



Cairo University
Faculty of Engineering
Systems and Biomedical Department
SBME 3048
Medical Equipment Design

FMRI Report

Submitted to

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Sec: 1 **BN:** 8

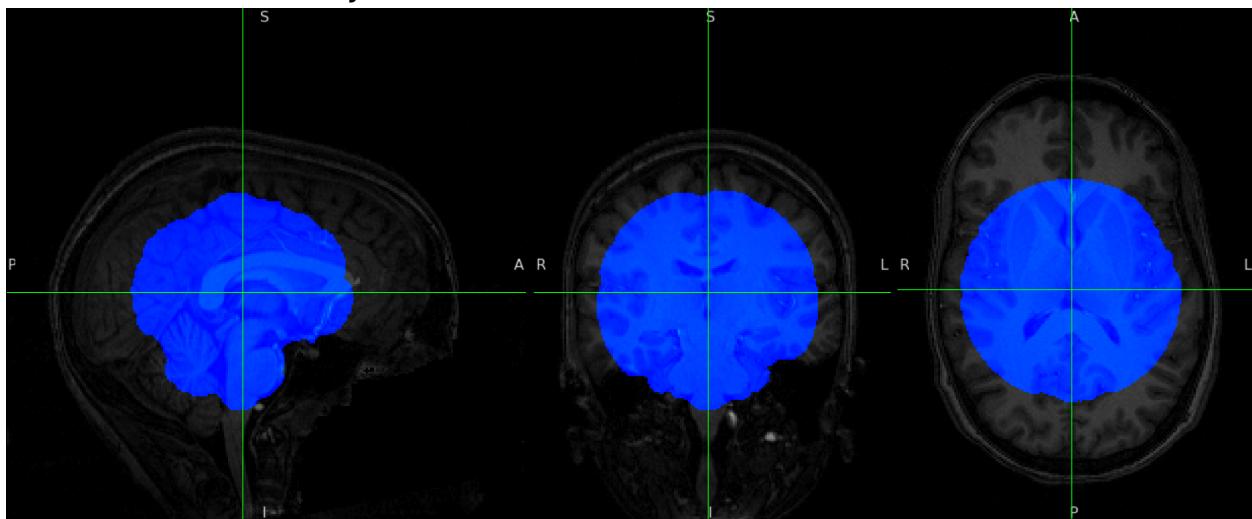
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1-BET Brain Extractions

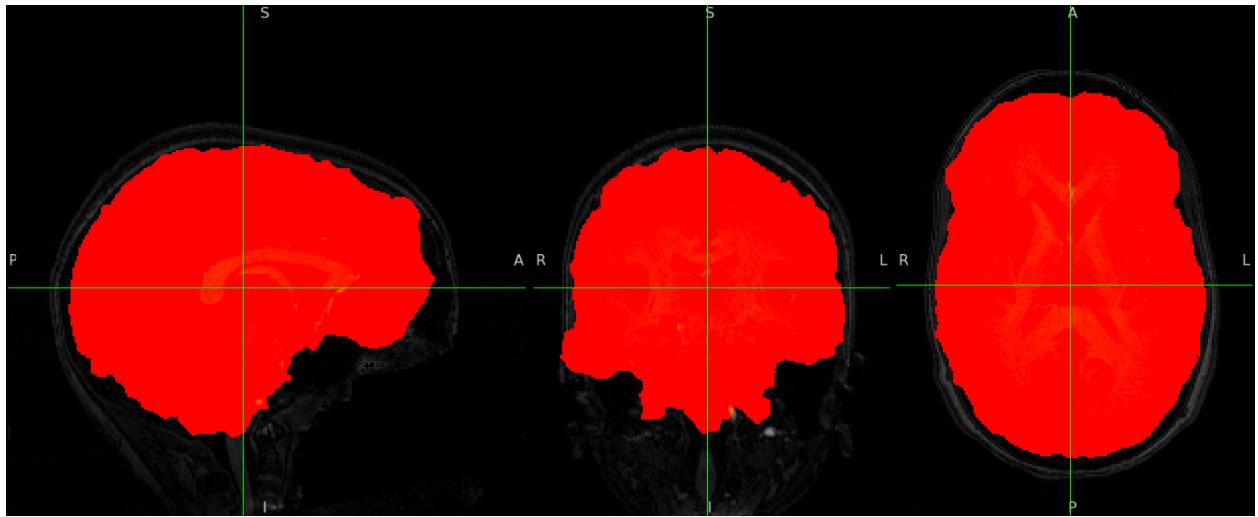
BET (Brain Extraction Tool) is a vital component within FSL (FMRIB Software Library), serving to automatically remove non-brain tissue from structural MRI (Magnetic Resonance Imaging) images. Its core function revolves around the process of skull stripping, where it accurately delineates the boundary between brain tissue and surrounding structures such as skull, skin, and other extraneous elements. This delineation is achieved through a combination of sophisticated mathematical algorithms and image processing techniques. BET is designed to be robust, capable of handling various MRI image types, contrasts, resolutions, and potential image artifacts with minimal user input. Its automation makes it highly efficient, facilitating the processing of large datasets or batch operations. Brain extraction, as performed by BET, is a crucial preprocessing step in neuroimaging analysis pipelines, laying the foundation for subsequent analyses such as registration, segmentation, and volumetric measurements, thus contributing significantly to neuroimaging research and clinical assessments conducted with FSL.

- **Exercise 1 on subject 08:**



Fig(1)

Fig(1) shows subject 08 with brain extraction with fractional intensity threshold of a high value of 0.9 which give us a mask with a very small region of the brain that is not sufficient to study the brain limiting our capabilities and wasting a lot of information



Fig(2)

Fig(2) shows subject 08 with brain extraction with fractional intensity threshold of a low value of 0.1 which gives a mask with very large region that covers the whole brain and more of it exceeding to the skull and unnecessary regions.

Conclusion:

In conclusion, the choice of fraction intensity threshold plays a crucial role in the analysis of functional MRI (fMRI) data. The selection of an appropriate threshold value balances the sensitivity and specificity of the results obtained from the analysis.

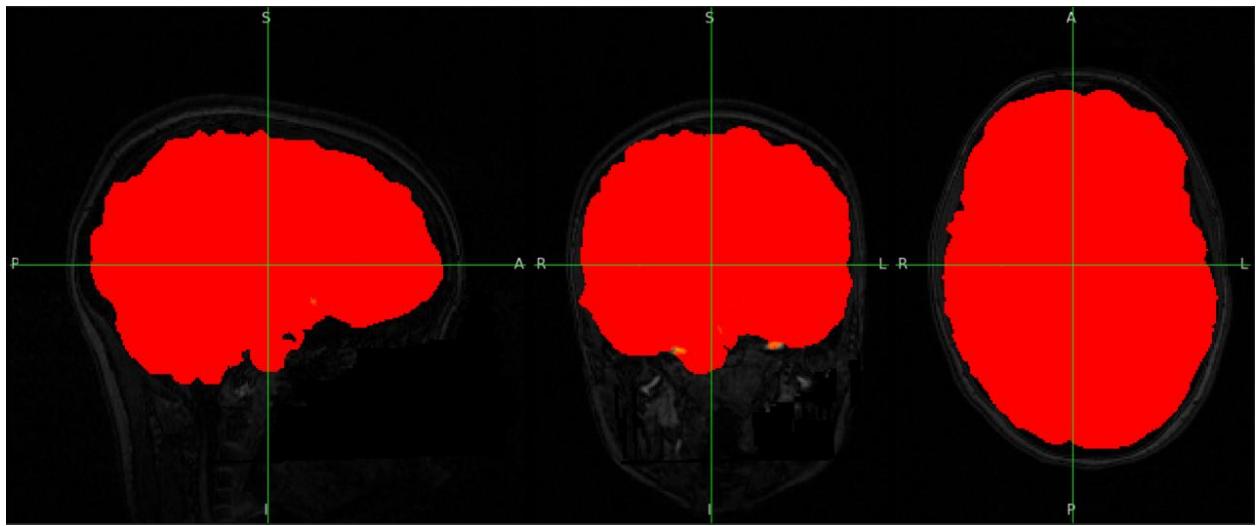
A lower threshold may enhance sensitivity by including more voxels in the analysis, potentially capturing subtle effects, but it also increases the risk of false positives. Conversely, a higher threshold may improve specificity by reducing the likelihood of false positives, but it might also lead to missed signals and reduced sensitivity.

Therefore, we should carefully consider the specific characteristics of their data, the research question, and the desired balance between sensitivity and specificity when determining the optimal fraction intensity threshold in FSL.

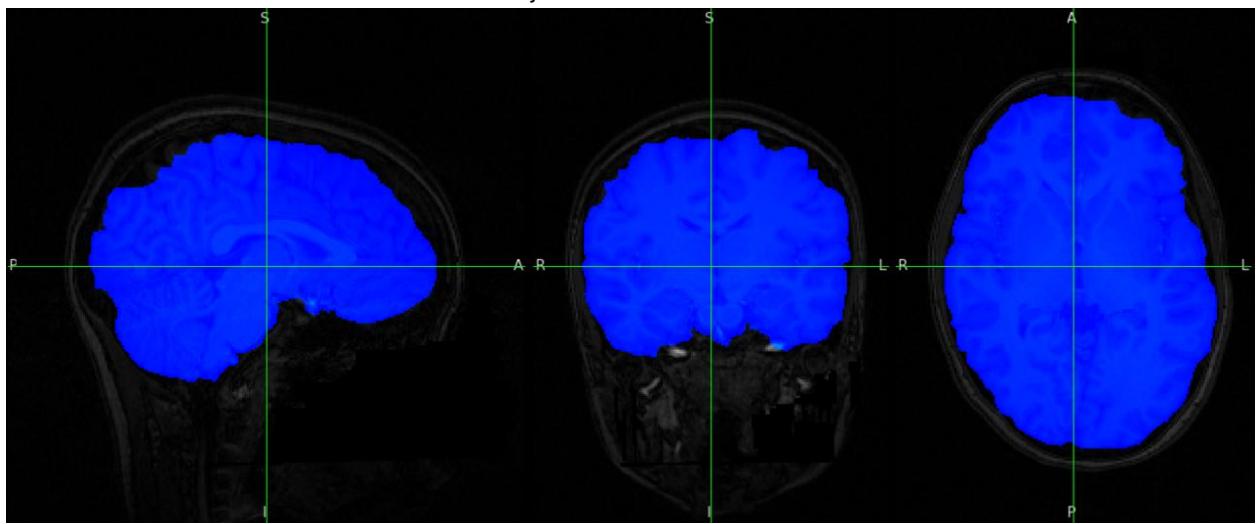
Additionally, conducting sensitivity analyses with different threshold values can provide insights into the robustness and reliability of the findings.

And with the above conclusion I will test on 5 subjects from sub15 to sub19 to find the best threshold, giving the following two examples for the criteria for threshold selection.

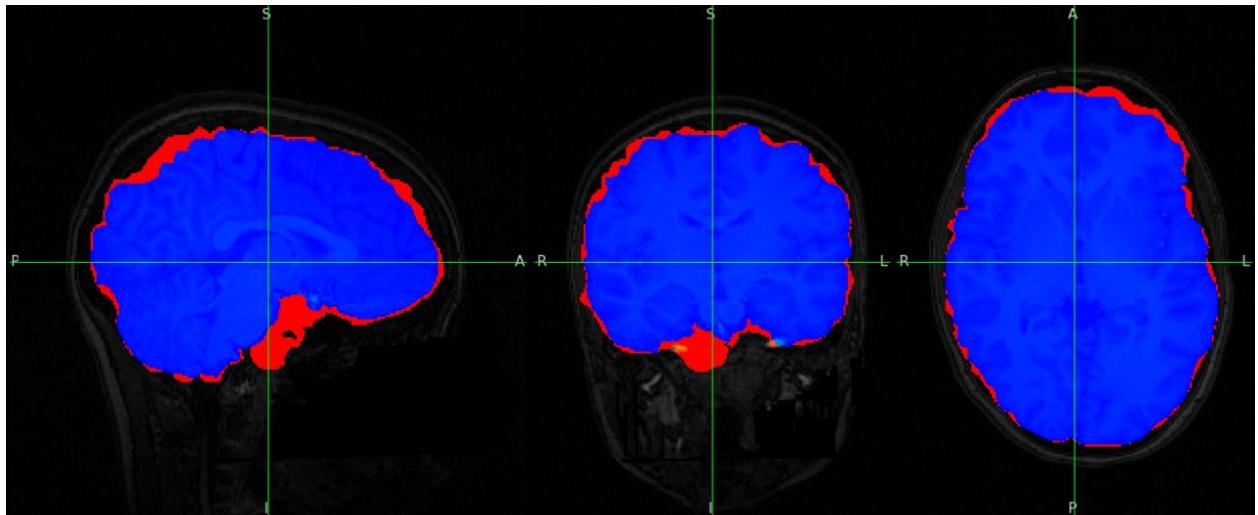
- Subject 18:



Subject 18 with threshold 0.2



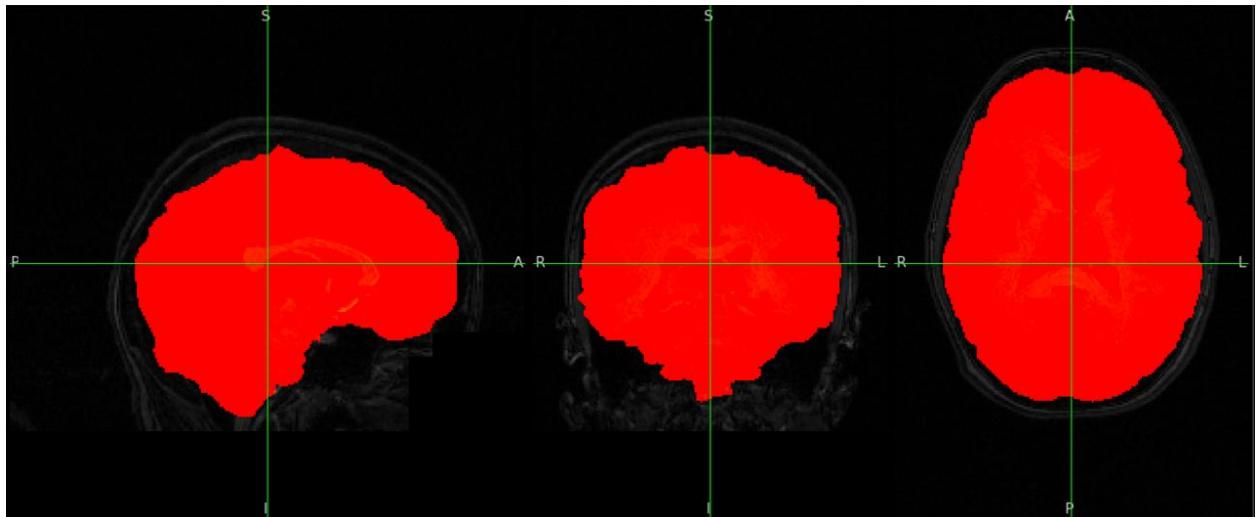
Subject 18 with threshold 0.5



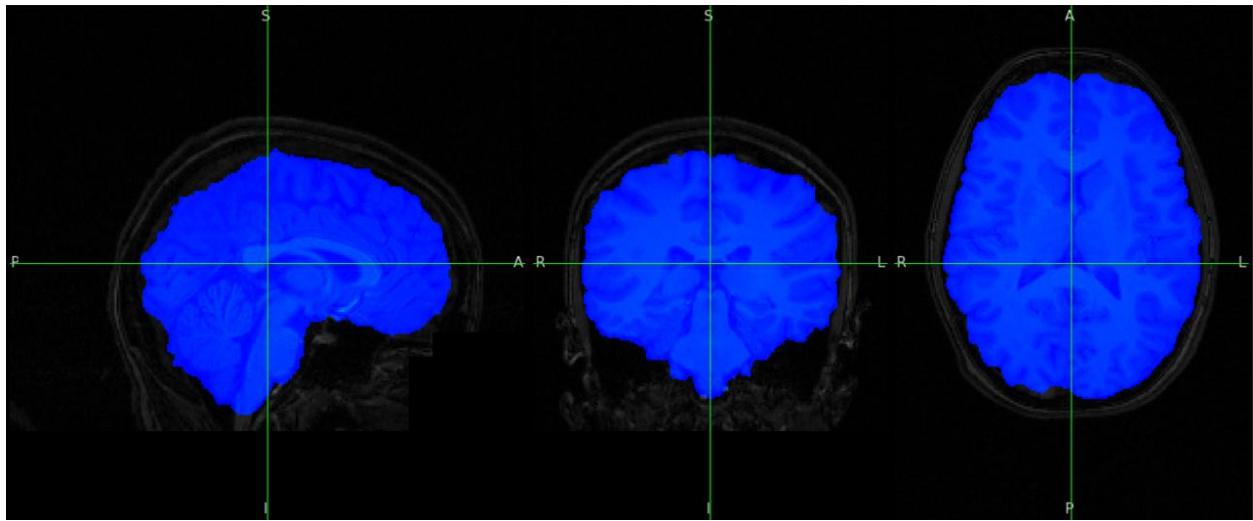
subject 18 with both extraction masks overlayed on the anatomical image

Observations on subject 18 are that using threshold of 0.2 (blue) is worse than with 0.5 (red) as it leaves a big part of the brain bottom part that the difference is well observed in the image of both masks overlayed showing the blue mask neglecting big part of the brain bottom and top.

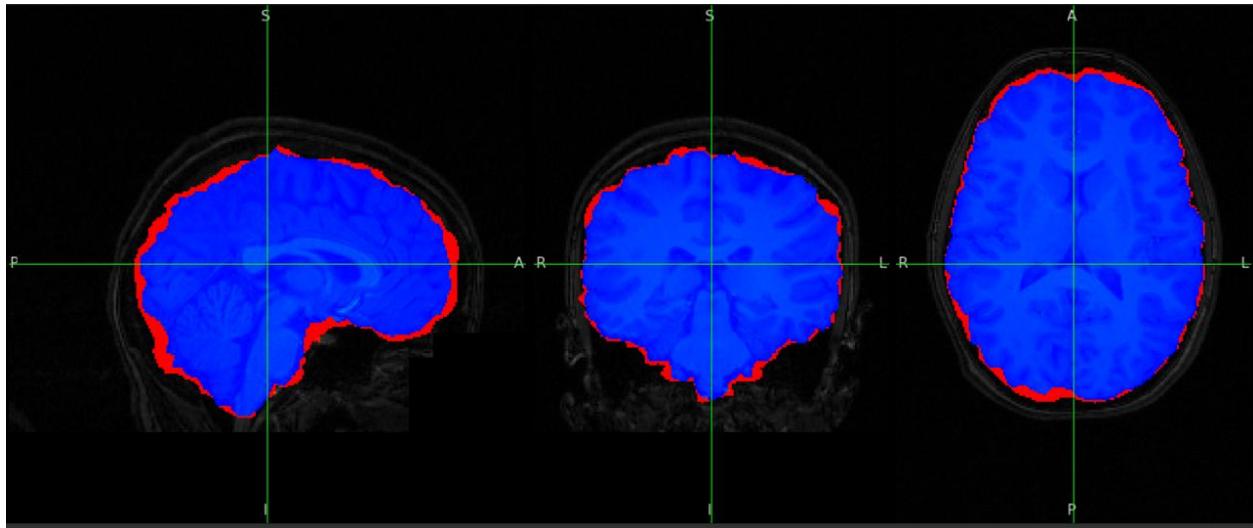
- Subject 19:



Subject 19 with threshold 0.2



Subject 19 with threshold 0.5



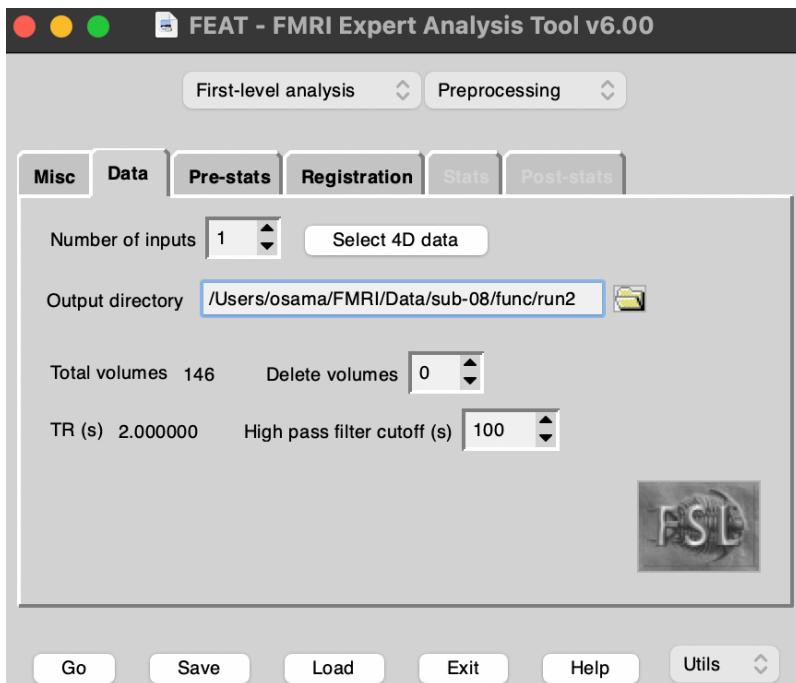
subject 18 with both extraction masks overlayed on the anatomical image

Observations on subject 19 are that using threshold of 0.2 (blue) is better as it shape the brain better leaving excess parts of skull and non-brain that got taken using threshold of 0.5 (red).

