Stimulus Dependent Dynamic Reorganization of the Human Face Processing Network

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Abstract

Using the “face inversion effect”, a hallmark of face perception, we examined network mechanisms supporting face representation by tracking functional magnetic resonance imaging (fMRI) stimulus-dependent dynamic functional connectivity within and between brain networks associated with the processing of upright and inverted faces. We developed a novel approach adapting the general linear model (GLM) framework classically used for univariate fMRI analysis to capture stimulus-dependent fMRI dynamic connectivity of the face network. We show that under the face inversion manipulation, the face and non-face networks have complementary roles that are evident in their stimulus-dependent dynamic connectivity patterns as assessed by network decomposition into components or communities. Moreover, we show that connectivity patterns are associated with the behavioral face inversion effect. Thus, we establish “a network-level signature” of the face inversion effect and demonstrate how a simple physical transformation of the face stimulus induces a dramatic functional reorganization across related brain networks. Finally, we suggest that the dynamic GLM network analysis approach, developed here for the face network, provides a general framework for modeling the dynamics of blocked stimulus-dependent connectivity experimental designs and hence can be applied to a host of neuroimaging studies.

Key words: dynamic connectivity, face processing, fMRI, functional connectivity, network analysis

Introduction

Likely as a result of its evolutionary and social significance, evident in everyday functioning, face perception is a highly honed visual perceptual skill. A wealth of behavioral literature posits that faces are processed in a qualitatively different fashion compared with other visual categories (Rossion 2013; Richler and Gauthier 2014). One hallmark of these unique mechanisms is the “face inversion effect” (Yin 1969), that is, the disruption of face processing due to inversion compared with the effect of this manipulation on other objects.

At the neuronal level, there is a growing realization that face perception is accomplished via the coordinated activity of a face processing network (Fairhall and Ishai 2007; Tsao et al. 2008; Kadosh et al. 2011; Davies-Thompson and Andrews 2012; Gschwind et al. 2012; Phillips et al. 2012; Pyles et al. 2013; Zhen et al. 2013; Hung et al. 2015; Wang et al. 2016). However, many functional magnetic resonance imaging (fMRI) studies still utilize a localized approach mostly focusing on “core brain regions” (e.g., fusiform face area—FFA, occipital face area—OFA, superior temporal sulcus—STS; Haxby et al. 2000)
often neglecting additional extended face regions (Kriegeskorte et al. 2007; Simmons et al. 2010; Nestor et al. 2011) and importantly the interaction between these different regions at the network level. This localized approach is also evident in most studies utilizing the face inversion effect (Aguirre et al. 1999; Haxby et al. 1999; Epstein et al. 2006). Nonetheless, the emerging view is that face-related representation associated with the face inversion effect is not only evident at the level of a single region either in the core (Yovel and Kanwisher 2005; Mazard et al. 2006; Pinsk et al. 2009; James et al. 2013) or the extended systems (Aguirre et al. 1999; Pinsk et al. 2009), but rather, that the activity of the entire face network, in parallel with non-face selective regions (Haxby et al. 1999; Yovel and Kanwisher 2005; Sadeh and Yovel 2010; Pitcher et al. 2011; James et al. 2013), mediates this effect (Matsuyoshi et al. 2015). However, so far, no study examined this notion explicitly by using a network-based approach (Smith et al. 2011).

The goal of the present study is to examine network mechanisms supporting face representation by tracking fMRI stimulus-dependent dynamic functional connectivity within and between brain networks associated with the processing of upright and inverted faces. We hypothesize that while upright faces would predominantly recruit the face network, inverted faces, while still processed by this network to some extent (Yovel and Kanwisher 2005), would also be processed by a complementary non-face-selective network and that the coupling between the face and non-face networks would increase while inverted faces are processed (Sadeh and Yovel 2010; Pitcher et al. 2011). We term this scenario the “between network hypothesis” in contrast to the “within network hypothesis” in which each visual category (i.e., upright or inverted faces) is being processed exclusively by either the face or the non-face networks.

