observer’s brain regions from the pMNS of the gesturer.

4.3.3 **BbGCM: gesturer to a random guesser**

To further test whether bbGCM is indeed identifying information flow based on the fine-grained temporal chain of behaviors that makes each social interaction unique, we recalculated bbGCMs while pairing each gesturer’s brain activity with that of a randomly selected guesser that had viewed different gestures of another gesturer (Fig. 4.3D and Fig. 4.6). Virtually no vertices (vertices refer to the nodes on the cortical surface, and are therefore similar to voxels except that they are on a cortical surface instead of a brain volume) demonstrated significant differential G-causality in this control analysis and there was significantly more G-causality from gesturer to guesser than from gesturer to random guesser (Fig. 4.3E and Fig. 4.7). Because the sequence of words used for each couple was randomized, the original guesser and his/her
randomly selected control guesser saw a different sequence of words being gestured to them. As a yet stricter control analysis, we therefore repeated this control analysis by substituting ‘word by word’ the time series of the original guesser with that of a randomly selected control viewing the same word being gestured to him/her but by someone else than the original gesturer (Fig. 4.8). Differential G-causality was again significantly stronger for the original gesturer-guesser pair. This provides direct evidence that the brain activity of the guesser was indeed an echo of the unique way in which his/her particular partner generated these gestures and suggests that bbGCM can indeed track the unique way in which two brains time lock onto one another during communication.

4.3.4 GLM: using instantaneous motion energy of the gestures as predictor

Here we used bbGCM to indentify brain regions involved in tracking the gestures of others. To test whether this new technique can unravel the involvement of brain regions that more traditional techniques do not, we compared bbGCM with a classical GLM in which we enter two regressors. One contains the timing of the gesture movies (as a boxcar function), and the other the fluctuation of instantaneous motion energy within each movie, both of which were convolved with the hemodynamic response function. This analysis shows that while the brain is strongly reacting to the on- and offset of the movies (see Schippers et al., 2009, their Figure 1a), it does not show a correlation between the fluctuations in the movement of the gesturer (as approximated using instantaneous motion energy in the movie) and fluctuations in brain activity of the guesser as the parameter estimates for the predictor motion-energy was not significantly above zero in any cluster (See Fig. 4.9).

4.4 DISCUSSION

In the present study, we introduced bbGCM to investigate to which degree two brains ‘resonate’ during gestural communication. We show that activity in the pMNS and the vmPFC of the guesser is Granger-caused by fluctuations in activity in the pMNS of the gesturer. These findings have three sets of implications. First, they show that pMNS regions indeed ‘resonate’ across brains, thereby providing evidence for resonance theories (Gallese and Goldman, 1998; Gallese et al., 2004; Rizzolatti et al., 2001). Second, they extend our understanding of the neural basis of gestural communication by providing evidence for a fine grained temporal interplay between regions involved in motor planning (pMNS) and regions involved in thinking about the mental states of others (vmPFC). Third, they demonstrate more generally that G-causality can be used to map directional information flow across brains during social interactions.
Figure 4.3: Specificity of G-Causality. (A) t-values for (gesturer → passive observer) - (passive observer → gesturer) differential G-causality. (B) Same as (A) but for the original active guessing condition as in Fig. 4.2. (C) Paired t-test of (B)-(A). (D) t-values for (gesturer → random guesser) - (random guesser → gesturer) differential G-causality. (E) Paired t-test for (B)-(D). All maps are statistically thresholded at p<0.05 corrected for multiple comparisons by using a Monte Carlo simulation-based cluster-size threshold adjustment (Forman et al., 1995; Hagler et al., 2006), and represent the summary of the results for the 8 seed regions. See Figures 4.4-4.8 for similar maps separately for each seed.
without the need for the experimenter to impose temporal structure on the social interaction.

Before going into details of each of these, we would like to discuss how such G-causality should be interpreted (see also Supplementary Information 2). Directed G-causality between brains has to be mediated by what the guesser can perceive: the observable gestures of the gesturer. BbGCM as we apply it is therefore not a method to determine direct causal interactions across brains, but a method to map brain regions that are at opposite ends of a longer indirect chain of causality that goes through the external world: neural activity in the gesturer causes both the execution of the gestures and the BOLD signal that we measure with fMRI. The video-taped gestures are seen by the guesser, which causes brain activity and an observable BOLD response that we again measure with fMRI. Keeping the indirect nature of the causal pathway in mind, our bbGCM maps brain regions in the receiver that echo the brain activity of the sender. By ‘echo’ in this context, we mean providing temporal information about the state of the other person’s brain region. In general, the directed bbGCM should be interpreted with a further issue in mind. Because it is calculated by contrasting bbGCM in two directions (Brain A → Brain B minus Brain B → Brain A), a value larger than zero is evidence for an influence of brain A on brain B, whereas the opposite is not evidence for a lack of influence from A → B. This is because, in addition to a potential lack of statistical power, negative findings with directed bbGCM could originate from two very different scenarios: because there is no significant information flow in either direction, or because the influence is significant but equally strong in both directions. In our experiment, the latter possibility is unlikely, as the guesser can view the gesturer but the gesturer cannot see the guesser. It is therefore unlikely that the guesser’s brain could influence the gesturer’s. The second scenario will, however, be more likely in future experiments in which two interacting partners might be able to mutually observe each other in real time.

