Materials and Methods

Participants

Two students: one male and one female (age range: 20-25; mean: 22.5), participated in the study. One participant was left-handed, and the other one was right-handed. They had normal or corrected to normal vision. They had no past neurological disorders like epilepsy or no past visual deficits. All the participants were informed about the study method and the MRI session. Participants were cautioned about the special safety instructions for being in an MRI investigation, like the items they shouldn't keep on them (metal objects, credit cards, etc.), noise, and possible claustrophobic atmosphere. Before the fMRI session, participants were demanded to sign an informed consent form. The Bilkent University Human Ethics Committee approved the protocols and procedures of the experiment.

MR image acquisition parameters

MRI data were obtained on a 3 Tesla MR machine (Magnetom Trio, Siemens AG, Erlangen, Germany) with a 32-channel array head coil in UMRAM. All MR sessions started with a short localizer to see the shape of the participants' head.

Structural images: After the short localizer each session continued with a structural run. Structural images were obtained with T1-weighted 3D anatomical sequence which lasted 4-5 minutes. (TR: 2600 ms, TE: 2920 msec, spatial resolution: 1mm3 isotropic, number of slices: 176). These structural images were utilized to adjust the domain considering the area for functional images.

Functional images: After structural runs, a functional scan was used for the visual ROI localizer scan. Functionality was house and face. This localizer is used to measure the retinotopic maps in the more ventral surface of the temporal lobe (FFA, PPA) for the purpose of the task and stimuli (which will be explained later). Slices shouldn't be so high, so the slices' orientation not changed and kept horizontal to see the ventral pathway only. The occipital and temporal cortex are covered and held in the frame. Functional images were obtained with a T2*-weighted gradient-recalled echo-planar imaging sequence (TR: 2000 msec, TE: 35 ms, spatial resolution: 3x3x3 mm3, number of slices: 28, slice orientation: parallel to fusiform gyrus and parahippocampal gyrus).

Functional Localizer: To measure the activation of FFA and PPA in the medial and inferior temporal lobes there were two functional runs that include stimuli. The scan session for each participant take approximately 20 minutes including functional and structural scan with two runs of house face task.

Experimental procedures

Visual stimuli were shown on an 32-inch LCD Monitor (T-32, Troyk Med A.S., Ankara,Turkey). The monitor was positioned around the back end of the scanner bore, and the participants viewed the stimuli from a space of 165 cm over a mirror attached on the head coil. Participants were told to perform a fixation task during the experiment. Around the fixation cross there appear either a face image or a house image to measure the boundaries between FFA and PPA. The experiment was of 16 active blocks, each block is for 16 s (TR=2000). Each run's total duration was 4 minutes 36 seconds with added initial and final blank periods. There was two runs for each participant.

Preprocessing and Analysis Methods