Most current fMRI studies define network connections (i.e., edges) by estimating functional coupling between regions from long BOLD time series. An implicit assumption underlying this approach is that the strength of these connections does not change across time. However, this assumption has been challenged by studies showing that the functional coupling between regions changes spontaneously over the time scale of seconds in resting state fMRI (Chang and Glover 2010; Sakoglu et al. 2010; Allen et al. 2014; Hutchison et al. 2013; Leonardi et al. 2013; Calhoun et al. 2014; Keilholz 2014). So far, only a few studies have explored fMRI dynamic connectivity during different tasks (Büchel et al. 1999; Bassett et al. 2011; Geerligs et al. 2015; Gonzalez-Castillo et al. 2015). This approach, in which topology and dynamics of large-scale brain networks are examined in the context of carefully tailored cognitive tasks (Hutchison et al. 2014), is highly suited for the investigation of the dynamics of the face processing system (Rosenthal et al. 2016). Hence, in the present study, we adapt, for the first time, the general linear model (GLM) framework classically used for univariate fMRI analysis (Friston et al. 1994) to capture stimulus-dependent fMRI dynamic connectivity of the face network.

We set out to determine whether under the face inversion manipulation, the face and non-face networks would have complementary roles evident in their dynamic and static connectivity patterns as assessed by measuring the network decomposition into components or communities. Specifically, we investigated the network pattern (i.e., a community) of upright compared with inverted faces. We found that the network for upright faces encompassed face selective regions, while in the inverted condition, the pattern changed and expanded to include non-face selective regions. These analyses were validated with a classical static network approach and a univariate analysis. Thus, using these techniques we establish “a network-level signature” of the face inversion effect and demonstrate how a simple physical transformation of the face stimulus induces a dramatic functional reorganization across related brain networks.

We suggest that the dynamic GLM network analysis approach, developed here for the face network, provides a more general framework for modeling the dynamics of blocked, stimulus-dependent dynamic connectivity experimental designs and hence can be applied to a host of neuroimaging studies.

General outline

The main focus of Study I is to capture the stimulus-dependent dynamic functional connectivity of the face network during the face inversion task using a GLM framework. This framework was implemented on a fine-grained parcellation of category selective cortex. In Study II, to validate the results and framework of Study I, the dynamic and static experiments were conducted with a separate independent group of participants and several complementary analyses were employed. These included: (1) a standard univariate ROI based GLM analysis, (2) a bivariate ROI static functional connectivity analysis and (3) a static functional connectivity analysis implemented on the same nodes used for the dynamic connectivity analysis (see Materials and Methods).

Materials and Methods

Study I—Stimulus-dependent Dynamic Functional Connectivity

Visual Stimulation

Stimuli were presented using the E-prime 2.0 software (Psychology Software Tools, Inc., Pittsburgh, PA, USA) and projected onto an LCD screen located in the back of the scanner bore behind the subject’s head. Participants viewed the stimuli through a tilted mirror mounted above their eyes on the head coil. Stimuli consisted of 124 pairs of grayscale line-drawings of upright and inverted faces in frontal view, with neutral expression, containing no diagnostic or salient cues, scanned from a 3D laser and obtained from Bülthoff and Troje (Max Planck Institute, Tubingen, Germany; Bülthoff and Bülthoff 1996).

MRI Setup

Participants were scanned in a 3T Philips Ingenia scanner equipped with a standard head coil, located at the Soroka Medical Center, Beer Sheva, Israel. fMRI BOLD contrast was acquired using the gradient-echo echo-planar imaging sequence with parallel acquisition (SENSE: factor 2.8). Specific scanning parameters were as follows: whole brain coverage 35 slices (2.61 × 2.61 × 3 mm³), transverse orientation, 3 mm thickness, no gap, TR = 2000 ms, TE = 35 ms, flip angle=90°, FOV = 256 × 256 and matrix size 96 × 96. High-resolution anatomical volumes were acquired with a T1-weighted 3D pulse sequence (1 × 1 × 1 mm³, 170 slices).

General Procedure

Nineteen healthy, right-handed individuals (six females) with normal or corrected-to-normal vision participated in the
stimulus-dependent dynamic study (mean age ± standard deviation (SD) = 26.6 ± 1.34). The data from one additional participant were discarded due to excessive motion (see Preprocessing of functional data in the Supplementary Materials for specific criterion).

Participants completed an fMRI scanning session which included a 3D anatomical scan, two resting state runs, one localizer run and five experimental runs of upright/inverted faces designed for stimulus-dependent dynamic connectivity analysis. The order of the upright/inverted runs was counterbalanced across participants. The rest and localizer scans were acquired for a different study and are not analyzed in the current study. All experiments, for this study as well as for the static study (see below) were approved by the Helsinki committee of the Soroka Medical Center, Beer Sheva, Israel.

