# Higher Level Analysis Task

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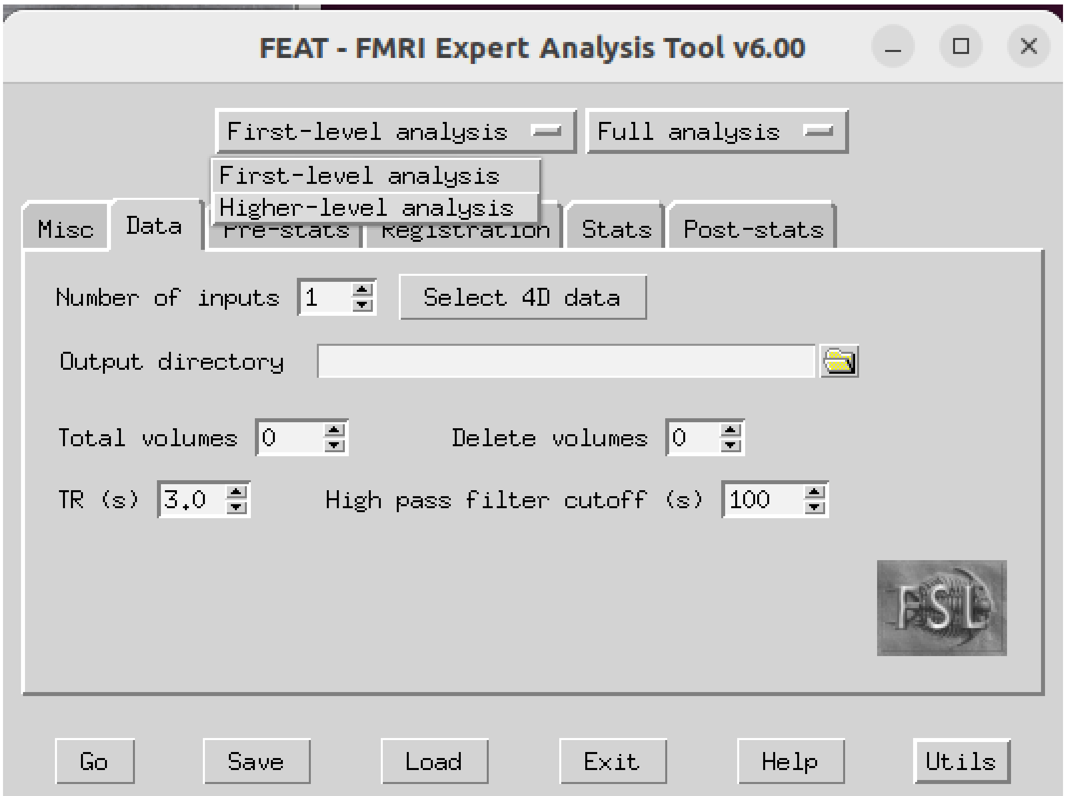
# 2nd Level

## Steps

1)open Fet gui and instead of choosing first level analysis , choose higher level analysis

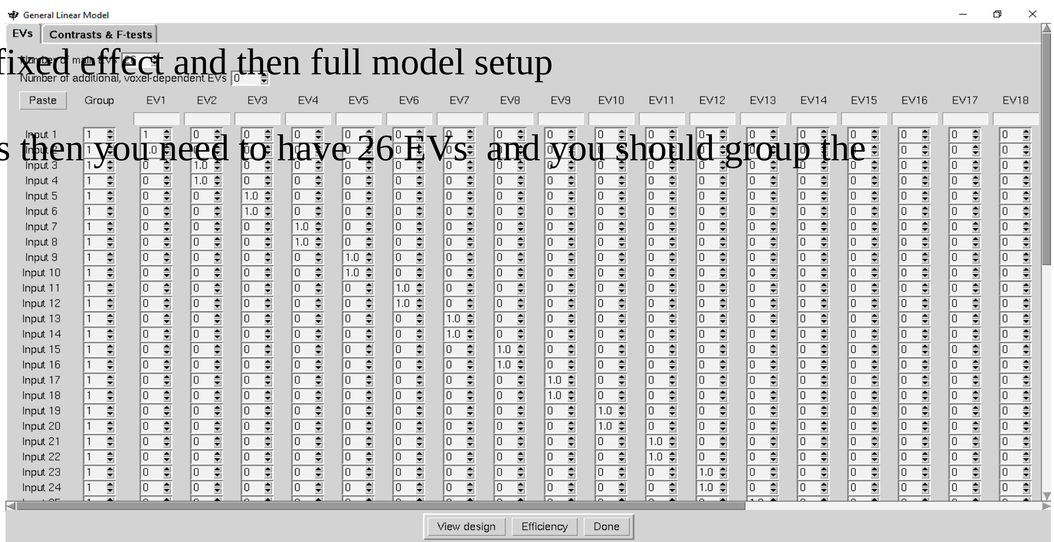



2)choose your fet directories as an input
here instead of manually selecting each directory , we will use wildcard selection to select all 52 fet at once by running the following command in the terminal
**ls -d$PWD/sub-??/func/run***

