Analyzing Task-Dependent Brain Network Changes by Whole-Brain Psychophysiological Interactions: A Comparison to Conventional Analysis

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Abstract: While fMRI activation studies contrasting task conditions regularly assess the whole brain, this is usually not true for studies analyzing task-dependent brain connectivity changes by psychophysiological interactions (PPI). Here we combine standard PPI (sPPI) and generalized PPI (gPPI) with a priori brain parcellation by spatially constrained normalized cut spectral clustering (NCUT) to analyze task-dependent connectivity changes in a whole brain manner, and compare the results to multiseed conventional PPI analyses over all activation peaks in an episodic memory recall task. We show that, depending on the chosen parcellation frame, the whole-brain PPI approach is able to detect a large amount of the information that is detected by the conventional approach. Over and above, whole-brain PPI allows identification of several additional task-modulated connections, particularly from seed regions without significant activation differences between conditions. *Hum Brain Mapp* 35:5071–5082, 2014. © 2014 Wiley Periodicals, Inc.

Key words: functional magnetic resonance imaging; connectivity; connectome; episodic memory; recall

INTRODUCTION

One of the main applications of functional magnetic resonance imaging (fMRI) is the identification of local brain activation with relatively high spatial resolution. However, an advantage of fMRI is the ability to measure hemodynamic responses from the whole brain within 1–2 s, which allows the study of distributed brain networks and the interaction of network nodes.

Studying these interactions by connectivity methods allows extracting additional information about the brain, which might not be apparent in regional brain activation alone. Particularly, when searching for brain imaging markers of diseases, measures of functional integration might be even more informative than measures of functional segregation. For example, in schizophrenia research, one of the best described and validated intermediate

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phenotypes is altered brain connectivity [Meyer-Lindenberg et al., 2001; Stephan et al., 2009]. Consistent with this view, it could be shown that a genome wide significant risk variant for schizophrenia and bipolar disorder (rs1344706 of the ZNF804A gene) influences the coupling between dorsolateral prefrontal cortex and hippocampus during a working memory paradigm, while no modulatory effect on regional brain activation was found [Esslinger et al., 2009].

Connectivity methods are often classified into functional and effective connectivity [Friston, 2011]. While in functional connectivity analysis the correlation between time courses of different brain areas are analyzed, effective connectivity analyses like dynamical causal modeling (DCM) [Friston et al., 2003] or Granger causality try to estimate the underlying causal structure of brain networks. Despite their advantages, both types of analysis have restrictions. While correlations are easy to compute, they do not take into account changes of connectivity during the analyzed period, for example those introduced by different tasks or conditions. DCM on the other hand is computationally expensive, and thus normally has to be restricted to specific models and clearly defined networks with only a few nodes, although it has recently been extended to larger brain networks [Seghier and Friston, 2013]. Granger causality tries to estimate causality by taking time lags into account, which is problematic for the relatively slow BOLD signal [Smith et al., 2011].

A method which is lying somewhere in the middle between stationary functional and dynamic effective connectivity, and that is probably best described as “directed functional connectivity,” is the psychophysiological interactions (PPI) method [Friston et al., 1997].

PPIs are a specific form of moderated multiple regression [Jaccard and Turrisi, 2003] that allows to study the contribution of one area to another with regard to the experimental condition by a regression model that incorporates an interaction term between the experiment (psycho) and the BOLD time course of a seed region (physiological). Recently, McLaren et al. [2012] introduced generalized PPI (gPPI), which extends the standard SPM implementation of PPI (sPPI) by allowing the use of more than one experimental term to give a more complete coverage of the experimental space. In simulations, this expansion has been found to increase the power of PPI to detect true effects [Cisler et al., 2014; McLaren et al., 2012].

However, a gap exists between activation and (PPI) connectivity analyses. While activation studies contrasting experimental and control conditions regularly assess the whole brain, this is normally not true for studies analyzing brain connectivity by PPI where only the identification of target but not of seed regions is done on a whole brain level. Any form of conventional PPI requires the *a priori* specification of one, or a few, seed regions from which analyses towards all other voxels of the brain are performed. This method is spatially precise in defining the varying contribution of a particular seed region to other

brain areas during different conditions of the experiment, but both, contributions of other areas to the seed region, as well as potentially important connectivity changes from areas other than the chosen seed to the rest of the brain might be missed.

