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Awareness of Observation Affects Resting State Brain Activity

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by

Jing Li

Committee in charge:

Professor Michael Miller, Chair

Professor Gregory Ashby

Professor Barry Giesbrecht

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The thesis of Jing Li is approved.

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Gregory Ashby

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Barry Giesbrecht

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Michael Miller, Committee Chair

June 2014

ABSTRACT

Awareness of Observation Affects Resting State Brain Activity

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Functional imaging studies have revealed the default mode network (DMN) activates when people are at rest. However, generally only minimal instructions were provided among those studies. Our goal in this study was to demonstrate how resting state activity varies with the knowledge of being watched. In this study, we used two distinct manipulations to address this question: first, we described two separate scans as being either anatomical or functional (with little additional detail), when in fact both were functional; and second, in a putatively separate experiment, we informed participants we were able to observe their thoughts, and after a more thorough description, carried out three more functional scans, one of which was again described as anatomical. Our results demonstrate there are systematic differences across several networks as a function of instructional differences. Most strikingly, there was a significant increase in the orbitofrontal cortex (OFC) when comparing the first functional scan to the first sham anatomical scan, and a substantial increase in functional connectivity within the DMN when comparing the second sham anatomical scan to the second and third functional scans. These results suggest the mere awareness that one is being watched causes significant changes in the patterns of activity across functional networks, including the

DMN. They also suggest the importance of using precise instructions in resting-state studies, because even slight variations in instruction can have substantial impacts on the brain's activity at rest.

Key words: DMN, resting state, fMRI, functional connectivity, awareness

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Resting state brain activity has been studied vigorously in the last 15 years or so. While there are a lot of studies regarding the neural aspects of resting state functional connectivity as well as the DMN activations (Raichle, 2009; Greicius et al, 2003; Fransson, 2005), the psychological construct of the "resting state" is not well defined and not measured or manipulated in any way. Among those studies with well-described instructions in their methods, generally only minimal instructions were provided, for instance, to lay awake in the scanner with eyes open or closed and to think as little as possible (Damoiseaux et al, 2006; Fransson, 2005). Our concern is the resting state functional connectivity and brain activations may change dramatically when the individual's psychological states change.

People's mental activities vary from person to person depending on what the instructions are and how they interpret them. How would the knowledge of observation play a role in the resting-state scans? Are there differences in brain activity when people think their brain activity is being observed versus not? A recent study found participants did not judge neuroimaging techniques to be of a privacy violation unless brain monitoring was described as providing access to self-relevant information (Baker et al., 2013). This study suggests that how brain-imaging techniques being described plays an important role in people's interpretations about how they actually work. Our goal in this study was to use a simple instructional manipulation to elucidate how resting state activity depends on the mere knowledge of being watched.

In this study, we used two distinct manipulations to address the question of resting state brain networks: first, we described two separate scans as being either anatomical or functional (with little additional detail), when in fact both were functional; and second, in

a putatively separate experiment, we informed participants that we had technology that would allow us to observe the contents of their thoughts, and after a more thorough description of the anatomical/functional distinction, carried out three more functional scans, one of which was again described to the participant as anatomical. Our assumption was when a scan was described as an anatomical scan, people would feel less concerned about their minds being watched given the description of what an anatomical scan was, while when a scan was described as a functional scan, people would feel more nervous or concerned about their minds being watched given the description of what a functional scan was. We predicted that the distinction between anatomical and functional scans would reveal activity caused by knowledge of observation, and that the distinction between the first and second sets of scans would reveal the degree to which this difference could be modulated by task instruction. After controlling for test-retest stability and other possible confounding factors, we found that a subject's knowledge of being observed had a significant impact on several, but not all, brain activations and functional connectivity. We argue that the psychological construct of the "resting state" needs to be better understood in order to make inferences about neural networks of resting states.

Method

Participants

30 healthy undergraduate students from the University of California, Santa Barbara (age range 18-22 years) who never had previous fMRI experience participated in this study, and were paid for their participation. We excluded data from one subject who

reported not feeling well during scanning. Of the 29 subjects whose data we collected, 10 were males and 19 were females. All the participants gave their informed consent. Our experiment was approved by the UCSB Institutional Review Board.

Procedure

As described above, the fundamental manipulation in this experiment was to describe scans as functional or anatomical with various details while they were actually all functional scans. We used a two-part design in order to demonstrate the effects of knowledge of observation to the fullest extent. As participants arrived at the brain imaging center, they were told they were going to participate in two separate experiments: the first experimenter was said to be collecting pilot data on a resting state project, and the second experimenter was said to work on another resting state project and would give the subject more information about the study when they were in the scanner. The instructions that the participants received in the scanner were as follows:

Study 1: After the participant was loaded up in the scanner, and received a localizer, there were two functional scans, one was described as functional and the other as anatomical, with order counterbalanced across subjects. The descriptions given to the participants before each of these two scans were minimal: “Now you are going to get a functional scan of your brain, which observes brain activity” or “Now you are going to get an anatomical scan of your brain, which only tells us your brain anatomy”. Besides these scan-specific instructions, participants were instructed to rest with their eyes open without moving. The purpose of study 1 was to see the observation effect under minimal instructions.

Study 2: There were three functional scans in study 2. The first scan was described functional with descriptions given to the participants as: “Hello, I am the researcher for the second experiment. I am working on testing some novel technology that allows reading people’s minds in real time with a functional scan. Also, just letting you know, based on previous experience in such studies, participants frequently had sexual or otherwise embarrassing thoughts. But you don’t need to be concerned because I am the only person that can see the monitor. Now you are going to get a functional scan, which will show your mental thoughts in real time.” The last two scans comprised one more scan described as functional and one described as anatomical with the order counterbalanced across subjects. The instructions the participants received for the second functional were: “Now you are going to get another functional scan of your brain. To recap, a functional scan measures your brain activity and the technology I am testing on tells me your mental activity in real time.” The instructions given to the participants described as an anatomical scan were: “Now you are going to get an anatomical scan of your brain. An anatomical scan will only show me your brain structure but won’t tell brain activity in real time”. Similar to Study 1, besides these scan-specific instructions, participants were instructed to rest with their eyes open without moving. The purpose of study 2 was to see the observation effect under conditions meant to maximize difference. Study 2 was described to the subjects as separate from study 1 to prevent subjects’ possible paranoid retrospective thinking about the part of study 1.

fMRI data acquisition

Scanning took place on a 3T Siemens Trio MRI scanner (12 channel phased-array head coil) equipped with high-performance gradients. The resting-state functional images

were acquired with the following parameters: TR = 2000 ms, TE = 30 ms, flip angle (FA) = 90◦, in-plane resolution = 64 × 64, FOV = 192 mm, Voxel size = 3.0×3.0×3.0 mm, 37 axial slices, thickness/gap = 3.0/.5 mm and 180 volumes (6 min).