We still know relatively little about the neural basis of mind reading and gestural communication. However, two sets of brain regions could play a role: pMNS areas and the vmPFC. Our findings support the idea that both play a role: we show that BOLD activity in functionally defined regions of the pMNS and in the vmPFC are G-caused by BOLD activity in the pMNS of the gesturer. This strengthens the idea that simulation and mentalizing both contribute to our interpretation of other people’s gestures (Keysers and Gazzola, 2007; de Lange et al., 2008; Thioux et al., 2008). Because of the constraints of traditional data analysis (e.g. GLM analysis), the degree to which the sequences of complex actions composing a gestural phrase (McNeill, 1992) would be parsed (Byrne, 2003) similarly by the gesturer and guesser has never been explored. Our finding of significant G-causality between the pMNS in the two brains using a temporal window of 3 (i.e. regressing the brain activity of the guesser onto the activity in the past 3 volumes = 4 seconds of the gesturer) provides the first evidence,
to our knowledge, that the two brain activities go up and down together in naturalistic gestural communication. Reducing the order to 1 (i.e. considering only the past 1.33 seconds of the gesturer) much reduces this differential G-causality (See Fig. 4.10). This shows that the pMNS of communicative partners indeed resonates with each other (in the loose sense used in this literature) as had been suggested by simulation accounts of mind reading and communication (Gallese and Goldman, 1998; Gallese et al., 2004; Rizzolatti et al., 2001; Rizzolatti and Craighero, 2004). Moreover, it informs us that this resonance is not most evident at the second-to-second time scale of time window 1, but at a more moderate time scale of several (~4s) seconds that is commensurable with the time it takes to plan, generate and perceive a gestural element (McNeill, 1992). BbGCM is therefore a powerful tool to test the temporal resonance phenomenon at the core of simulation accounts of communication. It furthermore shows that the pMNS indeed provides the time-resolved information about the state of the gesturer's motor system that would be required for motor simulation to be useful for communication in a naturalistic context. This adds to the evidence that the excitability of an observer's motor system fluctuates in synch with the repetitive wrist flexion of another individual by showing that the concept of resonance indeed applies to the complex, non repetitive and non rhythmical streams of gestures that are more typical of real mind-reading situations and may have been essential in the early state of language evolution (Rizzolatti and Arbib, 1998). Additionally, this finding dovetails with the observation that brain activity in both these regions predicts the accuracy with which participants can judge the moment to moment emotional state of another individual (Zaki et al., 2009).

The fact that vmPFC activity was also G-caused by pMNS activity in the gesturer is surprising. This region is well known to play a role in inferring mental states from written stories or cartoons (Amodio and Frith, 2006) and activity in this region is increased while participants try to interpret certain gestures (Montgomery et al., 2007). These findings suggest that the vmPFC might be involved in attributing mental states to others, and could do so during gestures and actions. However, a previously published preliminary GLM analysis of our data revealed that this region does not demonstrate more brain activity while guessing gestures than while fixating a cross (Schippers et al., 2009). In addition, during the free-viewing of a Hollywood movie, the vmPFC does not seem to synchronize across viewers (Hasson et al., 2004). The inferential nature of these processes seems to detach brain activity in this region from the exact timing of the stimulus, leading it not to synchronize across viewers and therefore also making it difficult to link activity in this region directly to the stimulus itself. Indeed activity in this region also does not simply correlate with the low level motion contained in the stimuli (see Fig. 4.9). Here, on the other hand, we show that activity in this brain region in a guesser does contain information (in the sense of Supplementary Information 2) about the time course of activity in the
regions involved in planning and executing gestures in the gesturer. This supports the idea that the pMNS and mentalizing brain areas may work in concert to derive mental states from observed actions (Keysers and Gazzola, 2007; Thioux et al., 2008). A traditional GLM analysis of the same data (Schippers et al., 2009) may have been unable to detect the involvement of the vmPFC because this region is also part of the default network (Raichle and Snyder, 2007; Spreng et al., 2009). The default network is a set of brain regions that demonstrate augmented metabolism during passive baseline conditions, supposedly because they contain neurons that are involved in the self-referential processes we engage in while not performing a particular task (Raichle and Snyder, 2007; Spreng et al., 2009). During guessing, these self-referential processes would have been suspended, lowering the BOLD in this brain region below baseline. The activity, in this region, of a smaller number of neurons engaging in the mental state attribution required by the game of charades would then have been masked by the concurrent reduction of self-referential activity. Our bbGCM can detect such activity nevertheless, because it examines not whether activity overall goes up or down relative to a baseline condition, but rather whether fluctuations in activity during the stream of gestures covaries with the past activity of the gesture execution system of the gesturer. This changes the interpretation of the same data set compared to a classical GLM analysis which showed that only the pMNS but not the vmPFC demonstrate augmented activity compared to baseline during the active decoding of gestures (Schippers et al., 2009).