These restrictions are not inherent to the method but consequences of its current implementations. From an algorithmic point of view, PPIs and closely related methods can easily be used to assess the whole functional connectome and analyze task-dependent connectivity changes in a whole-brain manner, for example for the assessment of large-scale brain-system dynamics related to cognitive control. (For a more detailed discussion of this point, see Cocchi et al. [2013b]).

Surprisingly, only few studies so far have used such whole-brain PPI approaches. For example, one approach has been presented by Dodel et al. [2005] who developed Correlation Modulation, a method closely related to PPI that detects network changes between a number of preselected ROIs by subtracting weighted condition-specific correlation networks. Recently, Minati et al. [2012] applied PPI analyses to connections between *a priori* chosen regions of interest based on the automated anatomical labeling (AAL) atlas [Tzourio-Mazoyer et al., 2002] in a decision-making paradigm. Fornito et al. [2012] developed an undirected correlational PPI (cPPI) approach that they used to analyze task dependent functional connectivity changes between large-scale network components identified by independent component analysis (ICA) during a contextual recollection task. Cocchi et al. [2013a] performed PPI analyses between 18 seed region defined by activation peaks to analyze the influence of relational complexity on brain networks in a deductive reasoning task.

Even if multiple seed regions are chosen for a PPI analysis, they are often restricted to areas displaying significant activations for the task at hand. However, the relationship between activation and connectivity is not straightforward. Although both should overlap to a certain extent, this is not necessarily exclusive. An area could change its activity between task conditions, but not its connectivity, and *vice versa*, the activity could stay the same, but the connectivity might change, e.g., by dynamic reconfiguration of large-scale brain networks. From this point of view, it makes sense to look for connectivity changes also in regions, which do not show condition related changes in activation. To do so, we use here *a priori* parcellations of the whole brain combined with PPI analyses to detect task-dependent connectivity changes without prior restriction to specific brain areas.

An essential aspect of performing whole-brain analyses is the definition of functional nodes of the assessed brain network [Zalesky et al., 2010a]. While often anatomy-based atlases are used for whole-brain analyses, this might not be the best way [Smith et al., 2011], and a clear gold standard is hitherto missing.

From a functional point of view, a promising way would be to parcellate the brain into distinct functional units and summarize the data within these units.

One recently developed method, spatially constrained normalized cut spectral clustering (NCUT) [Craddock et al., 2012] might provide means to approach this goal. NCUT is a parcellation approach based on functional data, results in spatially restricted ROIs, and allows the integration of single subject results into a second level group clustering. The number of ROIs is prespecified by the user, allowing comparison of different parcellations with ROIs of different number and size, which is one of the most important factors influencing the results of whole-brain analyses [Zalesky et al., 2010a].

In the present study, we combined NCUT clustering with PPI to extract task-dependent connectivity changes of the human functional connectome during an episodic memory recall paradigm between all brain regions and examined how the number and size of brain regions chosen for the parcellation influences the information that can be gained by whole-brain PPI.

To quantify the results, make them comparable between different parcellations, and test whether whole-brain PPI is able to detect the same and/or additional brain network changes as conventional PPI analyses, we compared the results of whole-brain PPI to those of multiseed conventional PPI with all nonoverlapping seed regions that showed significant activation or deactivation in our episodic memory recall data.

METHODS

Participants

The study sample consisted of 41 right handed healthy adults (24 female, mean age $\pm$ standard deviation 30.2 ± 9.2 years, age range 19–49 years). All subjects were eligible for MRI scanning and had no history of mental disorders, substance abuse, or severe head injury. Subjects were recruited from the local population by sending invitation letters to addresses randomly drawn from the population registry. Participants gave written informed consent and received 50 € compensation for participation. All procedures complied with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and were approved by the local ethics committee of the University of Heidelberg/Medical Faculty Mannheim.

Scanning and Recall Task

Subjects performed an episodic memory task [Erk et al., 2010] in a 3 T Siemens Trio TIM scanner at the Central Institute of Mental Health in Mannheim, Germany. Echo planar images (EPI) were acquired with a 32 channel head coil in 33 slices of 3 mm with 1 mm gap, with an in-plane resolution of 3×3 mm, FOV = 192 mm, TR = 1.8 s, TE = 30 ms, flip angle of 73° , and GRAPPA factor 2.

The task consisted of an encoding and a subsequent recall phase of which we only analyzed the latter here.

During the encoding phase, subjects learned 16 face profession pairs, which had to be remembered. The recall phase took of 3:47 min and had a simple block design with 4 recall blocks and 4 alternating control blocks. During the recall blocks the faces from the encoding phase were presented and subjects had to recall the corresponding profession and indicated by a left or right button press whether job training or a university degree is a prerequisite for the job. In the control blocks subjects had to indicate, which of the two ears of schematic heads was larger.