First, I downloaded the DICOM data for each subject. Then, I convert the DICOM files to NIFTI files for each subject using MRIcroGL. My final directory is in Figure 1 (the NIFTI file outlook). Converting DICOM files to NIFTI files is significant because NIFTI is a typically utilized neuroimaging data structure. Intake in NIFTI structure is demanded by most of the software packages for analyses. Regardless, raw data obtained from almost all machines are in a typical DICOM structure (as in the UMRAM fMRI machine used in this study). Consequently, DICOM files should be converted to NIFTI files to be analyzed best-fitted. The NIFTI file is the file we share with the world. It contains information that all the people can access. That's why the NIFTI file mustn't include any personal information of the participants so that they can be recognized to ensure the confidentiality of the participants. Because of this NIFTI file participant list doesn't contain the name or surnames of the participants. Also, when converting from DICOM to NIFTI files is done, still the face of the participant can be detected and lead to exposing the participants' real identity. To overcome this problem, I used *pydeface* commend on the terminal to deface the NIFTI files. By performing BIDS validation goodness of the BIDS dataset can be inspected. It is significant because it qualifies for recognizing possible mistakes or incoherence in the structure of the data. I have validated my NIFTI folder operating the BIDS validator successfully. A screenshot of the validator is shown in Figure 2.

```
NIFTI
 ├── README
 ├── dataset_description.json
 ├── participants.json
 ├── participants.tsv
 ├── stimuli
|   ├── sub04-ses1-ExperimentalProtocol.zip
|   ├── sub05-ses1-ExperimentalProtocol.zip
|   └── sub06-ses1-ExperimentalProtocol.zip
 ├── sub-04
|   └── ses-session1
|       ├── anat
|       |   ├── sub-04_ses-session1_T1w.json
|       |   └── sub-04_ses-session1_T1w.nii.gz
|       ├── fmap
|       |   ├── sub-04_ses-session1_dir-AP_run-01_epi.json
|       |   ├── sub-04_ses-session1_dir-AP_run-01_epi.nii.gz
|       |   ├── sub-04_ses-session1_dir-PA_run-02_epi.json
|       |   └── sub-04_ses-session1_dir-PA_run-02_epi.nii.gz
|       └── func
|           ├── sub-04_ses-session1_task-houseFace_run-01_bold.json
|           ├── sub-04_ses-session1_task-houseFace_run-01_bold.nii.gz
|           ├── sub-04_ses-session1_task-houseFace_run-01_events.json
|           ├── sub-04_ses-session1_task-houseFace_run-01_events.tsv
|           ├── sub-04_ses-session1_task-houseFace_run-02_bold.json
|           ├── sub-04_ses-session1_task-houseFace_run-02_bold.nii.gz
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|           └── sub-04_ses-session1_task-houseFace_run-02_events.tsv
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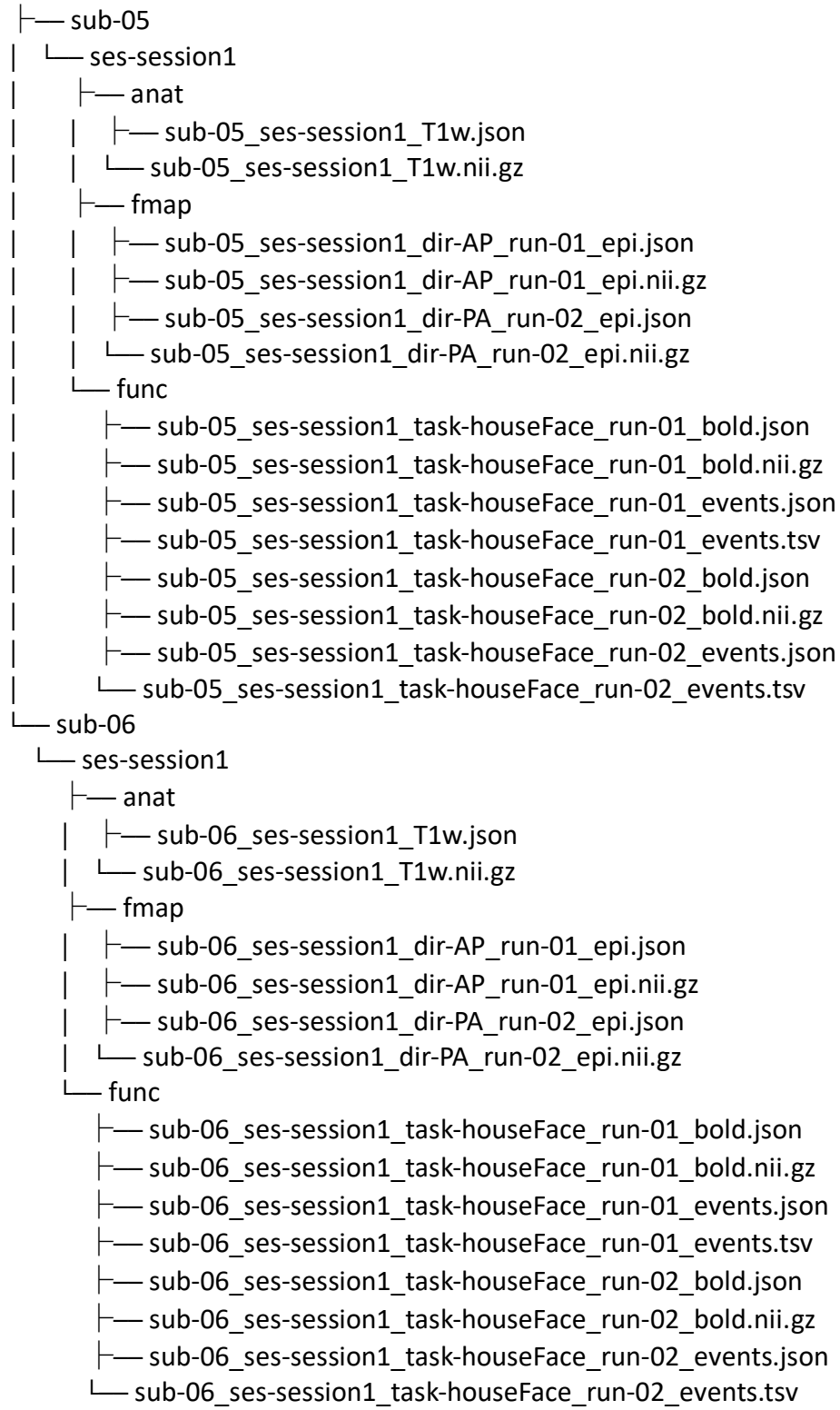

16 directories, 49 files

Figure 1: Structure of the NIFTI file

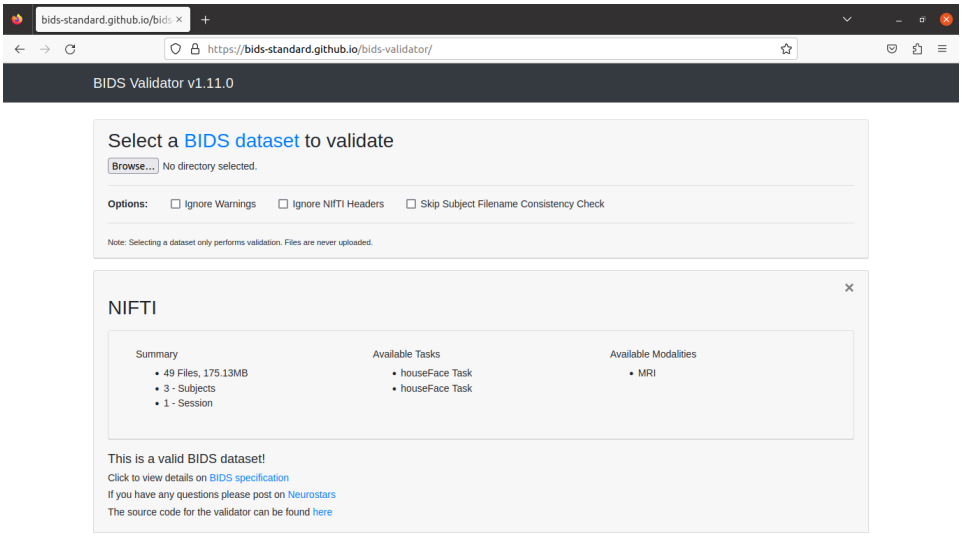

Figure 2: BIDS validation for NIFTI file

Anatomical preprocessing steps

I performed skull-stripping using the BET brain extraction function of FSL. The input image is a T1 weighted image, and the output image is the same image with a _brain attached to it (automatic). I used *robust brain center estimation* under advanced options, I used *output brain surface overlaid onto the original image* for better visualization, and the threshold is 0.5. The output image extracted a brain with no skull. When I inspect the image using *fsleyes*, the skull stripping is good. Too much stripping is not ideal because it holds the risk of losing the cortex. The resulting image doesn't show any lost cortex. Although smaller threshold values give a larger brain estimate because it strips less brain, I chose the threshold to be 0.5 because it is a reasonable threshold for not losing the cortex and not remaining too much of the skull.