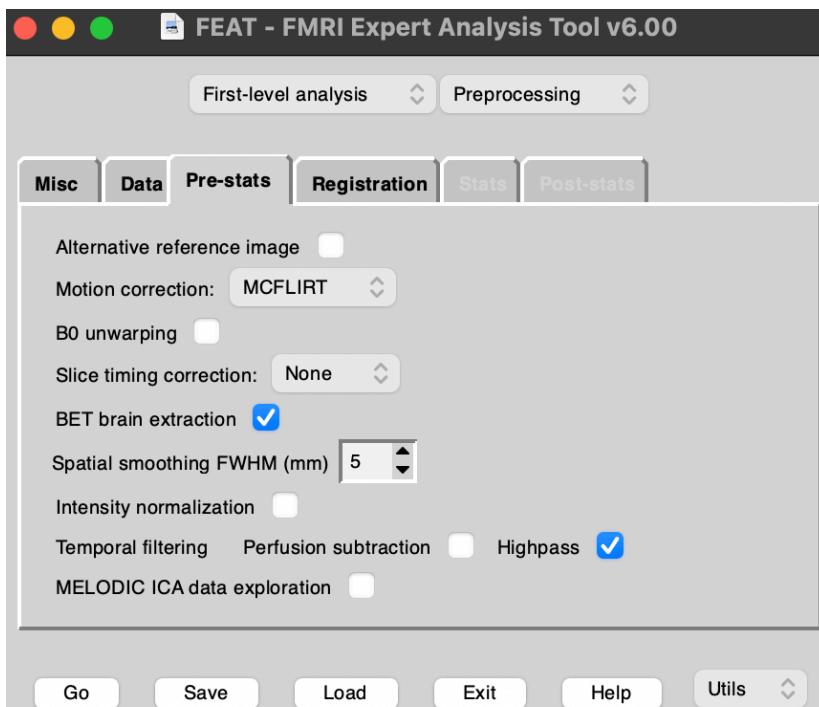
2-FEAT FMRI Analysis

FEAT (fMRI Expert Analysis Tool) is a crucial component of FSL, dedicated to the analysis of functional MRI data. It provides us with a user-friendly interface to perform various tasks essential for conducting robust and insightful fMRI analyses. FEAT enables preprocessing of fMRI data, including motion correction, spatial smoothing, and high-pass filtering to remove noise and artifacts. It facilitates statistical modeling of the data through the General Linear Model (GLM), allowing us to identify regions of the brain that exhibit task-related activity. FEAT also supports the creation of complex experimental designs, incorporating factors such as block designs, event-related designs, and parametric modulations. Moreover, FEAT offers visualization tools to interpret and communicate analysis results effectively. Overall, it streamlines the fMRI analysis process, empowering us to uncover meaningful insights into brain function and connectivity.

- Exercise 2
 - Preprocessing run 2 of the functional data using the FEAT GUI involves selecting the file "sub-08_task-flanker_run2.nii.gz" from the func directory and specifying the output directory as "run2". Ensure that the Preprocessing option is selected from the dropdown menu and keep all other settings the same as when you analyzed run 1. Once the preprocessing is completed, conducting the same quality checks as run 1. This includes examining various aspects such as motion correction, slice timing correction, spatial smoothing, and intensity normalization to ensure that the preprocessing steps were applied correctly and that the data quality is adequate for subsequent analysis. Additionally, I inspected the resulting preprocessed images visually using FSLeyes to verify that the preprocessing has effectively addressed any artifacts and enhanced the signal-to-noise ratio in the functional data. This ensures the reliability and validity of the data for further analysis and interpretation.

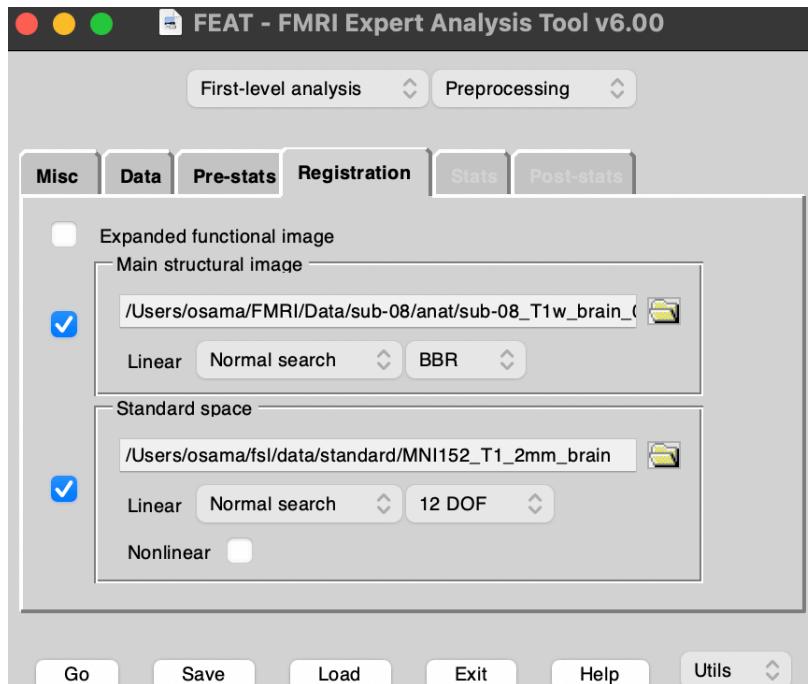


Start with selection 4D data of subject 8 and specifying the output directory to run2. Showing that the imaging was done using 146 volumes with TR equal to 2 sec.



Specifying Pre-Stats leaving it the same as run 1 and choosing MCFLIRT for motion correction for its proven effectiveness in mitigating motion artifacts while preserving data quality. Its advanced algorithms ensure accurate alignment of functional MRI volumes, reducing the impact of subject

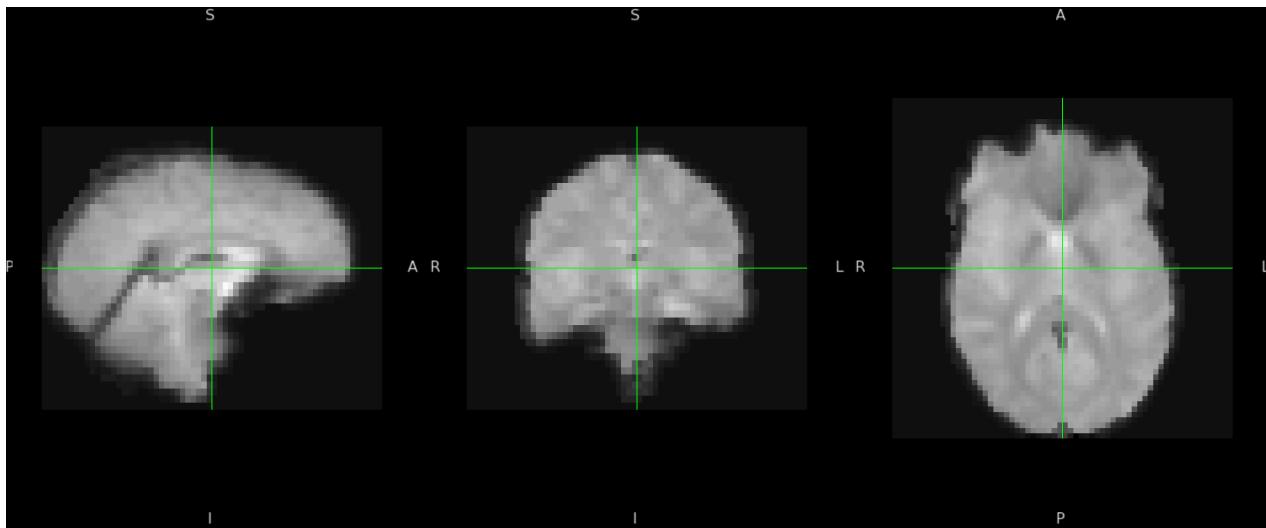
motion on subsequent analyses. Additionally, MCFLIRT's computational efficiency allows for timely processing of large datasets, making it an ideal choice for motion correction in neuroimaging studies.



Specifying the main structure image for registration to provide a high-quality anatomical reference for aligning functional data. This ensures accurate spatial correspondence between functional and structural images, enhancing the precision of subsequent analyses such as co-registration and normalization. Using a reliable structural image with the right mask detected previously from BET as the reference facilitates the identification of brain regions and improves the registration's robustness, ultimately enhancing the interpretability and reliability of the results.

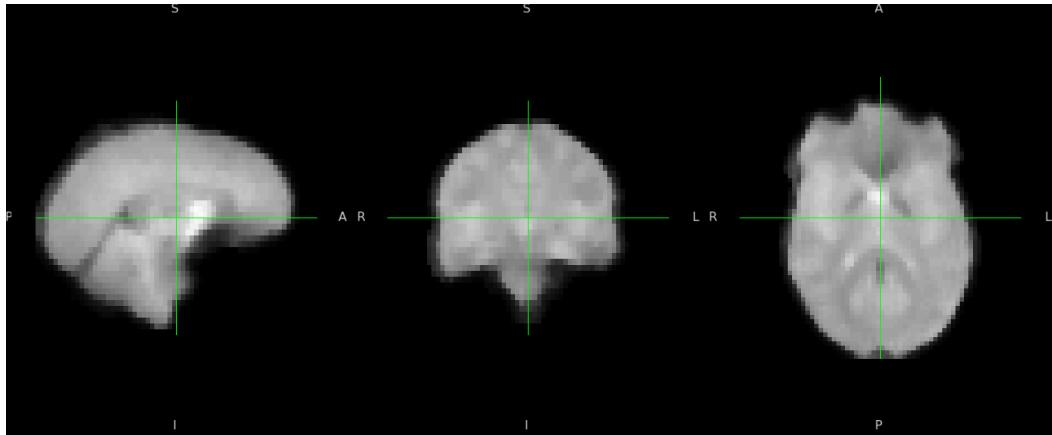
Also using MNI152 for registration to standardize the spatial coordinates of brain data across different subjects and studies. By aligning functional and structural images to the MNI template, we establish a common reference space that enables direct comparison and integration of findings across individuals and research cohorts. This registration process facilitates group-level analyses, allowing for the aggregation of data and the identification of consistent patterns or differences in brain activity or structure across populations. Additionally, registration to the MNI space enables the utilization of standardized anatomical atlases and templates for region-of-interest analyses, enhancing the interpretability and generalizability of neuroimaging results.

Results



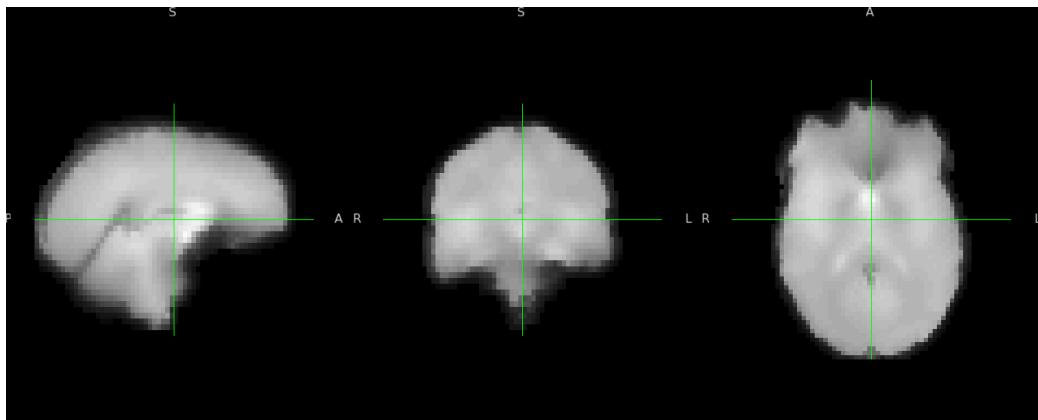
Result of filtering using the previous pre-processing settings on subject08.

- Exercise 3
 - Upon reprocessing run 1 with a 3mm smoothing kernel, I would expect the resulting preprocessed functional data to exhibit smoother images compared to the original data, retaining finer details while reducing noise. Conversely, running the analysis with a 12mm smoothing kernel would lead to more heavily smoothed data, sacrificing some finer details for overall smoother images. When loading the filtered images from each analysis into FSLeyes, I observed that the data processed with the 3mm kernel retains more fine-grained details and sharper features, while the data processed with the 12mm kernel appears more blurred and smoother overall. This comparison provides insights into the impact of different smoothing kernel sizes on the appearance and quality of preprocessed functional MRI data.



Run1 on kernel size of 3

Showing running run1 on preprocessing with kernel size of 3 showing that it retain fine details while reducing noise



Run1 on kernel size of 12

While running it on preprocessing with kernel size of 12 it become more blurred and smoothed which may lead to lose of detailed informations.

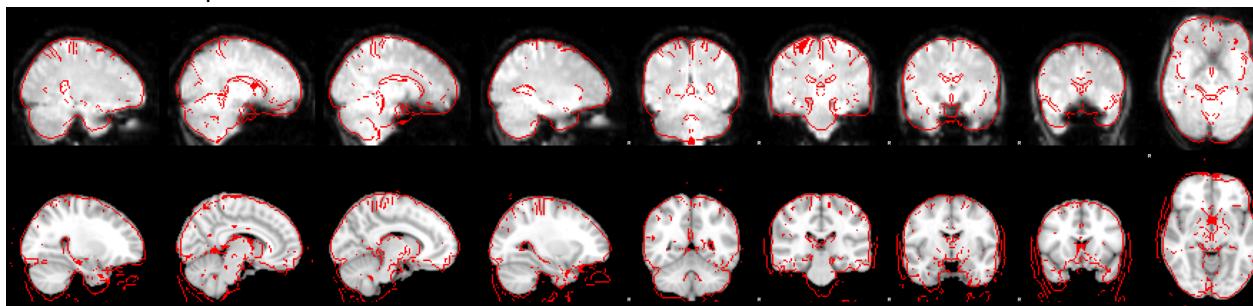
- Exercise 4 and 5

Registration and normalization checking

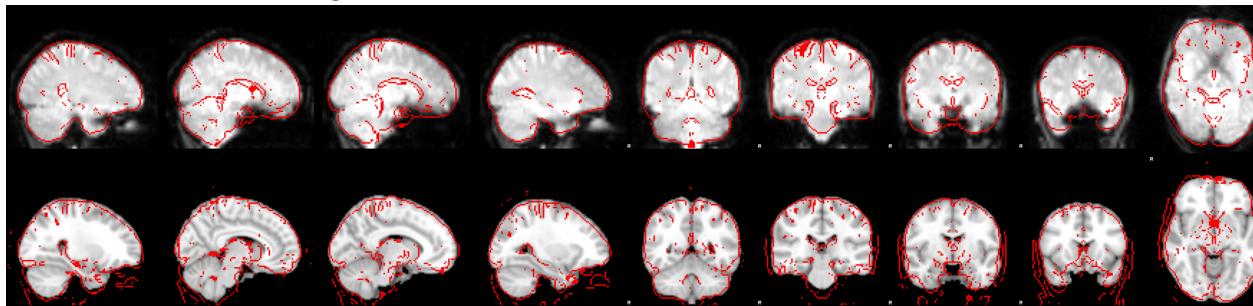
Registration and normalization involve aligning neuroimaging data to a common reference space for comparison and analysis. Checking and choosing different degrees of freedom (DOF) in registration refers to adjusting the flexibility of the transformation model used to align images, balancing accuracy and computational efficiency. Lower DOF may suffice for rigid transformations, while higher DOF allow for more complex deformations, accommodating variations in anatomy or pathology. The choice of DOF should consider the specific characteristics of the data and the desired balance between accuracy and computational resources.

Benefits of each DOF

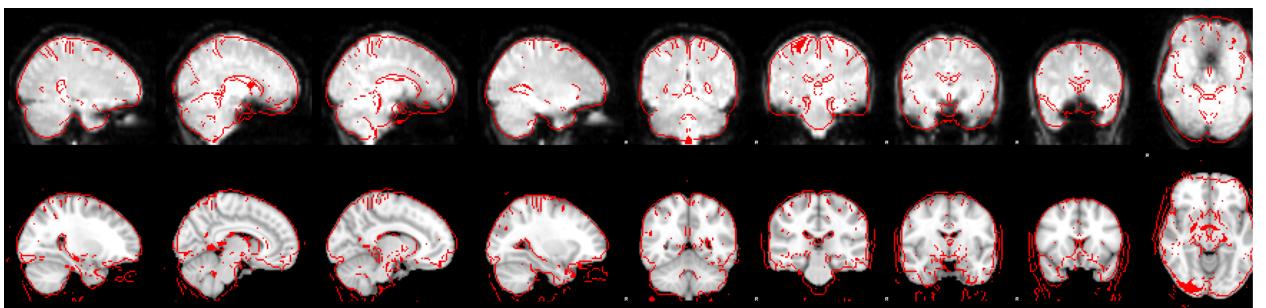
- 3 DOF Registration:
 - This method typically involves only translation in three dimensions.
 - It's the most basic form of registration and is often used when the images to be registered are already quite similar in terms of size and orientation.
 - It's fast but may not be sufficient for accurately aligning images with more complex differences



- 12 DOF Registration:
 - This method includes translation, rotation, and scaling in three dimensions, adding more degrees of freedom compared to 3 DOF.
 - It allows for more flexible alignment, accommodating for differences in size and shape between the images being registered.
 - 12 DOF registration tends to be more accurate than 3 DOF registration and is often used when images exhibit moderate differences in orientation and size.

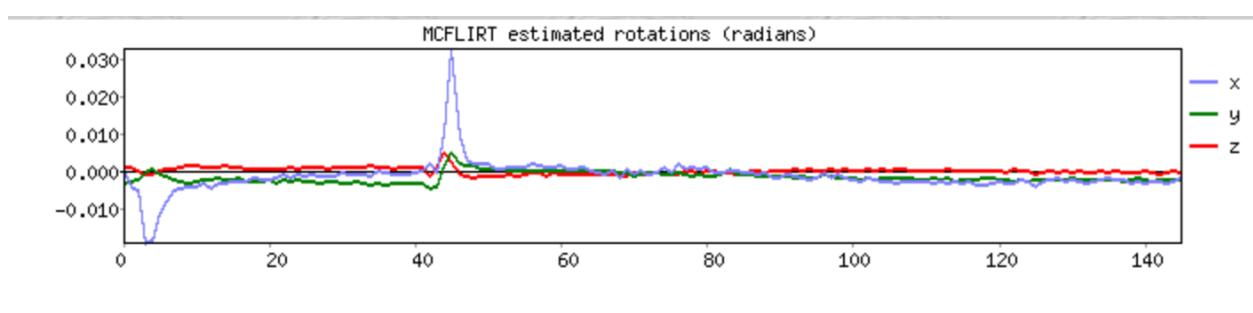


- BBR (Boundary-Based Registration):
 - BBR is a more advanced registration method offered in FSL.
 - Instead of relying solely on intensity-based registration, BBR utilizes the boundaries between tissue types in the images to guide the registration process.
 - It tends to be more robust and accurate, especially when dealing with images that have significant differences in intensity due to factors like varying contrast or image artifacts.
 - BBR is particularly useful for aligning structural images (e.g., T1-weighted images) where the tissue boundaries are clearly defined
 - It's slow but gets the most accurate aligning images with more complex differences

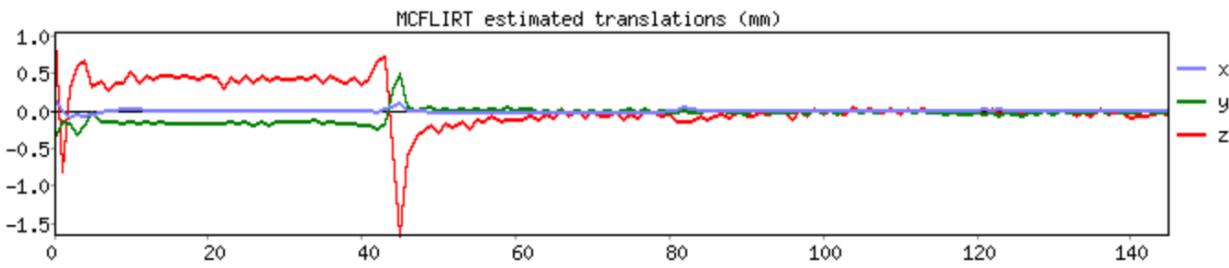


- In summary, the main differences between using 3 DOF, 12 DOF, and BBR registration methods in FSL lie in their flexibility, accuracy, and robustness. While 3 DOF registration is fast but less accurate, 12 DOF registration offers more flexibility and accuracy, and BBR provides the highest level of accuracy and robustness, especially when dealing with images with significant intensity differences or complex anatomical structures. The choice of method depends on the specific characteristics of the images being registered and the level of accuracy required for subsequent analysis.