The parameters for the T1-weighted structural image were: TR/TE = 1700/2.97 ms, FA = 9°, in- plane resolution = 256, FOV = 258 mm, Voxel size = 1.0×1.0×1.0 mm, and thickness = 1.0 mm.

fMRI data analysis

Our primary interest was in three different comparisons across scans, all designed to address variants of the question of how knowledge of observation influences the brain in the resting state. After preprocessing the data, we used two main techniques to assess neural activity—namely fractional amplitude of low-frequency fluctuations (fALFF) and parcel-based coherence—and then computed how these measures changed across scans. We additionally controlled for a number of confound variables, including counterbalance order and gender. Each of these steps is described in more detail below.

1. Preprocessing

The fMRI data were analyzed using the tools from the functional Magnetic Resonance Imaging of the Brain Centre (fMRIB) Software Library (FSL: http://www.fmrib.ox.ac.uk/fsl/). Image preprocessing involved the following steps. Using FEAT (FMRIB's Expert Analysis Tool) in FSL, the cerebrospinal fluid (CSF), white matter (WM) and grey matter were segremented by FAST (FMRIB's Automated Segmentation Tool) by thresholding the probabilistic maps at 90%, the images were motion corrected by MCFLIRT (Motion Correction using FMRIB's Linear Image Registration Tool), and non-brain structures were removed with BET (Brain Extraction

Tool). FSLmaths was used to regress the CSF and WM. Parameters, including translation and rotation of the x, y, z axes, and the CSF and WM, were extracted via MATLAB (matrix laboratory).

General Linear Model (GLM): there were eight explanatory variables (EVs): translation and rotation of the X, Y, Z axes, and the CSF and the WM. Temporal derivatives were added to the model. The GLM was then estimated using OLS<WLS and using FILM prewhitening. The functional images were registered to the structural scan using 6 degrees of freedom (DOF), and the structural scan to MNI-152 standard space, using 12 degrees of freedom (DOF), with warp resolution at 10 mm.

2. Whole-brain fALFF analysis

In order to investigate resting state activity, we used the fALFF method, which uses a normalized measure of low-frequency power to index neural activity (Zou et al. 2008). A 5 mm kernel was used for spatial smoothing. For each voxel, the time domain was transferred to the frequency domain using Fourier transform, and the power spectrum was obtained (Yu-Feng et al, 2007). The square root was calculated at each frequency of the power spectrum and the averaged square root was obtained across 0.011–0.075 Hz at each voxel. This averaged square root was taken as the ALFF (Yu-Feng et al, 2007). To obtain fALFF, the sum of amplitude across 0.011–0.075 Hz was divided by that across the entire frequency range (0.00277–0.25 Hz) (Zou et al, 2008). This measure was computed separately for each EPI.

3. Parcel-based coherence & correlation analysis

In addition to the influence of our manipulation on raw activity, we were interested in whether patterns of functional connectivity would be affected by our

manipulation. First, a 7 mm kernel was used for spatial smoothing followed by registering each preprocessed functional run to standard space using FSL's FNIRT (10mm warp, after initial FLIRT to anatomical using trilinear interpolation).

200 parcels were utilized across the whole brain (Craddock et al, 2012). Next, within each parcel, and separately for each subject and EPI, we computed average timeseries by taking the unweighted mean of all voxels within each parcel. Then we computed the coherence between every pair of parcels, yielding 19900 (200 choose 2) connectivity values per scan per subject. After computing contrast scores within each parcel for each of our contrasts, we sought to reduce the complexity by mapping each parcel into one of the intrinsic connectivity networks (ICNs) (Laird et al, 2011; Smith et al, 2009) (table 1). To this end, we used a winner-take-all clustering method to assign each parcel to the ICN with which that parcel had the most overlap (after excluding the two ICNs as being primarily noise).

We then used a model with subjects as random effects to find the mean difference score per ICN-pair. In addition to including ICN-pair indicators, this model included a measure of the distance between the member parcels of each parcel pair, which was meant to ensure that comparisons of changes in intra- and inter-ICN connectivity were unaffected by the fact that the average distance between parcels was lower when those parcels belonged to the same ICN as compared to when they came from different ICNs ($z = 56.28$, p ¡0.001 for difference in distribution of distances by Mann–Whitney U). This reduced our symmetric 200×200 matrix of difference scores to a symmetric 18×18 matrix.

The correlation analysis is the same as coherence except that we bandpass filtered the data between .01 and .08 Hz and computed a Pearson correlation, which we took the absolute value of prior to computing contrasts.

4. Measuring changes across scans

Our main research question involves how knowledge of observation influences the brain. To test this effect, we focused on three contrasts. The first contrast included scans only from the first "study," and was simply a direct comparison between the "anatomical" and "functional" scans. The second contrast was analogous, but for the second "study," and was a comparison between the average of the two "functional" scans and the "anatomical" scan. Finally, the third contrast was a comparison between the average of the two "functional" scans from the second half and the "functional" scan from the first half. Each of these contrasts was designed to address the question of what happens under knowledge of observation, and differ in terms of the theoretical magnitude of the difference between the "observed" and "unobserved" scans. Roughly speaking, the first contrast was the most subtle, and the second contrast the least subtle, with the third contrast falling somewhere between the two.

The statistic we used differed slightly between our two measures: whole-brain fALFF and parcel-based coherence. For fALFF, we used paired-sample t-tests for each contrast, substituting in the mean of the two second-half "functional" scans for those contrasts that included those scans. For parcel-based coherence and correlation, we used the standard test of regressor significance from the model we fit to calculate ICN-pair changes.

5. Confound correction

To eliminate any possible influence of a variety of confound variables, we removed from each contrast any voxels or parcel-pairs that showed a confound effect. We had a total of five binary confound variables, plus a whole-brain confound factor. The former included participant gender, counterbalance order for the first half of the experiment, counterbalance order for the second half, an interaction between these two, and an indicator of whether the participant's self-report (collected immediately after scanning) indicated understanding of the distinction between "anatomical" and "functional" scans. The latter made use of a fourth contrast, carried out in the same way as described above, comparing the two "functional" scans in the second half of the experiment—in other words, any voxel whose activity differed or parcel-pair whose connectivity differed between two putatively identical scans was excluded.

For each scan, we fit an ANOVA including indicators for each of the binary confound variables in each voxel (parcel-pair), and calculated the significance of each indicator, to identify voxels whose activity (connectivity) differs as a function of each confound variable. This gives us three or five maps (i.e., because we did not control for second counterbalance order or its interaction for the first two scans, which were necessarily unaffected by this variable, as described further below) for each of the five original scans, which reflect the magnitude of the difference between subjects grouped according to each confound variable. We established a threshold by choosing a value such that we got no false positives for an atemporal confound (namely, second counterbalance order and its interaction for the first two scans).

In addition to these binary confound variables, we considered the stability of resting state activity each voxel. For every voxel, we compared the difference observed in

each of our contrasts against the difference observed between the two "functional" scans in the second half of the experiment. Specifically, in any voxel whose activity differed significantly between those two "functional" scans, we masked out the voxel if a paired t-test comparing the magnitude of the difference scores for our confound of interest were not significantly larger than the difference scores for this confound contrast.