Preprocessing and Activation

Preprocessing was done with SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm8/>) running in MATLAB (R2011b). The functional images were slice-time corrected to the middle (17th) slice, realigned to the first image of the run, normalized into standard stereotactic space (MNI EPI template), rescaled to $3 \times 3 \times 3$ mm resolution, and smoothed with a FWHM = 6 mm Gaussian kernel. Contrast images for the recall condition versus the control condition (and *vice versa*) were obtained for each subject and entered into a second level group analysis with one sample *t*-tests to detect significant activations (Fig. 1).

Parcellation

For parcellation we used the normalized cut algorithm (NCUT) [Shi and Malik, 2000; Yu and Shi, 2003]. In general, for normalized cut the data are represented as an undirected weighted similarity graph $G = (V, E)$, where V represents the nodes and E represents the edges of the graph, reflecting the similarity between the nodes. This graph is then cut into different sets with the goal that similarity is high within a set, and low between sets [Shi and Malik, 2000].

To prevent clusters from containing only single voxels, a normalization is introduced in NCUT. For two sets A and B normalized cut minimizes the objective function [Shi and Malik, 2000]:

$$N_{cut}(A, B) = \frac{cut(A, B)}{assoc(A, V)} + \frac{cut(A, B)}{assoc(B, V)}$$

where $cut(A, B)$ is the cut cost associated with a partition, meaning the sum of the weights of eliminated edges, and $assoc(A, V)$ is the sum of edge weights from nodes in A to all nodes in the Graph, respectively for $assoc(B, V)$. This problem is solved by linear algebra.

Brain parcellation of fMRI data was performed by spatially constrained normalized cut spectral clustering implemented in a freely available toolbox [Craddock et al., 2012] (http://www.nitrc.org/projects/cluster_roi/) that has several appealing properties. It is based on functional data, results in spatially restricted ROIs, can give different numbers of ROIs, and allows for the integration of single subject results into a second level group analysis. We used

Figure 1.

Group level brain activation for the contrasts Recall > Control (left) and Control > Recall (right). $N = 41$, $P < 0.05$, FWE corrected. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

smoothed raw data to attenuate the influence of voxel-intrinsic random noise on voxel similarity measures.

In detail, first subject specific similarity matrices between voxels were constructed. As supposed by Craddock et al. [2012] we used the Pearson correlation of voxel time series as metric of similarity, and set correlations below a threshold of $r = 0.5$ to zero to exclude negative and weak correlations. The similarity matrices were sparse because only the correlation of a voxel to its 26 adjacent voxels (face and edge neighbors) were taken into account. These individual spatially restricted similarity matrices were then partitioned by NCUT into an *a priori* set number of ROIs, and the results were entered into a group analysis [van den Heuvel et al., 2008]. The resulting ROIs tend to be roughly of the same size [Craddock et al., 2012], and NCUT can result in single clusters containing no voxels, so the final number of ROIs can be smaller than specified.

For comparison, we also included the automated anatomical labeling (AAL) atlas consisting of 116 anatomical ROIs [Tzourio-Mazoyer et al., 2002] into our analysis.

After parcellation, the first eigenvariates of the time courses of all ROIs resulting from the parcellations were extracted, grand mean scaled to an overall mean of 100, and stored for PPI analyses.

Psychophysiological Interactions

PPI [Friston et al., 1997] is a specific version of moderated multiple regression [Jaccard and Turrisi, 2003] in which the regression model consists of the time course of the experiment (psycho), the time course of a seed region (physiological), an interaction term of both, and covariates. As the interaction takes place on the neural level, which underlies the blood oxygenation level dependent (BOLD) signal measured by fMRI, Gitelman et al. [2003] introduced a deconvolution of the seed area BOLD signal before calculating the interaction term, which is afterwards convolved again with the canonical hemodynamic response function (HRF). This procedure is implemented in the SPM software package.

McLaren et al. [2012] extended the standard implementation of PPI in SPM (sPPI) by the introduction of gPPI, which allows the use of more than one experimental term in the multiple regression model.