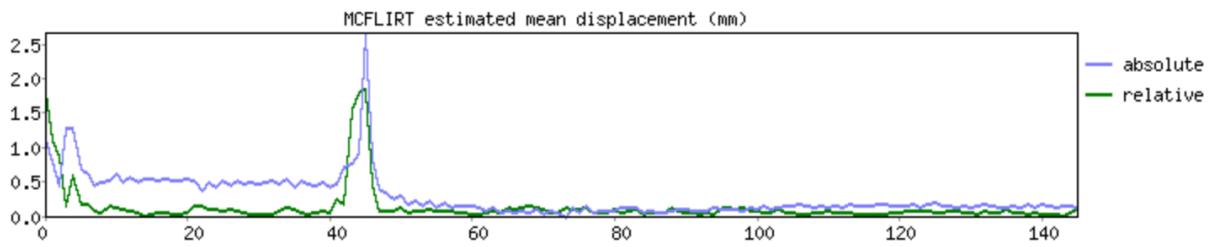
Motion Correction



The estimated rotations MCFLIRT analysis reveals rotational motion patterns, with the highest rotation observed in the x-direction reaching 0.03 radians. This rotational motion parameter offers valuable insights into the stability of the imaging acquisition session, assisting in evaluating potential sources of variability within the acquired data.



The findings from the motion analysis indicate a substantial translational movement along the z-axis, exceeding 1.5 mm. This displacement is greater than the size limit present in single voxel of the MNI standard space, raising significant concerns regarding potential effects on acquired data.



The mean displacement analysis indicates a maximum relative displacement of 2.0 millimeters and an absolute displacement of around 2.5 millimeters and with these high values they may lead to concern about the motion artifacts in the imaging session as this metric serves as a comprehensive indicator of the overall movement within the dataset, providing crucial insights into the extent of subject motion during the imaging.

3-Neuroanatomy

Neuroanatomy, the study of the nervous system's structure and organization, delves into the intricate complexities of the brain, spinal cord, and peripheral nerves. I will specifically focus on the brain and its major regions. Understanding the brain's anatomy provides insights into how it controls bodily functions, behaviors, and cognitive processes while also showing the corresponding part as a highlight in the MNI space using FSL.

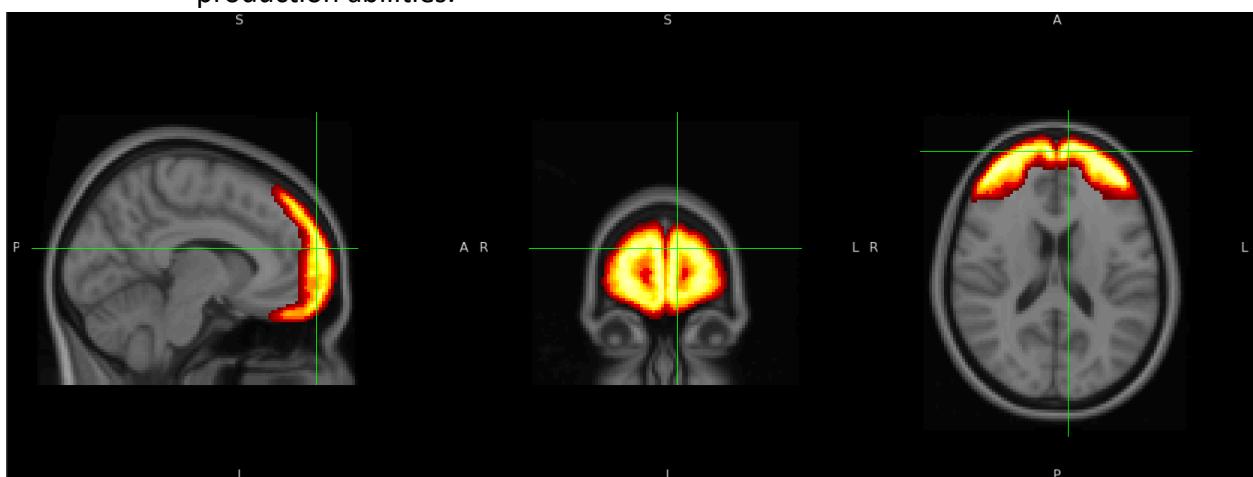
- Major Brain Regions

- Cerebrum :

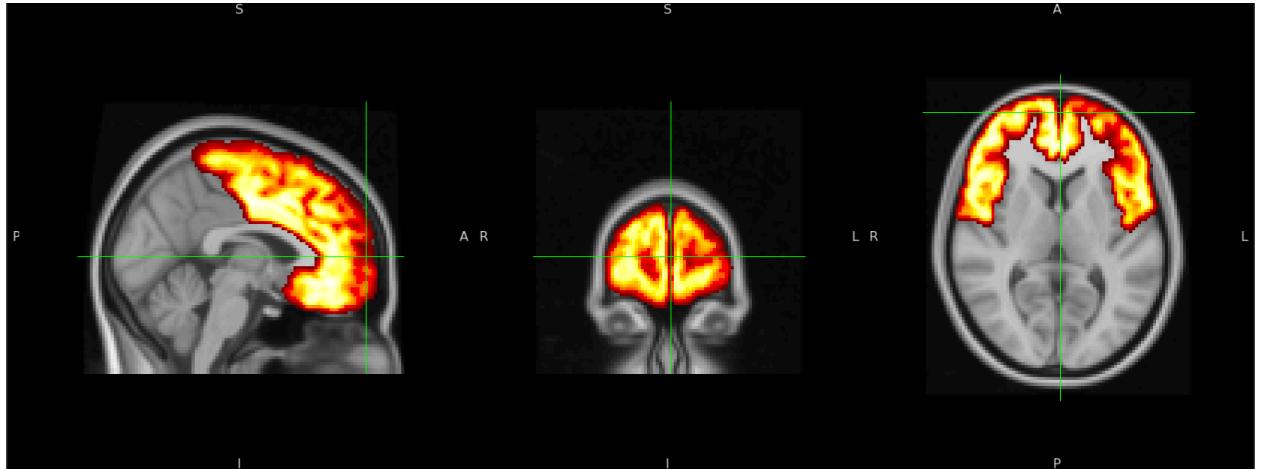
The cerebrum (front of brain) comprises gray matter (the cerebral cortex) and white matter at its center. The largest part of the brain (80% of the brain), the cerebrum initiates and coordinates movement and regulates temperature. Other areas of the cerebrum enable speech, judgment, thinking and reasoning, problem-solving, emotions and learning. Other functions relate to vision, hearing, touch and other senses.

- Frontal lobe:

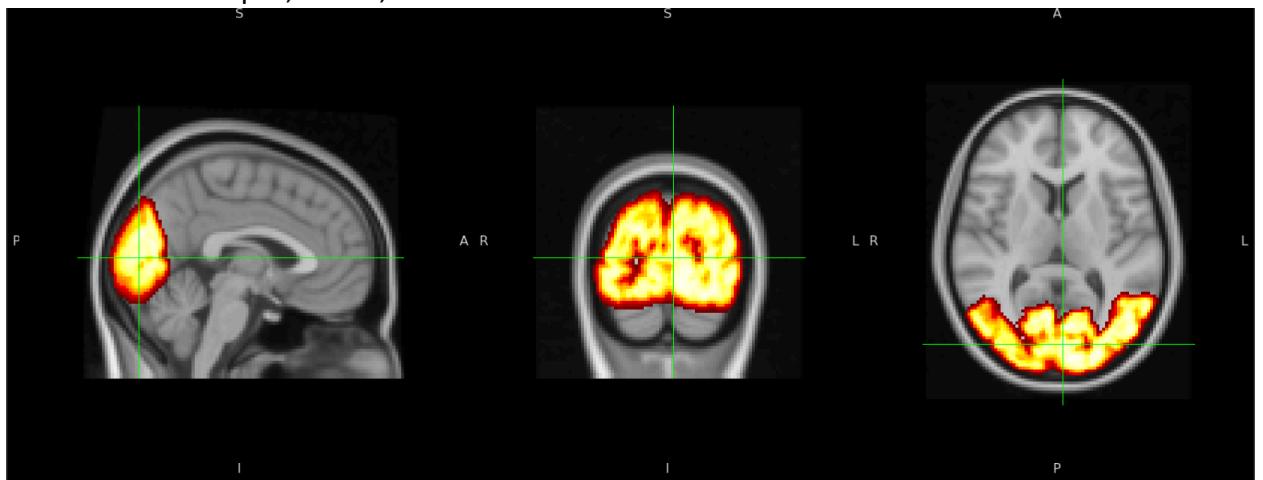
Positioned at the front of the head, represents the brain's largest lobe. It plays pivotal roles in shaping personality traits, facilitating decision-making processes, and coordinating movements. Additionally, olfactory recognition often engages regions within the frontal lobe. Notably, the frontal lobe harbors Broca's area, a critical region linked with speech production abilities.



- **Parietal lobe:**
the middle part of the brain, it plays a crucial role in object recognition and spatial perception, enabling individuals to understand their body's position relative to surrounding objects. Moreover, the parietal lobe is instrumental in processing pain and tactile sensations throughout the body. Within the parietal lobe lies Wernicke's area, which facilitates the comprehension of spoken language within the brain.



- **Occipital lobe:**
Located at the back of the brain, the occipital lobe is primarily responsible for processing visual information, including the perception of shapes, colors, and movement.



- **Temporal lobe:**

Situated on the sides of the brain, the temporal lobes play pivotal roles in various cognitive functions, including short-term memory formation, speech processing, recognition of musical rhythm, and to some extent, the perception of smell.



- **Pituitary gland:**

Referred to as the "master gland," the pituitary gland is a small, pea-sized structure located deep within the brain, behind the bridge of the nose. Its paramount role lies in regulating the function of other glands throughout the body, overseeing the secretion of hormones from the thyroid, adrenal glands, ovaries, and testicles. The pituitary gland receives chemical signals from the hypothalamus through its stalk and blood supply, facilitating the intricate coordination of hormonal activity within the body.

Can't find the atlas that contain it's information.

- **Hypothalamus:**

Situated above the pituitary gland, the hypothalamus serves as a vital control center for numerous physiological processes. It communicates with the pituitary gland through chemical messages, dictating its function. Among its diverse functions, the hypothalamus regulates body temperature, orchestrates sleep-wake cycles, modulates hunger and thirst sensations, and contributes to certain aspects of memory and emotion.

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- **Amygdala:**

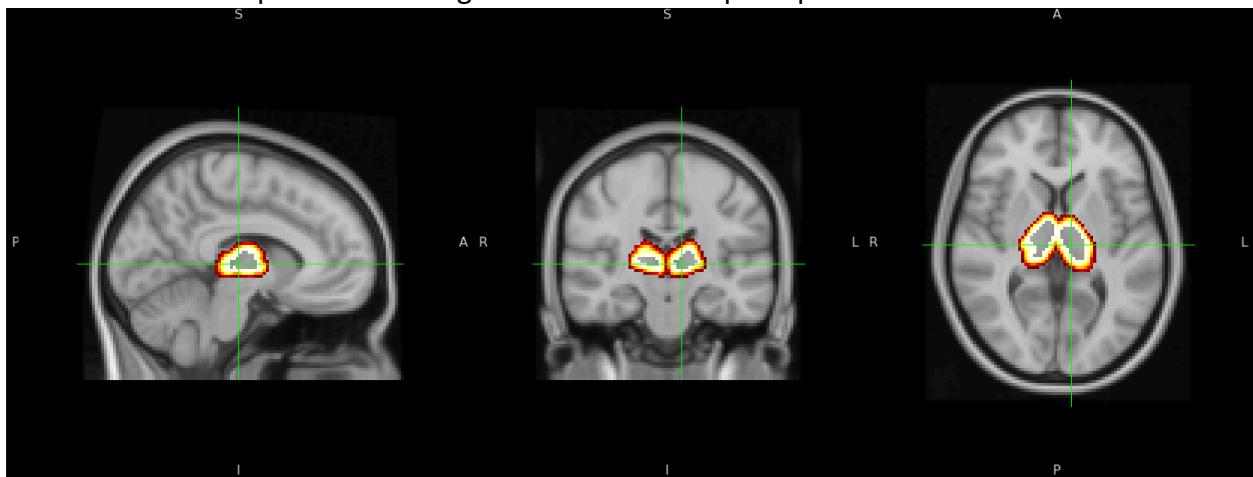
Nestled beneath each hemisphere of the brain, the amygdala is characterized by its small, almond-like shape. Integral to the limbic system, these structures play essential roles in regulating emotions and

memory. Moreover, the amygdalae are intricately linked with the brain's reward system, stress response, and the activation of the "fight or flight" reaction in response to perceived threats.

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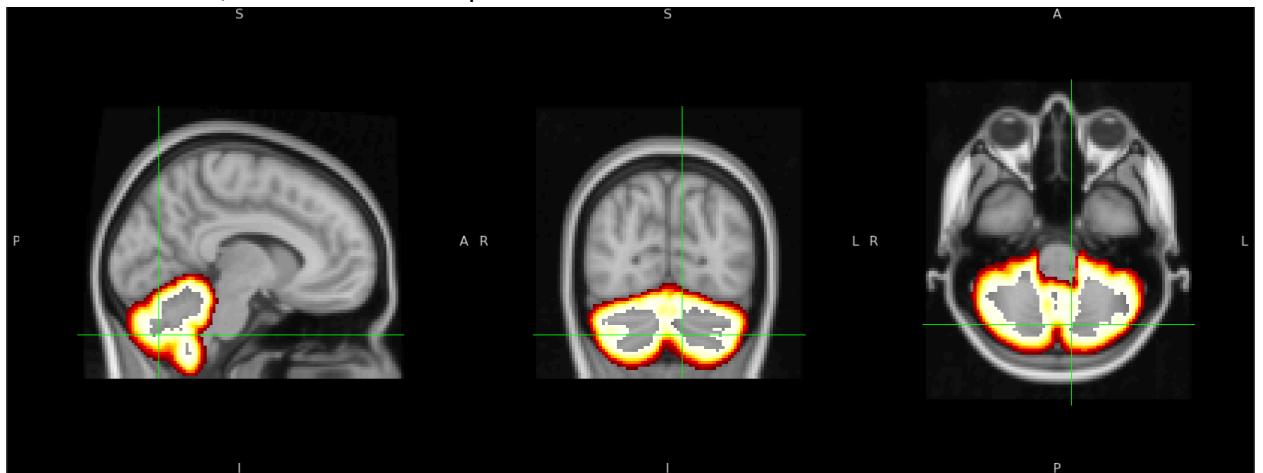
- Thalamus:

resembling two walnut-sized structures nestled within the brain's hemispheres, serves as a critical relay station for sensory and motor signals. Positioned atop the brainstem, this vital hub directs sensory information from various modalities, such as touch, taste, vision, and hearing, to the corresponding regions of the cerebral cortex for further processing. Additionally, the thalamus regulates states of consciousness, including alertness and sleep, by modulating neural activity across the brain. Its intricate network of connections facilitates the integration of sensory inputs and orchestrates responses to stimuli, making it indispensable for cognitive function and perception.



- Cerebellum:

The cerebellum ("little brain") is a fist-sized portion of the brain located at the back of the head, below the temporal and occipital lobes and above the brainstem. Like the cerebral cortex, it has two hemispheres. The outer portion contains neurons, and the inner area communicates with the cerebral cortex. Its function is to coordinate voluntary muscle movements and to maintain posture, balance and equilibrium. New studies are exploring the cerebellum's roles in thought, emotions and social behavior, as well as its possible involvement in addiction, autism and schizophrenia.

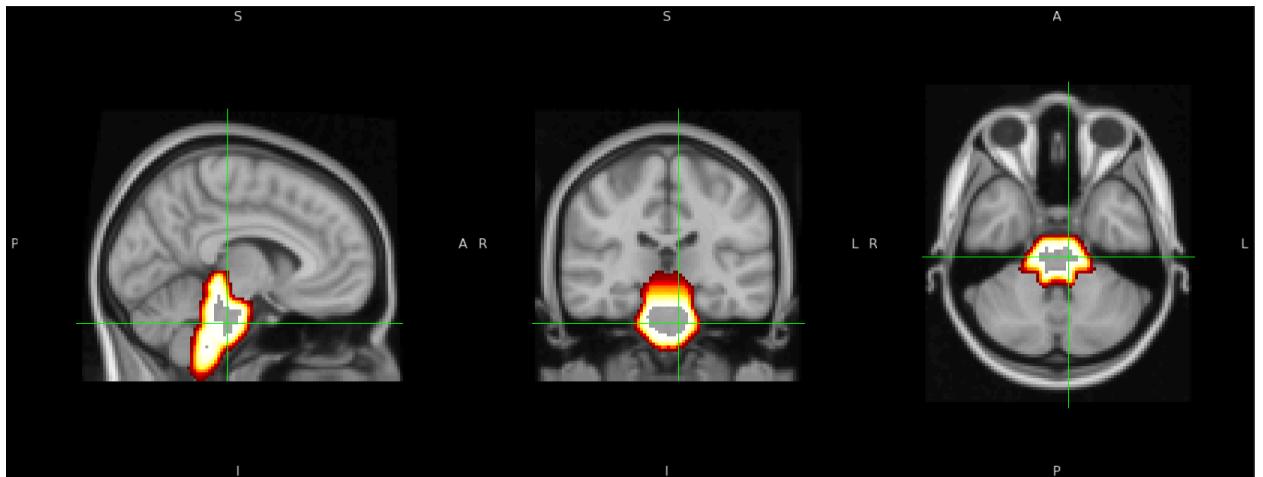


- Brainstem

The brainstem, positioned at the core of the brain, serves as the vital link between the cerebrum and the spinal cord. Comprising the midbrain, pons, and medulla, it orchestrates a symphony of essential functions crucial for sustaining life and facilitating bodily coordination.

- Midbrain: The midbrain, or mesencephalon, is a complex structure featuring diverse neuron clusters, nuclei, and colliculi, enabling functions from auditory processing to motor control. Notably, it houses the substantia nigra, crucial for movement and coordination.
- Pons: Named for "bridge," the pons links the midbrain and medulla, regulating activities like tear production, mastication, and facial expression.

- Medulla: Essential for survival, the medulla governs vital functions like heart rate, respiration, and reflexive actions such as sneezing and swallowing.
- Spinal Cord: Serving as a neural conduit between the brain and body, the spinal cord facilitates bidirectional communication for sensory input and motor commands.



Conclusion:

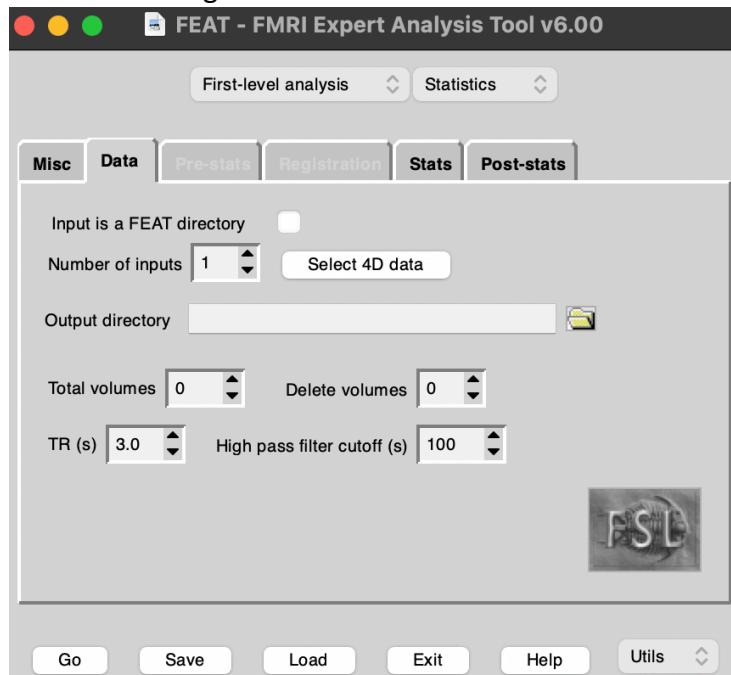
The brain's intricate parts and regions play indispensable roles in shaping our thoughts, behaviors, and bodily functions. Each component contributes uniquely to our cognition, emotions, and physiological processes. However, comprehending the exact locations and functions of these structures is a daunting task due to their complexity and the presence of fine details with specific tasks.