Finally, after getting the final mask for each confound variable, we combined across masks for each scan, and then applied to each contrast the masks associated with each of the constituent scans in that contrast, plus the whole-brain stability mask just described. All of our final results were computed on the subset of voxels or cells that survived this confound correction procedure.

6. Multiple comparison correction

After excluding voxels or cells according to the confound correction procedure described above, we assessed significance in our contrasts using standard multiple comparison corrections. In particular, for the whole-brain fALFF results, we used cluster-based thresholding as implemented by FSL, with a voxel threshold of 2.33 and a cluster threshold of 0.05. For the parcel-based coherence matrices, we used FDR, implementing the standard Benjamini-Hochberg algorithm with $q = 0.05$.

Results

Behavioral results

A total of 22 participants reported having understood the distinction between the terms "functional" and "anatomical," while 7 participants either admitted to not understanding, or else had the terms reversed. Two participants reported having become suspicious at some point during scan.

Whole-brain fALFF results

Figures 1–4 show the results of our three contrasts on the fALFF measure of resting state activity.

As shown in figure 1a and 1b, the frontal pole had significantly higher activation in F1 than in A1. The frontal pole is highly involved with executive functions (Duncan & Owen, 2000); it could be that people used more executive functions or cognitive control during F1 when they felt their minds were watched than during A1 when they didn't think their minds were being watched.

As shown in figure 2a and 2b, the medial prefrontal cortex (mPFC) was less active in F2/F3 than in A2. While mPFC is an important component of the DMN (Raichle et al., 2001), it could be that the DMN activity was more robust when people didn't think they were being observed during the second anatomical scan than during the second and the third functional scans when they felt they were being watched more.

As shown in figure 3a and 3b, higher activity was observed during F2/F3 than during F1 in the primary somatosensory cortex (S1) and the primary motor cortex (M1), as well as in the right primary visual cortex (V1).

As shown in figure 4, higher activity was observed during F3 than during F2 in a few brain areas including S1. This result indicates that with the identical instructions, brain activations were different. This could be due to passage of time and the possibility of A2 being inserted between F2 and F3.

Parcel-based coherence & correlation results

Figure 5–8 show the results of the contrasts on resting state connectivity; the connectivity coherence results are shown in the upper-left triangle of the figures and the connectivity correlation results are shown in the bottom-right triangles.

As shown in figure 5, the functional connectivity among the 18 ICNs did not change much from A1 to F1. This could be because the instructions about the first functional scan and the first anatomical scan were minimal.

As shown in figure 6, some of the intra- and inter- connectivity among the 18 ICNs changed from F2 to F3. Though the instructions that participants received in the beginning of F2 and F3 were identical, the brain networks were not functionally connected in the same way. This could be due to the passage of time, including people feeling fatigued after a few scans, and also the fact that A2 took place between F2 and F3 in half of the subjects.

As shown in figure 7, some of the intra- and inter- connectivity among the 18 ICNs changed from F2/F3 to A2. Most strikingly, the inter-connectivity between ICN 7 and ICN 13, which are the visuospatial reasoning network and the DMN, and the intra-connectivity within the DMN, increased from F2/F3 to A2. This indicates when people felt being observed less, the functional connectivity between the visuospatial reasoning network and the DMN, as well as the functional connectivity within the DMN, were more robust.

As shown in figure 8, some of the intra- and inter- connectivity among the 18 ICNs changed from F1 to F2/F3. Most interestingly, the inter-connectivity between ICN 7 and ICN 16, which are the visuospatial reasoning network and the audition network, increased from F1 to F2/F3. This implies that those two networks were more functionally

connected when the subjects knew more about functional scans and felt like they were being watched more.

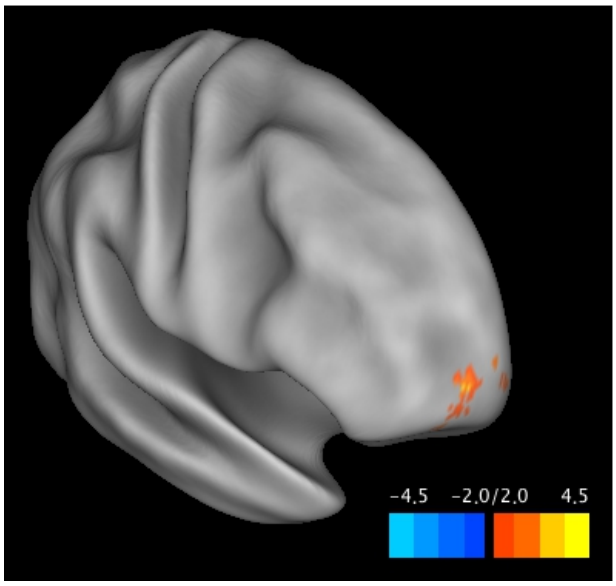

Figure 1a: Contrast of first "functional" and "anatomical" scans; areas in warm colors denote "functional" > "anatomical."

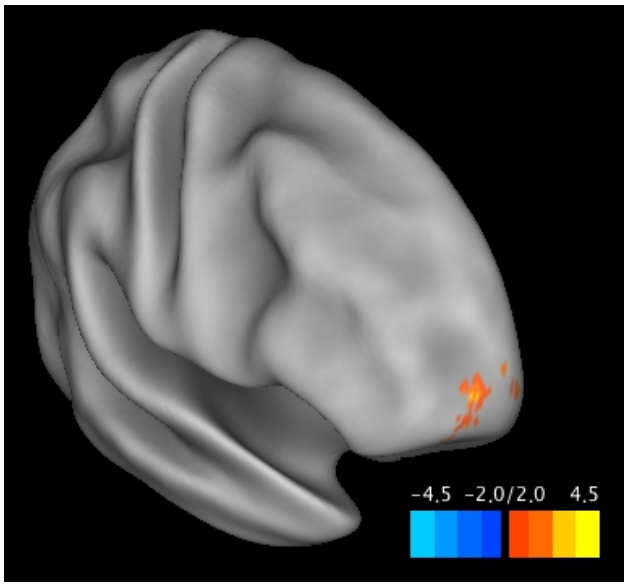

Figure 1b: The same contrast result as 1a but without masking out any confounds.