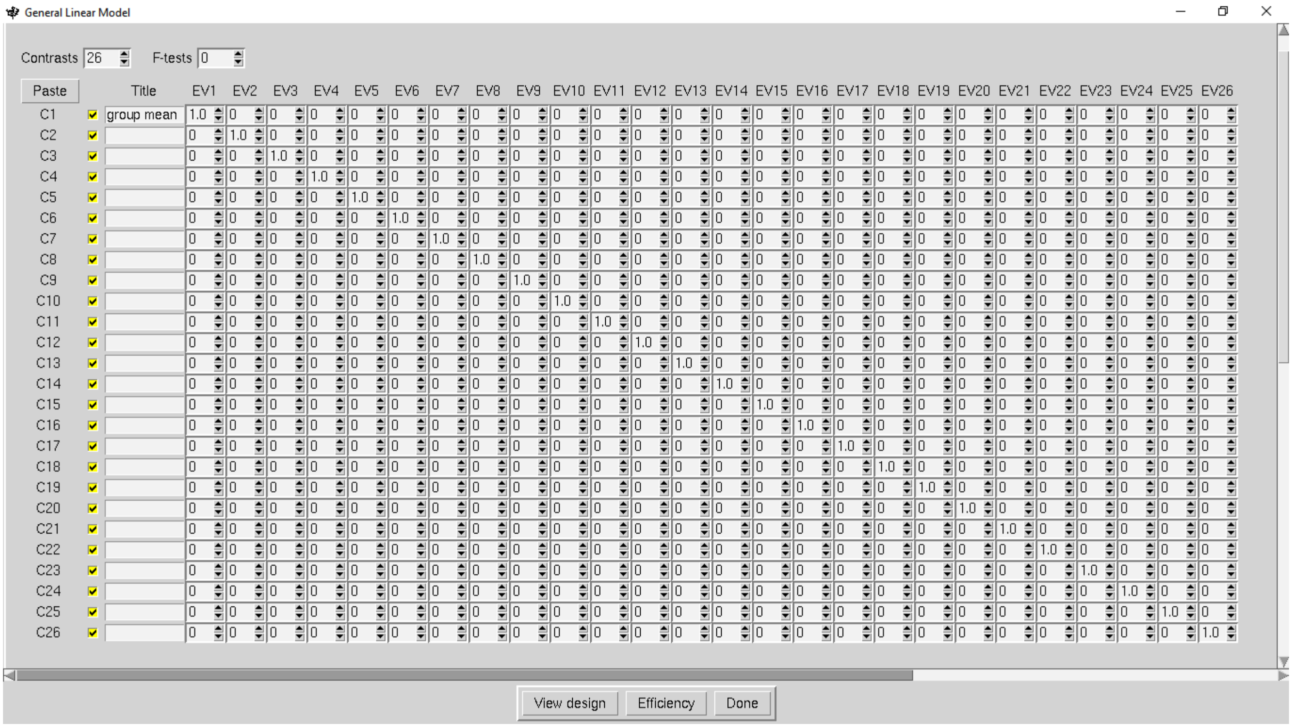
3)select your ouput directory

4) from the stats choose fixed effect and then full model setup

5) since you have 52 runs then you need to have 26 EVs and you should group the two runs of each subject



6) in the contrast tab, you have 26 contrasts and the output should be diagonally as follow:




7) click done and go.

# Inference Types

| Inference Type | Description |
|---|---|
| **Fixed effects** | Assumes that the data being analyzed comes from a single group or subject and estimates the effects of interest based on individual data. Considered more sensitive to activation than Mixed effects types |
| **Mixed effects: simple OLS** | Assumes that the data being analyzed comes from multiple groups or subjects and estimates both within-subject and between-subject effects. This method uses ordinary least squares (OLS) to estimate the model parameters |
| **Mixed effects: flame 1** | Uses a mixed-effects model to estimate both within-subject and between-subject effects while accounting for the correlation structure of the data. The model is fitted using a combination of OLS and restricted maximum likelihood (REML) methods. |
| **Mixed effects: flame 1+2** | Uses a mixed-effects model similar to flame 1, but also includes a variance smoothing term to model the spatial correlation of the fMRI data. |
| **Randomise** | A permutation-based method that involves randomly shuffling the data and re-analyzing the results to create a null distribution. This distribution is used to determine the significance of the observed results. |
| | |