4-GLM Modeling

The General Linear Model (GLM) is a foundational statistical approach used in functional magnetic resonance imaging (fMRI) analysis. In fMRI, the GLM models the relationship between experimental tasks and observed brain activity. It helps identify regions responding to specific stimuli or tasks by estimating regression coefficients.

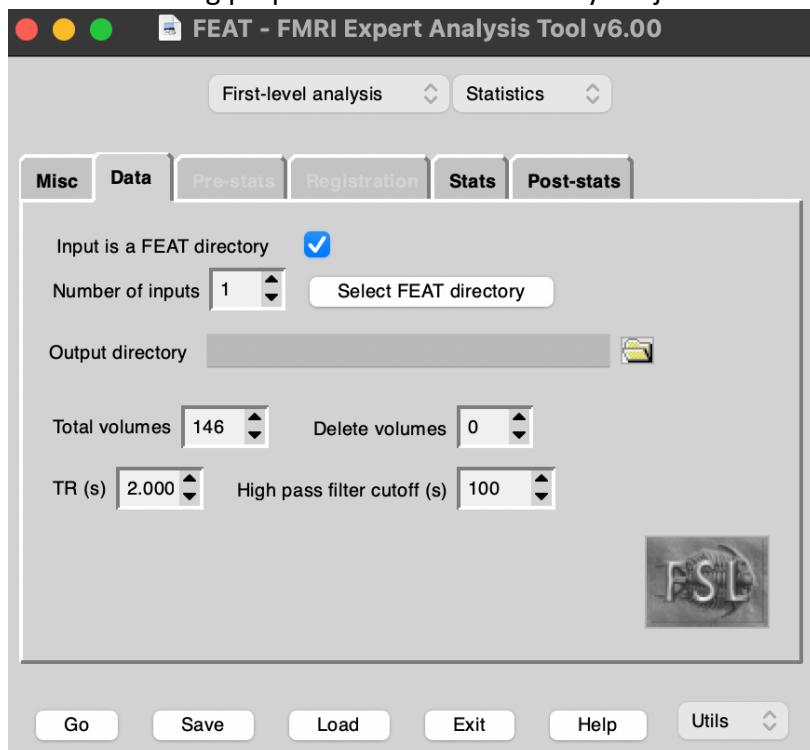
4.1 – Conducting First Level Analysis using FSL FEAT-GUI on subject 18.

4.1.1 – choosing statistics from GUI menu.



This step will ignore the preprocessing as we have done it before on 5 subjects in the previous task and will dim their options.

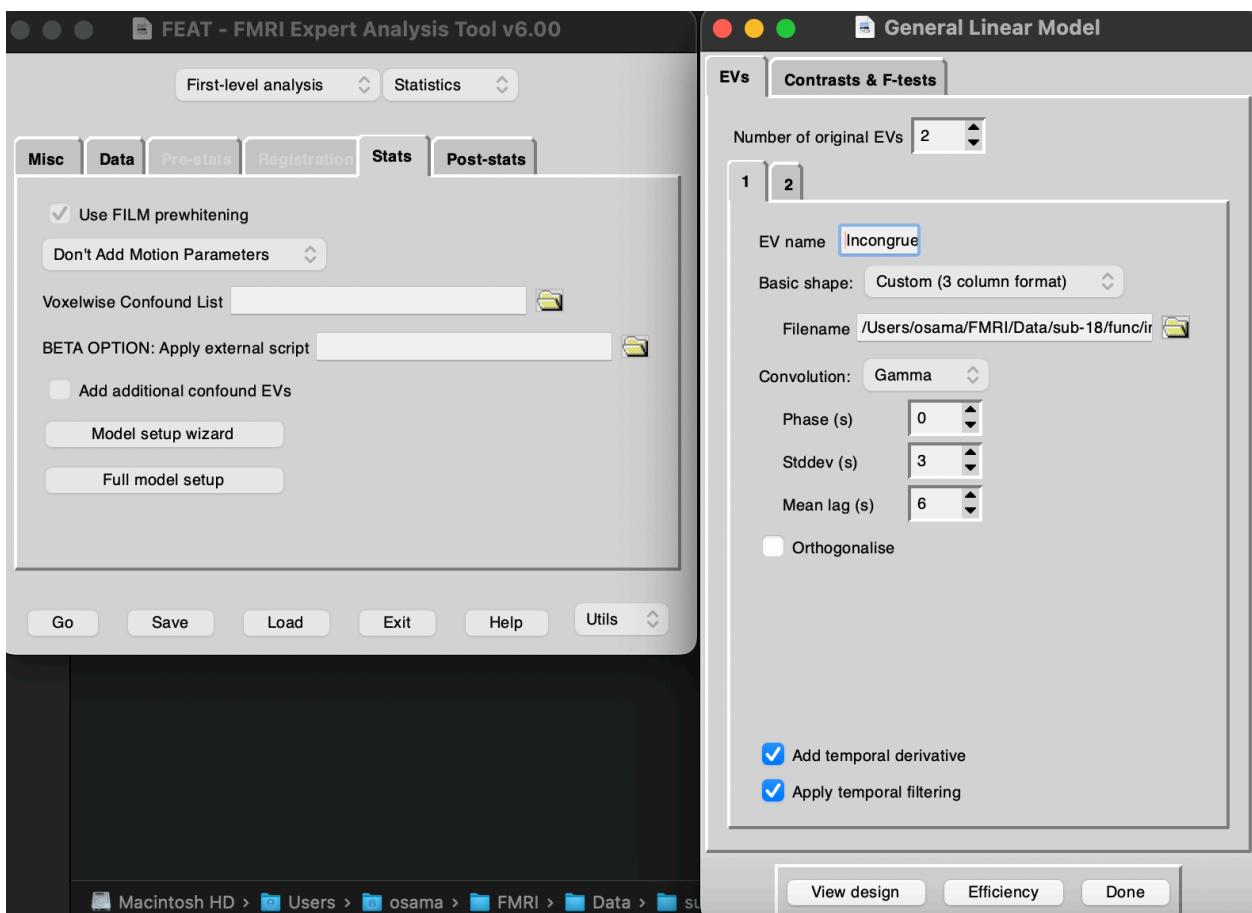
4.1.2 – Choosing preprocessed FEAT directory subject



Making sure to choose the “Input is a FEAT directory” to select the whole folder of preprocess we have done before, and select the folder by clicking the “Select FEAT directory”.

4.1.3 – Adding EVs

4.1.3.1- Creating 3 column format files from the data set by running a bash script on the event files withing the dataset, creating a txt files for each run with each regressor(class) having the first column as the timeseries, the second one to be it's duration (same as TR in our case) and the third column to be ones.



Moving to the “Stats” tab and selecting “Full model setup” we can start here defining our model, firstly by selecting the number of EVs which are our regressor where we have two in our case (Incongruent and Congruent) then selecting the 3 column file basic shape and adding the txt file extracted from the bash script run before, leaving other parameters as they are for now and doing the same with the second EV.

4.1.4 – Specifying COPE

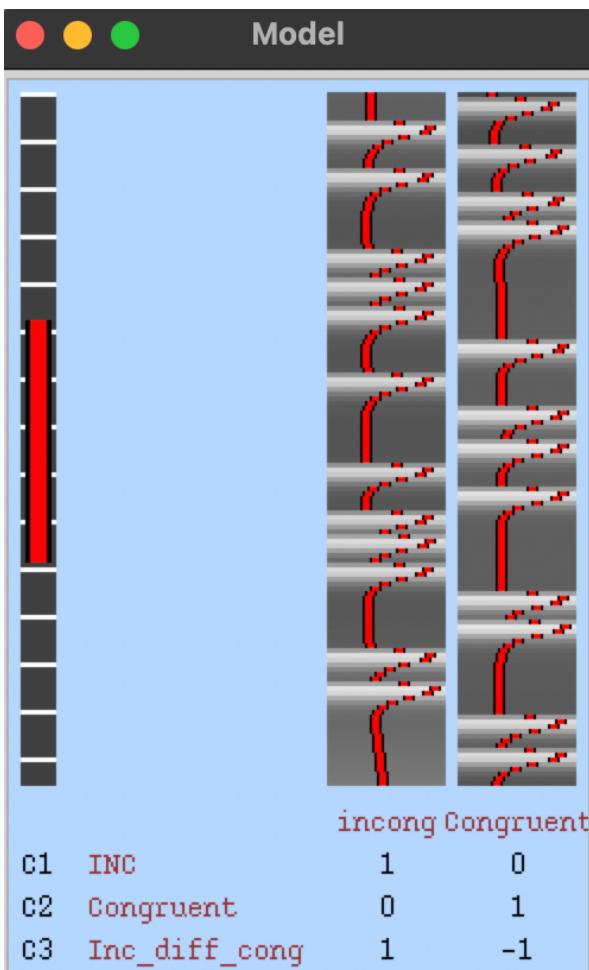
The screenshot shows the 'General Linear Model' dialog box with the 'Contrasts & F-tests' tab selected. The 'EVs' tab is also visible. The 'Setup contrasts & F-tests for' dropdown is set to 'Original EVs'. There are two spinners: 'Contrasts' set to 3 and 'F-tests' set to 0. Below these are three rows of contrast definitions:

	Paste	Title	EV1	EV2
OC1	<input checked="" type="checkbox"/>	Incongruent	1	0
OC2	<input checked="" type="checkbox"/>	Congruent	0	1.0
OC3	<input checked="" type="checkbox"/>	Inc_diff_cong	1.0	-1.0

At the bottom are three buttons: 'View design', 'Efficiency', and 'Done'.

Defining desired Contrast Of Parameter Estimates that we want to look at, here we specified one focusing on Incongruent only (1,0), one for Congruent only (0,1) and the last one for incongruent difference from congruent (1,-1). This is done by specifying 1 or 0 or -1 values for each EV.

4.1.5 – Model



GLM Model is define by the equation

$$Y = \beta * X + \epsilon$$

Where:

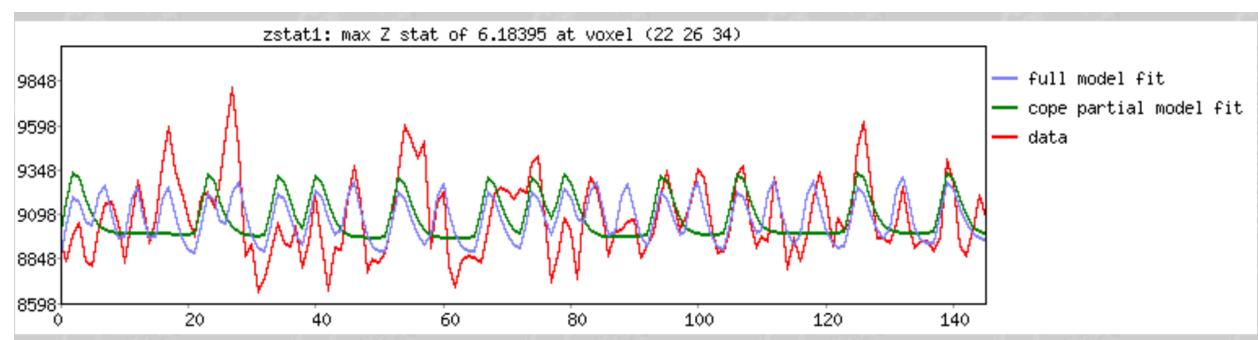
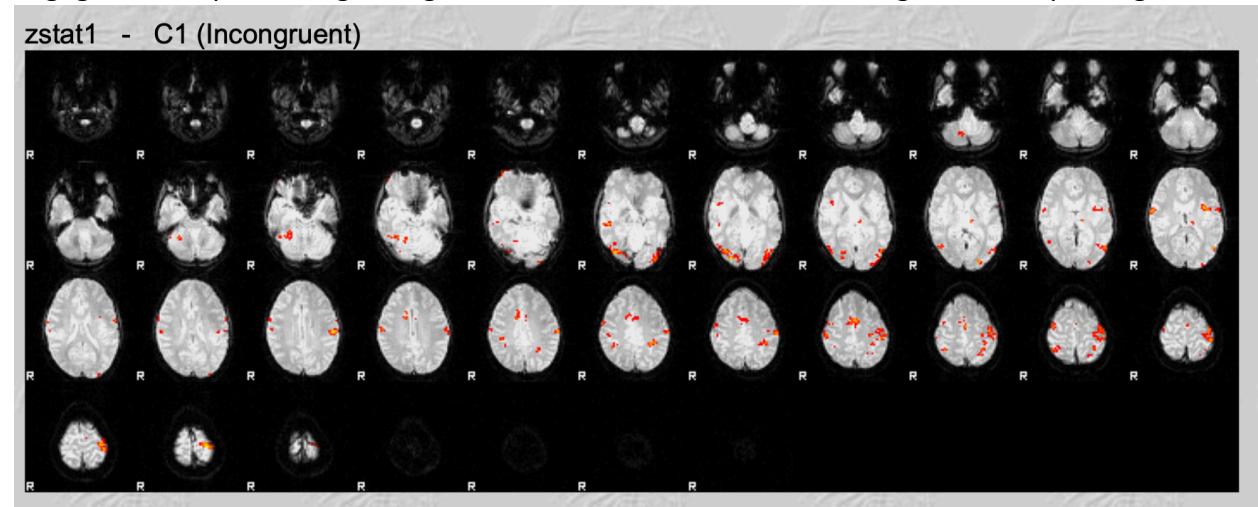
- Y : Bold time series (fMRI)
- β : Vector or Beta weights for each regressor
- X : Design Matrix (regressor) of the task
- ϵ : Vector residuals(error)

it represents the bold time series data obtained from fMRI scans, capturing brain activity over time. β denotes a vector of beta weights assigned to each regressor in the design matrix X, which represents the experimental tasks or conditions being studied created by convolution of HRF with timing onsets. The design matrix encodes the timing and characteristics of each task, allowing the model to estimate how each task affects brain activity. ϵ represents the vector of residuals or errors, accounting for variability in the fMRI signal that is not explained by the model. Essentially, this equation describes how the observed fMRI data can be explained by a combination of experimental conditions (as defined by the design matrix) and error terms.

4.2 - Output

1. C1 representing Incongruent Regressor :

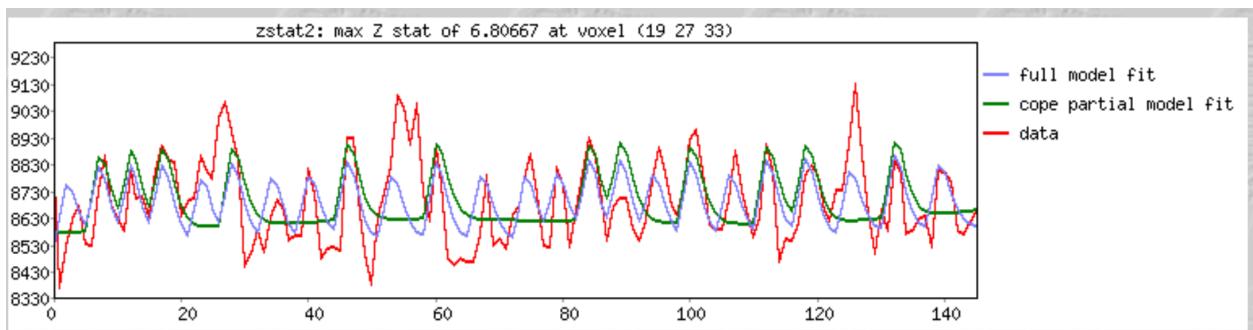
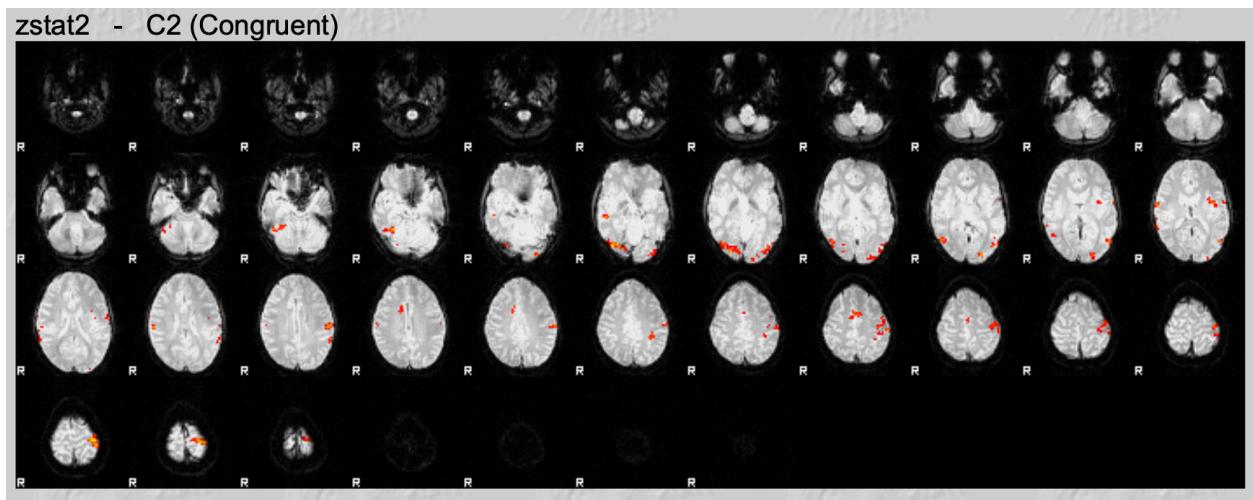
The image presented below provides a visual depiction of the activated brain regions observed during the performance of the incongruent task. In this representation, voxels within the brain exhibiting signal intensities surpassing a predetermined threshold are delineated and highlighted with red color, allowing for a clear identification of regions where neural activity significantly deviates from baseline levels. By applying a threshold to the voxel intensities, areas of robust activation are accentuated, offering insights into the neural circuits and networks engaged in the processing of cognitive conflict inherent to the incongruent task paradigm.



Timeseries Graph show the peak Z scores of 6.18395 at voxel (22 26 34)

2.C2 representing Congruent Regressor :

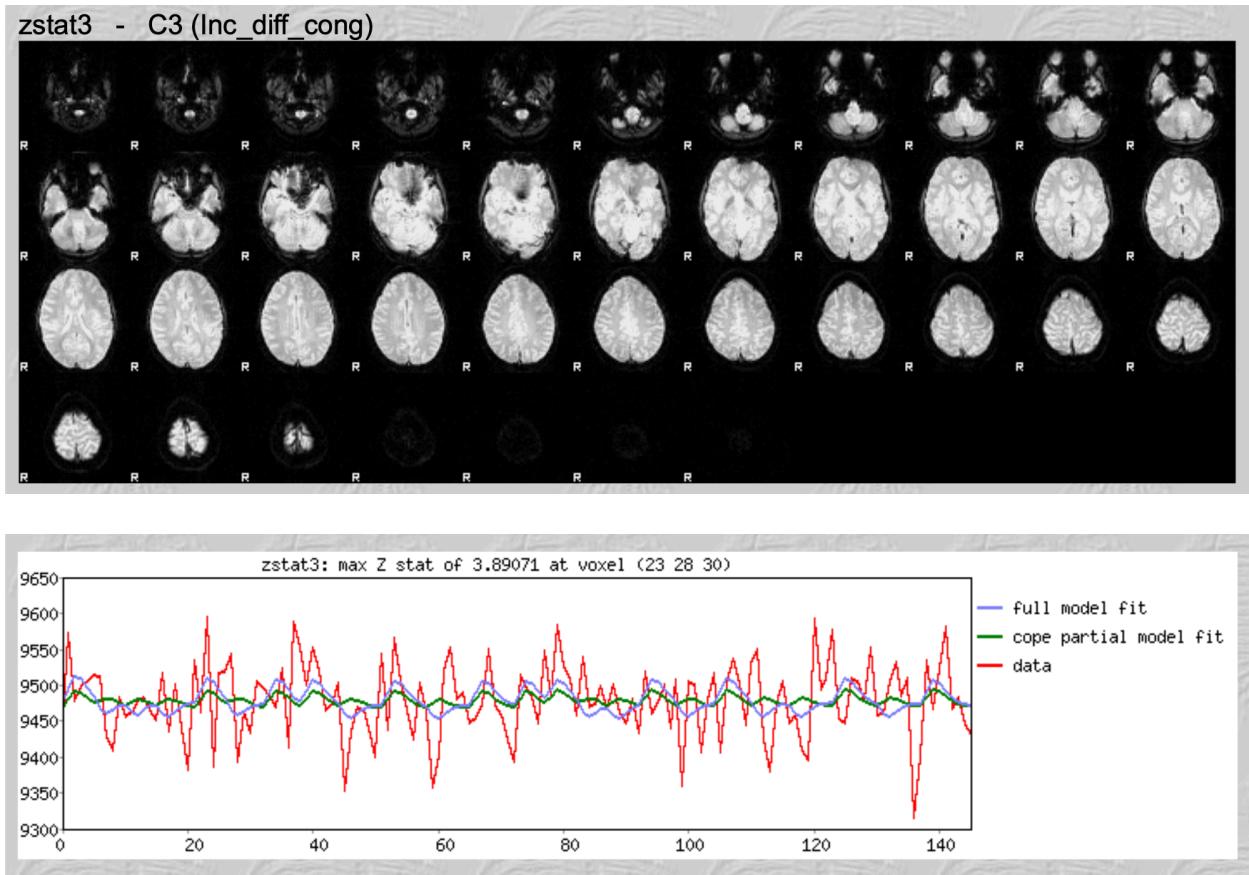
The image below highlights brain regions activated during the congruent task this time, where voxels exceeding a set intensity threshold are colored with the same principle of C1 image but with congruent task.



