Faculty of Engineering
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fMRI Full level analysis

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Health Informatics

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GitHub Repo

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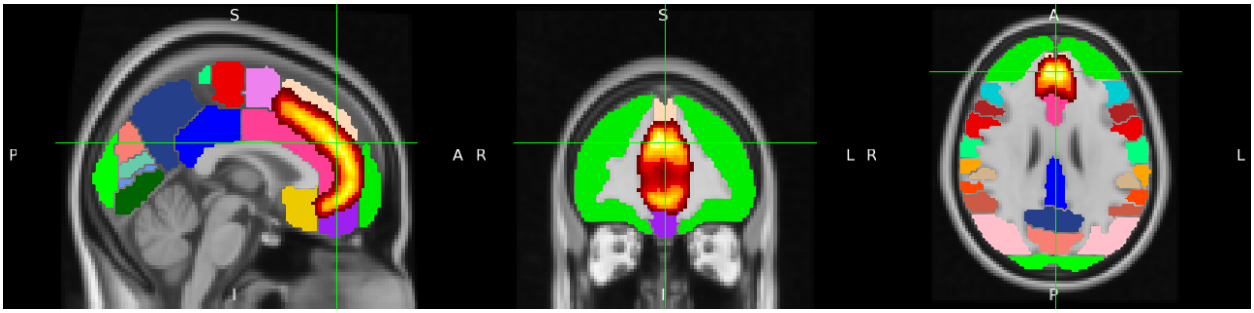


Contents

Overview

1.1 Introduction

Functional Magnetic Resonance Imaging (fMRI) is a commonly used method to study brain activity by measuring changes in blood oxygenation levels. fMRI full level analysis is a comprehensive approach that involves several steps to investigate and interpret the neural correlates of cognitive processes or experimental conditions.

In fMRI full analysis, the data undergo preprocessing steps to reduce noise and artifacts. These steps include correcting for motion, adjusting for differences in timing between image slices, smoothing the data, and skull stripping.

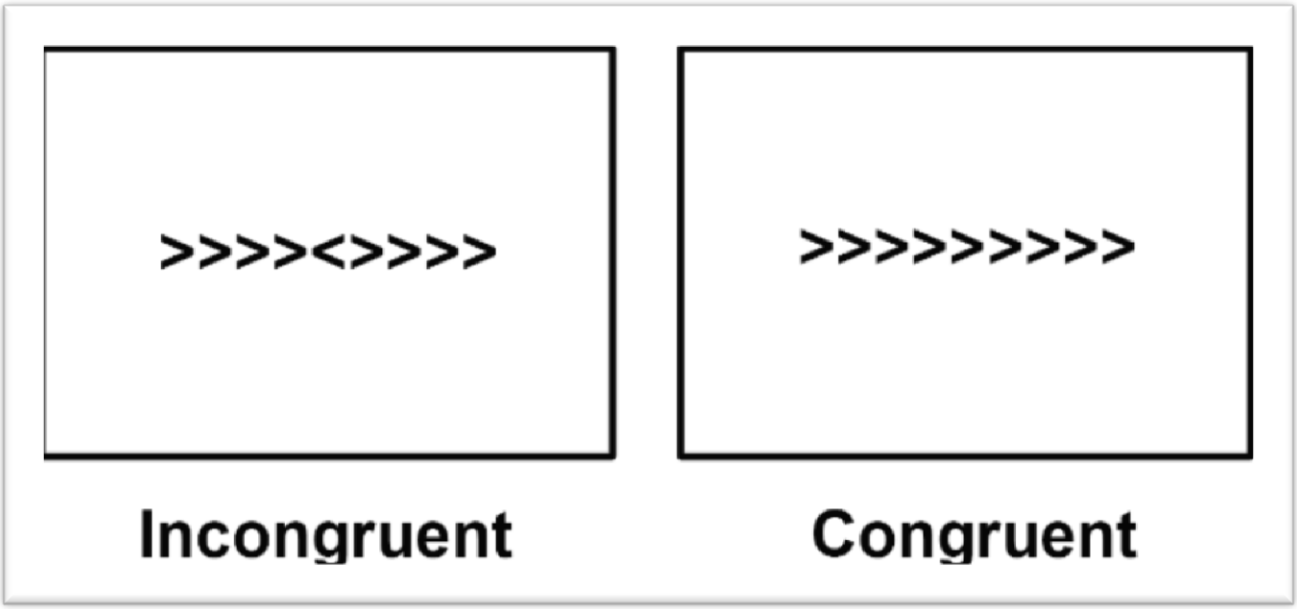
The first-level analysis creates a design matrix that represents the experimental conditions or tasks. Statistical tests, like the General Linear Model, are applied to estimate the effects of interest and generate statistical maps specific to each participant.

The second-level analysis combines the results from multiple participants to explore group-level effects. It allows researchers to make broader conclusions beyond individual participants. Group design matrices are constructed, and statistical tests are used to identify significant differences or activations across the group.

FSL, a popular software package, offers user-friendly tools like FEAT and FSLeyes. These interfaces make it accessible to researchers with limited programming skills and widely used in neuroimaging research.