Whole-Brain PPI

For whole-brain PPI analyses we adopted sPPI and gPPI data processing functions from SPM8 and the gPPI toolbox (<http://www.nitrc.org/projects/gppi>) on the level of brain regions. The pipeline for whole-brain PPI analyses was carefully designed to apply the same data processing steps as in the conventional approach, although the exact order differs due to our implementation of the functions for raw regional time series (Fig. 2). For each subject, the first eigenvariates of the ROIs were high-pass filtered with a cut-off of 128 s, prewhitened to account for serial autocorrelations of order 1, adjusted for an F-contrast over both experimental conditions, and deconvolved using the canonical HRF. The experiment was expressed as a single regressor with task condition minus control condition in sPPI, and as two regressors containing one condition each in gPPI. Conditions were modeled as blocks, including correct as well as incorrect trials. Interaction terms with the experiment were calculated at the neuronal level, and convolved with the canonical HRF. For each ROI, PPIs targeting all other ROIs were calculated with general linear models that included the convolved PPI interaction term(s), the seed region time course, the experimental regressor(s) convolved with the HRF, unconvolved movement regressors, and a constant. Beta values of the PPI interaction term (sPPI) or beta contrast between the interaction terms (gPPI) were saved in a full connectivity matrix between all ROIs (Fig. 2B) and used for second-level analyses with one-sample *t*-tests, in which we used a threshold of $P < 0.05$ Bonferroni corrected for the total number of directed connections between all ROIs. Significant connections were then plotted for each parcellation scheme as arrows from the center coordinates of the seed ROI to the center coordinates of the target ROI (Fig. 3) with BrainNet Viewer (<http://www.nitrc.org/projects/bnv/>).

Figure 2.

Simplified workflow for (A) multiseed conventional and (B) whole-brain PPI analyses. Specific data processing steps are mentioned at the step of their implementation. The whole-brain pipeline was carefully designed to apply processing equivalent to SPM; however, the exact order differs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Conventional PPI

To compare the results of the proposed whole-brain procedure to a conventional procedure we performed conventional sPPI analyses with SPM8 and conventional gPPI analyses with the gPPI toolbox by putting spherical seeds of 6 mm radius around all local maxima that were detected by significant task activation or deactivation ($P < 0.05$ FWE corrected, $k = 15$) in a way that the resulting spheres were non-overlapping (Supporting Information Table I). For each of these seeds we extracted the first eigenvariate adjusted for an F-contrast over both experimental conditions, set up separate PPI models with task condition minus control condition as task regressor (sPPI) or condition specific task regressors (gPPI), and performed PPI analyses with the interaction term(s), the seed time course, the task regressor(s), and the movement regressors as covariates. After single-subject PPI analyses, we performed second level analyses with one-sample t -tests for each of the analyses separately. In each of the second level SPM analyses we applied a significance threshold of $P < 0.05$ FWE corrected, and a minimum cluster size threshold of $k = 15$ voxels and counted voxel clusters surviving this threshold as significant connections. Please note that this is a strict threshold on the level of the individual analyses, but that we did not correct for the number of

different PPI analyses we set up. The results of the individual analyses were then combined and put into a common figure by plotting connections from the centers of the seeds to the peaks of significant clusters (Figs. 2A and 4A).

Comparison Between Conventional and Whole-Brain PPI

We compared the results of multiseed conventional PPI with whole-brain analyses on two levels: First on a coarse level of regional connections, and second on a fine-scaled voxel-to-voxel level. For regional connections we tested whether any connection between any voxel of a seed and any voxel of a significant target cluster was also detected by whole-brain PPI, i.e., whether a connection that was plotted for conventional PPI (Fig. 4A) could also be plotted for whole-brain PPI (Fig. 3). On the voxel-to-voxel level we compared for each voxel to which other voxels it was found to be connected by the approaches, and quantified the overlap. Thus, the regional level compares the existence of connections connecting different brain regions, while the voxel level compares the fine spatial scale of the overlap between the identified effects. In all analyses,

Figure 3.

Whole-brain PPI results for the different parcellation schemes. Arrows are drawn from the center of a seed ROI to the center of a target ROI for all connections with $p < 0.05$ Bonferroni corrected. Arrow directions indicate the directed nature of the underlying statistical models. Color codes indicate the contrast

whole-brain sPPI and gPPI results are compared to the respective conventional sPPI or gPPI analyses.