Timeseries Graph show the peak Z scores of 6.80667 at voxel (19 27 33)

3.C3 Incongruent – Congruent :

The image presented below showcases the difference in activated voxels between the incongruent and congruent tasks. By comparing activation patterns, it shows that there are no noticeable red highlights like previous two examples showing that maybe the parts of brain responsible for the two tasks are the same in some way resulting in canceling each other.



Timeseries Graph show the peak Z scores of 3.89071 at voxel (23 28 30)

5- 2nd-Level-Analysis

Which is the averaging together with each subject the parameter estimates and contrast estimates from the 1st-Level analyses, but first we need to run a script to create FEAT directories for all 26 subjects

- Creating FEAT directories for all the subjects
 - 1- Repeat process done for GLM modeling by specifying a subject 4D data, its BET, create its EVs and COPE and choosing its Timing files.
 - 2- Instead of going further for the GLM model by pressing “Go” we click “Save” button creating a design.fsf file for run1 which contain all the information about creating a model for run1 (for subject01 only for now)
 - 3- Repeat the same process for same subject but for run2.
 - 4- Once we finished the previous steps we would have 2 designs files each for specific run, so now we can run the following script

```
Dataset > $ Featscript.sh
1  for id in `seq -w 1 26` ; do
2      subj="sub-$id"
3      echo "====> Starting processing for $subj"
4      echo
5      cd $subj
6
7      #get a copy of the design files
8      cp ..../des_run1.fsf .
9      cp ..../des_run2.fsf .
10
11     #check for bet of f-0.2
12     if [ ! -f anat/${subj}_T1w_brainf02.nii.gz ]
13     then
14         bet2 anat/${subj}_T1w.nii.gz anat/${subj}_T1w_brainf02.nii.gz -f 0.2
15     fi
16
17     #change the subject number
18     sed -i.bak "s|sub-01|${subj}|g" des_run1.fsf
19     sed -i.bak "s|sub-01|${subj}|g" des_run2.fsf
20
21     #run feat
22     echo "====> Starting feat for run 1"
23     feat des_run1.fsf
24     echo "====> Starting feat for run 2"
25     feat des_run2.fsf
26     echo
27
28     echo "====> Finished processing of $subj"
29     echo
30
31     # Go back to the directory containing all of the subjects, and repeat the loop
32     cd ..
33 done
```

This script does the following steps:

- 1- Loop Setup: It iterates through a sequence of numbers from 01 to 26 (seq -w 1 26). The -w option pads the numbers with leading zeros, ensuring consistent formatting.
- 2- Subject Definition: Inside the loop, it sets the current subject identifier using the variable \$subj.
- 3- Processing Initialization: It prints a message indicating the start of processing for the current subject and navigates to the directory corresponding to that subject.
- 4-Design File Copying: Copies two design files (des_run1.fsf and des_run2.fsf) from a parent directory into the subject's directory.
- 5-Brain Extraction: Checks if a certain brain-extracted file bet2 command with a specific fractional intensity threshold (-f 0.2).
- 6-Subject Number Replacement: It modifies the copied design files (des_run1.fsf and des_run2.fsf) to replace occurrences of "sub-01" with the current subject identifier, this allow us to modify the desing parameter for each subject.
- 7-FEAT Analysis: It initiates FEAT analysis for both runs using the modified design files.
- 8-Processing Completion: Prints a message indicating the completion of processing for the current subject.
- 9-Loop Continuation: Moves back to the parent directory to prepare for the next iteration of the loop.

- Renaming Feat directories

After creating the Feat directories they are named by their subject names like "sub-08_task-flanker_run-1_bold.feat" which is the default at my situation for the naming but I need it to have a consistent naming to allow for the next steps of getting all the files paths, so I created a renaming script to do so,

```
Dataset > $ rename.sh
 1  for id in `seq -w 1 26` ; do
 2    subj="sub-$id"
 3    echo "====> Starting renaming of $subj"
 4    echo
 5    cd $subj
 6    cd func
 7
 8    if [ -e "${subj}_task-flanker_run-1_bold.feat" ];then
 9      mv "${subj}_task-flanker_run-1_bold.feat" "run1.feat"
10  fi
11
12  if [ -e "${subj}_task-flanker_run-2_bold.feat" ];then
13    echo "====> RENAMEEIN $subj"
14    echo
15    mv "${subj}_task-flanker_run-2_bold.feat" "run2.feat"
16  fi
17
18  cd ..
19  cd ..
20 done
21
```

This script simply enter every subject directory and then going to the “func” directory and changing the FEAT directory names to run1 and run2.

- Get all the directories path

This is the last step preparing for the higher-level analysis which is getting the paths for all the directories we have made which is a simple line in the terminal of the main directory as follows:

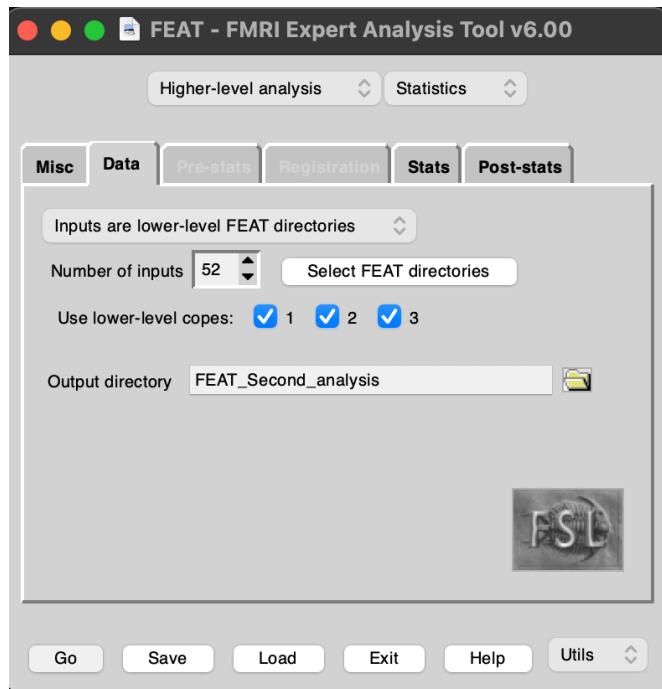
```
ls -d $PWD/sub-??/func/run*
```

this line just printout all the directory paths for both runs in each subject which we now take a copy for it for the next step.

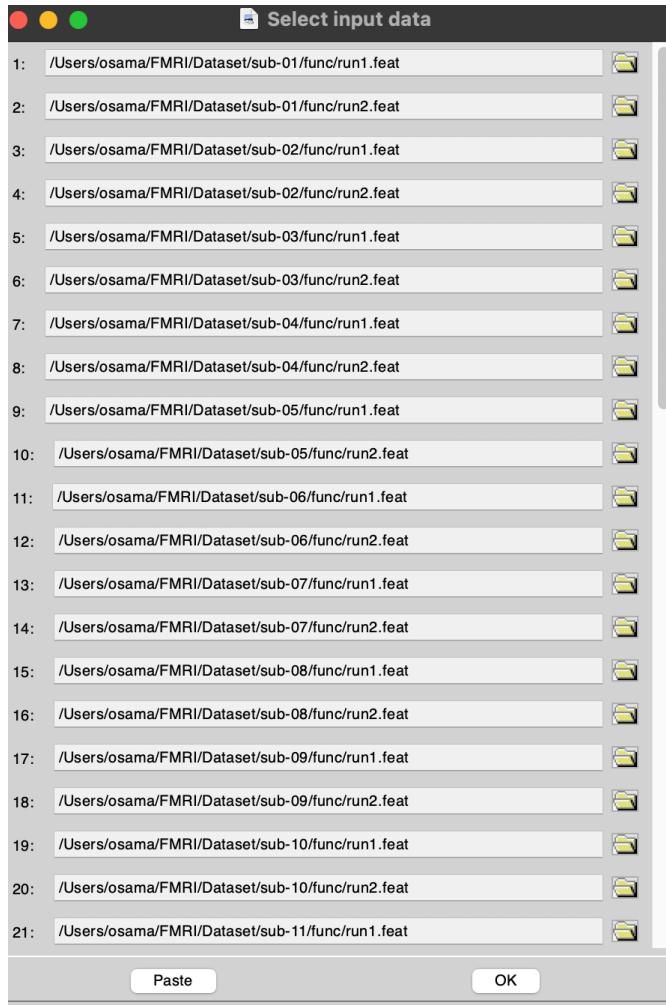
- Higher-Level Analysis

a- start by opening FSL and choosing from the top-left drop down menu “Higher-level analysis” instead of “First-level analysis”

b- Select number of inputs to be 52 which indicates the 2 runs for the 26 subject in our dataset.



c- Click on select FEAT directories and press on paste then just paste the paths that we have copied from previous step.



d- Go to stats tab and select “Fixed effects” from the drop down menu then head to the full model setup button, which will open a new window of the GLM setup

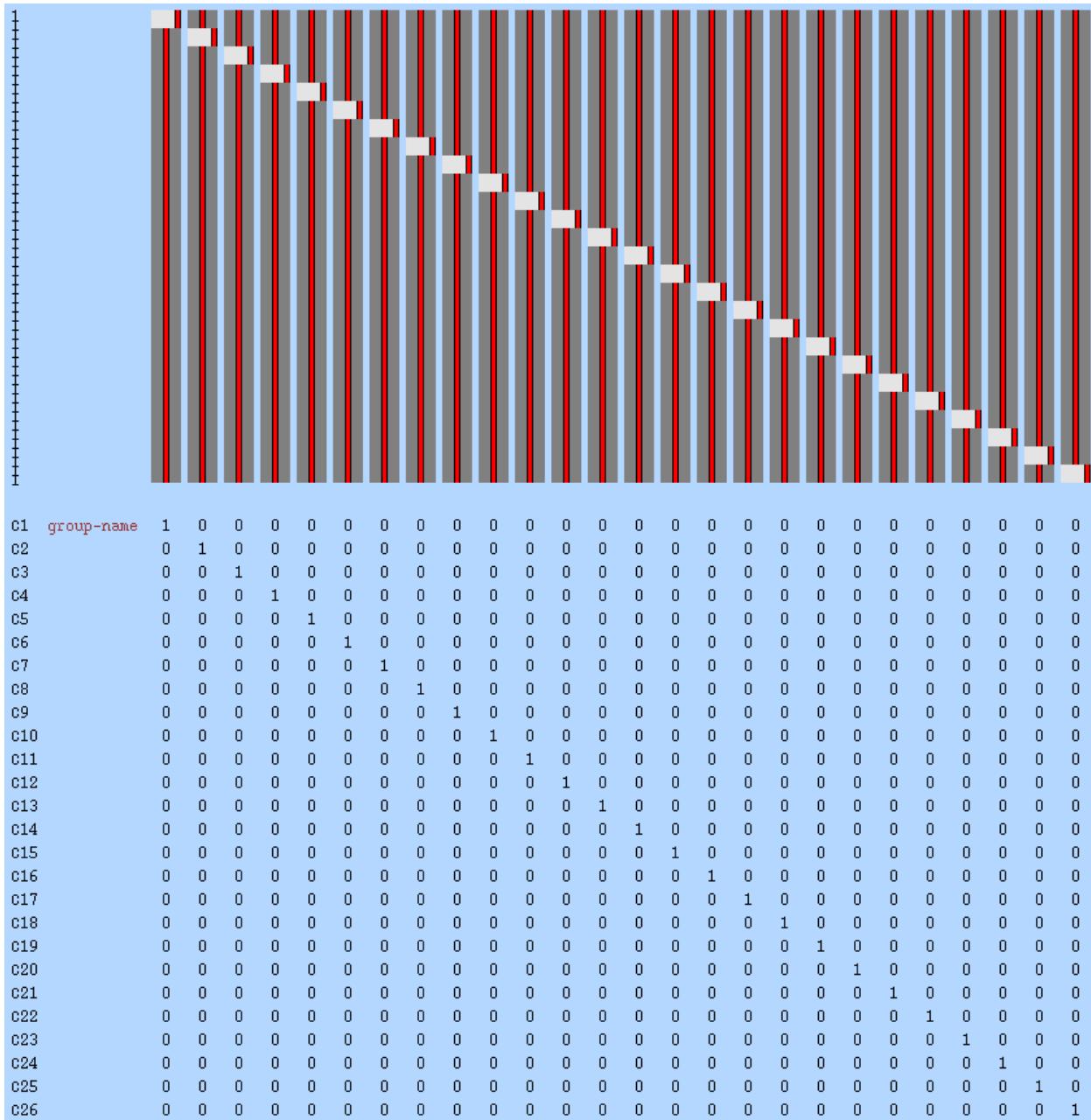
- Select number of main EVs to 26 which are our 26 subject
- Start filling the columns with 1 for the crossponding rows of each subject 2 runs, where each row indicate specific run for a specific subject like input 1&2 are run1 and run2 for subject 01 and so on, this is the view of the table after filling it

	Group	EV1	EV2	EV3	EV4	EV5	EV6	EV7	EV8	EV9	EV10	EV11	EV12	EV13	EV14	EV15	EV16	EV17	EV18	EV19	EV20	EV21	EV22	EV23
Input 1	1	1	0.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 2	1	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 3	1	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 4	1	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 5	1	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 6	1	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 7	1	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 8	1	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 9	1	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 10	1	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 11	1	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 12	1	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 13	1	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 14	1	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 15	1	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 16	1	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	
Input 17	1	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	
Input 18	1	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	
Input 19	1	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	
Input 20	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	
Input 21	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	
Input 22	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	
Input 23	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	
Input 24	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	
Input 25	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	
Input 26	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	
Input 27	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	

- After that we head to Contrasts & F-tests tap to choose our COPE, start by selecting Contrasts to be 26 and start filling 1 in the table in a diagonal pattern indicating that we want each contrast for each EV we specified before.

		General Linear Model																											
EVs		Contrasts & F-tests																											
Contrasts		26	F-tests		0																								
Paste	Title	EV1	EV2	EV3	EV4	EV5	EV6	EV7	EV8	EV9	EV10	EV11	EV12	EV13	EV14	EV15	EV16	EV17	EV18	EV19	EV20	EV21	EV22	EV23	EV24	EV25	EV26		
C1	group-name	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
C2		0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
C3		0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
C4		0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
C5		0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
C6		0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
C7		0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
C8		0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
C9		0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
C10		0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
C11		0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
C12		0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0	0		
C13		0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0	0		
C14		0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0	0		
C15		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0	0		
C16		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0	0		
C17		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0	0		
C18		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0	0		
C19		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0	0		
C20		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0	0		
C21		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0	0		
C22		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0	0		
C23		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0	0		
C24		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0	0		
C25		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0		
C26		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.0		

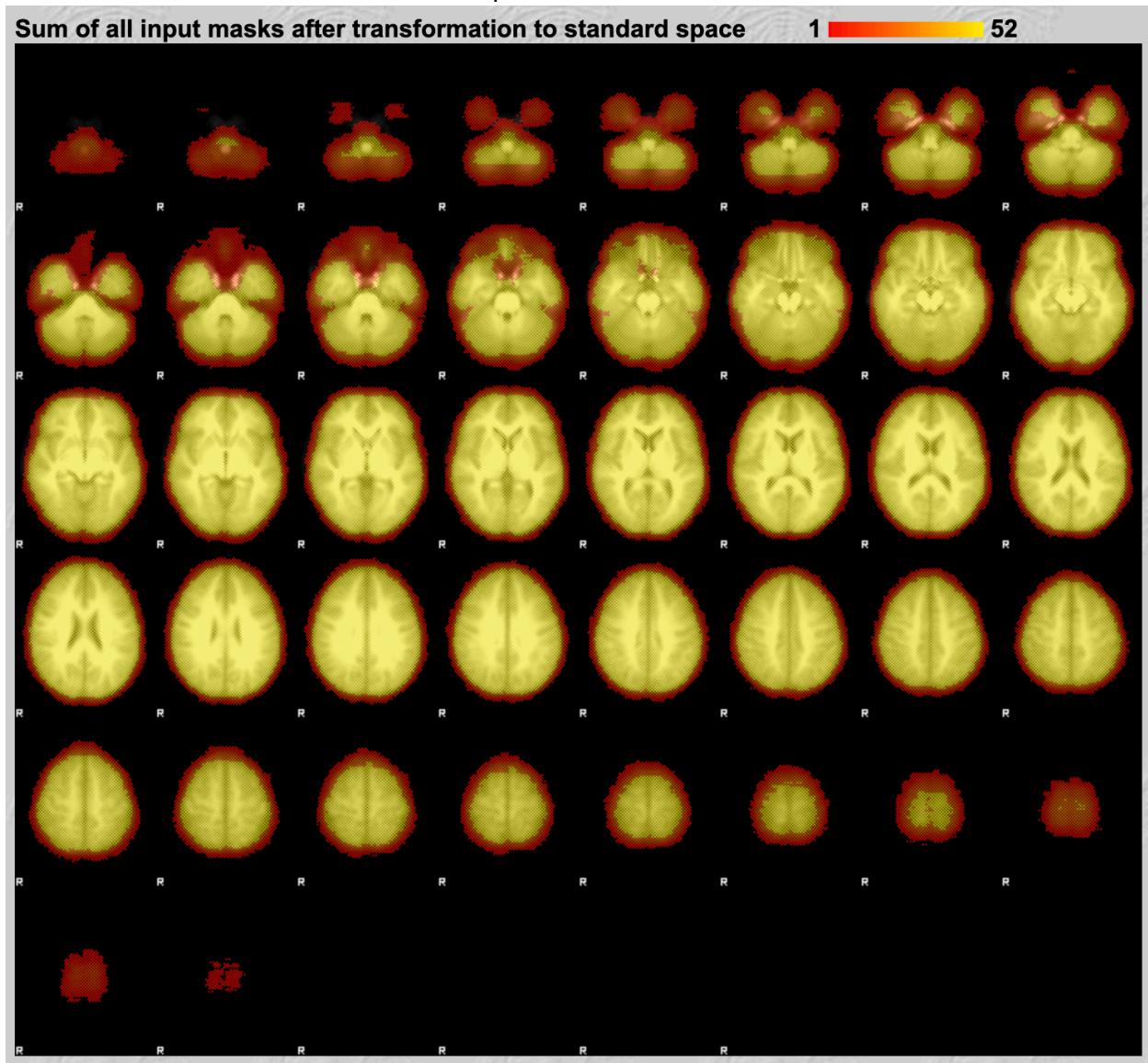
- After that we click on “Done” giving us the final GLM design matrix for all 26 subject averaging their runs.