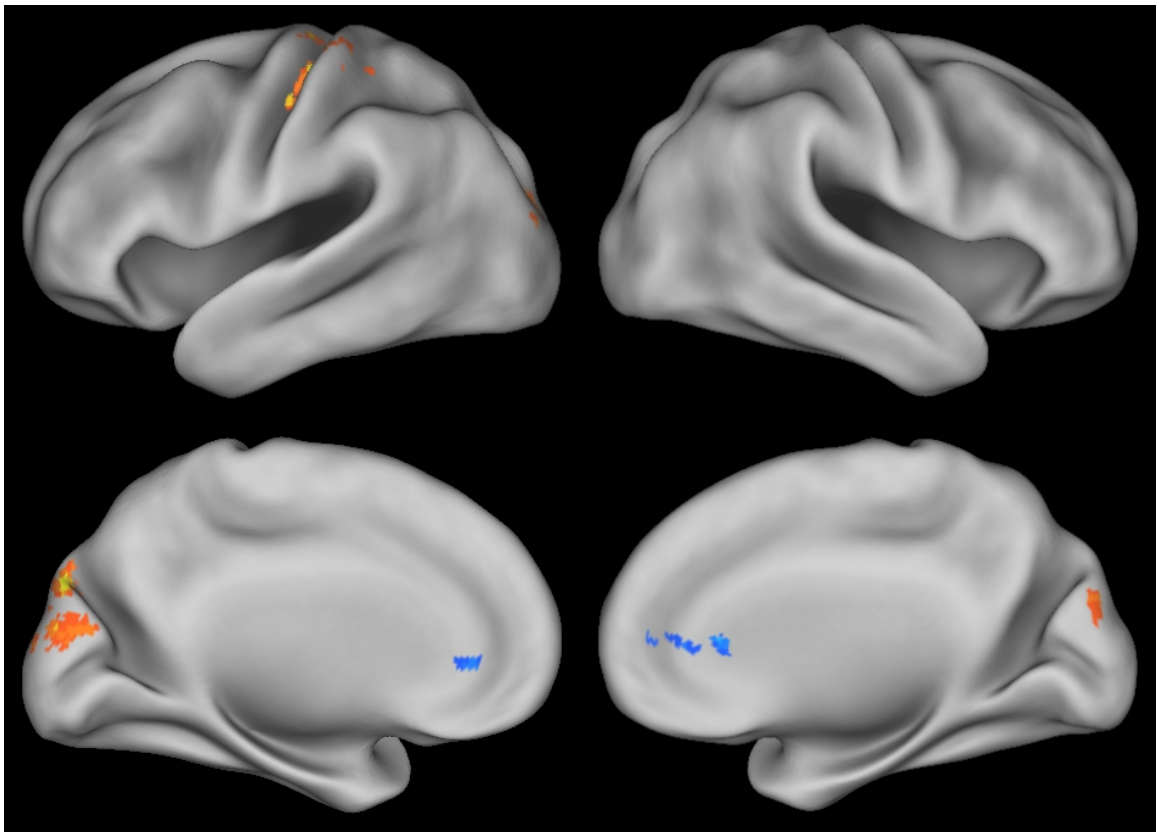

Figure 2a: Contrast of the average of the second two “functional” scans and the second “anatomical” scan; areas in warm colors denote “anatomical” > “functional.”

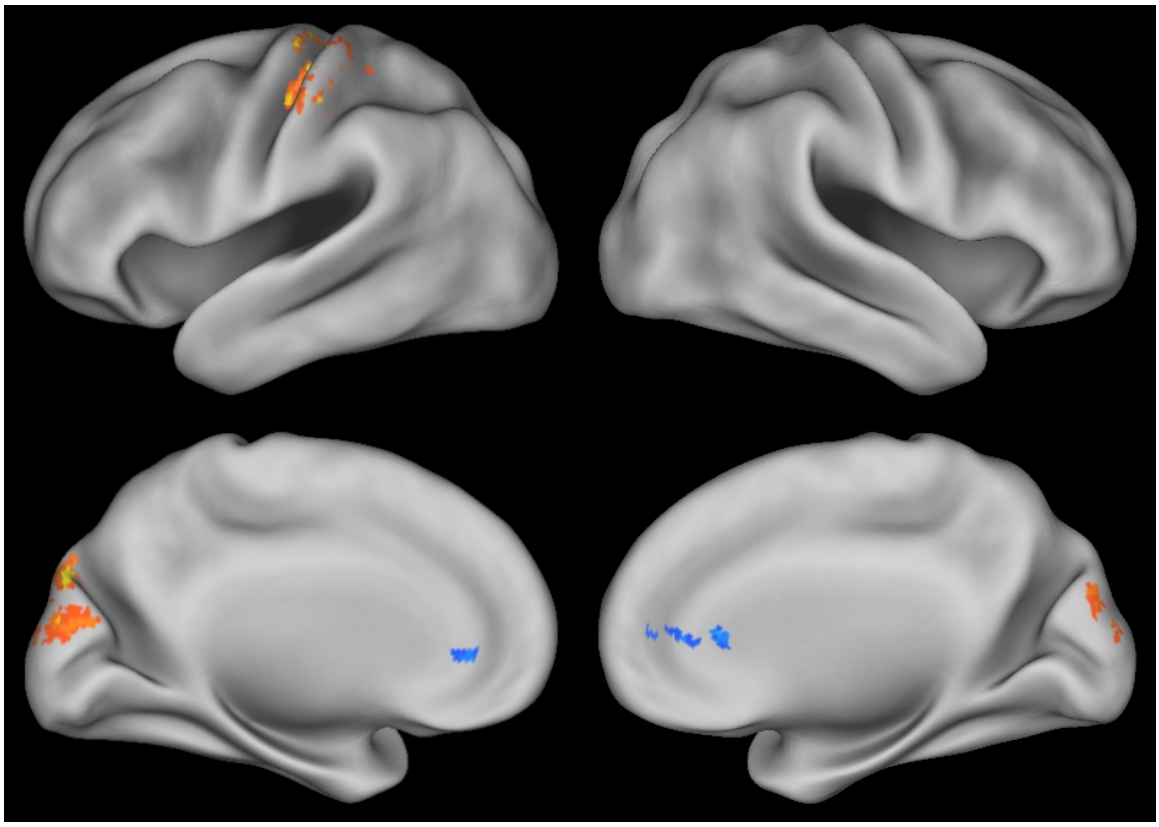

Figure 2b: The same contrast as 2a but without masking out any confounds.