Upright/inverted Faces—Stimulus-dependent Dynamic Functional Connectivity Design
Participants completed five runs in which following an initial fixation of 12 s, upright and inverted faces were presented in separate experimental blocks within each run. Resting blocks (black screen) were also embedded in each run and all blocks were 20 s long. Experimental blocks contained 10 trials, composed of 2 face stimuli presented sequentially, each for 250 ms with an inter-stimulus interval (ISI) of 500 ms. Following the presentation of the second stimulus within a trial, a 1000 ms inter-trial interval (ITI) was introduced (total duration of the trial was 2000 ms; Fig. 1). The two faces within a trial were either the same or different and participants responded accordingly by pressing designated keys on a response box with both RT and accuracy being recorded. The order of the transition between the different block types was pseudo-randomized such that each of the nine possible transitions between the different block types (for example rest block to upright faces block or inverted faces block to upright faces block) was presented 15 times across the 5 experimental sessions. Overall, across all 5 runs there were 47, 46 and 47 rest, upright and inverted blocks, respectively.

Data Analysis
To allow the application of methods from the field of network science, it is necessary to conduct a number of preprocessing steps (see Supplementary Materials) as well as to define nodes and edges which will serve as the backbone for the network as outlined below.

Definition of Fine-Grained Nodes
The definition of fine-grained nodes was done using the static localizer session from Study II (see details in Supplementary Materials for Study II). Briefly, to conduct network analyses, it is imperative to define a sufficiently large number of nodes that are internally functionally homogeneous and of comparable size or volume. To maintain the functional specificity of each node, a seed based correlation mask was constructed based on the FFA and LOC seeds located in the right hemisphere which was later subdivided into small spatially constrained clusters that served as the network nodes. This procedure preserved the original functional relation of each voxel, thus generating “functional tagging” which indicates the functional preference of each node (face selective, non-face selective, and nodes which are not selective to either of these stimuli).

Definition of Stimulus-dependent Dynamic Connectivity
For each subject and each of the five sessions, the time course of each of the 415 nodes as defined in Study II (see below) was extracted after standardization (zero mean and unit variance) and high-pass filtered using a cutoff of 1/128 Hz. Correlation matrices were constructed for each time point with a sliding window of 20 s using pairwise Pearson correlation coefficients between each pair of nodes after applying a Fisher r-to-z-transformation (Hutchison et al. 2013; Calhoun et al. 2014; Gonzalez-Castillo et al. 2015).

Reformulation of the GLM for Stimulus-dependent Dynamic Connectivity Analysis
The GLM is often used in classic univariate fMRI analysis to characterize changes in the BOLD signal in relation to an experimental manipulation. In this framework, the dependent variable is the measured BOLD signal and the independent variables are the expected responses for each experimental condition. The experimental conditions which are usually discrete (in the current study—either upright or inverted faces), are convolved with a canonical hemodynamic response function model (Glover 1999).

To assess the relation between the behavioral paradigms and the dynamics of the connectivity pattern, in the present study, the GLM framework was reformulated such that instead of the raw BOLD signal for each node, the fitted dependent
variable is the dynamic weight of an edge as quantified using the sliding window approach. Below we show that the analysis of the dynamic GLM contrasts and the derived networks enable us to establish a network-level signature of the face inversion effect.

At the participant level (first level), the beta weights are calculated using the GLM for each condition, and these can be contrasted to provide the difference between the experimental conditions (see Supplementary Materials). The relevant experimental contrasts are upright > inverted (upright contrast) and inverted > upright (inverted contrast). At the group level, the beta contrast could be evaluated for statistical significance using a second level GLM (evaluating one contrast results in a one sample t-test). Both the first level beta contrasts and the second level t-test scores are highly informative. For example, the beta weights could be correlated with behavioral measures at the individual subject level while the second level t-tests could be used to account for group level effects.

Modularity and Community Detection
An important attribute of brain networks is their community structure (Sporns and Betzel 2016) derived here by maximizing a measure of modularity, which captures the density of edges inside communities compared with edges outside communities (defined by a module partition). Once modularity is optimized (Blondel et al. 2008; Mucha et al. 2010), one can characterize the role of individual nodes within and between the different modules. Comparison of modularity assignments between different experimental conditions requires aligning the module partitions across different networks, or slices. Mucha et al. (2010) developed a framework for studying the community structure of multislice networks. In this framework each network forms a separate slice and a link is formed between corresponding nodes across slices, practically forming a composite network out of the different network slices. Thus, each slice can be regarded as a sub-network in the composite network. This approach facilitates the investigation of community structures in networks that have common nodes but different topology and hence different module partitions (see Supplementary Materials for technical details).