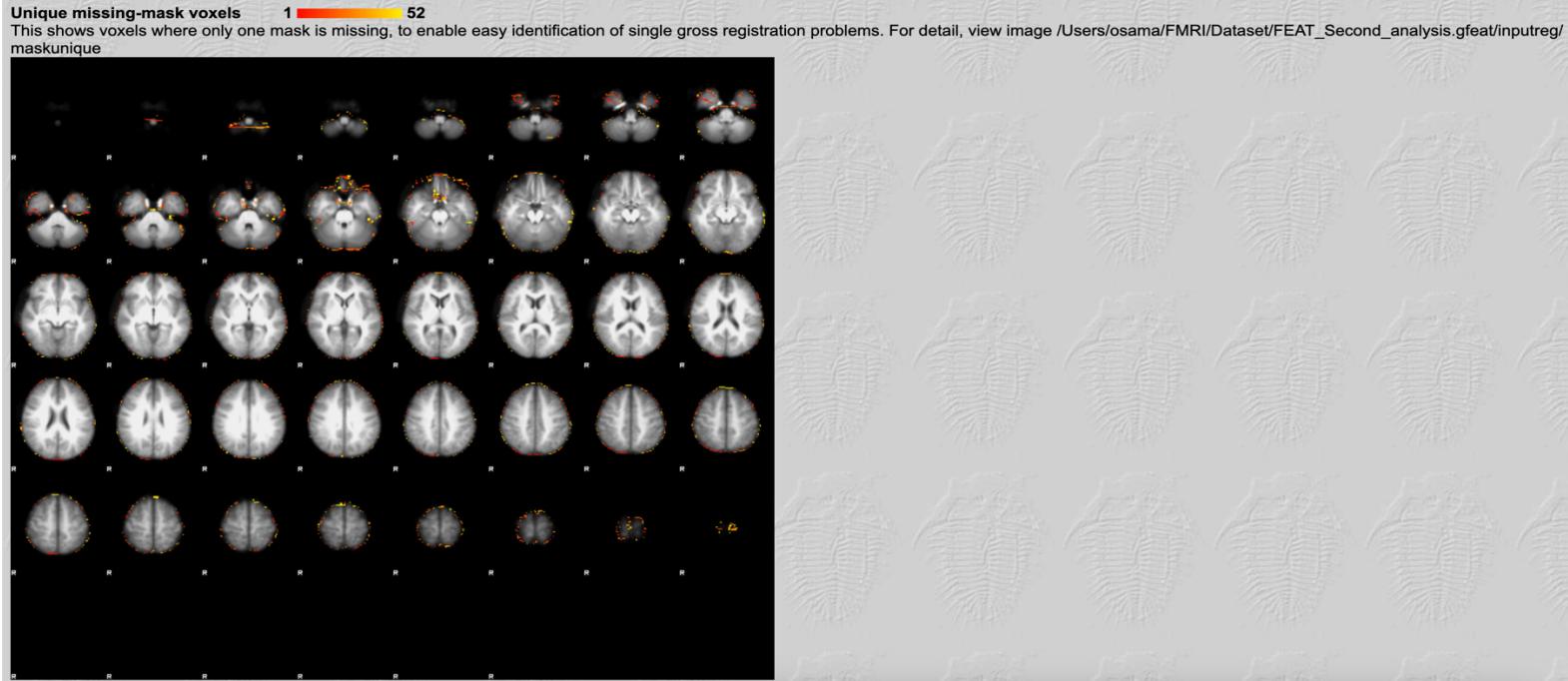


- After returning to the main FEAT wizard we just click go and wait for the analysis to finish

Results

After FSL finishes the Higher-Level Analysis we will be leaved by a html page containing all the information of our 26 subjects, starting from the inputs paths for all the runs FEAT ,also their registrations showing us all the 56 registrations for all 26 subjects for 2 runs with a sum of all masks after transformation to standard space as follows :





Also we would have a result tabs containing our Design Matrix and 3 external pages each for our 3 COPE we wanted in the lower level analysis(Incongruent- Congruent- Incongruent minus Congruent) showing us all the zstats and time series plots for all the 26 subjects.

Conclusions

FSL Higher-Level Analysis ensure that the errors that may happen with manual data extraction of BET, FEAT and GLM models are as minimum as possible allowing it to run everything by looping over each subject and just controlling the subject names by some scripting is a huge benefits for us allowing us to analyze huge numbers of cases with less error and more accurate results for better analysis and easier investigations.

Bonus Part (script to automate the filling of the EV file)

To automate the EVs filling we just need to create files with the values needed for FSL to use instead of doing it ourself, this would need to use Text2Vest tool which is bundled with FSL to convert the data into the format used by FSL.

Then we just can make the Design Matrix in any application like Notepad, Word or google docs, I chosen to make it using bash script and saving it as a txt file

```
3  # Define the dimensions of the matrix
4  num_rows=52
5  num_cols=26
6
7  # Create an empty matrix with all zeros
8  matrix=()
9
10 # Loop through each column
11 for ((col=0; col<num_cols; col++)); do
12     # Create an empty column with all zeros
13     column=()
14
15     # Loop through each row
16     for ((row=0; row<num_rows; row++)); do
17         # If the row is in the specified range for this column, set it to 1
18         if ((row >= 2*col + 1 && row <= 2*col + 2)); then
19             column+=("1")
20         else
21             column+=("0")
22         fi
23     done
24
25     # Add the column to the matrix
26     matrix+=("${column[@]}")
27 done
28
29 # Output the matrix to a text file
30 for ((row=0; row<num_rows; row++)); do
31     for ((col=0; col<num_cols; col++)); do
32         echo -n "${matrix[$((col*num_rows + row))]} " >> design_matrix.txt
33     done
34     echo >> design_matrix.txt # Add newline after each row
35 done
36
37 echo "Design matrix generated: design_matrix.txt"
```

After that we need to run the following line in terminal

```
Text2Vest design_matrix.txt design.mat
```

To change its format to .mat which the FSL can understand that this is the desing matrix it would use.

```

Dataset > $ COPE.sh
1  # Define the dimensions of the matrix
2  num_rows=26
3  num_cols=26
4
5  # Create an empty matrix with all zeros
6  matrix=()
7
8  # Loop through each row and column
9  for ((row=0; row<num_rows; row++)); do
10     for ((col=0; col<num_cols; col++)); do
11         # If it's the diagonal cell, set it to 1, otherwise set to 0
12         if ((row == col)); then
13             matrix+=(1)
14         else
15             matrix+=(0)
16         fi
17     done
18 done
19
20 # Output the matrix to a text file
21 for ((row=0; row<num_rows; row++)); do
22     for ((col=0; col<num_cols; col++)); do
23         echo -n "${matrix[((col*num_rows + row))]} " >> contrast.txt
24     done
25     echo >> contrast.txt # Add newline after each row
26 done
27
28 echo "Contrast matrix generated: contrast.txt"
29

```

Like the previous step we need to run the following command line

Text2Vest contrast.txt design.con

to change its format to .fts which tell FSL that this is the contrasts file

We can do the same for f-tests but we do not use for our higher-level analysis.

the previous steps would be creating both desing.mat and desing.con in our main directory, after creating them we can just run the Higher-Level analysis as we did previously and the matrices would be used automatically instead of entering them one by one.

6- 3rd-Level-Analysis

Third-level analysis in fMRI is pivotal for several reasons. It enhances the statistical power of studies by pooling data from individual subjects, thereby increasing the reliability and validity of the findings. This stage goes beyond individual variations and focuses on group-level effects, enabling researchers to identify common patterns of brain activity that are consistent across different individuals. This is particularly important in cognitive neuroscience, clinical research, and psychological studies, where understanding the generalizable aspects of brain function is essential.

Steps:

1- Loading the data

- Start by going into each COPE folder and get all the images located in stats subfolder of the COPE with the following command:
`ls$PWD/cope* | sort -V.`
- Open FSL FEAT and navigate to higher level analysis and select input are 3D COPE images and make the inputs 26 and paste the paths for the 26 subject
- Head to stats tap and create GLM model with Mixed Effects. This models the variance so that our results are generalizable to the population our sample was drawn from. FLAME 1 (FSL's Local Analysis of Mixed Effects) provides accurate parameter estimates by using information about both within-subject and between-subject variability and then select model setup to be single gorup average



Then head to post-stats and start choosing from the different thresholding techniques:

1- None (No Thresholding)

- Description: When no thresholding is applied, all statistical values are displayed, regardless of their significance level.
- Pros: This approach provides a complete view of the statistical map, showing all variations in the data.
- Cons: Without any thresholding, it can be challenging to differentiate between meaningful signals and noise, potentially leading to false-positive results.

2-Uncorrected

- Description: This technique involves setting a p-value threshold without any correction for multiple comparisons. A common uncorrected p-value threshold might be $p < 0.001$.
- Pros: It is straightforward and can detect strong signals.
- Cons: Since it does not account for the number of statistical tests performed, it increases the risk of type I errors (false positives), especially in whole-brain analyses where many comparisons are made.

3-Voxel

- Description: Voxel-wise correction adjusts the p-value threshold for multiple comparisons at the individual voxel level, using methods like the Bonferroni correction or False Discovery Rate (FDR).
- Pros: This method reduces the risk of false positives by controlling the family-wise error rate (FWER) or the proportion of false discoveries.
- Cons: It can be overly conservative, potentially missing true signals (type II errors), especially in areas with subtle effects.

4-Cluster

- Description: Cluster-wise correction identifies contiguous clusters of voxels that exceed a primary uncorrected voxel-level threshold (e.g., $p < 0.001$), and then tests these clusters for significance using permutation testing or Gaussian Random Field theory. The cluster p-value accounts for the size of the cluster.
- Pros: This approach balances sensitivity and specificity by focusing on spatially extended patterns of activity rather than isolated voxels. It is particularly effective in detecting true neural activations.
- Cons: The results can depend on the initial uncorrected threshold and the spatial smoothness of the data. Also, small but significant activations might be missed if they do not form large enough clusters.

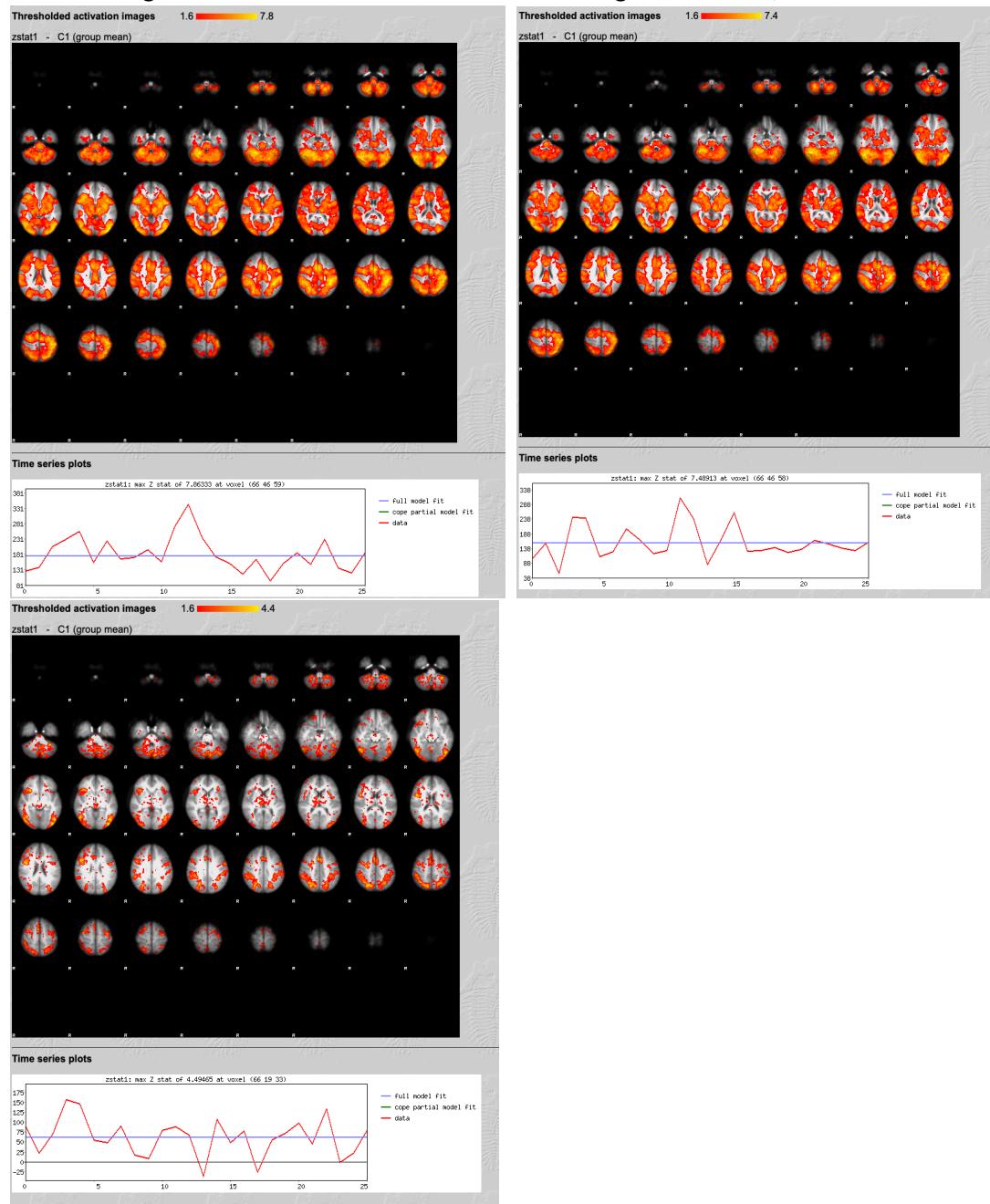
Results

1-None

It gives no image results as it does not differentiate between any signals in the brain
This suggests that thresholding is essential in filtering out noise and enhancing the detection of meaningful neural activations

2-Uncorrected

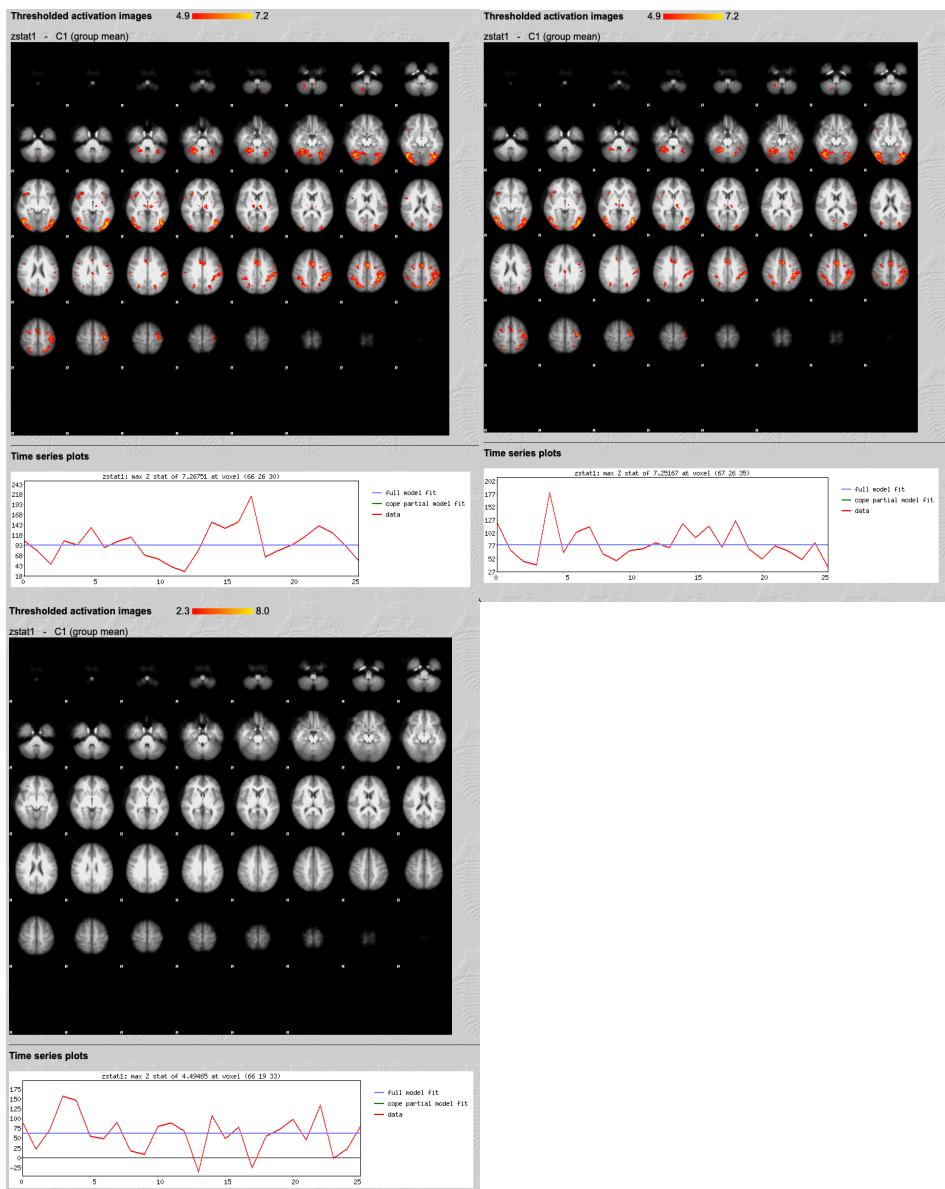
The following is the result of thresholded activation images for COPE 1, 2 and 3:



As this thresholding techniques implies no correction for multiple comparison the regions that are seemed to be activated occupies huge area of the brain that can lead to errors in the analysis, also uncorrected thresholding in our third-level analysis of the flanker task dataset yielded significant results for COPE 1 and COPE 2, identifying key brain regions involved in cognitive control and error processing. However, with COPE 3 underlying the difference between incongruent task and congruent one it's clearly showed where parts of the brain may give the difference during activation of the two tasks.

3-Voxel

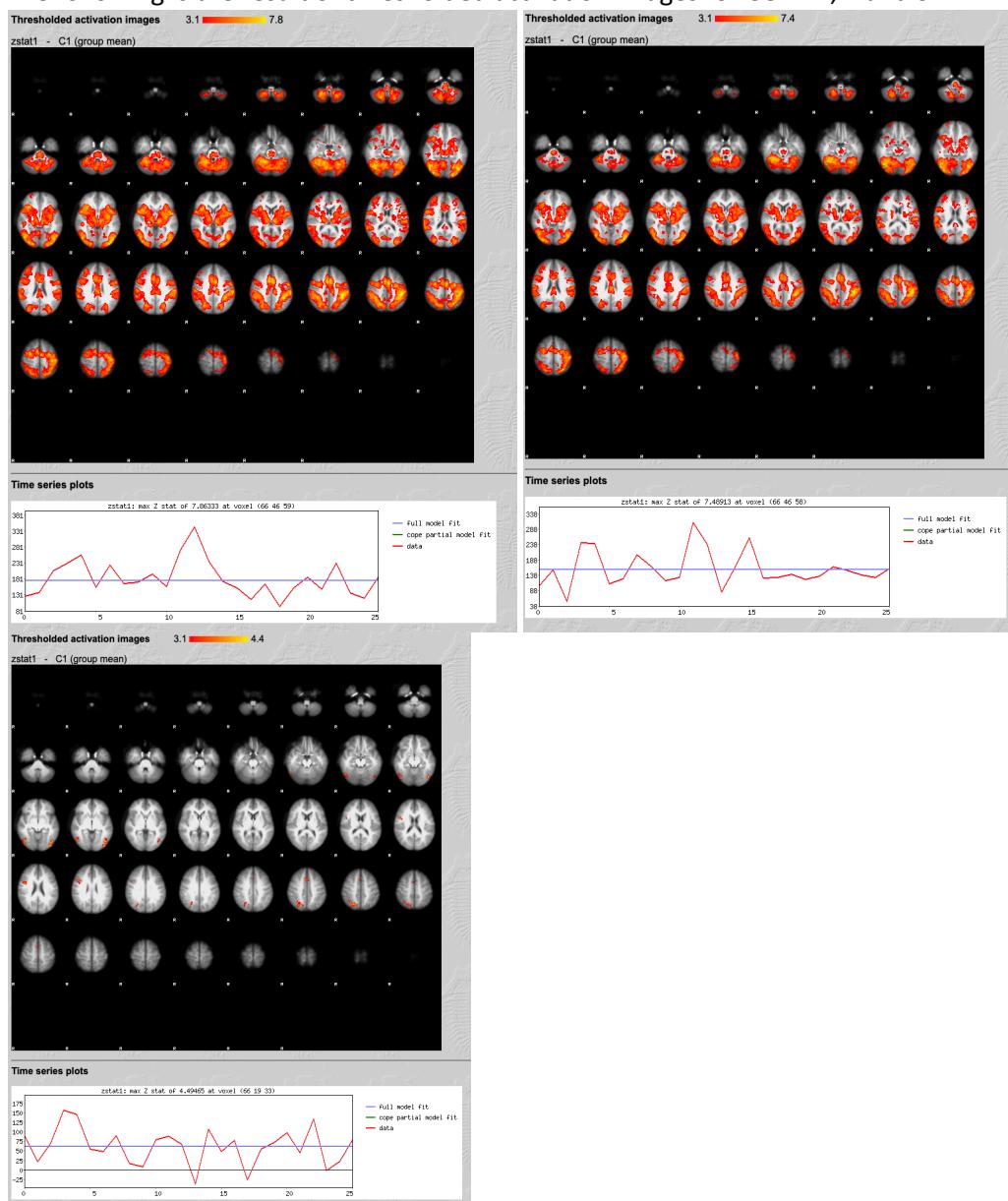
The following is the result of thresholded activation images for COPE 1 , 2 and COPE 3:



Here the use of voxel-wise thresholding in our third-level analysis provided a detailed and reliable map of brain activations associated with cognitive control and error processing in the flanker task. This method's ability to control for multiple comparisons at the voxel level ensured that the identified activations are statistically robust, minimizing the risk of false positives while maintaining sensitivity to detect true neural responses but that's achieved in tradeoff minimizing the amount of activation region detected which lead to missing out some regions which resulted in an empty activation regions in COPE 3 where the difference between voxel thresholded images of incongruent and congruent which were very similar lead to no activation regions appeared.