A number of control analyses served to establish that bbGCM indeed tracks a specific information flow between two communicating participants. When pairing the time course of a given gesturer with that of a randomly selected guesser, instead of the one that had actually observed the gestures, significantly less between brain influences were observed compared to the analysis with the active guessing condition (Fig. 4.3E). This suggests that bbGCM indeed revealed the specific effect of a particular pattern of gestures on the brain activity of the guesser. Furthermore, we found that active guessing, but not passive viewing of the same gestures leads to significant bbGCM of the pMNS and vmPFC (Fig. 4.3A). This shows that an instruction to actively decode the gestures increases the coherence between the activities in the two brains.

Given that the brain activity of our guesser is not directly caused by brain activity in the gesturer but by the pre-recorded movie of his/her gestures, one might argue that measuring the brain activity of the gesturer is not necessary to map brain regions involved in social information transfer. Instead, quantifying what is in the stimulus would suffice to localize those brain regions in the guesser’s brain that respond to that stimulus. We tested this approach by using instantaneous motion energy from the gesture movies as a predictor in a GLM analysis. Results show no correlation between their activity and the instantaneous motion energy from the movies, indicating that using this particular measure with a traditional GLM approach does not provide any
extra information. While alternative approaches to quantifying the content of the stimulus and introducing time-lagged versions of these predictors into a GLM may help, the fundamental problem with such a stimulus centered approach is that quantifying the relevant dimensions of a naturalistic stream of gestures is far from trivial: it is a highly multidimensional stimulus, and transforming it into univariate time series for a GLM requires knowledge of what aspects of the stimulus are relevant for the brain of the observer - a knowledge that we often lack. BbGCM has the elegant property of circumventing this problem altogether and thereby directly testing those theories, like the pMNS ‘resonance’ theory of mind reading (Gallese and Goldman, 1998; Rizzolatti and Arbib, 1998), which are formulated not as a link between a stimulus and a neural state, but between the neural states of two individuals.

As an alternative for this method, one might show the same gestures to many participants, and examine what brain regions synchronize across participants (Hasson et al., 2004). Between-viewer correlation also has the elegant property of circumventing the problem of quantifying the stimulus, and is conceptually related to our approach. It however has other limitations: it requires many viewers of the same stimulus and, in its standard form, only examines instantaneous dependencies between brain regions (i.e. it does not allow for time shifts between the brain activity of different viewers). BbGCM overcomes these limitations: It can be applied to pairs of interacting partners, with only one participant viewing any particular communicative episode, and is therefore more suited for studying dyadic communication. Additionally, it allows examination of dependencies between brain activity over a longer time period (the G-causality order, in our case 4s), which is more appropriate for the analysis of brain activity during communication, where several seconds can separate the planning of a gesture from its execution and perception. BbGCM and between-viewer correlation could be combined to a between viewer GCM: if the brain activity of one viewer contains information about the brain activity of another viewer, in the absence of direct communication between the viewers, this shared information has to be information about the stimulus. Between-viewer GCM would thus map regions containing information about the stimulus while allowing for slight time shifts across viewers.

More generally, for the field of social neuroscience, our findings show that it is possible to map the brain regions involved in the flow of information across individuals with fMRI without imposing a temporal structure on the social interaction and without depending on certain choices for the quantification of the information in a stimulus. A similar approach seems to be suited for analyzing EEG data during social interactions (Babiloni et al., 2006). Demonstrating bbGCM between one brain region in partner 1 and another region in partner 2 then allows a data driven identification of brain regions that could play a role in the information flow across participants. Much as for other data analysis techniques (Hasson et al., 2004), further experiments that control the content of the stimulus seen by participants are then needed to iso-
late which aspects of the complex interaction were encoded in the brain activity in these brain regions, and virtual lesions using transcranial magnetic stimulation will be needed to examine whether these brain regions are necessary for normal social interactions.

In conclusion, bbGCM has advantages over existing techniques: it can map the information transfer from brain to brain in pairs of participants without having to impose temporal structure on social interactions and without requiring knowledge about the relevant dimensions of the complex social stimulus. Here we used this approach to show for the first time that even for naturalistic streams of gestural communication, the core prediction of MNS theories of communication and mind reading hold: the pMNS of the guesser does indeed reflect moment to moment information about the state of the motor system of the gesturer. In addition, using this method, we narrow the gap between literatures exploring the pMNS and that exploring mentalizing by show that the vmPFC of the observer could add to this mirroring by also resonating with the motor system of the gesturer. More generally, we hope that this technique will enable and inspire the investigation of one of the most defining feature of human beings: their capacity to transfer knowledge from one person to another. In particular, the opportunity to leave the social interaction unconstrained will enable experiments to be more ecologically valid. This, in turn, could allow us to tap into the neural substrates of social deficits and ask questions like: Which neural substrates are responsible for the difficulty autistic individuals have in taking turns during communication?