Identifying Connector Hubs
Following the identification of the modularity community structure, we set out to identify "connector hubs" in each network using participation coefficient. These are defined as nodes that maintain a diverse set of connections across specialized modules (Sporns et al. 2007; see Supplementary Materials). We only considered high-degree nodes (nodes with a degree of at least 1 standard deviation above the mean), and nodes with a participation coefficient above 0.8 (kinless hubs; Guimerà and Nunes Amaral 2005; Sporns et al. 2007) (see Supplementary Materials for details).

Study II—Static Network Study
To validate the stimulus-dependent dynamic connectivity GLM framework described above and the findings obtained with this approach, we ran complementary statistical analyses using an independent group of participants. Specifically, we performed a univariate GLM analysis using an individual level ROI based approach, a bivariate connectivity analysis using the same set of ROIs and finally a network level analysis using a group level definition of the nodes used for the stimulus-dependent dynamic connectivity analysis. For a complete description of the methods used in Study II see Supplementary Materials.

Results
Study I—Stimulus-dependent Dynamic Connectivity Results
Group Level Dynamic GLM Results
Our goal was to test the stimulus-dependent dynamic connectivity during the face inversion effect manipulation. To this end, we utilized the GLM framework, and compared the different experimental conditions using fine grained nodes (for details on node definition see Supplementary Materials). First, we tested whether the face-related nodes were more dominant in the upright compared with the inverted condition. The upright and inverted contrasts network edges were defined as group level dynamic GLM connectivity t-test values comparing the dynamic connectivity in each of the corresponding contrasts. Multiple comparisons were corrected using an adapted variant (involving sign flipping) of the Network Based Statistic (NBS; Zalesky et al. 2010) at different values of the group level t-statistic (2.5–3.5; see Materials and Methods).

The statistically significant components of the upright contrast included nodes in the occipito-temporal ventral cortex and specifically face-related nodes encompassing the FFA, pSTS and amygdala (based on anatomical and functional considerations). Of the top 50 nodes found in this contrast (ranked in descending order by degree score), using a group level t-value threshold of 3.0, the percentage of the face-related nodes was 29% compared with 35% overall nodes (number of face nodes divided by overall number of nodes). The percentage of non-face nodes was 20% compared with 55% overall (number of non-face nodes divided by overall number of nodes). The differences between the observed percentage and the expected percentage was statistically significant with \( \chi^2(1, \ n = 94) = 9.6, \ P < 0.05 \) (Fig. 2). The same significant pattern emerged across the group level at 2.0 and 2.5 t-value thresholds thus indicating the robustness of the effect. In contrast, the statistically significant component of the inverted contrast contained nodes in occipito-parietal dorsal cortex including non-face selective nodes encompassing, but not limited to, the IPS, IFG and LOC regions. The percentage of non-face nodes at the 3.0 t-value threshold was 72% compared with 55% overall. The percentage of face nodes was 16%, compared with 35% overall. The differences between the observed percentage and the expected percentage was statistically significant with \( \chi^2(1, \ n = 90) = 8.43, \ P < 0.05 \). Again, the same significant pattern emerged across the 2.0 and 2.5 group level t-value thresholds. Thus, the face network was associated with the upright condition to a greater extent compared with the inverted condition and the opposite pattern was evident in the inverted compared with the upright condition.
determines the quality of a particular community structure in a network—see Supplementary Materials). The contribution of each module to its overall q score was relatively balanced across modules in the upright contrast face network (the most prominent modules contributed 28%, 27%, 21% and 17% each comprised of regions such as the STS, LOC, ventral temporal cortex and IPS). In contrast, when examining the inverted contrast, one module stood out accounting for 51% of the q score while the other modules accounted for only up to 15% of the q score. In this contrast, this module was composed of high-level...
visual nodes encompassing LOC, more dorsal nodes in the vicinity of the IPS as well as the ventral occipito-temporal nodes encompassing the FFA. Contrarily, in the upright contrast this same module was limited to nodes in the IPS. Thus, the pattern of modular segregation present in the upright contrast changed in the inverted contrast such that a single high-level visual cortex module is formed. This may reflect a more distributed pattern of integration of information in the inverted contrast involving high-level visual areas. In general, these stimulus-dependent changes in the modular organization of the face processing network support our “between network hypothesis” (Fig. 3).

Identifying Connector Hubs

The upright contrast revealed mainly nodes associated with the face network, including nodes encompassing the FFA, pSTS and amygdala. Contrarily, nodes obtained from the inverted contrast which had the highest participation coefficient mainly encompassed non-face-related regions including the IPS IFG and LOC (Fig. 4). Thus, as is evident, in the upright contrast the diversity of connections that are set across specialized modules is maintained by face network nodes, while in the inverted contrast the diversity is attributed to non-face nodes.