1.2 FLANKER dataset

The Flanker task is designed to assess cognitive control. In this task, participants are presented with arrows pointing left or right and are instructed to press corresponding buttons indicating the direction of the middle arrow. Cognitive control refers to the ability to ignore irrelevant stimuli and accurately perform the task. The middle arrow is surrounded by other arrows that either point in the same direction as the middle arrow or in the opposite direction. The dataset includes measurements of **26** participant responses and performance during this task, allowing researchers to investigate cognitive control processes and the impact of flanker stimuli on task performance.



Quality Control

Quality control is considered a pre step before you begin preprocessing your data and analyzing it. It mainly involves checking for motion correction or artifacts while performing the FLANKER task.

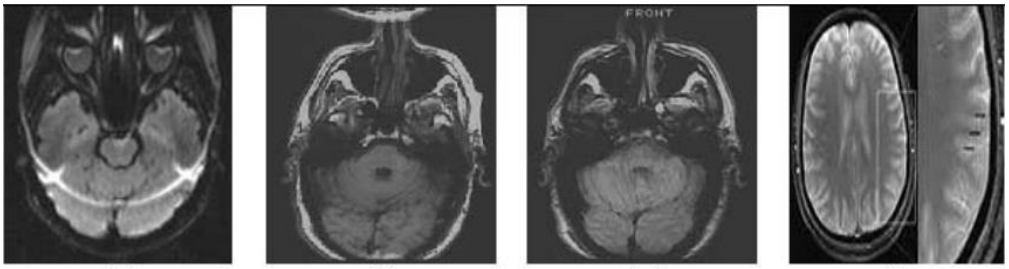
Some of the main artifacts include:

a. Chemical Shift Artifact: This happens when fat and water in an MR image have different frequencies, causing them to appear differently.

b. Aliasing/Wrap around Artifact: When the part being imaged is larger than the area captured, it can look like the image wraps around to the other side.

c. Black Boundary/Black Line Artifact: This is an artificial black line that shows up between fat and water areas, like where muscle and fat meet.

d. Gibbs Ringing/Gibbs Phenomenon/Truncation Artifact: This creates bright or dark lines near sudden changes in image intensity, like going from bright fluid to dark spinal cord (common in T2-weighted images). These lines can be distracting and affect image interpretation.



(a) [Courtesy: http://www.radiographics.rsna.org]

(b) [Courtesy: Ballinger, 2009]

(c) [Courtesy: http://www.bme.yonsei.ac.kr]

(d) [Courtesy: http://www.ajronline.org]

Steps to perform QC on your 26 subjects:

Open fsl using fsl command in your terminal then choose FSeyes and do the following:

- From Add, Choose add from file and Add your func run for your subject Ex: sub-01_task-flanker_run-1_bold.nii.gz

- Check for any artifact

- Check for motion correction in func (+ for low motion -> +++ high motion)

Name : Mahmoud Rabea
SEC:2 BN:25 ID: 9203396

Subject Names	QC			Motion Correction		Comments
		FUNC				
	T1	RUN 1	RUN 2	RUN 1	RUN 2	
Sub-01	good	good	good	+	+	
Sub-02	good	good	good	+	+	
Sub-03	good	good	good	+	+	
Sub-04	good	good	good	++	+	
Sub-05	good	good	good	++	++	
Sub-06	good	good	good	++	++	
Sub-07	good	good	good	+	+	
Sub-08	good	good	good	+	+	
Sub-09	good	good	good	+	+	
Sub-10	good	good	good	+	+	
Sub-11	good	good	good	+	++	
Sub-12	good	good	good	+	+	
Sub-13	good	good	good	+	+	
Sub-14	good	good	good	+	+	
Sub-15	good	good	good	+	+	
Sub-16	good	good	good	+	+	
Sub-17	good	good	good	+	+	
Sub-18	good	good	good	+	++	
Sub-19	good	good	good	+++	++	
Sub-20	good	good	good	+	+	
Sub-21	good	good	good	++	++	
Sub-22	good	good	good	+	++	
Sub-23	good	good	good	+	+	
Sub-24	good	good	good	+	+	
Sub-25	good	good	good	++	+++	
Sub-26	good	good	good	+	+	

Note that this was done by the human eyes so; excuse me if there was any artifacts I've missed ☺

Preprocessing

Preprocessing involves correcting for artifacts and noise, such as motion, slice timing, spatial smoothing and skull stripping, to enhance the quality and reliability of the data before we begin doing any sort of analysis.