RESULTS

Task and Brain Activation

Mean task performance was 72.7% with a standard deviation of 11.8%.

and whether a specific connection was detected by sPPI, by gPPI, or by both. Please see Supporting Information Figure 2 for a separate presentation of sPPI and gPPI results. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

The contrast recall > control revealed prominent cortical activation especially in frontal and parietal areas. In addition, activation was found in the occipital cortex, anterior insula, cerebellum, and substantia nigra (Fig. 1). The reverse contrast Control > Recall revealed activation mainly in pre- and postcentral gyri, as well as in temporo-occipital areas and posterior insula (Fig. 1). Within these results we found 46 task-positive (Recall > Control) and 60 task-negative (Control > Recall) local peaks separated by at

TABLE I. Results summary for parcellations and whole-brain PPI

Parcellation	ROIs	Mean ROI size Voxels $\pm$ StD	Connections (Regions) sPPI/gPPI	Connections (Voxel-to-voxel) sPPI/gPPI	Bidirectional connections (Regions) sPPI/gPPI
AAL	116	467 $\pm$ 348	16/30	4,867,213/9,095,135	1/2
116	116	550 $\pm$ 107	25/29	8,794,467/9,347,533	3/2
200	200	319 $\pm$ 56	33/41	3,866,062/4,922,091	5/4
400	399	160 $\pm$ 33	75/93	2,524,495/3,050,339	7/3
600	585	109 $\pm$ 24	85/87	1,345,991/1,365,053	9/7
1000	912	70 $\pm$ 18	75/69	485,168/426,244	4/1

Number of ROIs, mean size, and number of significant connections for the different parcellation schemes. Please note that bidirectional connections include two directed connections.

sPPI: whole-brain standard PPI; gPPI: whole-brain generalized PPI; StD: standard deviation.

Figure 4.

Results of conventional PPI analyses and comparison to whole-brain PPI. (A) Results of conventional sPPI (left) and gPPI (right) analyses. Arrows are drawn from the center of a seed to the peak voxel of a target cluster for effects above a threshold of $p < 0.05$, FWE corrected, $k = 15$ in any of the 106 conventional PPI analyses. Red: Recall > Control; blue: Control > Recall. (B and C) Comparison of conventional to whole-brain PPI results for the contrast Recall > Control. (B) Ratio of regional connections from A that were also detected by the respective whole-

brain PPI analyses for the different parcellation schemes. (C) Ratio of voxel-to-voxel connections from A that were also detected by the respective whole-brain PPI analyses. (D) Overlap of seed region and (E) Results between conventional and whole-brain sPPI (400 ROIs) for a single example seed in the precuneus. Yellow: conventional sPPI; Blue: whole-brain sPPI; Green: overlap. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

least 12 mm (Supporting Information Table I), and used them as seed coordinates in conventional PPI analyses.

Parcellation

We ran parcellations with *a priori* specified numbers of 116, 200, 400, 600, and 1000 ROIs.

The NCUT algorithm can lead to single ROIs being empty, so the number of regions as a result of the parcellation can deviate from the *a priori* specified. We found group-level parcellations with 116, 200, 399, 585, and 912 regions for the respective parcellation schemes. For ease of presentation and clarity, we label our results with the prespecified number of ROIs. Results of the parcellations are presented in Table I and Supporting Information Figure 1.

Whole-Brain PPI

All whole-brain analyses revealed significantly modulated connections ($P < 0.05$ Bonferroni corrected) in the task-positive, and all but the AAL and 116 ROI parcellation with gPPI also in the task-negative direction (Table I and Fig. 3).

In the task-positive direction, the number of significant regional connections increased with the number of ROIs up to the 600 ROI parcellation in sPPI, and up to the 400 ROI parcellation in gPPI, and decreased afterwards, while the number of identified voxel-to-voxel connections decreased continuously with the number of ROIs (Table I). In all but the 1000 ROI parcellation identified gPPI a higher number of connections that were significantly stronger during recall than sPPI.

The gross structure of the identified task-positive networks is remarkably similar across the parcellations, especially for the finer parcellations. Commonly identified by sPPI and gPPI (yellow connections in Fig. 3) were a right-lateralized fronto-parietal network, and a large-scale increase in connectivity of the precuneus, especially to parietal and occipital cortex, but also to prefrontal cortex (Fig. 4D,E). In addition, gPPI was able to detect more connections to the left hemisphere than sPPI, and thus detected more bilateral effects.

For the contrast Control > Recall, effects were restricted mostly to occipital and parietal cortex in the sPPI analyses. In contrast, gPPI detected very few connections that had significantly higher connectivity during the control task.

Further, we identified bidirectional connections with significant PPI effects in both directions; however, only a minority of connections displayed such behavior. Bidirectional connections included between 3% and 30% of all connections that showed higher connectivity during recall in the respective analyses in the different NCUT parcellation schemes (Table I).