4-Cluster

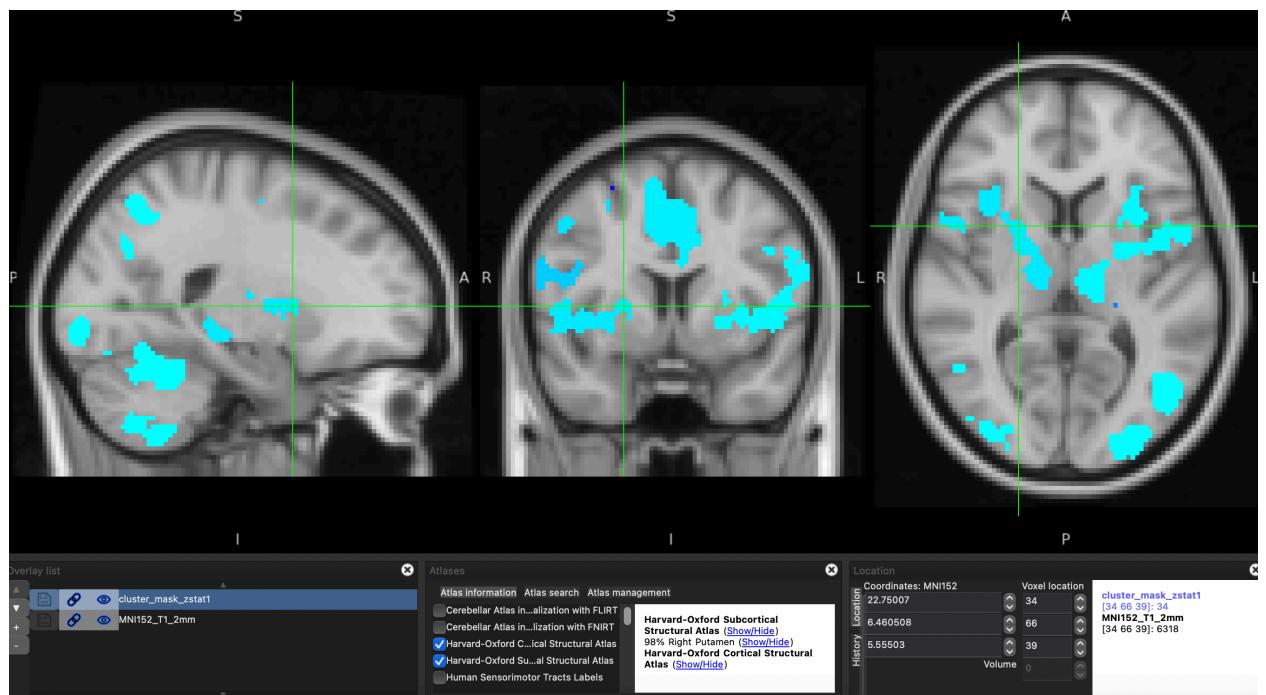
The following is the result of thresholded activation images for COPE 1, 2 and 3:



Using cluster thresholding facilitated the identification of robust activation patterns across all three COPEs, shedding light on the neural mechanisms underlying cognitive control, error processing, and other cognitive processes targeted by the flanker task. By accounting for spatially extended patterns of activity and controlling for multiple comparisons, cluster-wise thresholding offers a comprehensive approach to fMRI data analysis, enhancing the reliability and interpretability of the results, it also emphasizes the difference between both tasks in COPE 3 which appears very minimal using this threshold.

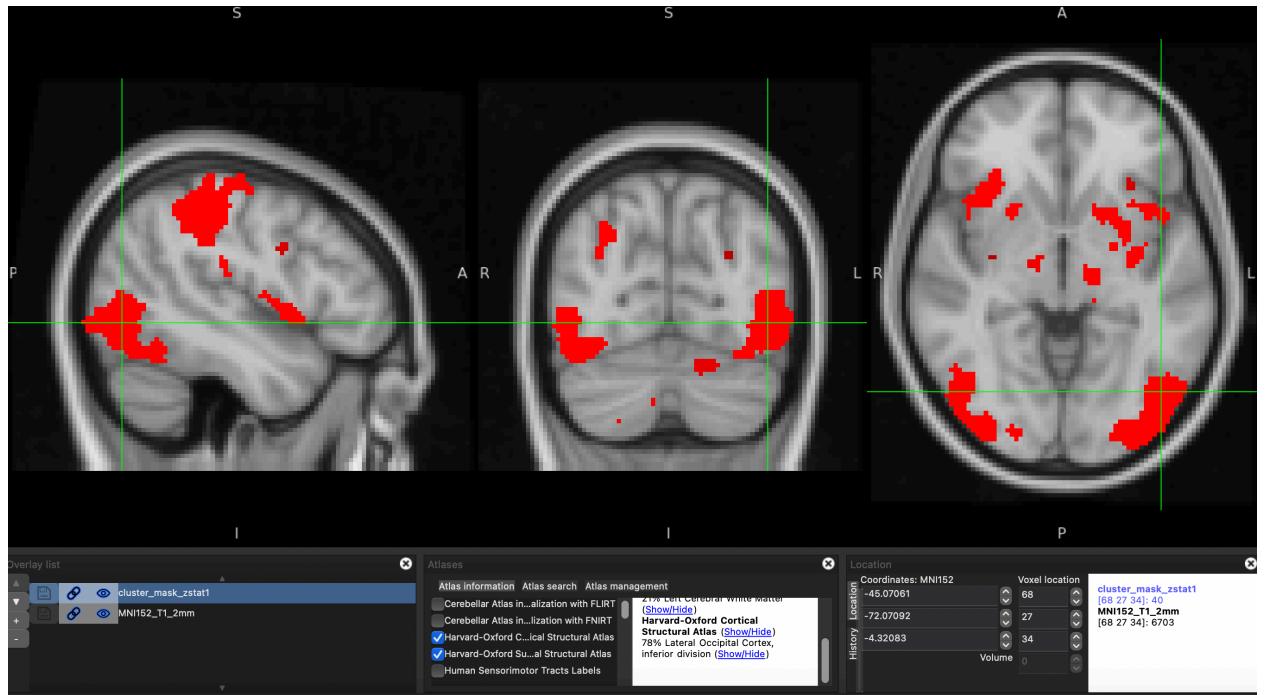
Regions

1-COPE 1



After viewing the results on FSLEYES using Voxel thresholding it showed that the region responsible for COPE1 which is for incongruent task is most likely to be the right and left cerebral cortex with some activation in regions like Right Putamen, and Lateral Occipital Cortex that's mainly can be because these regions are responsible for tasks like thinking, consciousness and problem-solving so that's make it activating trying to capture incongruent signal.

2-COPE 2



After examining the results in FSLEYES using voxel thresholding, it became apparent that the region responsible for COPE2, largely overlapped with the activation observed for COPE1, associated with incongruent tasks for its cognitive task ability similarity. Specifically, significant activity was predominantly observed in the right and left cerebral cortex especially in the lateral occipital cortex both the interior and inferior devision with also some regions activated in Cingulate Gyrus. This convergence of activation in these regions may be attributed to their involvement in high-order cognitive functions such as cognitive control, decision-making, and problem-solving.

3-COPE 3



For the difference between both task incongruent and congruent it is mainly showed that the main part the imphasize this difference is the Lateral Occipital Cortex and small percentage of some voxel suggest Paracingulate Gyrus showed in the FSLEYES it is the main part masked when using cluster thresholding because voxel thresholding is very conservative and only show highly activated regions in cope 1 and 2 so when the difference is calculated it showed no regions.

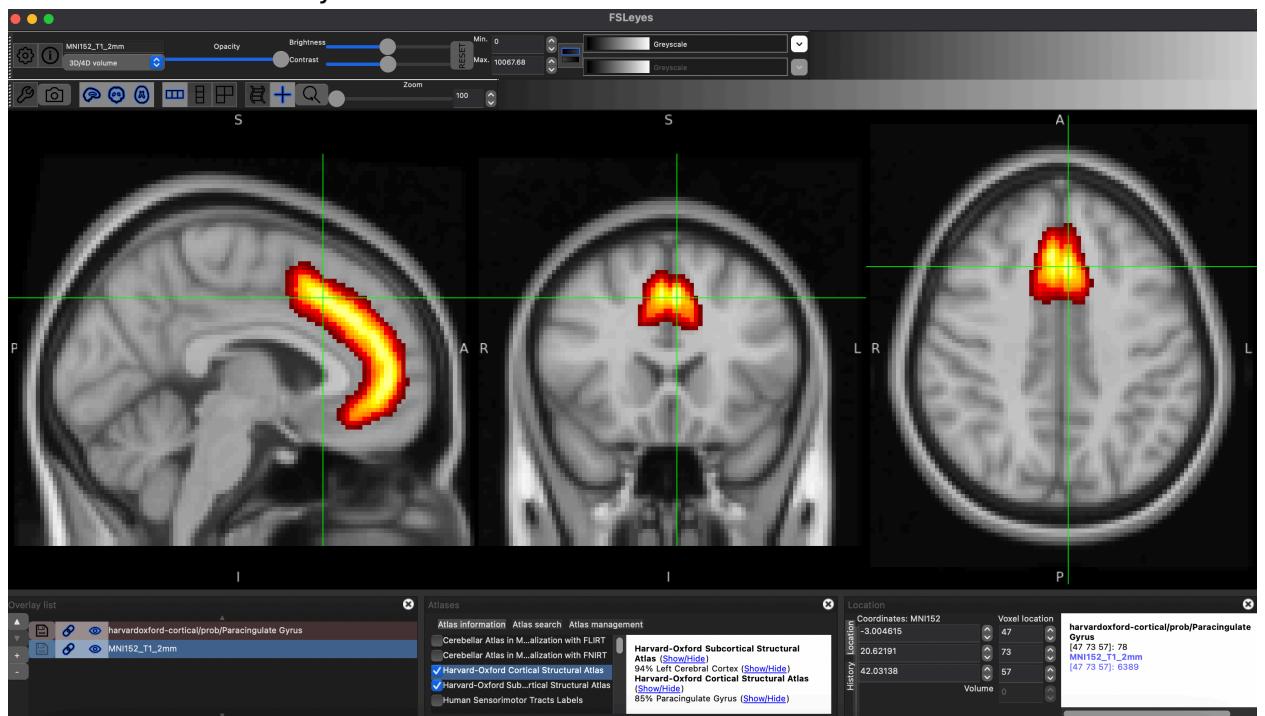
7- ROI

When many studies have been conducted on a specific topic, we can make more precise predictions about where we should find results in brain images. For instance, cognitive control has been extensively studied, and numerous fMRI studies have highlighted increased BOLD signals in the dorsal medial prefrontal cortex during cognitively demanding tasks. In a study like the Flanker task, we might focus our analysis on this region and only extract data from voxels within it. This approach is known as region of interest (ROI) analysis, and when we select a region based on prior studies, it's called a confirmatory analysis.

Whole-brain maps can sometimes obscure the details of the effects we're studying. For example, we might see a significant effect for incongruent versus congruent conditions, but we won't know if it's because incongruent is greater, congruent is lower, or a mix of both. ROI analysis helps us pinpoint what's driving these effects, which is crucial for understanding interactions and complex study designs.

1-Extract atlas mask

In our case and based on past studies we would focus on using the Paracingulate Gyrus as a mask, by heading to FSLEYES and opening our MNI and hovering over the wanted region (paracingulate gyrus here) and by saving that we would be having our PCG mask for ROI analysis.



2- Extract data from anatomical mask

We now need to get all the zstats produced by feat analysis and merge them to create one file for all zstates to calculate its mean with the PCG mask we have already save to do this we need to firstly head to the cope folder then stats then use the following fsl command to merge all those stats into one file:

```
fslmerge -t allZstats.nii.gz `ls zstat* | sort -V`
```

After doing that we can extract data from PCG mask by following command :

```
fslmeants -i allZstats.nii.gz -m PCG.nii.gz
```

For each cope we would do the same steps to compare all three at the end, but for this steps it should gave us 26 number one for each subject representation the contrast estimate for that subject averaged across all of the voxels in the mask like the following:

```
[osama@Osamas-MacBook-Pro Dataset % fslmeants -i allZstats.nii.gz -m PCG.nii.gz
1.364639
0.223197
1.055043
-0.213733
-0.095890
0.205869
0.712502
0.693325
0.263325
0.033409
-0.284475
0.252155
-0.077680
-0.129054
-0.107822
-0.398843
0.203575
-0.918502
-0.627403
-0.141979
-0.021796
0.362080
1.004431
-0.368109
-0.546998
0.207587

osama@Osamas-MacBook-Pro Dataset % ]
```

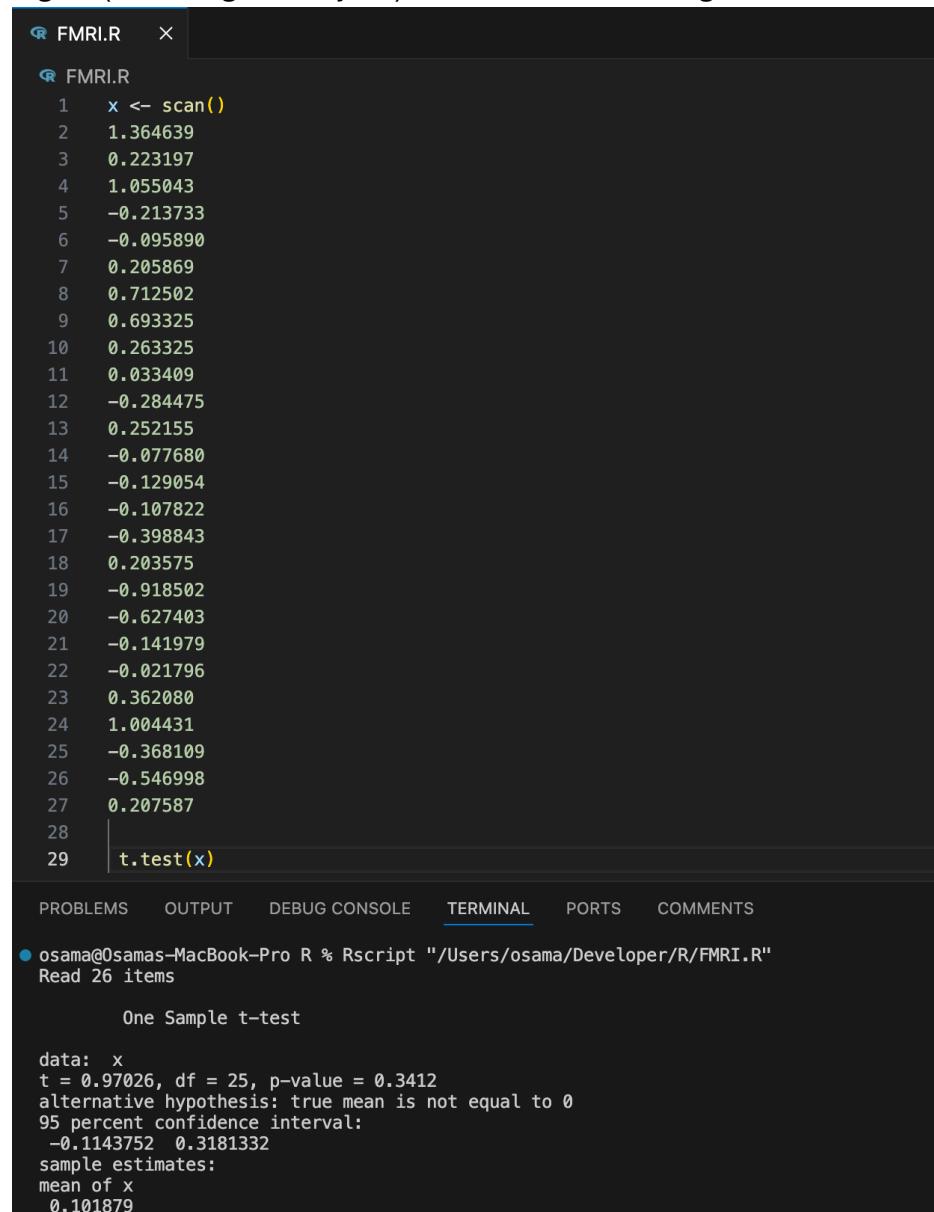
Here the positive values mean that the paracingulate gyrus shows a greater BOLD signal (i.e., more activity) during the incongruent task compared to the congruent task while negative values mean that, on average, the paracingulate gyrus is less active or deactivated region during the task which in this case of the snapshot it is during the

incongruent task compared to the congruent task(as this is for COPE 3) according to the BOLD signal.

Note: Deactivation in fMRI refers to a reduction in activity in certain brain regions when performing a specific task, compared to a resting state. This process enhances cognitive efficiency by suppressing non-essential activities, allowing for better focus and control. The patterns of deactivation also reveal how demanding a task is and which brain regions are downregulated to support performance.

Applying one sample t-test on the 26 values using R :

Start applying statistical analysis on the values using t test with null hypothesis that our region (Paracingulate Gyrus) is not activated during the task.



```
QM FMRI.R x
QM FMRI.R
1 x <- scan()
2 1.364639
3 0.223197
4 1.055043
5 -0.213733
6 -0.095890
7 0.205869
8 0.712502
9 0.693325
10 0.263325
11 0.033409
12 -0.284475
13 0.252155
14 -0.077680
15 -0.129054
16 -0.107822
17 -0.398843
18 0.203575
19 -0.918502
20 -0.627403
21 -0.141979
22 -0.021796
23 0.362080
24 1.004431
25 -0.368109
26 -0.546998
27 0.207587
28
29 t.test(x)

PROBLEMS OUTPUT DEBUG CONSOLE TERMINAL PORTS COMMENTS
osama@Osamas-MacBook-Pro:~/Developer/R$ Rscript "/Users/osama/Developer/R/FMRI.R"
Read 26 items

One Sample t-test

data: x
t = 0.97026, df = 25, p-value = 0.3412
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
-0.1143752 0.3181332
sample estimates:
mean of x
0.101879
```

The P-value output was equal to 0.3412 which is greater than threshold of 0.05, which mean that we cannot reject the null hypothesis that the region is not activated.

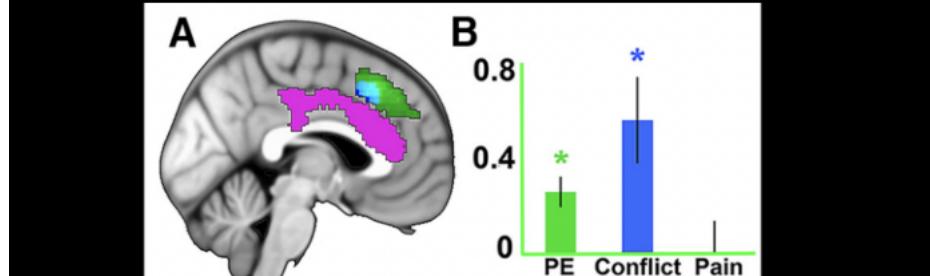
this result is mainly caused by the big area we choose for the comparision whichh included a lot of unactivated voxels which was more dominant over the real activated voxels.

3- Extract data from Sphere mask

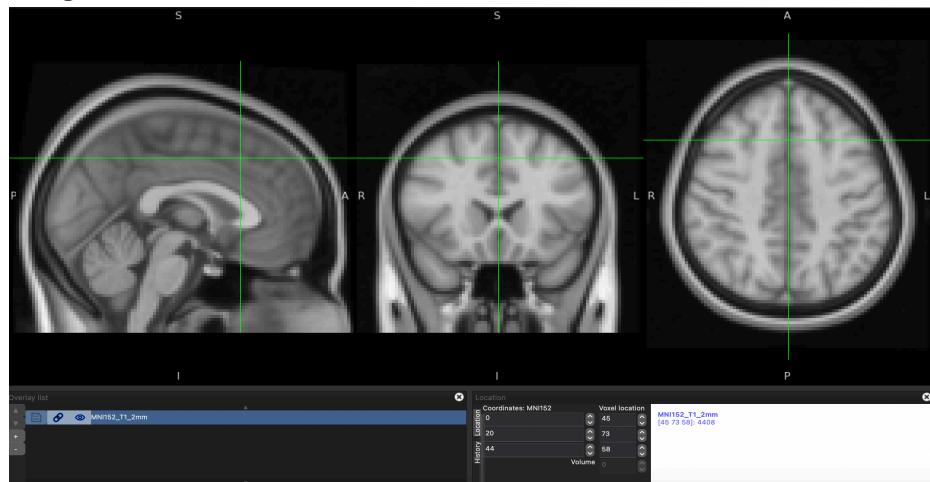
Here our aim is to extract ROI with smaller area than a whole region from atlas, we start by revising over past papers and search for related topic to shorten our search for proper area to start with.