Steps for preprocessing:

1) To apply Skull stripping

- open fsl using fsl command in your terminal then choose BET brain extraction
- Choose your subject anatomical image and click go;

the output will be as for example sub-01_T1w_brain_f02.nii.gz

2) To apply filtering to your func image:

open fsl using fsl command in your terminal then choose FEAT fMRI analysis

In the data tab:

- change Full analysis to Preprocessing
- select func file for your subject as an input

EX: sub-01_task-flanker_run1_bold.nii.gz

- Choose your output directory

In Registration tab:

- Check on Main structural image and place the anatomical image

For your specified subject EX: sub-01_T1w.nii.gz

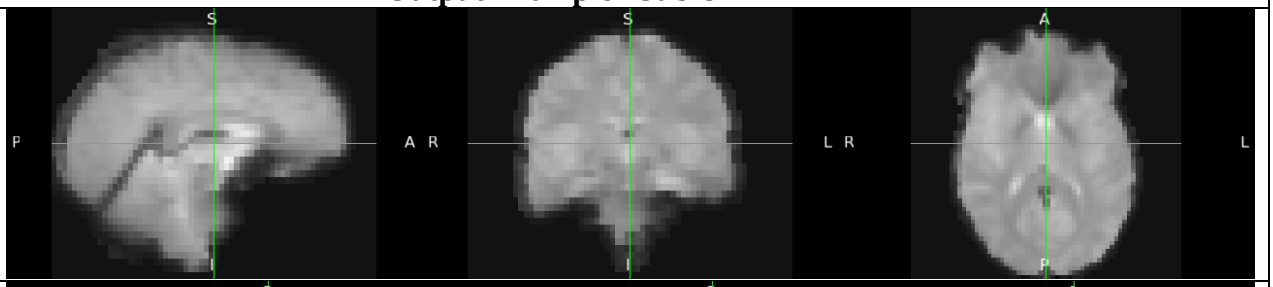
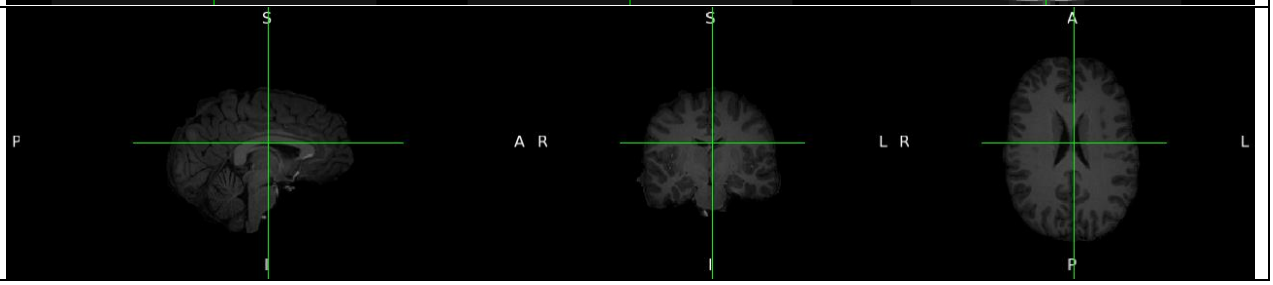
- In both main structural image and Standard space:
  - Change Normal Search to Full search
  - Change BBR to 12DOF

CLICK GO and all you'll have .feat folder containing all preprocessed files for your specified subject

Notes:

- When you click on go, it will take time to fully preprocess your subject
- The progress will be shown in an HTML log file
- To see your filtered image, open it using FSLeyes

EX: filtered_func_data.nii.gz

Output Example *Sub-8*	
Skull stripped image	
Filtered image	

1st Level Analysis

First-level analysis involves modeling the experimental conditions or our task for each participant, estimating effects of interest, and generating statistical maps specific to each individual. It helps identify brain regions activated during our task and provides subject-level information for group-level analyses. You can Run 1st level analysis either manually or using bash scripting for fast, efficient processing.

Step to do 1st level analysis for all your 26 subjects:

At first, you will need to create a design template that will be applied to all the subjects. Assuming that you haven't manually done GLM or Preprocessing, kindly follow these steps:

1) You need to place Make_FSL_Timings.sh in your Data folder and run it using bash command in your terminal

2) Open fsl using fsl command in your terminal then choose FEAT fMRI analysis

3) Do the following for 1st run of your Data:

In the data tab:

- Full analysis
- select 1st func Run file for your template subject as an inpu
- Choose your output directory EX: run1_Template

In Registration tab:

- check on Main structural image and place the anatomical image for your specified subject EX: sub-01_T1w.nii.gz

- in both main structrual image and Standard space:
  - Change Normal Search to Full search
  - Change BBR to 12DOF

In stats tab:

- click on Full model setup
- change the number of original EVs to 2
- in the 1 first tab: EV name -> incongruent

  Basic Shape -> Custom (3 column format)

  input file -> choose incongruent.txt file

  Check on Add Temporal derivative & Uncheck

  on Apply Temporal Filtering
- Repeat the previous step for 2 but replace incongruent with congruent
- Go to Contrast&F-Tests: Contrasts -> 3

  OC1 -> incongruent [1,0]

  OC2 -> congruent [0,1]

  OC3 -> incongruent-congruent [1,-1]

  CLICK Done

CLICK Save and all you'll have run1_Template files folder

we care about design_run1.fsf

4) Repeat the previous step for Run 2

After you prepare your design template for Both Run 1 & 2 , you will need to do the following:

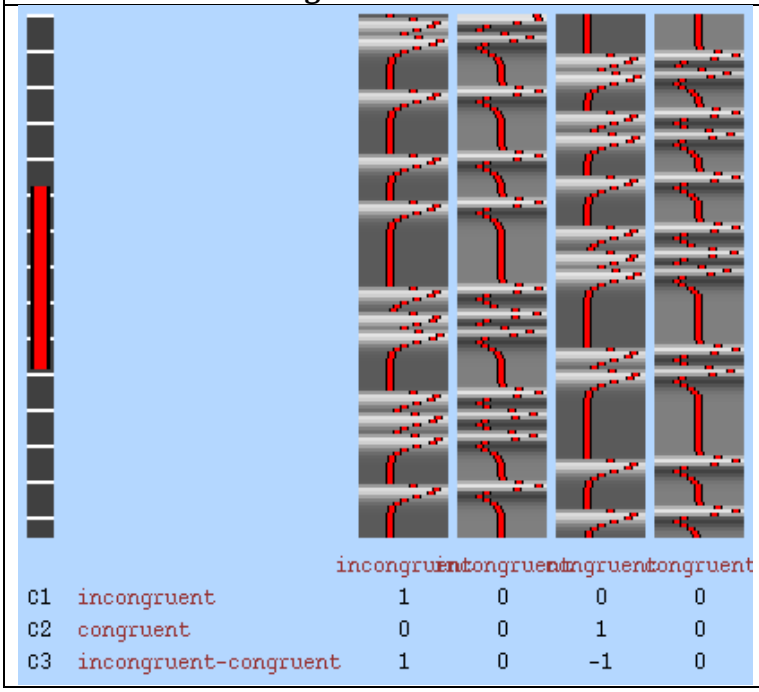
1) move design_run1.fsf , design_run2.fsf & run_1stLevel_Analysis.sh to your Data directory

2) Finally, Run run_1stLevel_Analysis.sh file in the terminal using bash command

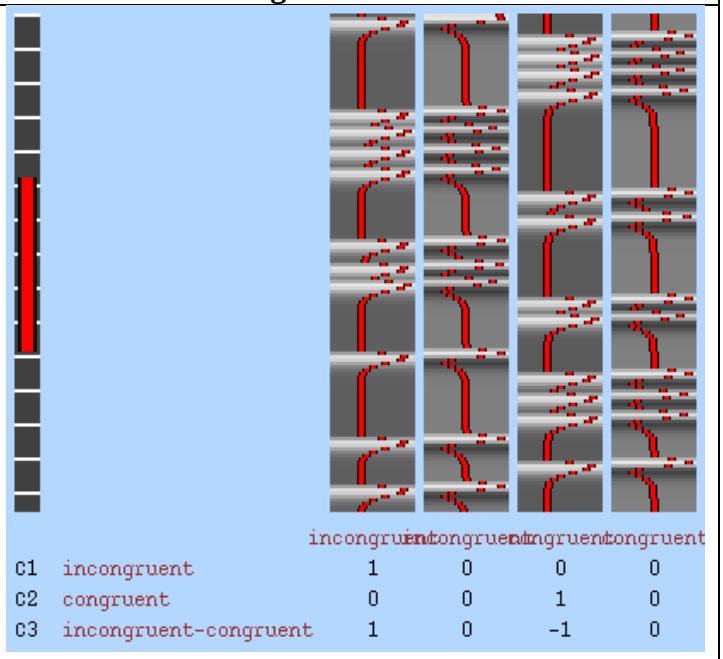
YOU can use Modified_1fsf.sh instead of run_1stLevel_Analysis.sh if you wish to have only one design file and loop over it :)

Note that it will take much time for the script to complete the 1st Level analysis on all the 26 subjects. You can track the progress from the log HTML files.

Design Matrix Run1



Design Matrix Run 2



Note that all .sh files are attached with the report

Higher Level Analysis

5.1 2nd level analysis

Second-level analysis combines results from multiple participants to examine group-level effects by averaging the two runs of each subject into single coepe.

Steps:

1) Open fsl using fsl command in your terminal then choose FEAT fMRI analysis

2) Instead of choosing first level analysis, choose higher level analysis

3) Choose your 52 input fet directories

Instead of choose each fet manually,

do wildcard selection by running the following command: ls -d$PWD/sub-??/func/run*

4) Choose your output directory

5) Go to the stats tab:

- Choose fixed effect inference

- Choose full model setup =>

- choose 26 EVs and average the two runs of

each subject by placing 1 at only these specified runs for this subject

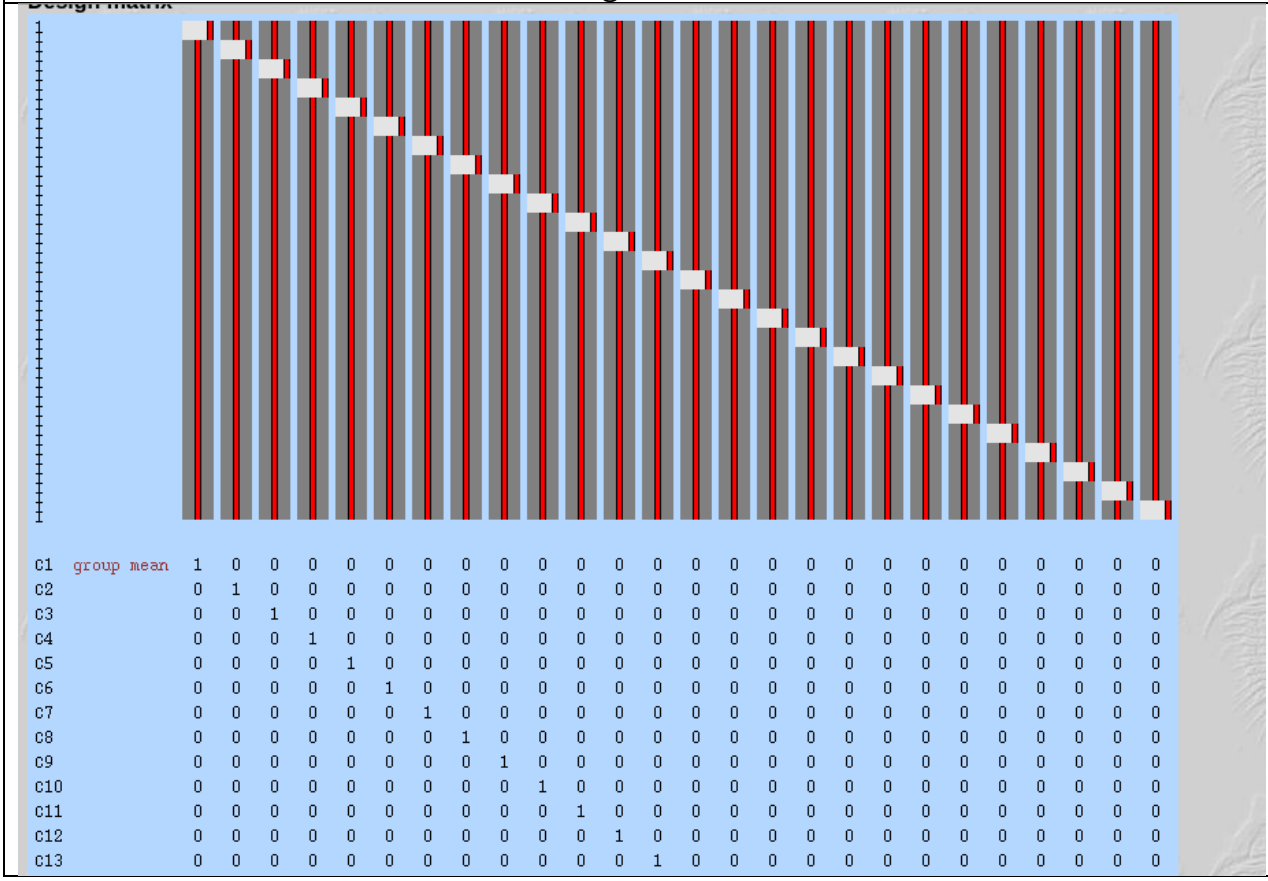
EX => EV3 input5: 1.0 input6: 1.0 and the rest 0

- go to contrast and select 26 contrasts and place 1 in only the main diagonal

6) Click done and GO

Note that 2nd level analysis is computationally expensive.

Design Matrix



5.2 3rd level analysis

3rd level analysis involves combining results from multiple second-level analyses to finally get the activated clustered.

Steps:

1) Open fsl using fsl command in your terminal then choose FEAT fMRI analysis

2) Instead of choosing first level analysis , choose higher level analysis

3) Choose 26 3D cope images as an input

instead of choose each cope manually , do wildcard selection by running the following command in cope3.feat/stats:

ls $PWD/cope* | sort -v

4) Choose your output directory

5) Go to the stats tab:

- choose mixed effect Flame 1 inference

- choose model setup wizard and check on single group average then process

6) go to post stats and make sure to choose cluster thresholding with:

Z-threshold => 3.1 P-threshold => 0.05

7) Click done and GO and after you do so, repeat for cope1&cope2

To view the significant clusters, follow these steps:

-Open fsleyes

-Open the MNI template

-overlay the harvard-oxford ATLAS to check significant clusters in different brain regions.