Then I performed FAST of FSL, segmenting white matter and grey matter. The input image is the betted image I obtained before from BET. As an output of FAST, several images formed: *pve0* showing the liquid fluid CSF, *pve1* showing the grey matter, and *pve2* showing the white matter. These FAST files, when inspected using *fsleyes*, show a probabilistic map. The probabilistic value of estimation appears. The meaning of the values is how much FSL is sure it is CSF, white matter, or grey matter with different images. FAST also creates a file named *pveseg*. This file is kind of binary, one thresholded with one image. When inspected with *fsleyes*, this file shows no probability estimation but tells us this is grey matter, white matter, and CSF. The selected participant's skul-stripped (native) brain and different matters: white matter, gray matter, and CSF, are shown in Figure 3. The blue place is the CSF, the pink place is the white matter and the red place is grey matter. CSF – white matter – grey matter from outer place to inner place order. The segmentation can be seen clearly.

Then, I performed FLIRT on FSL. This step aligns the participants' data with the standard data MNI 152. The reference image was chosen as the MNI 152 T1 weighted 1mm brain (NIFTI). The input image is the best-betted image. The resulting image is the participants' brain but aligned with MNI 152 standard as if there is a frame and they are inside the same frame. Not every sulcus and every gyrus are exactly aligned, but the overall shape is aligned with the standard. The same participant's brain aligned with MNI 152 template can be seen in Figure 4.

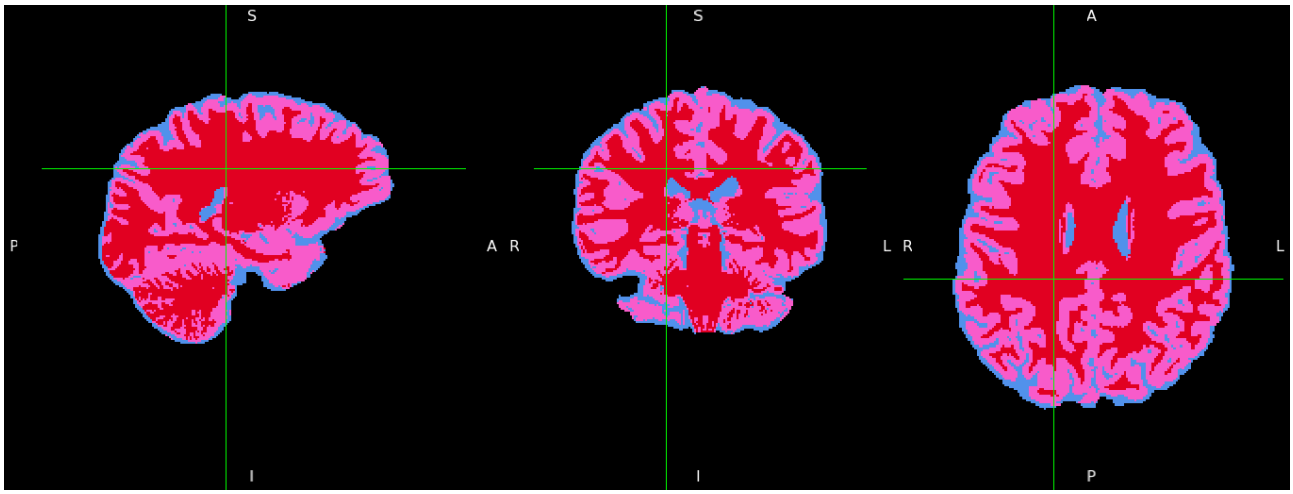

Figure 3: Sub-04's skul-stripped (native) brain and different matters therein (white matter, gray matter, and CSF)

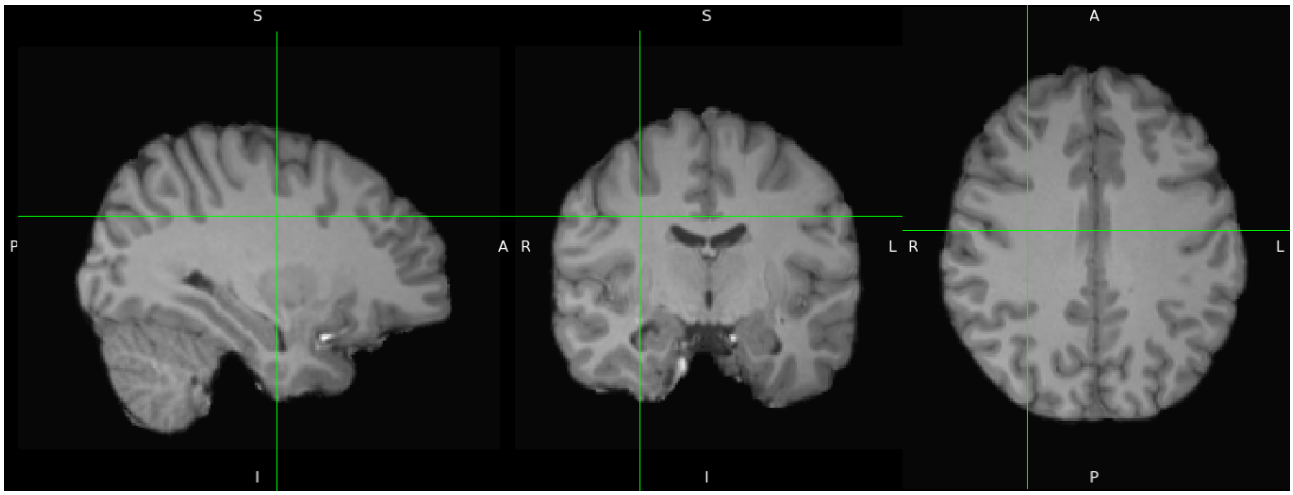

Figure 4: Sub-04's brain aligned with MNI 152 template

Functional preprocessing step

There is magnetic field inhomogeneity. This magnetic field inhomogeneity must be corrected because it can create distortions in the data. It is especially critical for multiband series. Magnetic field inhomogeneities were corrected using the FSL's function *topup*. But the first thing is merging the files AP and PA using: *$fslmerge* -t and name of output houseFace_merged_AP_PA_epi.nii.gz than the file names I want to merge. "t" means it is merging it in the time axis. The resulting merged image is from the same brain same position. Very different images are formed because of magnetic field inhomogeneity. Those distortions should ideally be corrected. The merged one has two volumes and two-time points in the same file because I merged two files (AP and PA). For topup, a helping file is needed, which tells us encoding direction and total-read out times datain.txt.