Based on the study done in 2016 by Jahn et al who ran a similar research, it is found that there is a Conflict effect for a Stroop task - a distinct but related experimental design also intended to tap into cognitive control - with a peak t-statistic at MNI coordinates 0, 20, 40.

the dACC (MNI -2, 30, 14; $k = 788$; peak z-value = 5.17; $p < 0.001$, cluster-corrected; Fig. 4). In contrast, an analysis of conflict effects revealed a significant cluster more dorsally within the mPFC (MNI 0, 20, 44) $k = 536$ voxels; peak z-value = 3.62; $p < 0.05$, cluster-corrected). Together with our previous analysis of



So we head to FSLEYES and start from that MNI space to navigate it into the Voxel space using our 2mm MNI152_T1.



From this we can conclude that the required region in Voxel space would be (45,73,58) which we would need to create a new ROI around starting from running the following scripts :

1-

```
"fslmaths $FSLDIR/data/standard/MNI152_T1_2mm.nii.gz -mul 0 -add 1 -roi 45 1 73  
1 58 1 0 1 Jahn_Paper_ROI_dmPFC_0_20_44.nii.gz -odt float"
```

This creates a file with the specified coordinates in voxel space.

2-

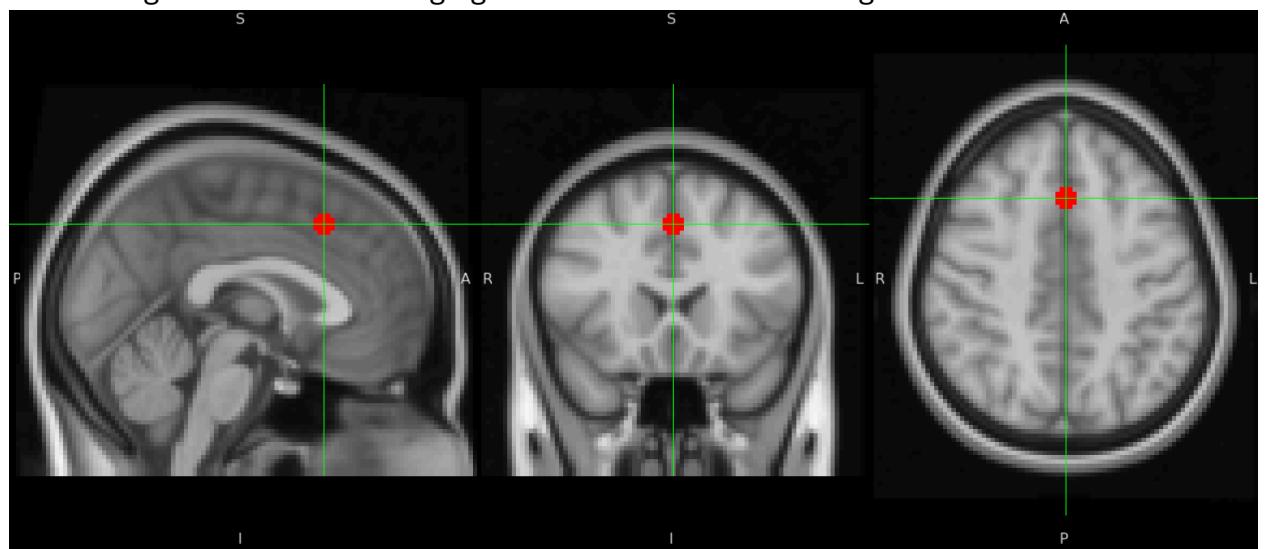
```
"fslmaths Jahn_Paper_ROI_dmPFC_0_20_44.nii.gz -kernel sphere 5 -fmean  
Jahn_Paper_Sphere_dmPFC_0_20_44.nii.gz -odt float"
```

This will expand our point into a sphere with 5mm radius which would be our new ROI.

3-

```
"fslmaths Jahn_Paper_Sphere_dmPFC_0_20_44.nii.gz -bin  
Jahn_Paper_Sphere_bin_dmPFC_0_20_44.nii.gz "
```

This will binarize the sphere making its values 1 and any other value into 0 as they were having different values ranging from 0 to 1 before binarizing.



4-

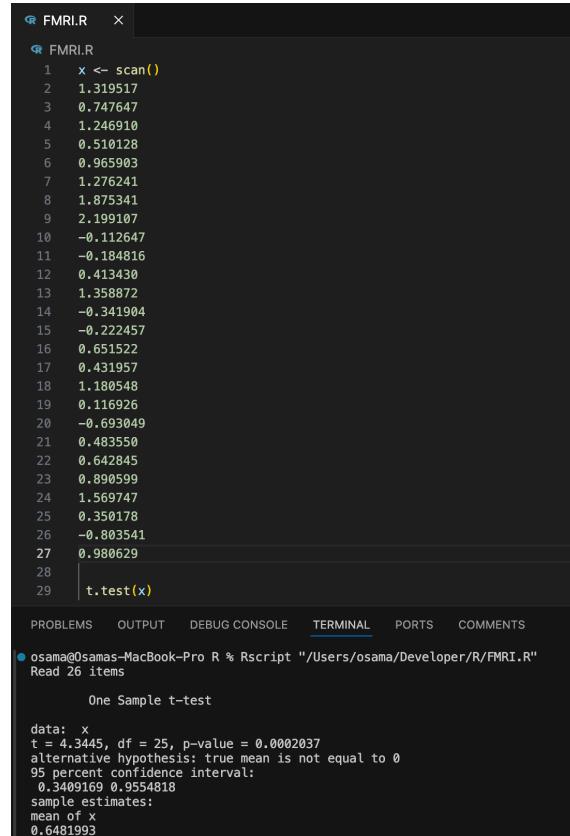
"fslmeans -i allZstats.nii.gz -m Jahn_Paper_Sphere_bin_dmPFC_0_20_44.nii.gz"

Which is the same we did for the atlas based but now with the new ROI to create the 26 values as follows :

```
[osama@Osamas-MacBook-Pro Dataset % fslmeans -i allZstats.nii.gz -m Jahn_Paper_Sphere_bin_dmPFC_0_20_44.nii.gz
1.319517
0.747647
1.246910
0.510128
0.965903
1.276241
1.875341
2.199107
-0.112647
-0.184816
0.413430
1.358872
-0.341904
-0.222457
0.651522
0.431957
1.180548
0.116926
-0.693049
0.483550
0.642845
0.890599
1.569747
0.350178
-0.803541
0.980629

osama@Osamas-MacBook-Pro Dataset % ]
```

Lastly we apply the same statistical t-test on the new ROI



```
FMRI.R  ×
FMRI.R
  1 x <- scan()
  2 1.319517
  3 0.747647
  4 1.246910
  5 0.510128
  6 0.965903
  7 1.276241
  8 1.875341
  9 2.199107
 10 -0.112647
 11 -0.184816
 12 0.413430
 13 1.358872
 14 -0.341904
 15 -0.222457
 16 0.651522
 17 0.431957
 18 1.180548
 19 0.116926
 20 -0.693049
 21 0.483550
 22 0.642845
 23 0.890599
 24 1.569747
 25 0.350178
 26 -0.803541
 27 0.980629
 28
 29 | t.test(x)

PROBLEMS   OUTPUT   DEBUG CONSOLE   TERMINAL   PORTS   COMMENTS
osama@Osamas-MacBook-Pro R % Rscript "/Users/osama/Developer/R/FMRI.R"
Read 26 items
One Sample t-test

data: x
t = 4.3445, df = 25, p-value = 0.0002037
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
 0.3409169 0.9554818
sample estimates:
mean of x
0.6481993
```

This time using the sphere concluded from the paper we got a new p-value of 0.00020237 which is less than the threshold of 0.05 which mean that we reject the null hypothesis this time that the mask is activated during the task.

Now we just start doing the same steps for both COPE 1 and 2 to find the difference in activation for both the atlas gyrus and the sphere we created and plot the difference between them:

COPE1 – Paracingulate Gyrus :

```
osama@Osamas-MacBook-Pro FEAT_Second_analysis++.gfeat % cd cope1.feat/stats
osama@Osamas-MacBook-Pro stats % fslmerge -t allZstats_cope1.nii.gz `ls zstat* | sort -V`
osama@Osamas-MacBook-Pro stats % mv allZstats_cope1.nii.gz ../../..
osama@Osamas-MacBook-Pro stats % cd ../../..
osama@Osamas-MacBook-Pro Dataset % fslmeans -i allZstats_cope1.nii.gz -m PCG.nii.gz

0.704541
1.149841
0.320686
0.567860
1.395177
-0.300650
0.251096
0.881863
0.515790
0.738673
0.939408
1.128993
0.364283
-0.782193
1.250096
-0.328332
0.667445
-1.091730
0.733176
0.208956
1.084516
0.162191
0.533900
0.427807
-0.069033
0.461587

osama@Osamas-MacBook-Pro Dataset %
```

```
FMRIR.R
x <- scan()
2 0.704541
3 1.149841
4 0.320686
5 0.567860
6 1.395177
7 -0.300650
8 0.251096
9 0.881863
10 0.515790
11 0.738673
12 0.939408
13 1.128993
14 0.364283
15 -0.782193
16 1.250096
17 -0.328332
18 0.667445 |
19 -1.091730
20 0.733176
21 0.208956
22 1.084516
23 0.162191
24 0.533900
25 0.427807
26 -0.069033
27 0.461587
28
29 t.test(x)

PROBLEMS OUTPUT DEBUG CONSOLE TERMINAL PORTS COMMENTS
osama@Osamas-MacBook-Pro R % Rscript "/Users/osama/Developer/R/FMRIR.R"
Read 26 items
One Sample t-test
data: x
t = 3.8484, df = 25, p-value = 0.0007305
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
0.212447 0.7618948
sample estimates:
mean of x
0.4571518
```

P-value Reject null hypothesis which means Paracigulate Gyrus is activated during the task

Mean of x = 0.4571518

COPE 1 – Sphere:

```
[osama@Osamas-MacBook-Pro Dataset % fslmeants -i allZstats_cope1.nii.gz -m Jahn_Paper_Sphere_bin_dmPFC_0_20_44.nii.gz
1.888848
2.743580
1.739639
1.746230
4.013843
1.902670
3.071899
2.727784
0.948608
1.389931
3.097099
2.903181
0.504833
1.148228
3.441200
0.166108
2.997341
1.048559
0.380633
0.967466
2.140696
1.260420
2.820580
0.438855
-0.465793
1.459452

osama@Osamas-MacBook-Pro Dataset % █
FMRI.R ×
FMRI.R ...
2 1.888848
3 2.743580
4 1.739639
5 1.746230
6 4.013843
7 1.902670
8 3.071899
9 2.727784
10 0.948608
11 1.389931
12 3.097099
13 2.903181
14 0.504833
15 1.148228
16 3.441200
17 0.166108
18 2.997341
19 1.048559
20 0.380633
21 0.967466
22 2.140696
23 1.260420
24 2.820580
25 0.438855
26 -0.465793
27 ★ 1.459452
28 1.459452
29 t.test(x)

PROBLEMS OUTPUT DEBUG CONSOLE TERMINAL PORTS COMMENTS
osama@Osamas-MacBook-Pro R % Rscript "/Users/osama/Developer/R/FMRI.R"
Read 26 items
One Sample t-test

data: x
t = 7.0577, df = 25, p-value = 2.588e-08
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
1.325873 2.250457
sample estimates:
mean of x
1.787765
NO NEW NOTIFICATIONS
```

P-value Reject null hypothesis which means this sphere region is activated during the task .

Mean of x = 1.787765

COPE 2 - Paracingulate Gyrus :

```
[osama@Osamas-MacBook-Pro FEAT_Second_analysis++.gfeat % cd cope2.feat/stats  
[osama@Osamas-MacBook-Pro stats % fslmerge -t allZstats_cope2.nii.gz `ls zstat* | sort -V`  
osama@Osamas-MacBook-Pro stats % mv allZstats_cope2.nii.gz ../../..  
osama@Osamas-MacBook-Pro stats % cd ../../..  
[osama@Osamas-MacBook-Pro Dataset % fslmeants -i allZstats_cope2.nii.gz -m PCG.nii.gz  
[-0.687041  
0.869012  
-0.821125  
0.767785  
1.639780  
-0.538804  
-0.382690  
0.184617  
0.256845  
0.700286  
1.228531  
0.948611  
0.433232  
-0.592130  
1.352840  
0.041448  
0.491625  
-0.230412  
1.382420  
0.347535  
1.077982  
-0.254735  
-0.598868  
0.806127  
0.522121  
0.274130
```

```
osama@Osamas-MacBook-Pro Dataset % █

④ FMR1.R
1 x <- xcom()
2 =0.587841
3 0.859012
4 -0.82125
5 0.74125
6 1.639788
7 -0.530884
8 -0.382690
9 0.184617
10 0.256845
11 0.700286
12 1.228531
13 0.948611
14 0.433232
15 -0.592130
16 1.352840
17 0.641448
18 0.491625
19 -0.230412
20 1.382420
21 0.347535
22 1.877982
23 -0.254735
24 -0.598868
25 0.806127
26 0.522121
27 0.274138
28
29 t.test(x)

PROBLEMS OUTPUT DEBUG CONSOLE TERMINAL PORTS COMMENTS █
osama@Osamas-MacBook-Pro R % Rscript "/Users/osama/Developer/R/FMR1.R"
Read 26 items
One Sample t-test

data:
t = 2.544, df = 25, p-value = 0.01752
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
0.0675245 0.6416387
sample estimates:
mean of x
0.3545816

NO NEW NOTIFICATIONS
```

P-value Reject null hypothesis which means Paracigulate Gyrus is activated during the task

Mean of x = 0.3545816

COPE 2 – Sphere:

```
osama@Osamas-MacBook-Pro Dataset % fslmeants -i allZstats_cope2.nii.gz -m Jahn_Paper_Sphere_bin_dmPFC_0_20_44.nii.gz
0.631664
1.912791
0.358934
1.145323
3.359457
0.623910
1.364576
0.449410
1.065207
1.587001
2.713154
1.694218
0.823099
1.361667
2.894989
-0.299998
1.937514
1.038504
1.172523
0.536567
1.330621
0.361845
1.101824
0.064491
0.435510
0.539241
```

```
osama@Osamas-MacBook-Pro Dataset %
```

The screenshot shows a terminal window at the top with command-line output and a code editor below it.

Terminal Output:

```
osama@Osamas-MacBook-Pro Dataset % fslmeants -i allZstats_cope2.nii.gz -m Jahn_Paper_Sphere_bin_dmPFC_0_20_44.nii.gz
0.631664
1.912791
0.358934
1.145323
3.359457
0.623910
1.364576
0.449410
1.065207
1.587001
2.713154
1.694218
0.823099
1.361667
2.894989
-0.299998
1.937514
1.038504
1.172523
0.536567
1.330621
0.361845
1.101824
0.064491
0.435510
0.539241
```

Code Editor:

```
FMRI.R
x <- scan()
1 0.631664
2 1.912791
3 0.358934
4 1.145323
5 3.359457
6 0.623910
7 1.364576
8 0.449410
10 1.065207
11 1.587001
12 2.713154
13 1.694218
14 0.823099
15 1.361667
16 2.894989
17 -0.299998
18 1.937514
19 1.038504
20 1.172523
21 0.536567
22 1.330621
23 0.361845
24 1.101824
25 0.064491
26 0.435510
27 0.539241
28 t.test(x)
```

The code editor interface includes tabs for PROBLEMS, OUTPUT, DEBUG CONSOLE, TERMINAL, PORTS, and COMMENTS. Below the code, the R console output is displayed:

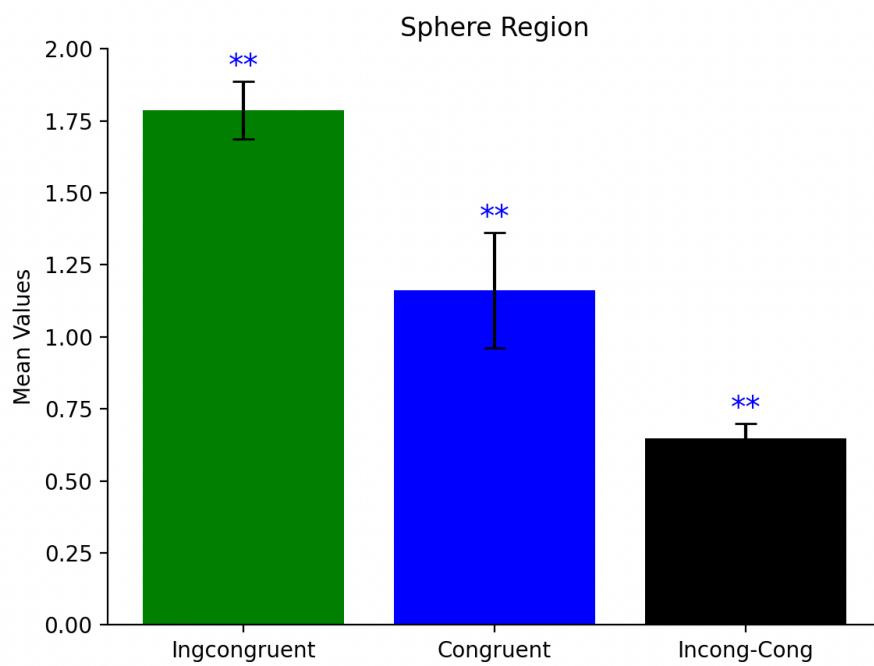
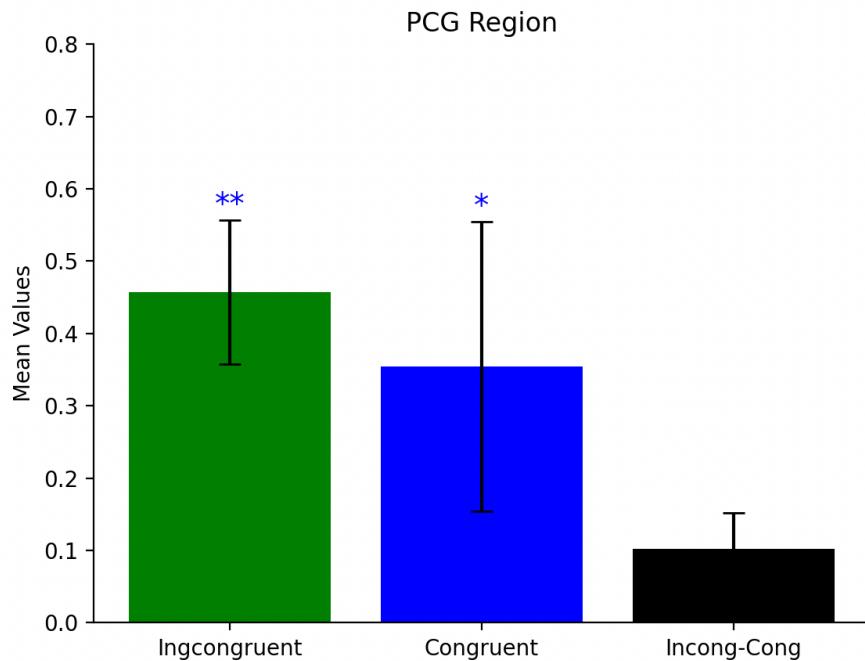
```
osama@Osamas-MacBook-Pro R % Rscript "/Users/osama/Developer/R/FMRI.R"
Read 26 items
One Sample t-test

data: x
t = 6.7712, df = 25, p-value = 4.264e-07
alternative hypothesis: true mean is not equal to 0
95 percent confidence interval:
 0.8083493 1.5150386
sample estimates:
mean of x
1.161694
```

P-value Reject null hypothesis which means this sphere region is activated during the task .

Mean of x = 1.161694

Plotting the Mean of x for each task we see the following :



Firstly the plot where made using Matplotlib with some modification applying ** for p-value < 0.001 and * for p-value < 0.05 .

These plots complete our statistical analysis finding the right regions and how strong we can agree on it.

At the end, analyzing regions of interest (ROIs) using both anatomical and spherical masks provided crucial insights into the neural mechanisms involved in cognitive control tasks. Although the anatomical mask did not yield significant results, the spherical ROI approach uncovered significant activation in the Paracingulate Gyrus. This discovery underscores the importance of selecting precise and appropriate ROIs in fMRI studies. The findings highlight the Paracingulate Gyrus and other related brain regions as key players in conflict monitoring and cognitive control, thereby deepening our understanding of the neural processes that support these functions.