Behavioral Results of the Face Inversion Effect during fMRI Scanning

In accordance with previous studies (Yovel and Kanwisher 2004, 2005; Mazard et al. 2006; Pinsk et al. 2009; James et al. 2013), the behavioral face inversion effect was evident in the stimulus-dependent dynamic sessions. Accuracy was higher for upright compared with inverted condition (86% and 78% respectively; $F(1,18) = 57.8, P < 0.00001, \eta^2 = 0.76$) and RT was faster for the upright compared with the inverted condition (578 ms and 598 ms respectively; $F(1,18) = 35.4, P < 0.00001, \eta^2 = 0.66$).

Study II—Static Connectivity Results

Behavioral Results of the Face Inversion Effect During fMRI Scanning

Similar to the stimulus-dependent dynamic sessions and to previous studies (Yovel and Kanwisher 2004, 2005; Mazard et al. 2006; Pinsk et al. 2009; James et al. 2013), the face inversion effect was evident in both the univariate and connectivity sessions. As expected, accuracy was higher for the upright compared with the inverted condition in both the univariate session (88% and 79% respectively; $F(1,15) = 60.88, P < 0.00001, \eta^2 = 0.80$) and static connectivity session (0.88 and 0.75 respectively; $F(1,15) = 54.56, P < 0.00001, \eta^2 = 0.78$). Additionally, RT...
was faster for the upright compared with the inverted condition in both the univariate (550 ms and 574 ms respectively; $F(1,15) = 7.9$, $P < 0.05$, $\eta^2 = 0.34$) and connectivity session (615 ms and 674 ms respectively; $F(1,15) = 7.09$, $P < 0.05$, $\eta^2 = 0.32$).

**fMRI Univariate Analysis**

This analysis was conducted as a baseline for estimating the BOLD response for upright compared with inverted faces. A GLM analysis was first conducted separately for each region of interest in each participant using an individual level ROI based approach (see Supplementary Materials). Next, the mean of the normalized beta weights was calculated separately for all the face and non-face selective regions (see Materials and Methods). Repeated measures ANOVA with region selectivity (face/non-face selective), face orientation (upright/inverted) and hemisphere (left/right) as within subject independent variables revealed a significant two-way interaction between region selectivity and face orientation [$F(1,15) = 68.06$, $P < 0.000001$, $\eta^2 = 0.81$]. Probing of this interaction revealed both greater activation for upright compared with inverted faces [$F(1,15) = 7.99$, $P < 0.05$] in face selective regions and reduced activation for upright compared with inverted faces [$F(1,15) = 73.6$, $P < 0.0000001$] in non-face selective regions (Fig. 5a; see individual region comparisons in supplementary Figure 1 and Table 1). The three-way interaction was not significant [$F(1,15) = 3.75$, $ns$, $\eta^2 = 0.2$].

**Functional Connectivity Analysis**

To further explore the “between network hypothesis”, the functional connectivity between the predefined ROIs which were used in the univariate static analysis of the face and non-face selective networks was examined. Functional connectivity was measured using the correlation coefficients of the activation profile of each ROI with the activation profiles of all other ROIs. To measure the difference between the upright and inverted conditions in the face and non-face networks, the correlations within each network (face and non-face selective regions) were averaged and the mean correlation was tested using a repeated measures ANOVA with orientation (upright/inverted) and network (faces/non-faces) as within subject independent variables. A two-way interaction between orientation and network was not significant, but showed a strong trend [$F(1,15) = 60.78$, $P = 0.057$, $\eta^2 = 0.21$] (Fig. 5b).

The relatively coarse definition of the individual level ROIs does not allow conducting a more refined investigation of the possible differences in the network topology between the two experimental conditions. Thus an optimized pipeline was constructed to examine the network topology of the upright compared with the inverted faces conditions using high-resolution network which was also used in Study I (see Materials and Methods for details of the network construction).

**Network-wise Correspondence Between Node’s Selectivity and the Face Inversion Effect**

Similarly to the stimulus-dependent dynamic network analysis, we tested whether the non-face-related nodes are more dominant in the inverted compared with the upright condition. The inverted contrast network was defined using an edge-wise t-test at varying thresholds. After thresholding, the node degree, reflecting the number of edges connecting to a node, was calculated (Rubinov and Sporns 2010; see Fig. 6a, b for an example of the networks in two representative thresholds). Of the top 50 nodes (ranked in descending order by degree score), the percentage of non-face nodes at 3.5 t-value threshold was 70%, compared with 55% overall (number of non-face nodes divided by overall number of nodes). The percentage of face nodes...
The objective of the FDR procedure is to reduce the number of false positive edges in the network. In each iteration, the model was fit using a portion of the data (14 subjects) and the remaining two subjects. Performing this procedure 10 000 times yielded a mean t-test distribution of 10 000 permuted samples. Edges that were significantly higher in the original t-test compared with the bootstrap distribution (while controlling for FDR) were further analyzed.