ACKNOWLEDGEMENTS

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BIBLIOGRAPHY


SUPPLEMENTARY INFORMATION 1

MATERIALS AND METHODS IN DETAIL

Participants

Twelve couples (total: 24 participants) were scanned while playing the game charades. Four participants had moved more than the voxel-size during the gesturing phase, which lead us to exclude 3 couples from the data analysis that contained these participants. All the analyses in this paper are performed on 18 participants. The mean age of the participants was $27.5 \pm 3.8$ years. Each couple consisted of a man and a woman involved in a romantic relationship for at least 6 months. We included this criterion for two reasons. First, we expected that the participants would be more motivated and more at ease during gesturing if they knew it was their partner who had to interpret their gestures. Second, we expected them to have a better or faster understanding of each other’s gestures since they knew each other better than a stranger would. Our aim was not to study specifically romantic processes, but simply to let the participants feel as comfortable as possible during the game. Participants were prescreened to exclude those with a history of neurological or psychiatric illness. Participants were also asked not to drink coffee before scanning commenced. The participants freely consented to participating in the study by signing an informed consent form and were scaled for their right-handedness on the Edinburgh Right-handedness scale (Oldfield, 1971). This entire study was approved by the Medical Ethics Committee of the University Medical Centre Groningen (2007/080).

Task / Experimental Design

The experiment consisted of two separate sessions on different days. In the first session, the couple was required to play the game of charades. In the second, detailed anatomical scans and a passive observation control condition were acquired. For the game of charades, participants took turns going into the scanner, alternating gesturing and guessing of words. Words were either objects (for example nutcracker, watch, pencil sharpener) or actions (for example painting, knitting, shaving, see Table 4.1). Each participant performed two gesture and two guess runs in which they gestured 14 words and guessed 14 words in total (7 per run). The set of words used was the same for each couple, but word order was randomized between participants.
After the last gesture-session, a T1-weighted anatomical image was acquired. See Fig. 4.11 for a schematic overview of the experiment.

**Gesture run:** during a gesture run, the participant was presented with a word on the screen and was instructed to communicate this word to his or her partner by means of gestures (see Table 4.1 for an overview of the words). Every word had to be gestured for 90 seconds. Prior to scanning participants were trained not to repeat the same gesture over and over again, but to keep generating new gestures to provide their partner with multiple sources of information. The participant could see how much time he/she needed to keep gesturing by a progress bar on the screen. A fixation cross was presented for 20 s after each word, which served as our baseline (see Fig. 4.11 for a detailed overview). The gestures were recorded from the control room of the MR-scanner with a video camera (Sony DSR-PDX10P). After the participant had gestured seven words, he/she was taken out of the scanner and went into the waiting room, while his/her partner went into the scanner to guess what he/she had gestured. During this changeover, the experimenter cut the recording of the gestures into movies of 90s in which the participant gestured a word. To ensure that the movies were cut at exactly the moment the word was presented to the gesturing participant, the stimulus computer’s sound card emitted a sound at the beginning of word presentation. The output of the sound card was connected to the audio input of the video camera, thus allowing the auditory signal to serve as a marker for cutting. To minimize the amount of head motion in the participants, the upper arms of the participant were fixed to the bed by means of a Velcro strap band. This left the participant free to gesture with his lower arms and fingers, which still allowed 86% percent correct gesture recognition.

**Guess run:** during a guess run, the participant was shown the movies that were recorded in the gesture run of their partner. The task they had to perform was to guess what their partner was trying to gesture to them. Participants were asked to consider the gestures for at least 50 seconds before committing to a specific interpretation.

<table>
<thead>
<tr>
<th>Actions</th>
<th>Objects</th>
</tr>
</thead>
<tbody>
<tr>
<td>peel fruit</td>
<td>fold</td>
</tr>
<tr>
<td>ride a bike</td>
<td>drive a car</td>
</tr>
<tr>
<td>shuffle cards</td>
<td>play the piano</td>
</tr>
<tr>
<td>polish nails</td>
<td>squeeze fruit</td>
</tr>
<tr>
<td>juggle</td>
<td>paint</td>
</tr>
<tr>
<td>knit</td>
<td>light fireworks</td>
</tr>
<tr>
<td>throw a snowball</td>
<td>shave</td>
</tr>
<tr>
<td></td>
<td>nutcracker</td>
</tr>
<tr>
<td></td>
<td>drive a car</td>
</tr>
<tr>
<td></td>
<td>pencil sharpener</td>
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<td></td>
<td>electric eel</td>
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<tr>
<td></td>
<td>watch</td>
</tr>
<tr>
<td></td>
<td>board game</td>
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<tr>
<td></td>
<td>glove</td>
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<tr>
<td></td>
<td>telephone</td>
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<tr>
<td></td>
<td>winding stairs</td>
</tr>
<tr>
<td></td>
<td>ashtray</td>
</tr>
<tr>
<td></td>
<td>bow</td>
</tr>
<tr>
<td></td>
<td>handcuffs</td>
</tr>
<tr>
<td></td>
<td>glove</td>
</tr>
</tbody>
</table>