For correcting magnetic field inhomogeneities, I used topup using the command:
$topup –imain=houseFace_merged_AP_PA_epi.nii.gz --datain=datain.txt - -out=houseFace_topup.
After applying topup we have two new files.
After this I apply this topup to actual bold file that I want to analyze later using command:
$applytopup --imain=../func/sub-04_ses-session1_task-houseFace_run-01_bold.nii.gz --inindex=1 --method=jac --datain=datain.txt --topup=houseFace_topup –out=../func/sub-04_ses-session1_task-houseFace-unwarped_run-01_bold.nii.gz.

Motion correction, slice acquisition time correction, filtering, coregistration:

FEAT function of fsl used for first level analysis preprocessing misc default data select 4D data as unwarped one total volume=168 TR=2.0000 output directory fsl-func. Under the prestats tab, MCFLIRT is used for motion correction. I use slice timing correction interleaved (0,2,4..) BET extraction on no spatial smoothing temporal filtering, just high pass registration. I did coregistration by registrating standard MNI 152 and aligning the functional with the MNI, so I chose a 2mm, courser one because 1mm is high-resolution functionals are 3mm low resolution, so it is closer to 2mm and better for this analysis. Full search with 12 DOF, aligning to the standard space. Nonlinear option on aligning the functional to the native brain original brain from the participant, not MNI 152 pick up best skull stripped betted image use full search BBR. One participant's functional-native anatomical (Figure 5), functional-MNI152 alignments (Figure 6), and estimated mean displacement plots (Figure 7) shown below.

Figure 5 shows the aligned functional image to the anatomical image. This is done to guarantee that functional data correspondes with anatomical structure spatially. In Figure 5, it can be seen that the functional image aligns nicely with the high-resolution anatomical image. The primary brain structures are correctly registered.