The upright contrast yielded three significant edges, all of which connect to nodes in the vicinity of the FFA. The inverted contrast yielded six significant edges, each connected to one non-face selective node. In contrast, the other connected node in this comparison was not selective to any category (faces/non-faces) (Fig. 6c,d and supplementary Table 2). Moreover, the non-face selective nodes were not constrained to one particular ROI (e.g., the LOC or the IPS; Matsuyoshi et al. 2015), but rather they were spread across multiple non-face selective regions such as the LOC, TOS and FFA.

**Correspondence Between the Behavioral Inversion Effect and Individual Network Edges**

Finally, we tested whether differences in individual edges between the upright and inverted face conditions play a role in the behavioral face inversion effect by examining the correlation between these two measures. Specifically, a multiple regression was used to test whether the difference in the strength of the individual edges between upright and inverted face conditions can predict the behavioral scores of the face inversion effect. As the number of predictors is relatively large (nine individual edges) compared with the number of samples (16 subjects), the model might be overfitting the data and would hence have a poor predictive performance. To avoid this limitation, we calculated the r-squared value using cross validation in an iterative fashion. In each iteration, the model was fitted using a portion of the data (14 subjects) and the r-squared value was calculated using the remaining two subjects. Performing this procedure 10 000 times yielded a mean r-squared value of 0.57 (95% confidence interval between 0.38 and 0.7). These results indicate that the strengths of the significantly different individual edges between the upright and inverted conditions are associated with the behavioral face inversion effect.

**Discussion**

By utilizing a series of analysis procedures ranging from stimulus-dependent dynamic connectivity of large-scale networks to univariate single region level modeling, we were able to gain insight into the network organization of brain regions which are involved in the representation of upright vs. inverted faces and to obtain a network-level signature of the face inversion effect. Our research reveals, for the first time, that the changes associated with viewing upright compared with inverted faces are widespread and involve reconfigurations of functional connections that are distributed across multiple face and non-face-related regions. Previous studies examining face representation and the face inversion effect have generally utilized a localized approach, mostly focusing on FFA and OFA. Moreover, despite the simplicity of this task, results vary substantially across studies with some showing neural face inversion effect (i.e., a stronger response for upright compared with inverted faces) in the FFA (Yovel and Kanwisher 2004, 2005; Mazard et al. 2006; Pinsk et al. 2009; James et al. 2013), while others do not (Aguirre et al. 1999; Haxby et al. 1999; Epstein et al. 2006). Moreover, whether or not the neural and behavioral face inversion effect are correlated in this region or in other regions is still controversial (Yovel and Kanwisher 2005; James et al. 2013). Another major controversy related to the face...

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**Figure 5.** Analysis of the univariate signal and functional connectivity for upright and inverted faces. (a) Univariate analysis (standard GLM) revealed a significant two-way interaction between network selectivity (face/non-face) and stimulus orientation (upright/inverted). (b) Functional connectivity analysis measuring correlation coefficients between ROIs revealed a significant two-way interaction between network type (face/non-face) and stimulus orientation. Using the 3.5 t-value threshold was 26%, compared with 35% overall (number of face nodes divided by overall number of nodes). The differences between the observed proportions and the expected proportions was statistically significant with $\chi^2(1, n = 84) = 7.75, P < 0.05$ (the same significant pattern emerged across the 1.5 and 2.5 group level t-value thresholds). Thus, the non-face network was more strongly associated with the inverted condition compared with the upright condition. Comparing the opposite upright contrast revealed a difference only at the 1.5 t-value threshold where the percentage of nonface nodes was 38% compared with 55% overall and the percentage of the face nodes was 42% compared with 35% overall $\chi^2(1, n = 76) = 3.9, P < 0.05$.

**Individual Edge Differences Between Upright and Inverted Faces**

Next, to examine the role of individual edges in the network, we assessed the differences between the connectivity patterns of individual edges of the networks while correcting for multiple comparisons. A bootstrapped FDR analysis with 10 000 iterations was conducted utilizing the network-based statistics toolbox. (Note that here we use the FDR procedure which is implemented in the NBS toolbox rather than the NBS correction; Zalesky et al. 2010.) The objective of the FDR procedure is to reduce the number of false positive edges in the network. Briefly, for each edge, the obtained pairwise t-test difference between upright and inverted condition was compared with the t-test distribution of 10 000 permuted samples. Edges that were significantly higher in the original t-test compared with the bootstrap distribution (while controlling for FDR) were further analyzed.