Table 4.1: Action and object words used in the charades
of the gestures. This was done to ensure at least 50 seconds of data in each trial to examine the time course of activity using between brains Granger causality. This was done by showing a progress bar below the movie, changing from red to green after 50 seconds, indicating the beginning of the period (50–90s post stimulus onset) during which participants could decide on their interpretation of the gestures, whenever they felt confident, by pressing a button on their 4-button button-box, triggering the appearance of a multiple choice screen. In the multiple-choice menu they had to choose the correct word from five alternatives. One of the alternatives was always ‘none of the above’ and the correct answer was always present in the multiple-choice menu. The correct answer was never the option ‘none of the above’. This marked the end of a trial. Two consecutive trials were separated by 20s of a white fixation cross against a black background, which served as our baseline (Fig. 4.11).

**Passive observation run:** As a control condition for the guess run, the participants watched the movies they had seen during the guessing condition again. This time, they were instructed not to guess what was gestured, but only to passively view them. We are aware that such instructions cannot ensure that participants entirely stopped to interpret the gestures, but at debriefing, participants reported having interpreted the gestures at least less consistently than during the guess run. To keep the run exactly the same as the original guess run, the movie stopped at the moment the participant during the original run had pushed the button. The same multiple-choice menu then appeared and the participant had to answer again. This time, however, they had to select the word written in green letters. The green word was the correct answer. A fixation cross was presented between two consecutive trials for 20 seconds and served as our baseline.

**Data Acquisition**

Functional imaging data was recorded with a Philips 3.0T MR scanner, using gradient echo planar imaging (EPI) and an 8-channel head coil using SENSE technology. T2* weighted images revealed changes in blood oxygen level. Volume repetition time (TR) was 1.33 seconds. The whole brain was scanned in 28 (axial) slices with a thickness of 4.5mm. Further imaging parameters include echo time (TE) 28 ms, field of view 224 x 224 mm, 64 x 62 matrix, SENSE acceleration factor 2.4, ensuing 3D voxel size 3.5 x 3.5 x 4.5mm. This set of imaging parameters were chosen to cover the entire neo-cortex while at the same time providing a TR short enough to expect sufficient power in a Granger Causality analysis (Roebroeck et al., 2005). In the first session, a fast structural image (“fast anatomy”) was acquired of the participant’s brain, while in the second session an additional structural image of higher resolution was acquired. Both were structural, T1-weighted images acquired with a T1TFE sequence (echo
time 3.5 ms, repetition time 7.6 ms, 224 x 160 x 256 matrix, 1 x 1 x 1 mm3 voxels).

Data pre-processing

All analyses and preprocessing was performed in BrainVoyager QX 1.10 along with custom written C++ code for the between brains Granger causality analyses. The pre-processing steps included slice scan time correction, 3D motion correction and temporal filtering (consisting of linear trend removal and a high pass filter with a cut-off at 0.004Hz). The images were not smoothed spatially. Functional images were co-registered with the structural images and morphed into Talairach space.

The structural images were corrected for inhomogeneity (to improve segmentation results), normalized into Talairach space after which the cortical grey matter/white matter boundary was segmented into a topologically correct surface representation. After segmentation, the cortical surface representations of all subjects were aligned using a cortical curvature based alignment procedure. This procedure aligns the sulci and the gyri of the different brains using their cortical curvature-maps (Fischl et al., 1999). Included in the cortex-based alignment was also the Colin (27) brain (Holmes et al., 1998). The fMRI time courses were resampled on the curvature aligned cortical surface representations.

Behavioral Results

During guessing the participants were asked to consider each movie for at least 50 seconds. After the 50s they could push the button when they thought they knew what was being gestured to enter the multiple-choice menu. The average latency to response was 58 seconds. Participants were equally accurate on both categories: 82.5% of the object words were guessed correctly against 86.5% of the action words (t(17)=-1, p>.33). We did not find a significant difference between the two types of gestures, neither in terms of latency to respond (58.7s ± 6.5s for action and 60.8s ± 6.8s for object words, t(17)=1.16, p>.26) nor in terms of accuracy (6.06 ± 0.73sd correct out of 7 action and 5.78 ± 1.11sd correct out of 7 object words, t(17)=-1, p>.33). Words that were guessed incorrectly were watched significantly longer than words that were guessed correctly: 58s ± 5s for the 289 correct guesses versus 68s ± 12s for the 47 incorrect guesses (t(16)=-4.41, p<.0005).

Granger causality analyses

Granger causality analyses were performed as described in Roebroeck et al. (2005) but applied here to data from different brains Supplementary Information 2. In
short, given two time-series (for a seed and another point on the cortical surface), autoregressive models are estimated that quantify G-causality. Given a seed in the gesturer, maps are created that specify G-causal influence from the seed in the gesturer to all of the guesser's brain, as well as influence in the reverse direction, i.e. from anywhere in the guesser's brain to the seed in the gesturer's brain. These two directions of G-causality are then subtracted from each other to generate differential G-causality maps, such that positive values indicate more G-causality from the gesturer to the guesser than from the guesser to the gesturer.