Conventional PPI

One hundred six conventional sPPI and gPPI analyses with spheres of 6 mm radius around the activation peaks as seeds were run. By applying a threshold of $P < 0.05$ FWE corrected, minimal cluster size $k = 15$ voxels in the individual PPI analyses, we were able to detect a total of 27 significantly modulated connections between seed spheres and result clusters in sPPI and 22 connections in gPPI. Of those, 9 showed negative (Control > Recall) task-modulation in sPPI, but only one in gPPI (Fig. 4A).

It has recently been shown that head movement can influence connectivity estimates [Power et al., 2012]. However, as PPI assesses task-dependent connectivity, it should mainly be affected when movements are confounded with the experimental design. We thus tested whether movements systematically differed between conditions. Neither relative frame-to-frame displacement nor any of the individual movement parameters did significantly differ between conditions (paired t -tests, P -values = 0.13–0.58).

Comparison

Comparing the results of the whole-brain analyses to the multiseed conventional results allows us to quantify the overlap between both approaches, evaluate the parcellation schemes based on the ratio of the conventional results that they allow to discover, and identify the additional results that whole-brain PPI is able to detect. We restrict the comparison to the contrast Recall > Control, as only one single significant connection was identified in the other contrast direction with conventional gPPI. However, comparisons for both contrasts in sPPI can be found in Supporting Information Figure 3.

In general, the whole-brain PPI approach is able to detect a substantial part of the results obtained by conventional analysis in our data. However, this overlap strongly depends on the chosen parcellation scheme. When comparing regional connections, whole-brain PPI with the different parcellations found between 33.3% and 72.2% of the conventional connections, with the 400 ROI NCUT parcellation giving the highest rate of 72.2% in sPPI as well as 71.4% in gPPI (Fig. 4B).

In the comparison on the voxel-to-voxel level whole-brain PPI based on the 400 ROI parcellation shows a clear peak with 54.2% (sPPI)/57.2% (gPPI) and thus again detects most similar information on a fine-scale level as conventional PPI (Fig. 4C). An example for the overlap between conventional and whole-brain PPI is illustrated in Figure 4D,E for an overlapping seed region in the precuneus.

By applying whole-brain PPI we were able to detect several additional significant connections compared to conventional PPI (Fig. 5). In addition to connections from ROIs containing significant activations or deactivations we also detected significantly modulated connections from seed ROIs that did not display task-dependent activation or deactivation, for example a higher connectivity from rostral anterior cingulate cortex (ACC) to inferior parietal cortex (Fig. 5B) in the recall condition.

To test for divergence between approaches, we also tested for overlap of significant connections with different signs. We did not identify a single voxel-to-voxel connection in any analysis where conventional and whole-brain PPIs yielded opposite significant results.

DISCUSSION

We compared whole-brain sPPI and gPPI analyses based on spatially constrained normalized cut spectral clustering with different spatial scales to multiseed conventional sPPI and gPPI analyses, respectively in an episodic memory recall task in order to investigate how much of the information that can be detected by conventional PPI analyses is reflected in the results of the whole-brain approach, how much this information depends on the chosen parcellation scale, and whether whole-brain PPI results exceed those of conventional PPI.

By using whole-brain PPI we identified increased connectivity mainly in a right lateralized frontoparietal, and in a parietal network with the precuneus as center. These results match those of Fornito et al. [2012], who demonstrated by correlational PPI that higher task-dependent connectivity between the default mode network and a right lateralized frontoparietal network was associated with faster reaction times in a recollection task.

We are able to demonstrate that whole-brain PPI can detect a fair amount of the information that is detected by conventional PPI, and also allows detection of additional information that would not have been detected by

Figure 5.

Whole-brain results that were not detected by the respective conventional PPI analyses for the 400 ROI parcellation. **(A)** All connections that were not identified by conventional PPI analyses. **(B)** Significantly task-modulated connections from seed regions that did not contain significant activation differences between con-

ditions. Color codes indicate the contrast and whether a specific connection was detected in sPPI, gPPI, or both. Please see Supporting Information Figure 4 for a separate presentation of sPPI and gPPI results. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

standard analysis. In addition, we show that the results depend on the chosen parcellation scheme and the intended spatial acuity.

The selection of seed regions in conventional PPI is often done by choosing activation peaks of the contrast of interest. However, this implicitly assumes that only

regions, which change their activation between conditions, change their connectivity. It is also plausible that a region might not show a significant difference in activation, but nonetheless changes its connectivity, e.g., by dynamic reconfiguration of the brain networks it is integrated in. Indeed, we are able to demonstrate here that regions,

which did not show significant task-related activation do change their connectivity significantly between task conditions (Fig. 5B).