The upright contrast yielded three significant edges, all of which connect to nodes in the vicinity of the FFA. The inverted contrast yielded six significant edges, each connected to one non-face selective node. In contrast, the other connected node in this comparison was not selective to any category (faces/non-faces) (Fig. 6c,d and supplementary Table 2). Moreover, the non-face selective nodes were not constrained to one particular ROI (e.g., the LOC or the IPS; Matsuyoshi et al. 2015), but rather they were spread across multiple non-face selective regions such as the LOC, TOS and FFA.

Finally, we tested whether differences in individual edges between the upright and inverted face conditions play a role in the behavioral face inversion effect by examining the correlation between these two measures. Specifically, a multiple regression was used to test whether the difference in the strength of the individual edges between upright and inverted face conditions can predict the behavioral scores of the face inversion effect. As the number of predictors is relatively large (nine individual edges) compared with the number of samples (16 subjects), the model might be overfitting the data and would hence have a poor predictive performance. To avoid this limitation, we calculated the r-squared value using cross validation in an iterative fashion. In each iteration, the model was fitted using a portion of the data (14 subjects) and the r-squared value was calculated using the remaining two subjects. Performing this procedure 10 000 times yielded a mean r-squared value of 0.57 (95% confidence interval between 0.38 and 0.7). These results indicate that the strengths of the significantly different individual edges between the upright and inverted conditions are associated with the behavioral face inversion effect.

**Discussion**

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inversion effect relates to whether inverted faces are processed exclusively by mechanisms that are face-selective or whether other behavioral processes (Barton et al. 2003) and brain regions devoted to non-face objects are also recruited by these stimuli, as has been implicated in fMRI (Haxby et al. 1999; Yovel and Kanwisher 2005) transcranial magnetic stimulation (TMS) (Pitcher et al. 2011) and Electroencephalography (James et al.) studies. Contrarily, the current study suggests that face inversion induces a dynamic reconfiguration of information flow between complementary networks, one involved in the representation of upright faces and the other with the representation of inverted faces. That is, the face inversion effect is not evident only at the level of a single region, but rather, the activity of the entire face network, in tandem with non-face selective regions, mediates this effect (Matsuyoshi et al. 2015).

As predicted, consistently across all experiments and analyses, face-related regions were implicated in upright face processing whereas processing of inverted faces, while activating the face network to some extent, additionally involved a complementary network of non-face regions. Using the stimulus-dependent dynamic connectivity analysis, when examining the network structure obtained for upright compared with inverted faces, we revealed multiple network modules which contributed relatively equally to the modularity score. One of these modules encompassed the IPS. Contrarily, in the inverted compared with upright faces contrast, this same module expanded to yield a unique compounded network module which encompassed the IPS all through the LOC and the ventral occipito-temporal cortex. Moreover, the IPS was also found to be a connector hub in the inverted contrast network, such that it mediated the exchange of information between specialized modules. Evidence from connectivity studies using TMS and fMRI indicates that the IPS exerts top-down modulation on the activity of visual areas during spatial orienting of attention (Bressler et al. 2008; Ruff et al. 2008; Vossel et al. 2014). Thus, we suggest that the excessive involvement of the IPS in the inverted compared with the upright condition may reflect mechanisms of spatial attention which induce a network-wide topological functional reorganization of the visual system.

In accordance with previous studies (Yovel and Kanwisher 2005; Pitcher et al. 2011; Matsuyoshi et al. 2015) the LOC seemed to serve as a hub for the inverted contrast both in the analysis of influential nodes obtained from stimulus-dependent dynamic connectivity as well as the univariate and static connectivity analyses. As LOC is associated primarily with object perception, it appears that inverted faces may exert, at least to some extent, a processing mechanism which is commonly associated with the processing of non-face stimuli. Moreover, similarly to previous studies, the FFA (Yovel and Kanwisher 2004, 2005; Mazard et al. 2006; Pinsk et al. 2009; James et al. 2013) was associated with the upright contrast together with other face-related regions such as the pSTS (Yovel and Kanwisher 2005) and Amygdala. Nevertheless, in contrast with previous studies, we have shown that the inversion effect is not limited to these isolated regions. Rather, these regions seem to serve as hubs with potentially important roles in the large-scale topology of stimulus-dependent networks of interdependent regions.