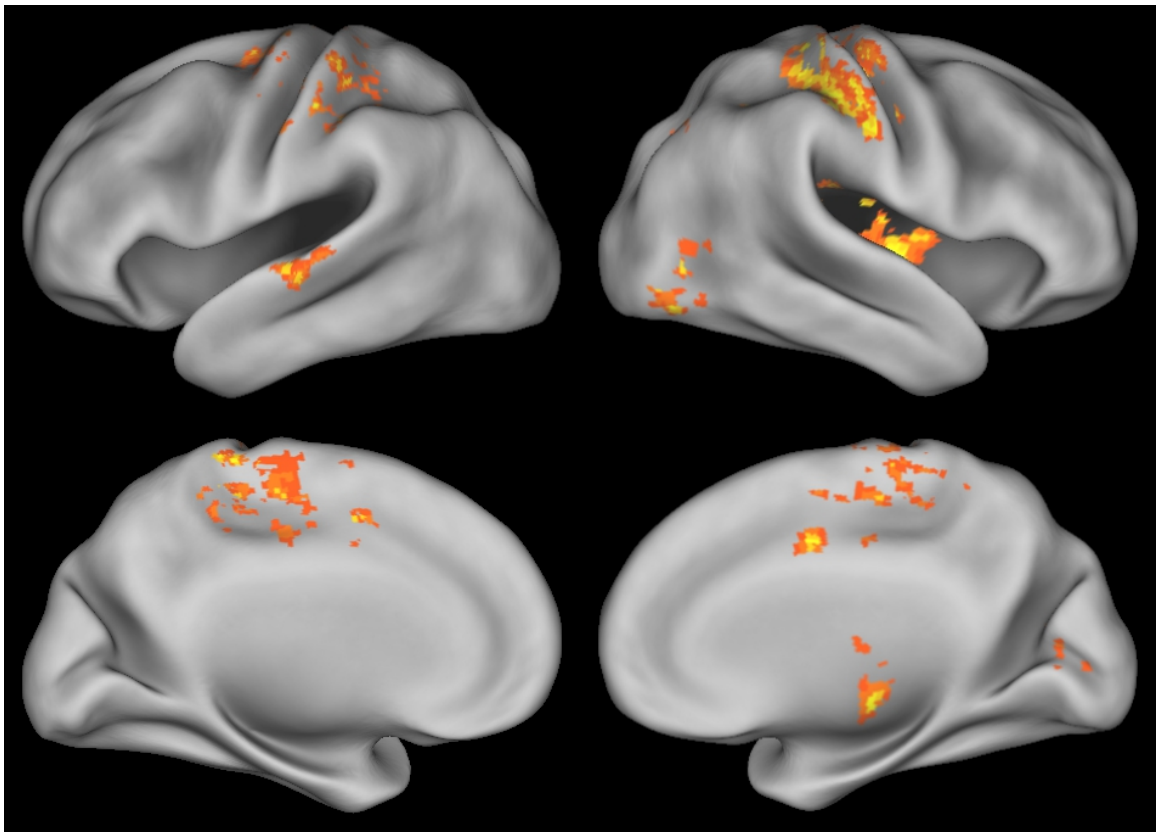

Figure 3a: Contrast of the average of the second two “functional” scans and the first “functional” scan; areas in warm colors denote “second half” > “first half.”

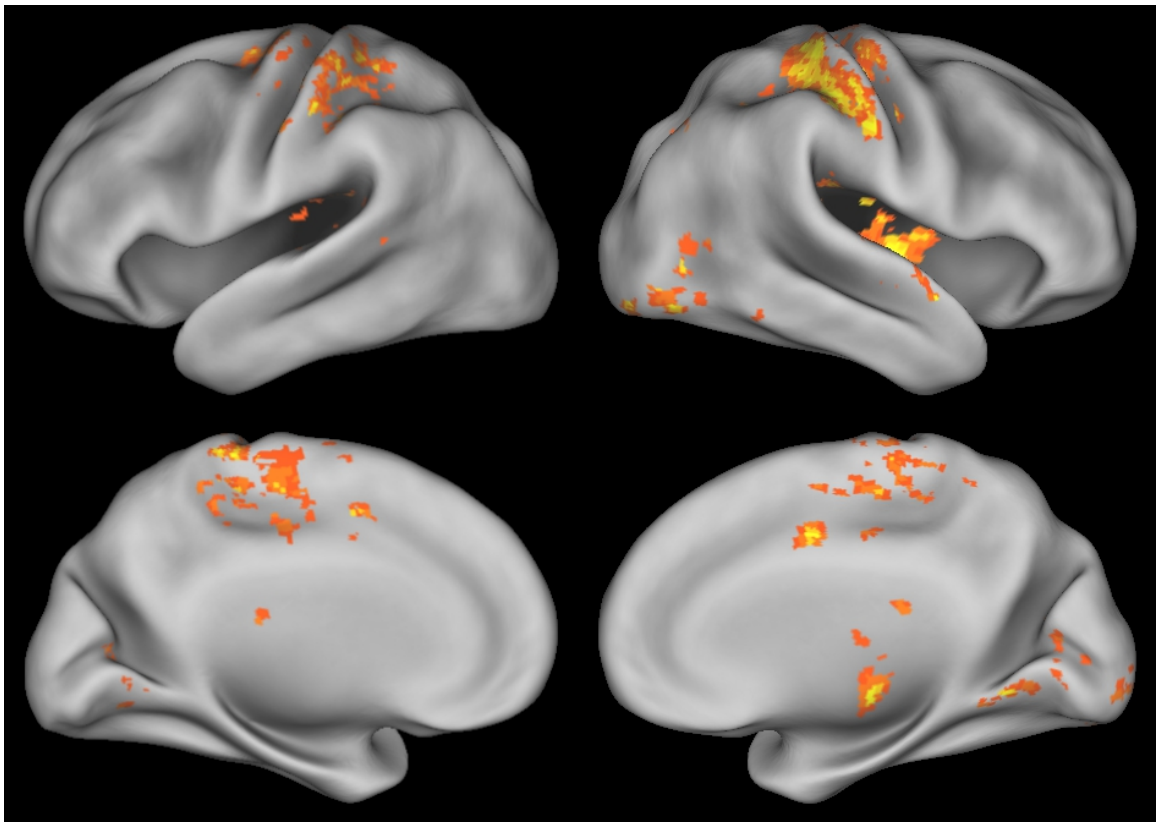

Figure 3b: identical to 3a but without masking out any confounds.