This differential G-causality measure was used for three reasons. First, it generates values that are approximately normally distributed, with a mean of zero under the null hypothesis of an absence of (indirect) causal relationship at the neural level between the two brains, and are thus suitable for parametric testing at the second level (see below and Roebroeck et al., 2005). Second, given that the guesser saw the gesturer but not the other way around (one-way video feed), we know that there should be more information flow gesturer $\rightarrow$ guesser than guesser $\rightarrow$ gesturer (the latter could only be due to anticipatory neural computations), allowing a directed hypothesis testing. Thirdly, it has been shown that when the BOLD signal is used to estimate G-causality, the differential G-causality (i.e. $X \rightarrow Y - Y \rightarrow X$) is more robust than testing the individual components due to the filtering properties of the hemodynamic response and the relatively low sampling rate of fMRI (Roebroeck et al., 2005). A separate differential G-map was calculated for each of the 8 seed regions (see below) for each gesturer. These differential G-causality maps were then taken, separately for each seed region map, to the second level (see below) and thresholded for multiple comparisons at $p < 0.05$ using a cluster threshold determined by a Monte Carlo simulation method (Forman et al., 1995; Hagler et al., 2006). The order of the estimated autoregressive models was 3, i.e. the 3 preceding time points are taken into account to predict the current activity, corresponding to $\sim$4 seconds (3TR). This interval was chosen a priori because it roughly covers the time it takes for a typical gestural phrase to unfold (McNeill, 1992), and would therefore permit the analysis to include time points involved in planning a gestural phrase in the regression of time points involved in perceiving the end of the phrase. In addition, an early exploratory analysis confirmed this expectation. Performing the analysis with orders of 1TR or 5TR revealed similar but weaker effects (Fig. 4.10, due to too little relevant history used in the order-1 models and power loss due to the increased amount of estimated parameters in the order-5 models. It is likely that other experimental paradigms may need different bbGCM orders, depending on the time scale of the semantic units involved.
Seed ROIs

The ROIs that were used as seeds in the between brains Granger causality analysis were defined as those ‘mirror’ areas that were active both during gesturing and guessing using a traditional GLM analysis on the same data (Schippers et al., 2009). The GLM for gesturing was estimated using the entire period in which the gesture was executed as the only predictor. The GLM for the guess runs included two predictors: 1) the period from onset of the movie in which the gesturer was shown until the time of button press and 2) from button press until the participant had given a response. All predictors were convolved with canonical hemodynamic response functions. The mean parameter estimates of the contrasts gesturing versus baseline and guessing versus baseline were tested at the second level using a one-sample t-test. Both results of the second-level random effects analysis of gesturing and guessing versus baseline were thresholded at \( p < 0.0001 \). We used this stringent criterion to reduce the size of our seeds. The resulting maps were binarized (i.e. contained value 1 at above-threshold vertices and 0 at below-threshold vertices). The binary maps were multiplied and the resulting clusters contained the middle temporal gyrus (LH: 69, RH: 256 vertices), the ventral (LH: 42, RH: 123 vertices) and dorsal premotor cortex (LH: 69, RH 147 vertices) and a larger cluster in the parietal lobe. To reduce the size of our parietal lobe seed, we used only that part of the ROI that overlapped with area BA2 (LH: 529, RH: 552 vertices), because BA2 was found to be the most consistent location of mirror voxels in a previous analysis of the execution and observation of goal directed behavior (Gazzola and Keysers, 2008). The location of BA2 was defined by projecting the maximum probability map of BA2 from the anatomy toolbox (Eickhoff et al., 2005) onto a cortical surface segmentation of the Colin brain (Holmes et al., 1998). These particular ROIs were chosen because our primary aim was to examine the resonance theory of mind-reading which suggests that the pMNS of to people resonate with each other, with the pMNS in the gesturer involved in triggering the gestures, which are then viewed by the guesser, triggering activity in the pMNS of the guesser.

Time series

The input of the Granger causality analysis consisted of the average time course of the seed of the gesturer during gesturing as well as all the corresponding time courses of the vertices of the guesser’s brain during guessing. The time courses were truncated to contain only those parts that reflected the steady-state part of either the gesturing or the guessing. We excluded 1) 15TR from the beginning and 5TR from the end of both the gesturing and the observation, thereby removing the on- and offset transients (Fig. 4.1, green blocks), 2) the period from the button press of the guesser until the
onset of the next gesture (Fig. 4.1, red and grey blocks), and 3) the baseline fixation cross period between two trials (Fig. 4.1, blue blocks). Additionally, information about the beginning and end of each separate gesture-part was taken into account in the Granger causality analysis, such that autoregressive model estimation was pooled over calculations on separate blocks rather than calculated over a single time-course with all blocks concatenated. On average, participants watched the gesture movies for 58 seconds, which corresponds to \( \pm 43 \) TR. This means that on average \( 43 - 15 - 5 = 23 \) TR per trial were included in the Granger analyses, cumulating to \( 23 \times 14 = 322 \) TR per participant.