Our findings support the “between network hypothesis” according to which in the inverted compared with the upright condition, faces are processed by face, as well as non-face-selective networks and the synchrony between these networks increases while inverted faces are presented (Sadeh and Yovel 2010; Pitcher et al. 2011).
The stimulus-dependent dynamic connectivity analysis captures the changes in connectivity between regions over time during a task. If second-order attentional mechanisms which modulate the activity of visual areas are involved in the processing of inverted faces, it is plausible that the pattern of connectivity between face and non-face networks would be non-stationary and hence be better captured by the stimulus-dependent dynamic connectivity analysis compared with more conventional tools.

Although stimulus-dependent dynamic connectivity captures fundamentally different information compared with univariate region-based activation and static connectivity, to complement and validate the stimulus-dependent dynamic connectivity results and framework, we also performed a number of more conventional static analysis procedures. Overall, the within network differences between upright and inverted faces were replicated in the static experiments and analyses while the stimulus-dependent dynamic connectivity analysis framework was more suitable to capture the between network connectivity hypothesis in addition to the within network patterns as suggested above. Specifically, first we used a univariate analysis of the face and non-face ROIs which were defined using the localizer sessions. We found that face-related regions such as the right FFA were more strongly activated by upright compared with inverted faces. The opposite, inverted contrast revealed activation in non-face selective regions such as the right LOC and left LOC. Several previous studies did not find a consistent activation pattern using upright and inverted faces (Aguirre et al. 1999; Haxby et al. 1999; Yovel and Kanwisher 2004, 2005; Epstein et al. 2006; Mazard et al. 2006; Pinsk et al. 2009; James et al. 2013) and the reliable effects found in the present study may be attributed to the statistical power obtained by the employment of relatively long blocked design experiments. Next, using bivariate connectivity analysis, a strong trend of interaction between orientation (upright/inverted) and network (faces/non-faces) was revealed. This finding implicates that not only the BOLD signal within specific regions is associated with face inversion, but rather the coherence of time series and statistical dependencies between these regions are also of significance. Next, to capture a more refined characterization of this connectivity pattern, we identified a high-resolution parcellation of the high order visual cortex using smaller nodes which were labeled by their selectivity (face selective, non-face selective or non-selective; note that the same fine grained node definition was used in the stimulus-dependent dynamic connectivity study). Utilizing this scheme, we identified large-scale patterns revealing that face nodes had higher degree in the upright contrast and the opposite pattern emerged for inverted contrast. Finally, we showed that the network structure can also account for the behavioral variance of the face inversion effect, thus relating the neural network level with the behavioral outcome.

These converging findings, obtained across several analytical approaches, support our adaptation of a GLM framework for stimulus-dependent dynamic connectivity analysis. However, while standard methods of fMRI connectivity assume that functional connections are static over a task or a resting session, dynamical formulations of connectivity (Hutchison et al. 2013) and specifically the dynamic GLM framework which was utilized in the current study do not make this assumption. Moreover, the stimulus-dependent dynamic connectivity analysis had more statistical power to reveal the different components of the network as evident by the modularity and NBS results, whereas the static-large-scale connectivity pattern was not statistically significant following the application of a more stringent criterion (i.e., correction for multiple comparisons). Finally, one crucial result which unfolded only in the stimulus-dependent dynamic connectivity analysis was the between-network patterns of connectivity in the inverted contrast. This result is qualitatively different compared with the results which were apparent in the static analysis. We propose that the GLM dynamic connectivity procedure introduced here can be applied to any fMRI experiment with sufficiently long blocks in which changes in dynamic connectivity over time occur in correspondence with the experimental conditions. Our methodology could potentially fit research designs in other behavioral and cognitive domains in which the temporal evolution of the data is critical. These include for example the different phases of working memory such as encoding or decoding, the temporally evolving neural correlates of language processing, and aspects of social judgment that are important for an accurate interpretation of dynamic scenes.

In the current study we show that the inversion of face stimuli not only induces a change in connectivity patterns within the face and non-face-related networks, but also between these networks. Hence, a simple change in the physical stimulus causes a profound functional reorganization of an extended network of cortical regions. Thus, while upright faces serve as optimal stimuli for the face network, the presentation of inverted faces, which are non-optimal for this network, triggers the recruitment of a non-face network, possibly reflecting increased demands on integrative processing which is required for representing these non-optimal stimuli.

Authors’ Contributions
G.R., O.S. and G.A. designed the research; G.R. and G.A. conducted the research; G.R., O.S. and G.A. developed the data analysis approach, analyzed the data and wrote the paper.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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