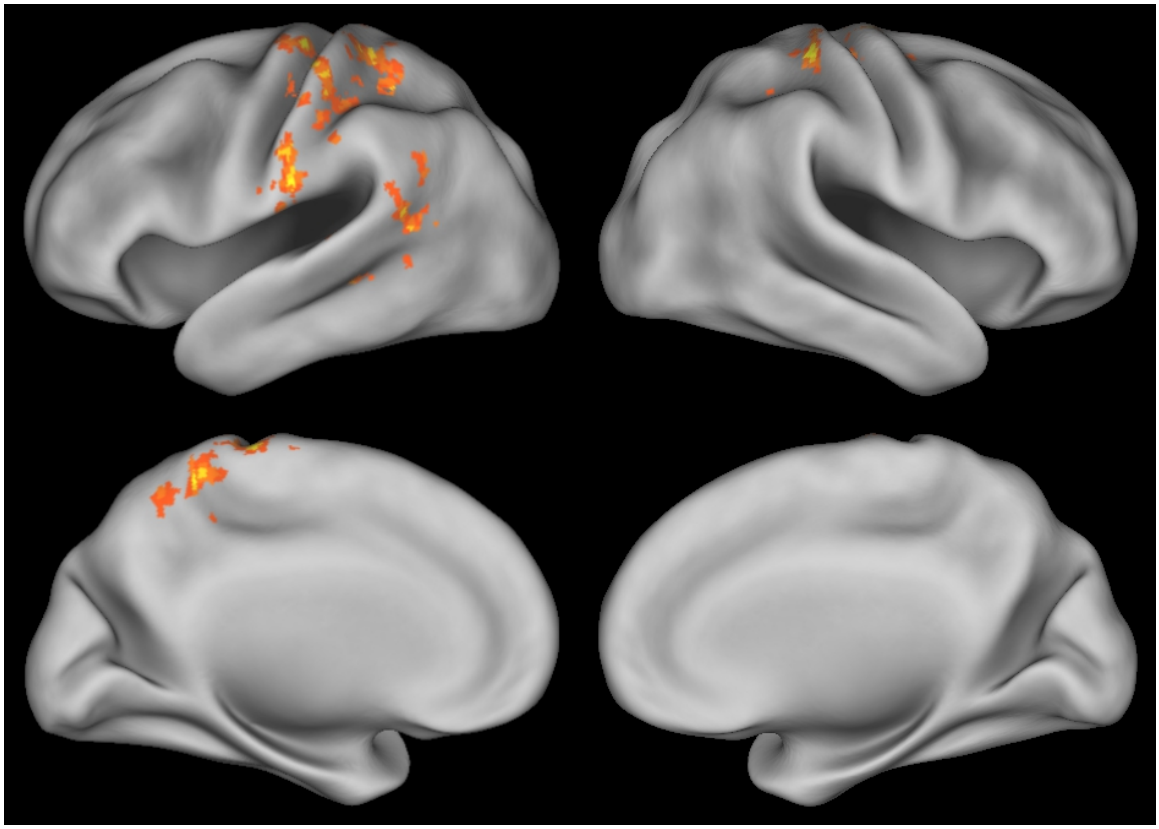

Figure 4: Contrast of third “functional” and second “functional” scans; areas in warm colors denote third “functional” > second “functional.”

ICN #	Location	Function
1	Limbic, medial temporal	Emotional perception
2	Subgenual ACC, OFC	Reward, thirst
3	Basal ganglia, thalamus	Emotion, interoception
4	Insula, anterior midcingulate	Transitional: emotion-cognition
5	Midbrain	Interoception
6	SFG, MFG	Motor planning, timing
7	MFG, SPL	Visuospatial reasoning
8	Central sulcus, cerebellum	Action, somesthesis
9	SPL	Motor learning, execution
10	Middle, inferior temporal gyri	Viewing complex stimuli
11–12	Posterior occipital cortex	Visual processing
13	mPFC, PCC	Default mode network
14	Cerebellum	Varied
15	Right fronto-parietal	Reasoning, inhibition, memory
16	Transverse temporal gyri	Audition
17	Dorsal precentral gyrus	Mouth sensorimotor function
18	Left fronto-parietal	Language

Table 1: ICN descriptions.

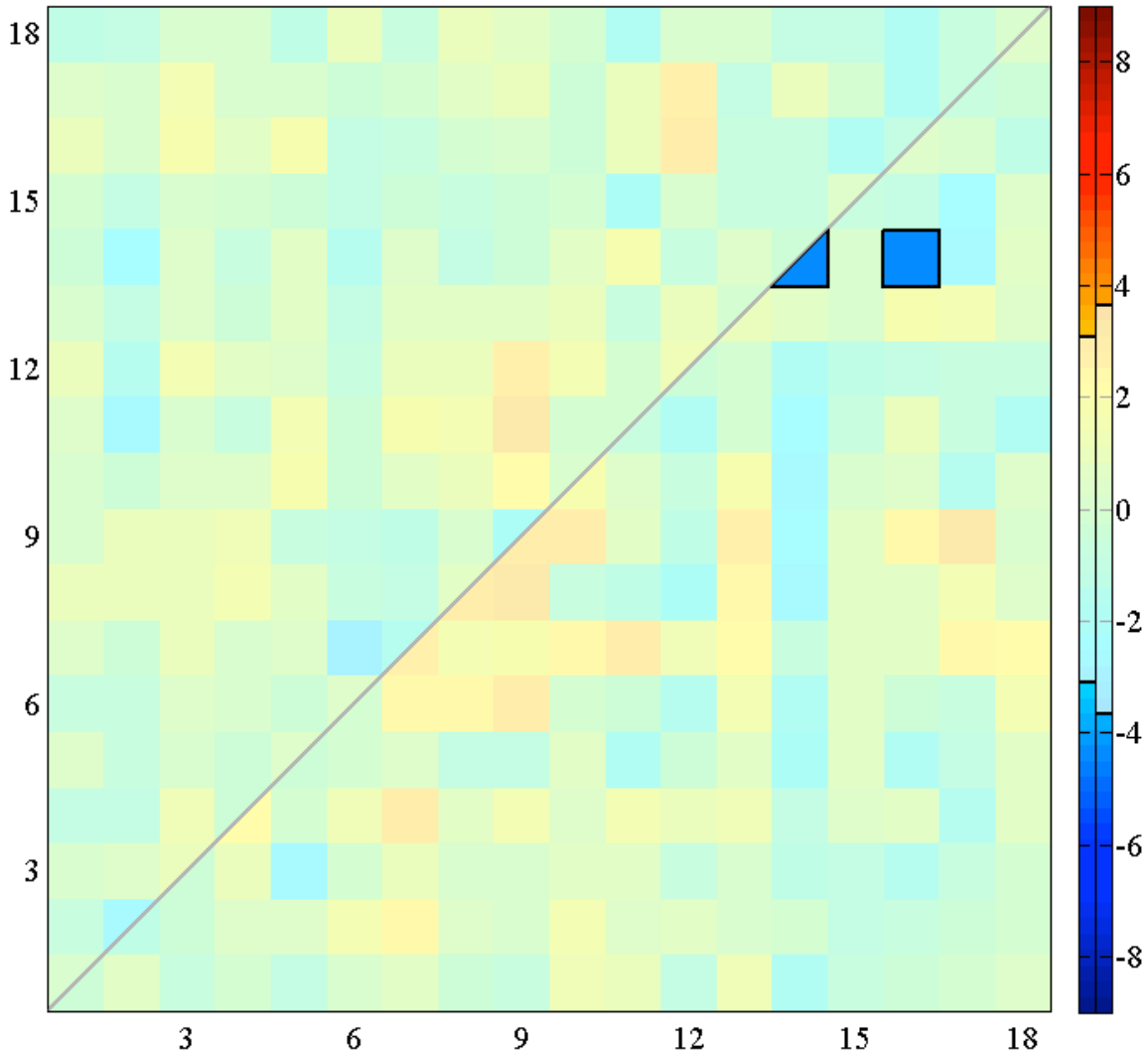

Figure 5: Contrast of the first “functional” scan and the first “anatomical” scan; areas in cold colors denote “anatomical” > “functional.”