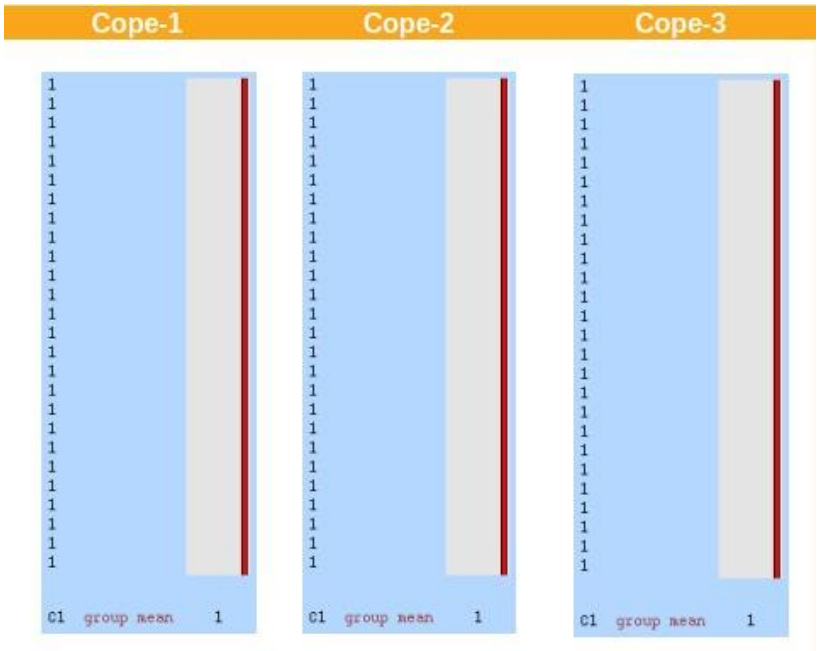
-load the thresh_zstat.nii.gz

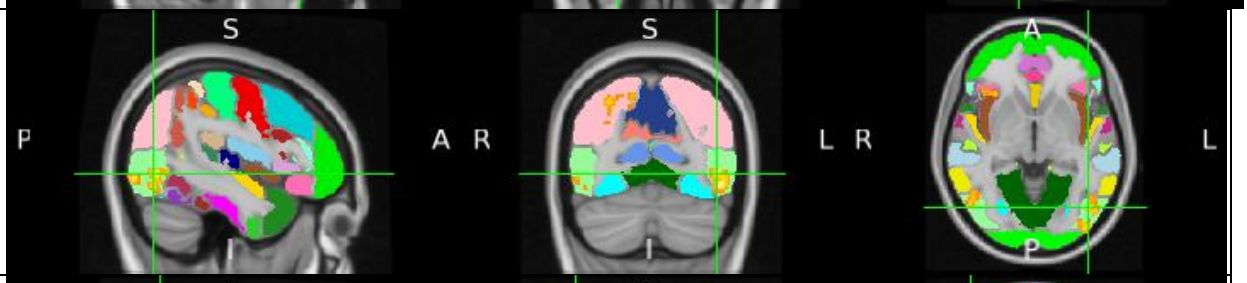
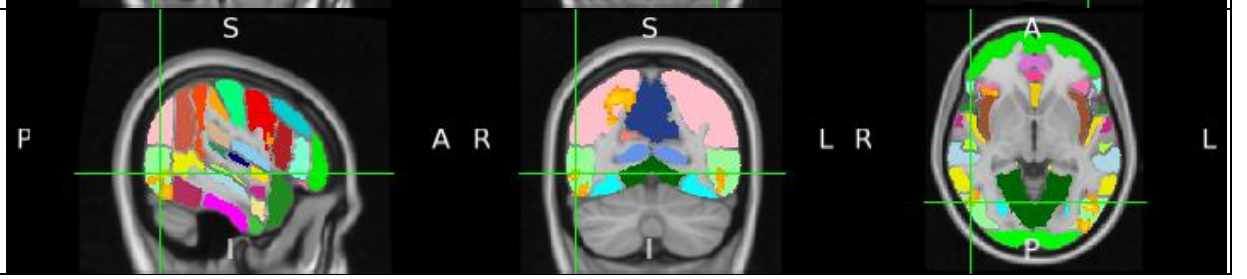
-change the contrast to see the significant clusters Ex: Red-yellow

-click on the gear icon and change the interpolation configuration to be linear

-from view,layouts choose FEAT mode.

3rd Level Design matrices



Significant clusters Examples	
52% inasular cortex & 29% Frontal Orbital Cortex	
79% Lateral Occipital Cortex, inferior division	
87% Lateral Occipital Cortex, inferior division	

ROI analysis

Region of Interest analysis involves focusing on specific brain regions or areas of interest to examine their activity. It allows researchers to extract and analyze data from predefined regions rather than the whole brain. ROI has two main methods Anatomical & Spherical.

1) Anatomical ROI:

Anatomical ROI analysis focuses on specific anatomical brain regions to investigate their structural properties or measure volumes.

Steps:

1) Create your anatomical mask:

-Open fsleyes and from standards open MNI152_T1_2mm.nii.gz

- From settings ortho view, open atlases , harvard oxford cortical atlas

- Choose paracingulate gyrus and save the template as PCG.nii.gz

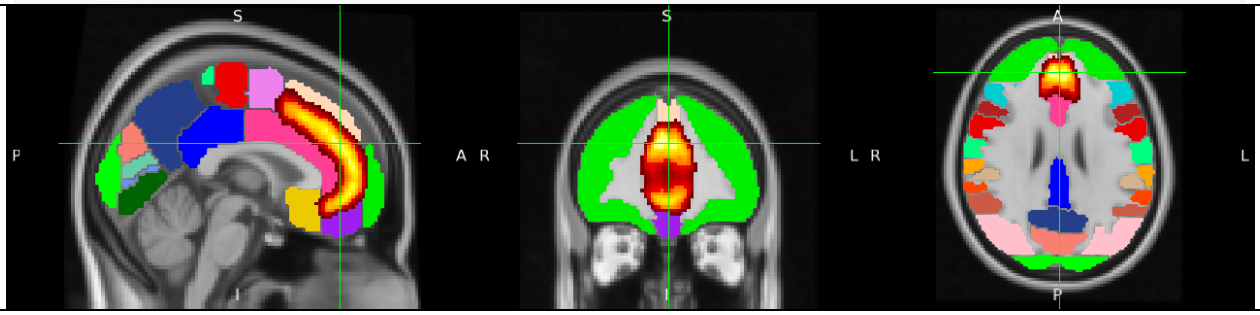
2) Merge all zstats of Cope3 by running the following command in

cope3.feat/stats fslmerge -t allZstats.nii.gz `ls zstat* | sort -V

3) Run the following command in order to get the means

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

4) Now do one sample t-test using R language to obtain the results

One sample t-Test	Rscript /tmp/VlTHIk0Ola.r One Sample t-testdata: my_data t = 1.2174, df = 27, p-value = 0.234alternative hypothesis: true meanis not equal to095percent confidence interval: -0.08678481 0.34003117 sample estimates: mean of x 0.1266232
PCG anatomical Mask	

2) Spherical ROI:

Spherical ROI analysis involves using spherical regions of interest to investigate brain activity.