We compared whole-brain to conventional PPI on a regional and a voxel-to-voxel connectivity scale. On both spatial scales the 400 ROI parcellation allows detection of the highest number of conventional results. However, the results reflect a trade-off between capturing an effect and correcting for multiple comparisons and seem to be specific for the analyzed task and contrast in the data set at hand, and should not be misunderstood as a generally valid result. In fact, results do already look different if the opposite contrast direction is considered (Supporting Information Fig. 3), which suggests that the results depend strongly on the structure of the underlying effect.

It is important to note that we assume that an overlap of the results is an indicator of quality of the whole-brain results, but that we do not expect that both approaches provide exactly similar results. In conventional PPI we used only spheres of 6 mm radius around significantly activated or deactivated peak voxels as seeds, while whole-brain PPI used all brain regions. Conventional PPI is spatially more exact because it works with voxels as dependent variables, so it might detect modulation of connectivity to smaller areas, while whole-brain PPI integrates information over brain areas and might thus have more power to detect widespread connectivity changes. Importantly, that an analysis based on a specific parcellation does not detect a large part of the results of conventional PPI does not mean that the results obtained are not valid. They might just represent another aspect of the contained information on another spatial scale.

Conventional PPI does of course also not reveal the ground truth of the underlying connectivity changes, particularly, as mentioned before, because it relies on detected activation clusters as seed regions. By our comparison we are thus not able to make any statement about false positive results in the whole-brain PPI analyses. To further target this problem, one of the most important next steps would be to use whole-brain PPI in a test-retest analysis to analyze the reliability of the method.

Results of whole-brain sPPI and gPPI showed substantial overlap, but also clear differences. Specifically, gPPI seemed to detect more bilateral effects, while sPPI found more effects in the negative contrast direction (Control > Recall). The main difference between sPPI and gPPI is a specific assumption about the nature of the analyzed effect incorporated in the sPPI model. By combining experimental conditions with opposite signs in a single regressor, sPPI models a change of connectivity from negative to positive (or *vice versa*) around zero between conditions. If the data exactly corresponds to this assumption, the results of sPPI and gPPI analyses would be the same, but if the data deviates from this assumption, results can differ [McLaren et al., 2012]. We can only speculate that in our data, under the assumption that the detected effects are correct, gPPI was more sensitive to effects violating the

assumptions of sPPI in the left hemisphere, while sPPI might have gained some power to detect weak effects in the negative contrast direction that to a certain degree complied with its model assumptions.

In the regional and voxel-to-voxel comparisons to the respective conventional analyses, the 400 ROI parcellation allowed detection of the highest number of conventional results in both, sPPI and gPPI. While a clear overlap between PPI methods was found for the effect of the parcellations on the fine-scale voxelwise mapping of the PPI effects (Fig. 4C), numbers differed in the regional comparison for parcellations with few and many regions (Fig. 4B).

Another interesting aspect our study revealed is that connectivity changes are mostly unidirectional, with only a minority of the connections showing significant effects in both directions (Table I). This is consistent with the results of a recent computational study demonstrating that effective connectivity between brain areas shows dynamic reconfigurations despite fixed bidirectional structural connectivity [Battaglia et al., 2012]. However, the notion of “directionality” in PPI is an important topic that often causes confusion. In regression analysis dependent and independent variables are strictly separated. If the variance of both is the same, similar regression coefficients are obtained when they are exchanged in simple regression, but the incorporation of the interaction term in PPI leads to different models for the influence of area A on area B, and for B on A, and thus to different results. This “direction” in PPI is sometimes interpreted as effective connectivity, while other authors count PPI as a measure of functional connectivity. We suppose that directions in PPI analysis should not be overinterpreted but should rather be seen as what they are: the results of directed statistical models characterizing the task dependent influence one area exerts over another without the incorporation of a test for the direction. In this sense, we present data with directed edges to indicate the significant models without the intention to make strong claims about the true directionality of the underlying causal structure. On the other hand, Minati et al. [2012] provide preliminary evidence by comparing PPI to DCM that PPI analysis matches results of much more elaborated methods that incorporate tests about the underlying causal model. As already mentioned by Friston et al. [1997], a promising possibility to get closer to the underlying causal structure with PPI and estimate more specifically the task-dependent effect one area exerts on another could be to correct for the influence of other areas and partialize out their effect before calculating the PPI [Friston et al., 1997; see also Marrelec et al., 2009]. Despite the computational demands of this procedure, whole-brain PPI would be well suited for this type of analysis, as all relevant information is directly available.