Figure 6 shows alligned functional image to standard MNI template. This is done for the aim of normalizing the functional data spatially to a standard permitting for group-level anaylses. Like we look at Figure 5 when we look at Figure 6 the allignment of functional to standard is good in that the central regions and brain structures alligned nicely.

Figure 7 shows the intermediate share of head motion among the whole fMRI scan session. The mean displacement doesn't exceeds a the threshold of 0.5 which indicates that there is no dramatic head motion that can cause disturbances in the data and may harm the analysis proces. So the head motion of the participant is okey.

The magnitude of head motion at each time point can be seen in the absolute displacement curve. By looking at Figure 7, we can state that participant move their head at the beginning and end of the session little (not a dramatic motion); other than that, the head of the participants is relatively stable.

The relative curve demonstrates the general measure of cumulative motion. Then again, by looking at Figure 7, we can conclude that there are no dramatic head movements at specific time points or in the general course of the run.

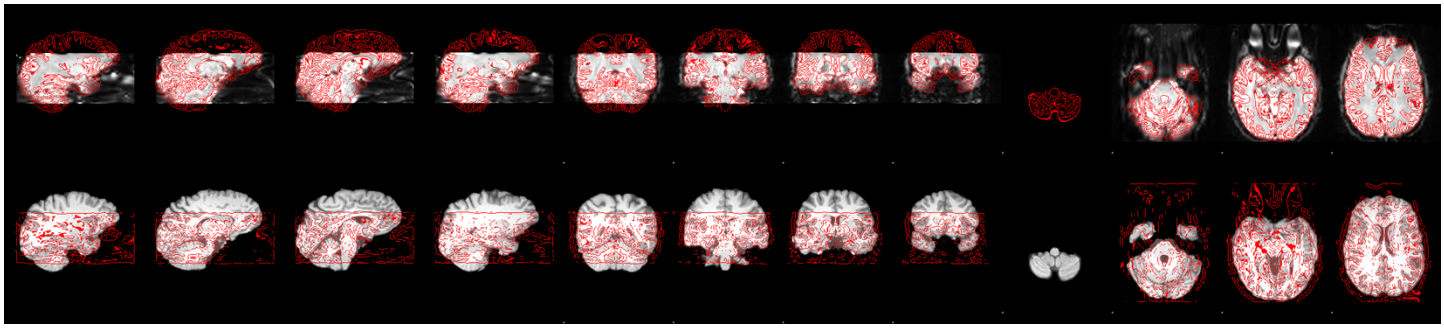

Figure 5: functional-native anatomical for sub-06 run-01 - Registration of example_func to highres

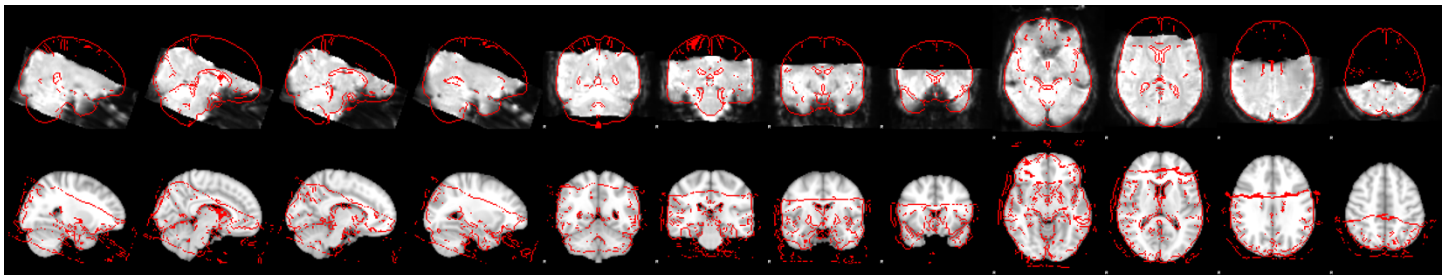

Figure 6: functional-MNI152 alignments for sub-06 run-01 - Registration of example_func to standard

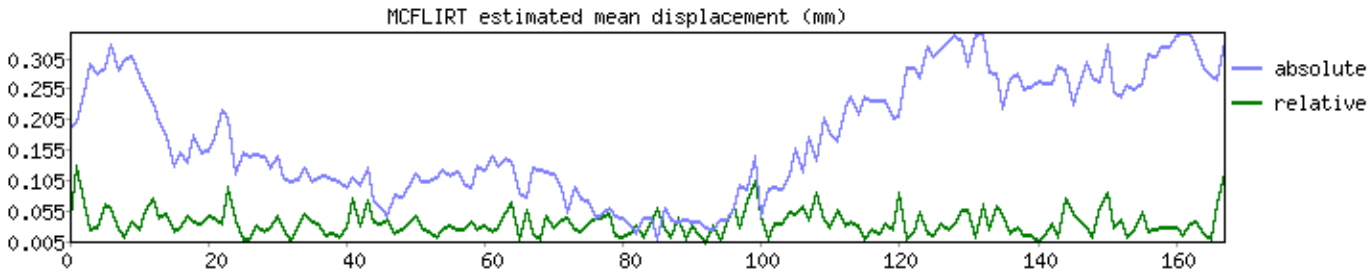

Figure 7: estimated mean displacement plot for sub-06 run-01

GLM analyses

First Level GLM Analysis:

FEAT is used for the first level GLM analysis. First-level analysis and statistic options were used. Under the data tab: the input is a FEAT directory. I already performed preprocessing steps, and it created FEAT directories. I selected those FEAT directories. The parameters come from design.fsf file. Under the stats tab, I checked “use FILM prewhitening" and chose “don't add motion parameters." Under the stats tab, full model setup option, I used custom (3-column format) as the basic shape and 'double-gamma HRF' for convolution. I have two EVs, EV1: house and EV2: face. I have to put a file that includes the beginning of each condition and how long that condition goes. These are blocks of events. Under the Contrast & F-test tab, I set up contrasts for 'house,' 'face,' ‘house-face’, and 'face-house.' Under post-stats, the voxel threshold is used because I want to see p values. After that, I go back to misc and click on the estimate high pass filter option.

Second Level Analysis:

For the second level analysis, I used FEAT, but this time with a higher level analysis option from the drop-down menu. Second-level GLM analysis combines two runs of the same participants. Under the data tab, I choose the option 'Inputs are lower-level FEAT directories". For this study number of inputs is 2 because we have two runs for each participant. I used fixed effects because, for using mixed effects, at least 3 data sets are needed, but there are only 2 for this study. These datasets come from the same participant anyway, so a fixed effect is sufficient for this study. Under Contrasts and F-tests, all inputs are 1 because we are taking input from both runs equally weighted; there is no difference between the two. Under post-stats, the voxel is chosen with a p-value of 0.05. Figure 8 showing houses>faces activation contrasts superimposed on anatomical images for each participant. Figure 9 showing faces>houses activation contrasts superimposed on anatomical images for each participant.