Steps at Voxel location [0, 20, 44]:

1- Open MNI template in fsleyes and get the voxel location.

2- In the terminal go to the FLANKER directory and type the following which shall give you a centroid of these voxel locations:

```
fslmaths $FSLDIR/data/standard/MNI152_T1_2mm.nii.gz -mul 0 -add 1 -roi 45 1 73 1 58 1 0 1 Jahn_ROI_dmPFC_0_20_44.nii.gz -odt float
```

3- Run the following command to expand this single voxel with specific raduis 5mm:

```
fslmaths Jahn_ROI_dmPFC_0_20_44.nii.gz -kernel sphere 5 -fmean Jahn_Sphere_dmPFC_0_20_44.nii.gz -odt float
```

4- Run the following command to binarize the results:

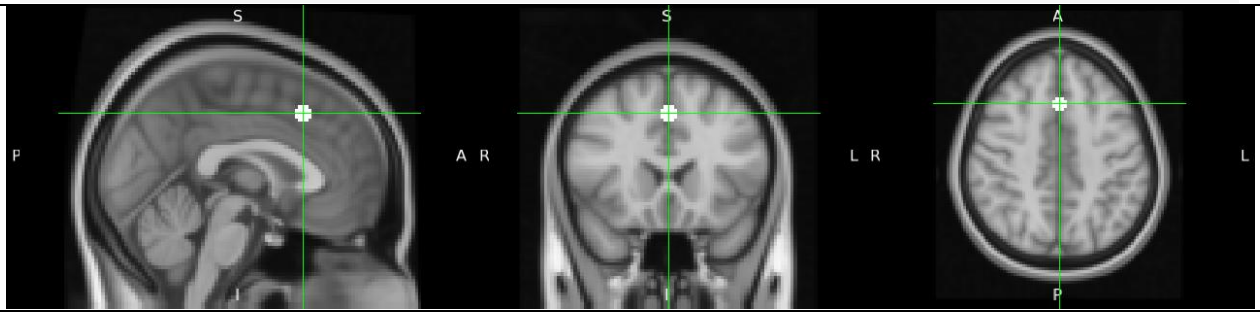
```
fslmaths Jahn_Sphere_dmPFC_0_20_44.nii.gz -bin Jahn_Sphere_bin_dmPFC_0_20_44.nii.gz
```

5- Get the means of this voxel location by running the following command:

```
fslmeants -i allZstats.nii.gz -m Jahn_Sphere_bin_dmPFC_0_20_44.nii.gz
```

6- Finally, do one sample t-test using R to get your results

One sample t-Test	`Rscript /tmp/VlTHIkOOla.r`
	`Read 26 items`
	`One Sample t-test`
	
	`data:  my_data`
	`t = 4.3729, df = 25, p-value = 0.0001893`
	`alternative hypothesis: true mean is not equal to 0`
	`95 percent confidence interval:`
	`0.3505255 0.9746588`
	`sample estimates:`
	`mean of x`
	`0.6625921`

[0,20,44] Spherical mask	

Conclusion

To sum up, the usage of FSL for FLANKER full level analysis offers researchers a comprehensive approach to examine attention and cognitive control processes. Through FSL's tools and statistical techniques, researchers can explore group-level effects and acquire valuable insights into the underlying neural mechanisms involved in the FLANKER task.

Appendix I [Brain regions using atlases]

By using FSLeyes and the Harvard-Oxford atlas, researchers can examine brain regions and their activation patterns. FSLeyes provides visualization tools, while the atlas offers predefined anatomical regions for analysis, helping in the exploration of specific brain regions and their functions.

Steps:

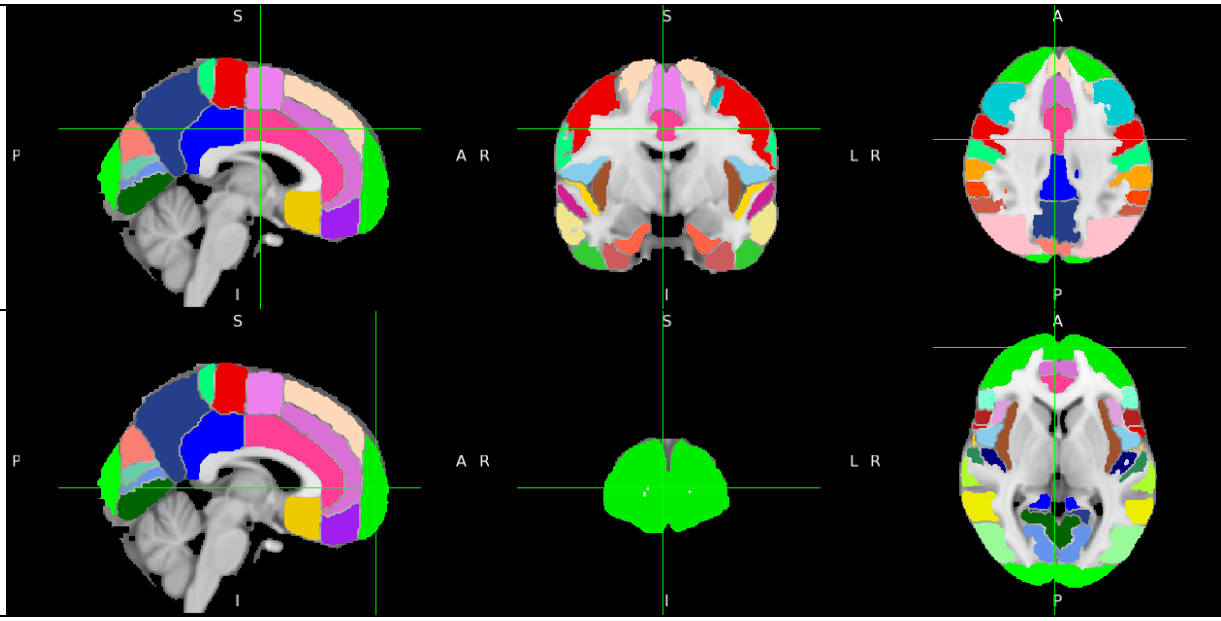
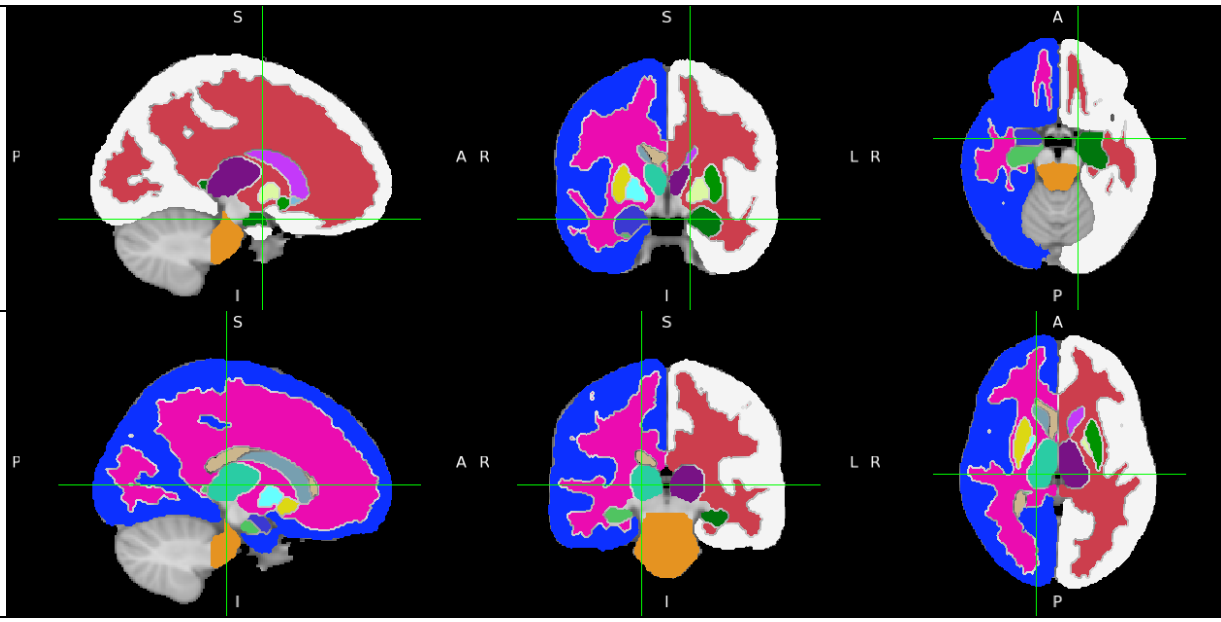
Open fsl using fsl command in your terminal then choose FSLeyes and do the following:

-From Add, Choose add standard and add your MNI152 Standard Ex: MNI152_T1_1mm_brain.nii.gz

- From Settings Ortho View 1, Choose Atlases

- Check on Harvard-Oxford Atlas and start Examine the brain regions!

Note that examining both the cortical & sub-cortical regions at the same time would be complex and not appealing for your eyes so, Show/Hide each one of them individually for better examining!.

Examples of Cortical regions		
Region	**Function**	**Screenshot**
Cingulate Gyrus, anterior division	**Emotional processing & Decision making**	
Frontal pole	**Decision making & Attention control**	
Examples of Sub-Cortical regions		
Region	**Function**	**Screenshot**
Left Amygdala	**Emotional processing &Emotional Memory**	
Right Thalamus	**Sensory relay & processing of sensory information**	

For more detailed information about each step, kindly check each Task's report