A shortcoming of the present study is that the scope of the reported analyses is rather limited. We analyzed only a specific task with a specific combination of methods. Notably, we used only the AAL atlas and spatially

constrained normalized cut spectral clustering for brain parcellation, but several other methods with resembling properties do exist [e.g. Blumensath et al., 2013; Hagmann et al., 2007; Thirion et al., 2006; Ward, 1963; Zalesky et al., 2010a] and might give different results. A specific assumption of the NCUT algorithm is that brain modules contain only neighboring voxels, which might not be true. We are considering voxels that touch only at their corners as neighbors, which could result in voxels that are almost exclusively surrounded by voxels of an area, but still belong to another area. However, if a single or few voxels are completely separated from another larger part of the same module they might be missed by this approach. On the other hand, we use the ROI data in connectivity analyses afterwards, which should compensate for this to a certain degree by being able to detect connectivity between two separated parts of the same module when they have a sufficient size. We restricted ourselves to NCUT because our main goal was to assess the influence that number and size of ROIs do have on the obtained results, which seems to be one of the most important factors influencing whole-brain results independent of the exact method chosen [Craddock et al., 2012; Zalesky et al., 2010a]. However, it is an important and still unsolved issue, which parcellation method fits best to a specific purpose. The differences between the results for the contrast directions in sPPI suggest that an algorithm that produces clusters of a similar size might lead to different performance based on the analyzed aspect of the data, and show that further improvements in clustering methods are necessary.

The comparison to conventional PPI we describe here can be used to compare whole-brain PPIs based on different clustering approaches by their results, and might thus help in examining this topic.

We use here a stringent Bonferroni corrected statistical threshold for our whole-brain PPI analyses with the goal to treat connectivity analysis in a way comparable to activation analysis, and are able to detect significant results. However, when effects are more subtle this strict correction might be too conservative. Another promising approach for family-wise error correction in this type of data is the network-based statistic (NBS) method [Cocchi et al., 2012; Zalesky et al., 2010b, 2012], which has also been applied to multiseed PPI results [Cocchi et al., 2013a]. NBS uses a permutation approach to tests for the extent or the intensity of network components, and is especially suitable when power is low, or when additional (e.g., graph-theoretical) analyses, are going to be based on the results.

PPI itself has several inherent restrictions, and most of its drawbacks also apply to whole-brain PPI. Notably, PPI does lack power, increasing the probability of false negative results, but can also produce spurious results, for example when brain activity does not closely resemble model assumptions [O'Reilly et al., 2012]. We analyzed our data with a simple block design model with two con-

ditions including correct and incorrect trials and used PPI functions implemented in SPM 8 and the gPPI toolbox because our main goal here was to compare whole-brain PPI to the conventional implementation in a direct way. More complex analyses, e.g., considering error-specific connectivity, would also be interesting but exceed the scope of this article.

Another limitation of our study is that we were not able to detect significant relationships between task performance and PPI results in our data, which might probably be due to a poor psychometric quality of the performance measure.

Despite these restrictions, we think whole-brain PPI has great potential to discover unknown relationships in the brain by allowing the analysis of task-dependent connectivity changes taking the whole brain into account. For example, whole-brain PPI would allow the integration of task-dependent activation and connectivity analyses in a single study, providing a more complete picture of brain function than each of the methods by itself could (see Supporting Information Fig. 5 for an illustration). Another application of whole-brain PPI could be the identification of task-related networks, which can then be studied further by means of DCM, which tests for the underlying causal structure of neural networks, but is restricted in the number of brain regions it can take into account. Last but not least, the results of whole-brain PPI could be used as input for graph theoretical analysis of task-dependent changes of the human functional connectome.

CONCLUSION

Whole-brain PPI allows the identification of task-modulated connectivity changes in distributed brain networks without a restriction to specific seed regions and is able to pick up a fair amount of the information that is also detected by conventional PPI. Furthermore, whole-brain PPI allows the detection of additional information due to higher power from averaging over areas and by taking seed regions into account that do not show significant activation differences between task conditions. The results of whole-brain PPI depend on the applied parcellation scheme, and the best choice of a parcellation scheme seems to depend on the intended spatial acuity, the data at hand, and the specific task-contrast of interest.

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