**Second level Granger analysis**

Random effects’ testing was performed by t-tests at the second level. Two-tailed one-sample t-tests were computed for a single differential G-causality maps, with a null-hypothesis of a zero value (i.e. there is as much G-causality from Gesturer \( \rightarrow \) Guesser than from Guesser \( \rightarrow \) Gesturer). Two-tailed paired t-tests were computed when differential G-causality maps were compared between conditions/situations, with a null-hypothesis of a zero value for the difference of the maps. Random effects t-maps were then statistically thresholded at \( p < 0.05 \) and corrected for multiple comparisons by using a Monte Carlo simulation-based cluster-size threshold adjustment (Hagler et al., 2006).

**Motion Energy GLM**

We extracted motion energy from the gesture movies using Matlab. For two consecutive frames of the recorded movies, motion energy was quantified in every pixel as the sum of the squared differences in the red, green and blue channels and then summed over all pixels. This time course was then mean corrected, convolved with the hemodynamic response function and sampled at the acquisition rate of the fMRI signal (TR = 1.33s). The GLM included three predictors: 1) a boxcar having values 1 for the period from onset of the movie in which the gesturer was shown until the time of button press and zero elsewhere 2) a boxcar having values 1 from the time of button press until the end of the response procedure and zero elsewhere, 3) the mean corrected, convolved and sub sampled motion energy time course. Predictors 1 and 2 were then also convolved with the canonical hemodynamic response functions. The mean parameter estimates of predictors 1 and 3 were then averaged for each participant and tested at the second level using a one-sample t-test (see Fig. 4.9). Predictor 1 but not 3 showed above threshold clusters.
G R A N G E R C A U S A L I T Y A N D I T S A P P L I C AT I O N T O F M R I

Granger causality or G-causality was proposed by Clive Granger (Granger, 1969, 1980) and partially based upon earlier ideas of Norbert Wiener (Wiener, 1956). The aim was to give an operational definition of what causality or influence could mean for observations, structured in time, of multiple variables of interest. Granger clearly did not mean to equate such a definition to an interventional notion of causality that demands that one can only establish true causality if a process is actively interfered with and the consequences, observed (c.f. Pearl, 2000). Rather, G-causality quantifies a very useful, pragmatic statistical notion of information transfer (see below) between two stochastic processes.

A stochastic process \( X(t) \) is a random variable that is observed repeatedly in time in an orderly fashion. The general idea of G-causality is that \( Y(t) \) G-causes another process \( X(t) \) if the prediction of \( X \)'s values improves when we use past values of \( Y \), given that all other relevant information is taken into account. Here, all other relevant information is understood to contain at least the past values of \( X \) itself. The vehicle most often used to make predictions in this context is the linear autoregressive (AR) model. Two regressions are compared: one including only the past of \( X \) itself, and one additionally considering the past of \( Y \):

\[
X_t = \sum_{i=1}^{3} \alpha_i X_{t-i} + \epsilon_t
\]

\[
X_t = \sum_{i=1}^{3} \alpha_i' X_{t-i} + \sum_{i=1}^{3} \beta_i Y_{t-i} + \epsilon_t
\]

The residual error (variance) reduction from \( \sigma^2(\epsilon) \) to \( \sigma^2(\epsilon') \) quantifies how much \( Y \) G-causes \( X \). In the present study, the index \( i \) runs from 1 to 3 (the so-called autoregressive order) which determines how far into the past we look for useful information to predict \( X \)'s values. The autoregressive order needs to be carefully chosen (see Methods and Fig. 4.10). When G-causality is applied to fMRI, the rich spatial structure in the fMRI signal can be used. fMRI gives 10's of thousands of independent time-courses (for each volume element or voxel) of activity throughout the brain. The technique of fMRI Granger Causality Mapping (GCM) explores all regions in the brain that interact with a single selected reference region using G-causality as a measure of directed
influence or information flow. By employing an autoregressive model containing the reference region and, in turn, every other voxel in the brain, the sources and targets of influence for the reference region can be mapped (Roebroeck et al., 2005).

Here, Granger causality mapping was applied to data from different brains in between brain Granger Causality Mapping (bbGCM). Given a seed selected in one brain (the gesturer), maps are created that specify G-causal influence from the seed in the gesturer to all of the guesser's brain, as well as influence in the reverse direction, i.e. from anywhere in the guesser's brain to the seed in the gesturer's brain. In this way we quantify information transfer (in the Granger causal or information theoretic sense, see below) between two brains during gestural communication (see Figure 4.2). Simulations have shown that if two populations of neurons influence each other strictly in a $X \rightarrow Y$ direction, if BOLD is used to make inferences about the neural populations, the low-pass filtering effect of the hemodynamic response function and the low sampling rate of fMRI lead to a certain cross-talk between the $X \rightarrow Y$ and $Y \rightarrow X$ G-causality, such that the $Y \rightarrow X$ direction in the BOLD is no longer zero. The same simulations have however also shown that the difference between $X \rightarrow Y$ and $Y \rightarrow X$, called the 'differential G-causality', remains positive (Roebroeck et al., 2005). When applying G-causality to BOLD signal, it is therefore advisable to interpret the differential G-causality, and limit inferences to stating that $X$ influences $Y$ more than the other way around or vice versa.