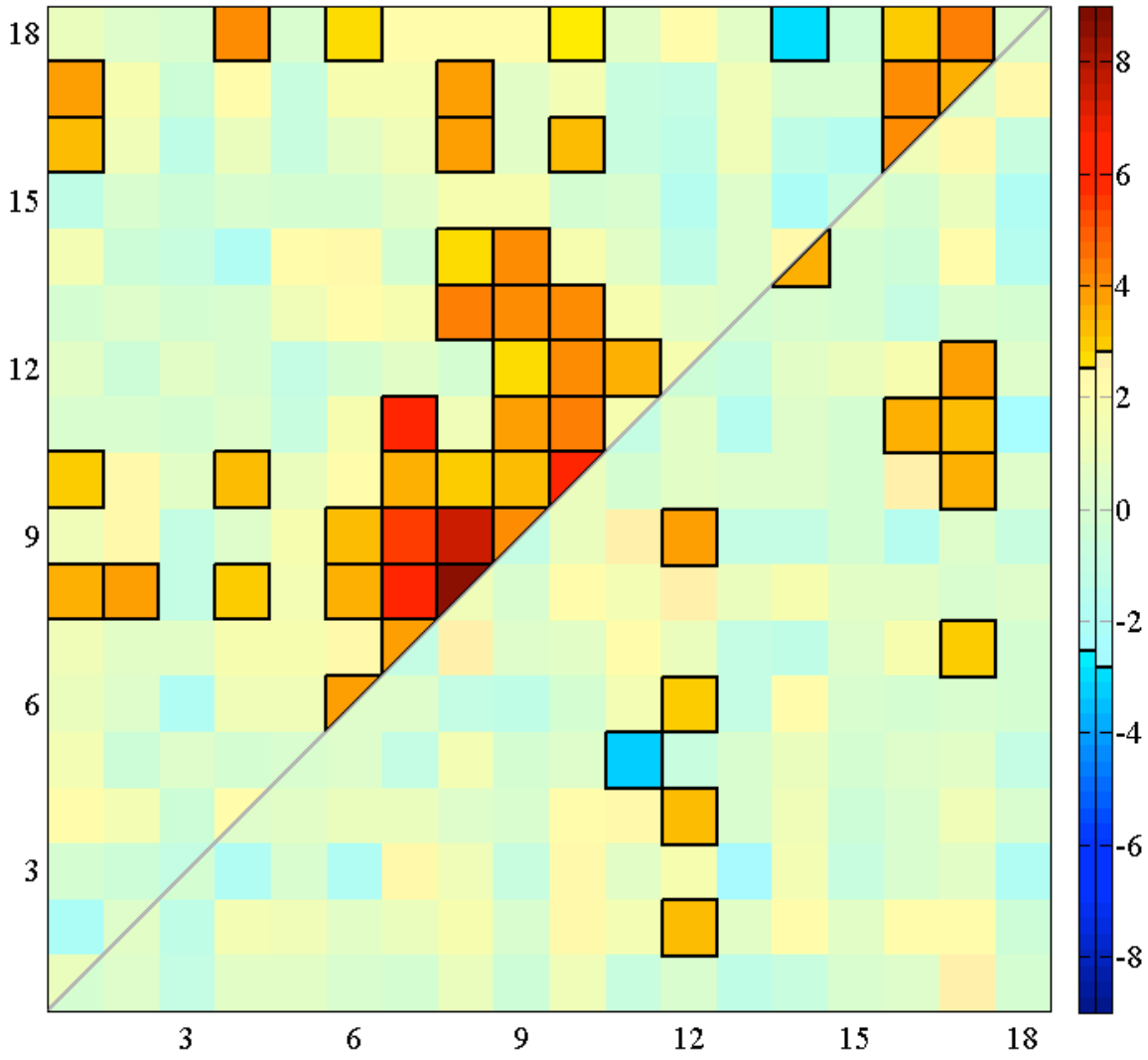

Figure 6: Contrast of third “functional” and second “functional” scans; areas in warm colors denote third “functional” > second “functional.”

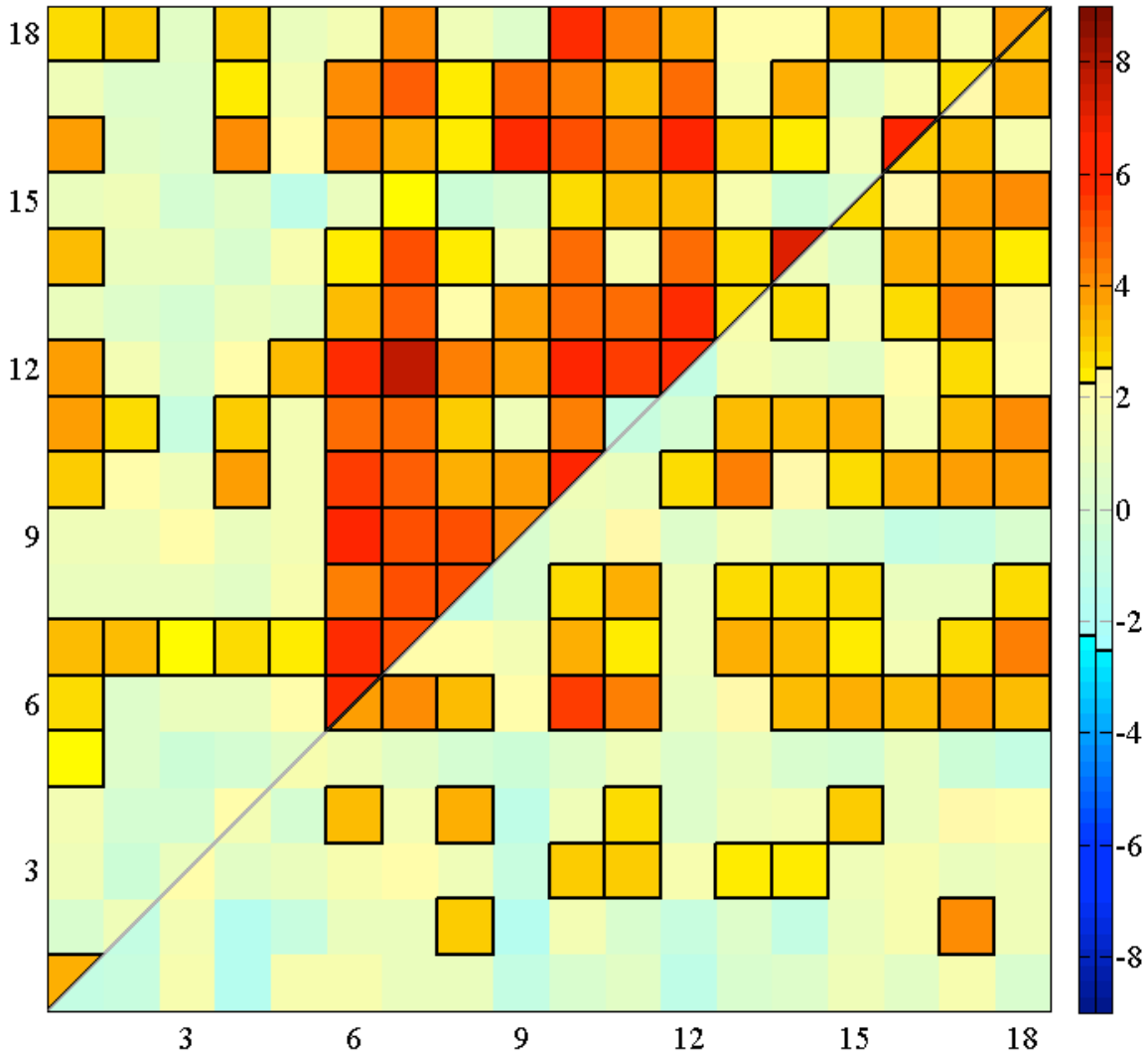

Figure 7: Contrast of the average of the second two “functional” scans and the second “anatomical” scan; areas in warm colors denote “anatomical” > “functional.”