In Figure 8 the PPA activation is higher than the FFA activation. The PPA activation can be seen in the temporal lobe. Because PPA is the region that process mostly the scenes and the place it’s activation is more in the house>face condition. In Figure 9 the FFA activation is higher than the PPA activation. The FFA activation can be seen in the temporal cortex. Because FFA is responsible for face processing it’s activation is more in the face>house condition. Besides these there is also some activity on other brain parts but not that clear and straightforward that it overshadow FFA or PPA activation.

Figure 8: House > Face

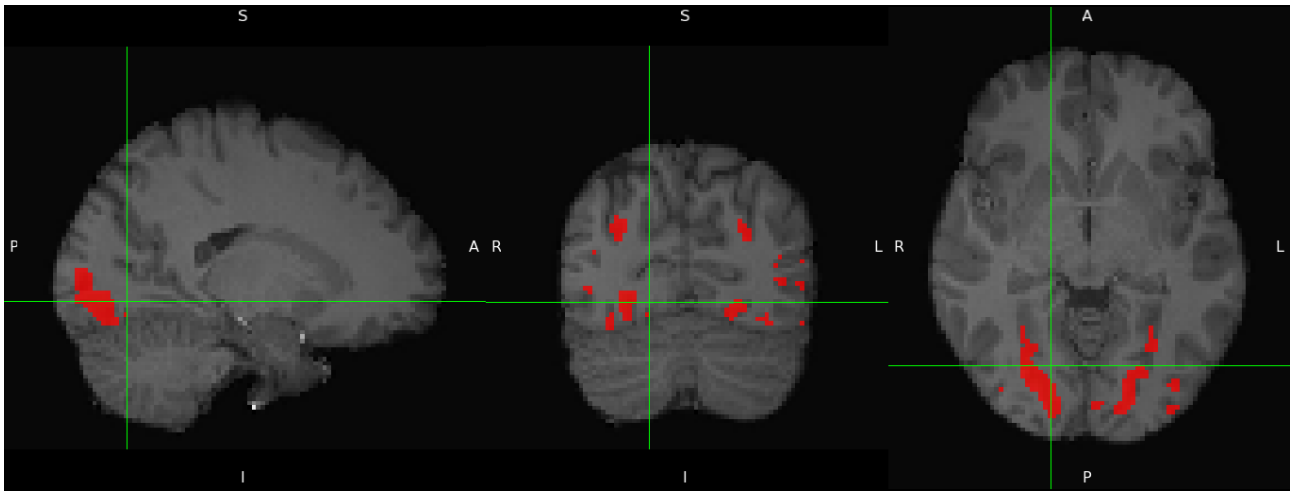

Figure 8.1: sub-04

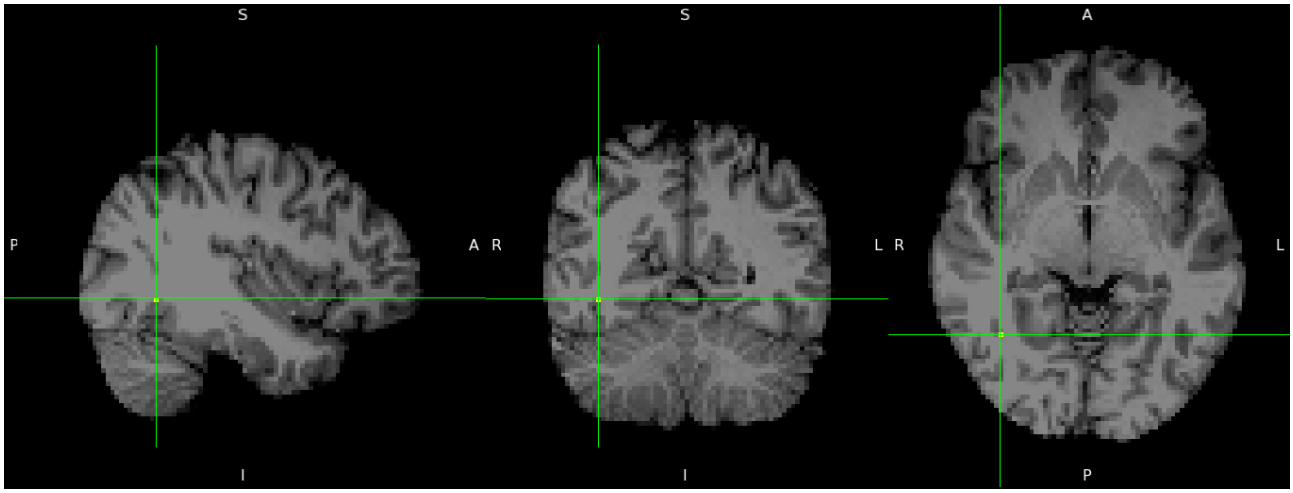

Figure 8.2: sub-05

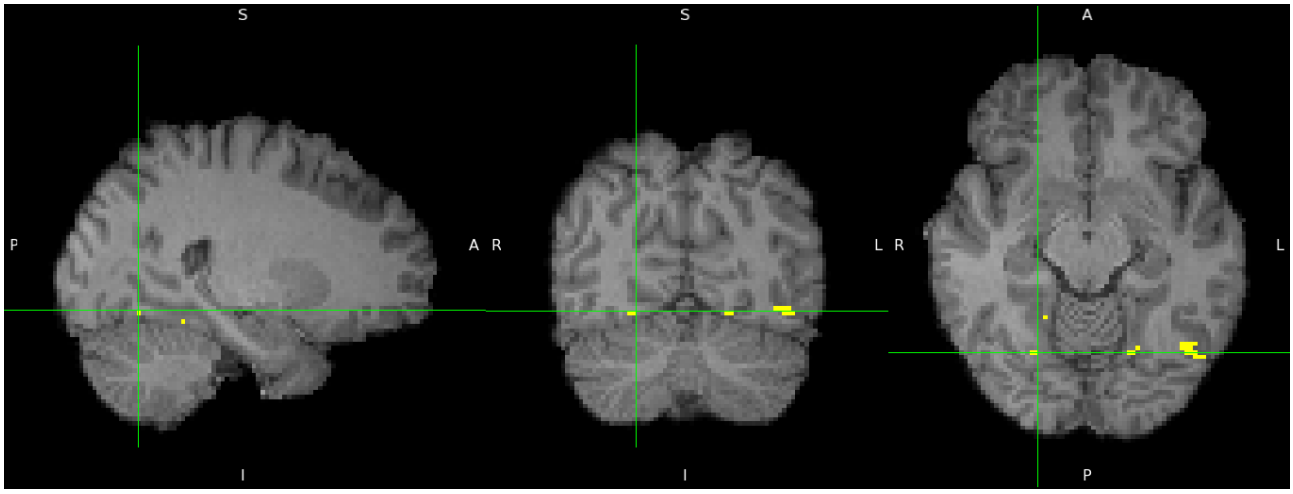

Figure 8.3: sub-06

Figure 9: Face > House

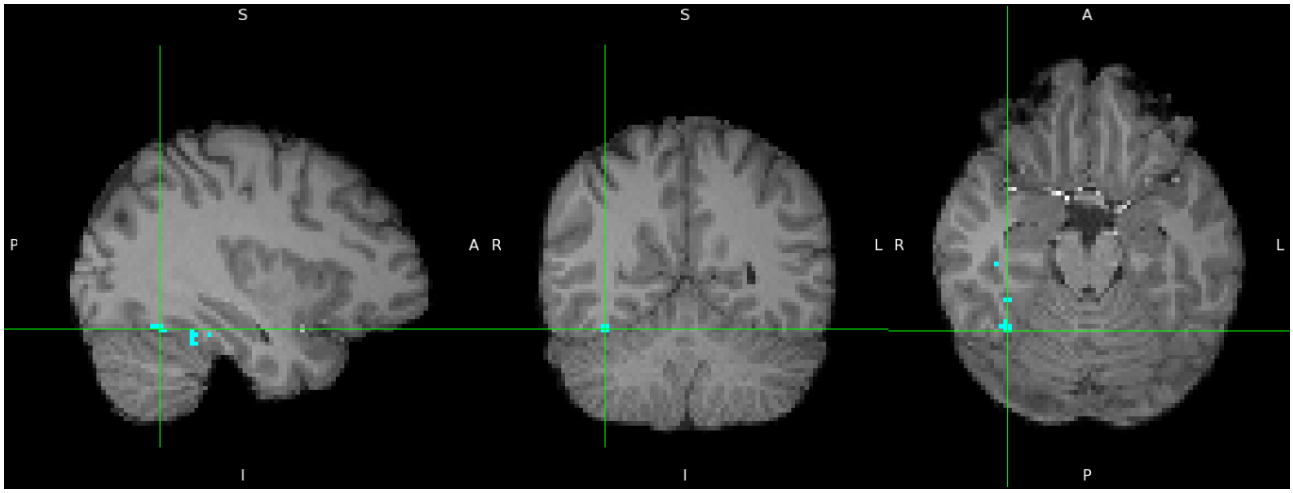

Figure 9.1: sub-04

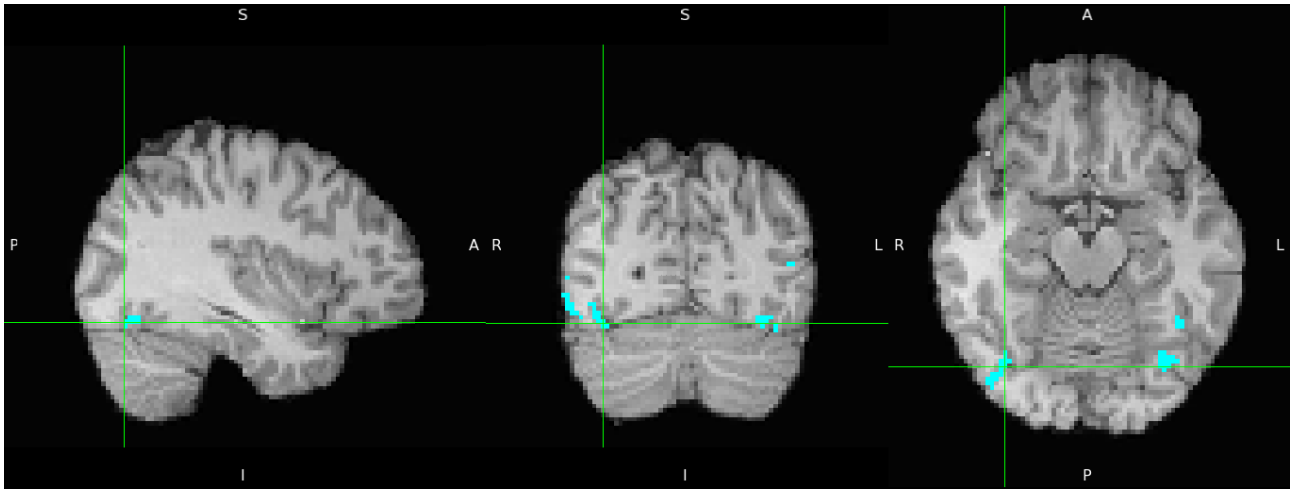

Figure 9.2: sub-05

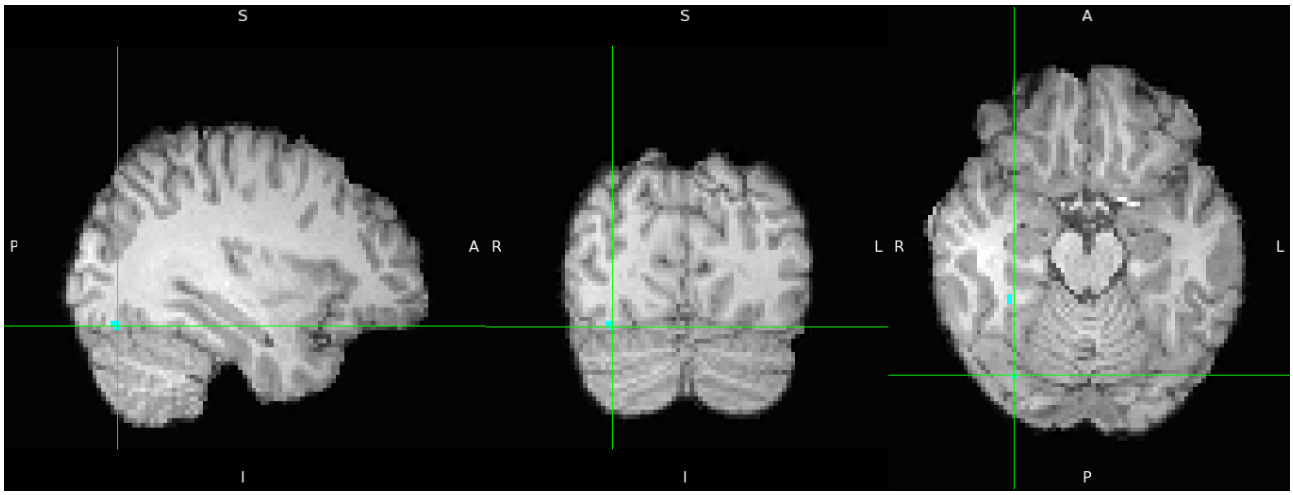

Figure 9.3: sub-06

Third Level Analysis:

Third level analysis is the group level analysis. This analysis combined different participants' data under the derivative-subGroup directory. FEAT is used for this analysis. The number of inputs is 3 because there are 3 participants. I select cope images that are formed from second-level analysis as input. The fixed effect is used. Typically, the fixed effect isn't preferred for third-level analysis because it is group-level, and the participants should be treated as an independent variable. But because we have just 3 participants, it is too low for mixed effect or random. Under the post stat tab, different from first and second-level analysis cluster is selected to limit the minimum cluster size to address the multiple comparison problems by using a minimal cluster size approach, allowing only clusters above a certain number of voxels. The resulting images shown in Figure 10 and Figure 11.