In addition to detecting potential directed causal influences, G-causality has been shown to be a way to quantify information transfer between two variables. In information theory, a branch of mathematical statistics, Mutual information is used as a mathematical quantity that measures how much one random variable $X$ tells us about another $Y$ (Cover and Thomas, 1991; Shannon and Weaver, 1949). Specifically, mutual information measures to what degree the uncertainty about the value of one random variable $X$ is reduced if we know the value of another random variable $Y$. Here uncertainty is mathematically quantified as entropy: the higher the entropy of $X$, the more uncertain we are about $X$'s value. Schreiber (2000) used these information-theoretic concepts to define the notion of transfer entropy as a measure of directed (time-asymmetric) information transfer between stochastic processes. Transfer entropy from $Y(t)$ to $X(t)$ is the degree to which $Y(t)$ disambiguates (i.e. reduces the uncertainty about) the future of $X(t)$ beyond the degree to which $X(t)$ already disambiguates its own future. Interestingly, one can show that, if $X(t)$ and $Y(t)$ values have a Gaussian distribution at each time point, transfer entropy is mathematically equivalent to G-causality quantified within an autoregressive model (Barnett et al., 2009).

In the context of social neuroscience, a key question is to study what brain regions are involved in information transferred from one brain to another. Given that G-causality quantifies time-directed information transfer, bbGCM should be seen as a tool to map which regions of one brain show evidence, in the measured signal
and at the time scale used, of receiving information from (or sending information to) the seed selected in the other brain. Finding significant differential G-causality between regions in two brains then suggest that a causal chain of events (in the stricter interventional sense see Pearl, 2000) may connect these two brain regions. This causal chain of event is necessarily indirect (i.e. mediated through the bodies of the agents and the world, here the gesture and their observation). Such G-causality is then an invitation to:

- Test whether these regions showing G-causality are indeed linked by a causal chain in the interventional, strong sense of causality tout-court, for instance by using TMS to manipulate the region of one brain and measure the effect on the other.
- Manipulate the visual/auditory channels connecting the two individuals to identify how one brain influences the other.
**Supplementary Figures**

Figure 4.4: Results of second-level bbGCM for gesturer to passive observation (Random Effects, n=18). Conventions as in Fig. 4.2, but using brain activity during passive observation instead of guessing for the guesser.
Figure 4.5: Comparison (paired t-test) between gesturer → guesser bbGCM and gesturer → passive observation bbGCM (Random Effects, n=18 participants). Warm colors indicate higher differential Granger values for the original analysis (active guessing). Further conventions as in Fig. 4.2.
Figure 4.6: bbGCM using brain activity from a randomly selected guesser that had seen the gestures of a different gesturer (Random Effects, n=18). The right side therefore represents the guesser's brain showing t-values of the paired t-test between gesturer → random guesser versus random guesser → gesturer G-causality. Conventions as in Fig. 4.2.
Figure 4.7: Comparison gesturer → guesser bbGCM vs gesturer → random guesser bbGCM (Random Effects, n=18 participants). Warm colors indicate higher differential Granger values for the gesturer → guesser analysis. Conventions as in Fig. 4.2.
Figure 4.8: Comparison gesturer-to-guesser bbGCM vs one in which the activity of the gesturer was paired with a patchwork of episodes in which a different, randomly selected guesser (that might be different for each word) saw the same word as the original guesser being gestured by a different gesturer (Random Effects, n=18 participants). Conventions as in Fig. 4.2.r
Figure 4.9: Activation maps of all 18 subjects for the GLM with motion energy and the guessing period as regressors. (a) Main effect guessing-baseline. (b) Main effect motion energy-baseline. All images are thresholded at $t=3.58$ which corresponds to an uncorrected $p<0.001$.

Figure 4.10: bbGCM at temporal windows 1, 3 and 5 (Random Effects, n=18). Only the summary representation is shown for each order, all other conventions as in Fig. 4.2.
Figure 4.11: Example time line of the paradigm of the Charades experiment. Orange signals times in which the female is in the scanner, blue times in which the male is in the scanner. A hand represents gesturing runs, and eye, guessing runs. Each change of color signifies that one partner went out of the scanner and the other was placed into the scanner. Gesture runs were composed of 7 words, and each word had to be gestured for 90s, as signaled by a progress bar. Guess runs were composed of 7 movies of gestures, and the participant could only provide his/her answer once the progress bar had crossed the 50s mark. Twenty seconds of rest with a fixation cross always separated two words or movies.