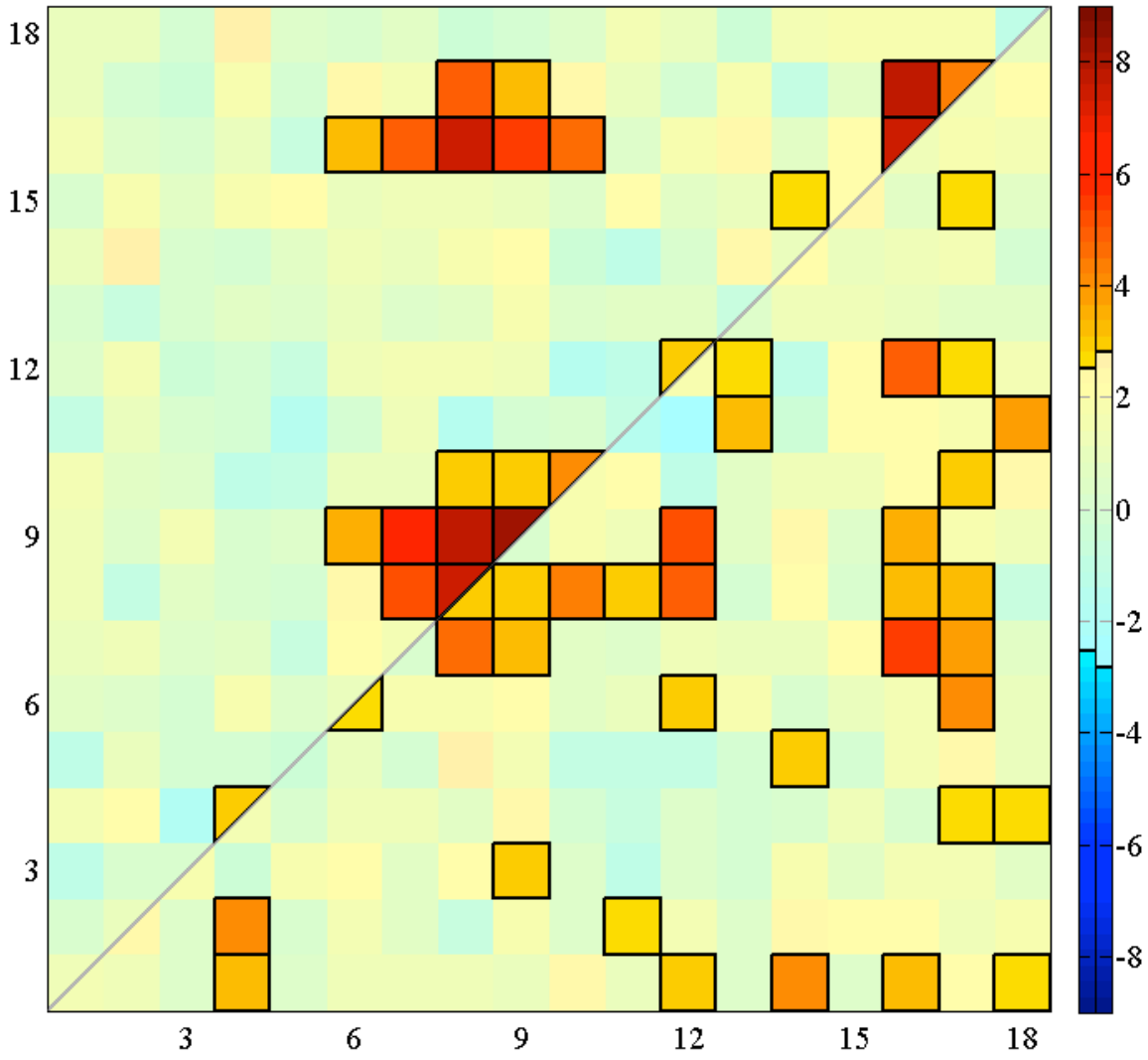

Figure 8: Contrast of the average of the second two "functional" scans and the first "functional" scan; areas in warm colors denote "second half" > "first half."

Discussion

Our results demonstrate that there are systematic differences across several networks as a function of simple instructional differences. As shown in figure 1, there was a significant increase in the OFC when comparing the first functional scan to the first sham anatomical scan. While the OFC is found to be highly associated with decision-making and expectation (Kringelbach, 2005), it's possible people were more struggling

and trying to control their thoughts when told it's a functional scan as opposed to an anatomical scan. As shown in the upper-left triangle of figure 7 (when using parcel-based coherence analysis), a substantial increase was observed in functional connectivity within the DMN when comparing the second sham anatomical scan to the second and third functional scans. This indicates that when people assumed it was an anatomical scan, the intra-DMN connectivity was increased.

The results we obtained indicate that the DMN is not as stable as people think or claim (Damoiseaux et al, 2006), and instructional manipulation does make a difference in the raw brain activations as well as functional connectivity between and within networks. In other words, when provided with different instructions or knowledge about what type of scan subjects were going to receive and the information of what the scan was going to measure, their brains reacted differently rather than only demonstrating DMN activity. Furthermore, as can be seen from the fALFF and functional connectivity results, the effects were selective, i.e., certain brain areas and functional connectivity were more affected than the others. For instance, the DMN was relatively more stable across the scans than other networks. Since the effects were selective, in other words, some networks were more affected than others; this means the results we obtained were not just due to motion artifacts but they tell us something meaningful about what's going on in the brain when people received different instructions about resting.

In fact, when collecting a resting state scan using fMRI, it matters if the resting scan is collected pre or post functional scans, because the task involved at the functional scans may make a difference in how people perform at the resting state scan. Moreover, an fMRI scan with the purpose of finding neural basis of a behavioral effect, the scan

result can consist of an ideal functional scan measuring the behavioral effect only, plus the effect of being watched. For instance, if a study investigates some personality trait and has found some results, the results could be due to the fact that people are being alert or self-conscious during the scan. Therefore, all fMRI results need to be interpreted as not only the functional scan alone, but also the awareness that occurred within individuals and across people. Particularly, fMRI results of any cognitive demanding task are not just the results of the task per se, but can also be influenced by the allocations of shifting their cognitive resources.

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