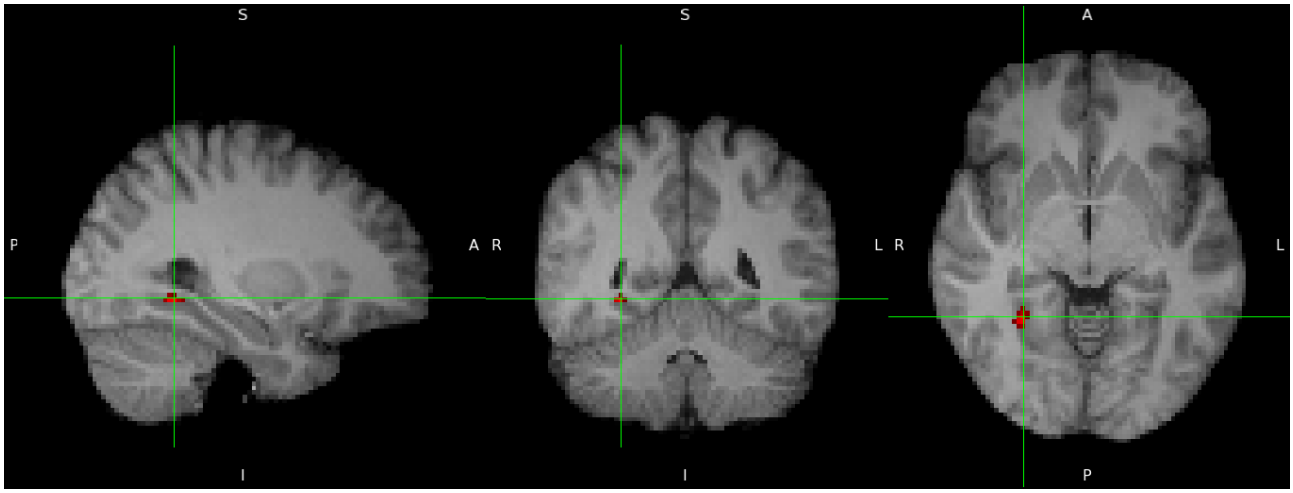

Figure 10: house>face, at the group level.

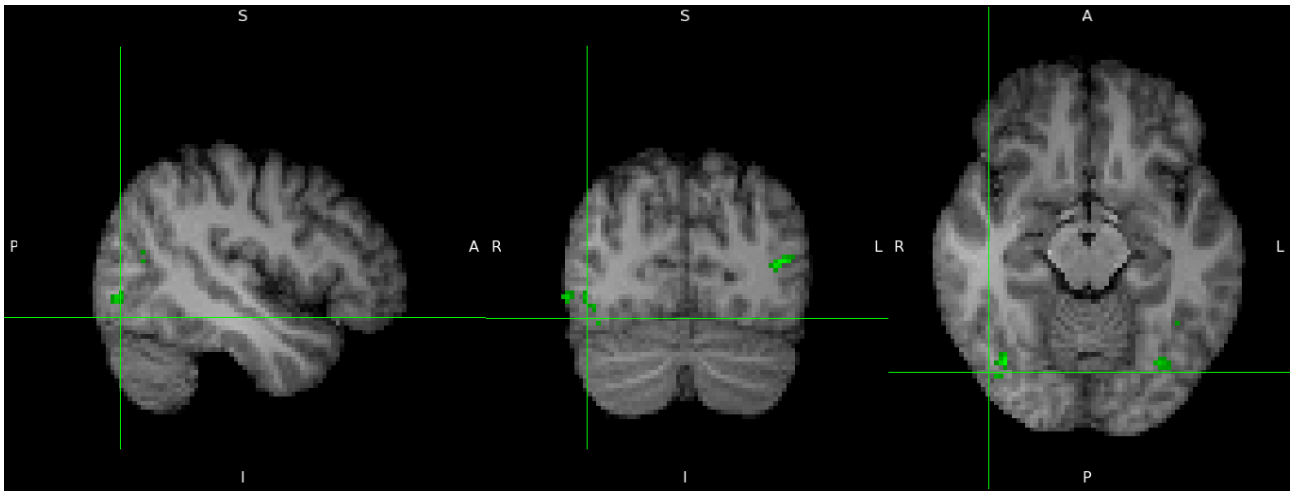

Figure 11: face>house, at the group level

In the figure 10 PPA activation is higher and in the figure 11 FFA activation is higher as described above for Figure 8-9. Because this is the group level the activations are all on averaged bases.

Discussion and Conclusion

This study's findings suggest that FFA is responsible for face processing and recognition. When the participants were shown face image stimuli, the increased activity of FFA and the decreased activity of PPA can be seen in Figure 9 and at group level Figure 11. Also, this study confirms the suggestion that PPA is responsible for object and scene processing. When the participants were shown house image stimuli, the increased activity of PPA and the decreased activity of FFA can be seen in Figure 8 and at the group level in Figure 10. This study's findings are consistent with the result of existing literature. Kanwisher et al. (1997) resulted that This study uncovered a region in the fusiform gyrus that reacted extremely more intensely during passive viewing of the face than object stimuli. Also, Epstein and Kanwisher (1998) found that the PPA examines the shape of the regional environment, which is essential to our capacity to decide where we are. This finding is one step away from our study in that it highlights the effect of PPA on navigating, whereas the focus of PPA in this study is scene processing. In general, our findings are consistent with the literature.

References

Epstein, R., & Kanwisher, N. (1998). A cortical representation of the local visual environment. *Nature*, *392*(6676), 598–601. https://doi.org/10.1038/33402

Kanwisher, N., McDermott, J., & Chun, M. M. (1997). The fusiform face area: A module in human extrastriate cortex specialized for face perception. *The Journal of Neuroscience*, *17*(11), 4302–4311. https://doi.org/10.1523/jneurosci.17-11-04302.1997