**Advantages and disadvantages:**

| Inference Type | Advantages | Disadvantages |
|---|---|---|
| **Fixed effects** | Simple and easy to implement. Suitable for within-subject designs. | Assumes equal within-subject and between-subject variances, which may not always be true. |
| **Mixed effects: simple OLS** | more powerful than fixed effects models. | Assumes equal within-subject and between-subject variances, which may not always be true. |
| **Mixed effects: flame 1** | - more powerful than fixed effects models.<br>-takes into account the correlation structure of the data. | Assumes equal within-subject and between-subject variances, which may not always be true. |
| **Mixed effects: flame 1+2** | -more powerful than fixed effects models.<br>-takes into account the correlation structure of the data.<br>- takes into account models spatial variability. | Assumes equal within-subject and between-subject variances, which may not always be true. |
| **Randomise** | Can handle complex correlation structures and unequal variances across groups or subjects. Permutation-based, which provides a strong control of the family-wise error rate. | -Computationally intensive<br><br>-Require a large number of permutations to achieve adequate statistical power. |

The main choice here is between fixed effects (FE) and mixed effects (ME) higher-level modelling. FE modelling is more "sensitive" to activation than ME, but is restricted in the inferences that can be made from its results; because FE ignores cross-session/subject variance, reported activation is with respect to the group of sessions or subjects present, and not representative of the wider population. ME does model the session/subject variability, and it therefore allows inference to be made about the wider population from which the sessions/subjects were drawn.

The FE option implements a standard weighted fixed effects model. No random effects variances are modelled or estimated. The FE error variances are the variances (varcopes) from the previous level. Weighting is introduced by allowing these variances to be unequal (heteroscedastic). Degrees-of-freedom are calculated by summing the effective degrees-of-freedom for each input from the previous level and subtracting the number of higher-level regressors.

We now discuss the different ME options.

OLS (ordinary least squares) is a fast estimation technique which ignores all lower-level variance estimation and applies a very simple higher-level model. This is the least accurate of the ME options.

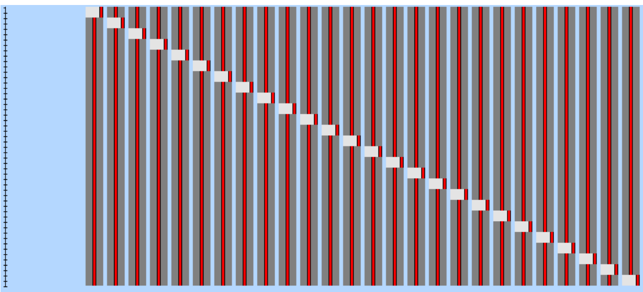
For the most accurate estimation of higher-level activation you should use FLAME (FMRIB's Local Analysis of Mixed Effects) modelling and estimation. This is a sophisticated two-stage process using Bayesian modelling and estimation (for example it allows separate modelling of the variance in different subject groups, and forces random effects variance to be non-negative).

The first stage of FLAME is significantly more accurate than OLS, and nearly as fast. The second stage of FLAME increases accuracy slightly over the first stage, but is quite a lot slower (typically 45-200 minutes). It takes all voxels which FLAME stage 1 shows to be near threshold and carries out a full MCMC-based analysis at these points, to get the most accurate estimate of activation.

We generally recommend using "FLAME 1", as it is MUCH faster than running both stages, and nearly as accurate. The added value from running full "FLAME 1+2" is most significant in a highest-level analysis when you have a small number of subjects (say <10).

If you are carrying out a mid-level analysis (e.g., cross-sessions) and will be feeding this into an even higher-level analysis (e.g., cross-subjects), then you should not use the "FLAME 1+2" option, as it is not possible for FLAME to know in advance of the highest-level analysis what voxels will ultimately be near threshold. With respect the question of whether to use fixed-effects or mixed-effects for such mid-level analyses, it could be argued that a mixed-effects analysis should be done at the mid-level. A mixed-effects analysis would assume that the sessions are randomly sampled from a "population" of sessions that that subject could produce. This includes estimation of each subject's session-to-session variance. However, it is common for only a small number of sessions to be collected for each subject, making estimation of each subject's session-to-session variance impractical. One solution to this is to assume a common session-to-session variance for all subjects, thereby providing enough data for the

# Matrix Design



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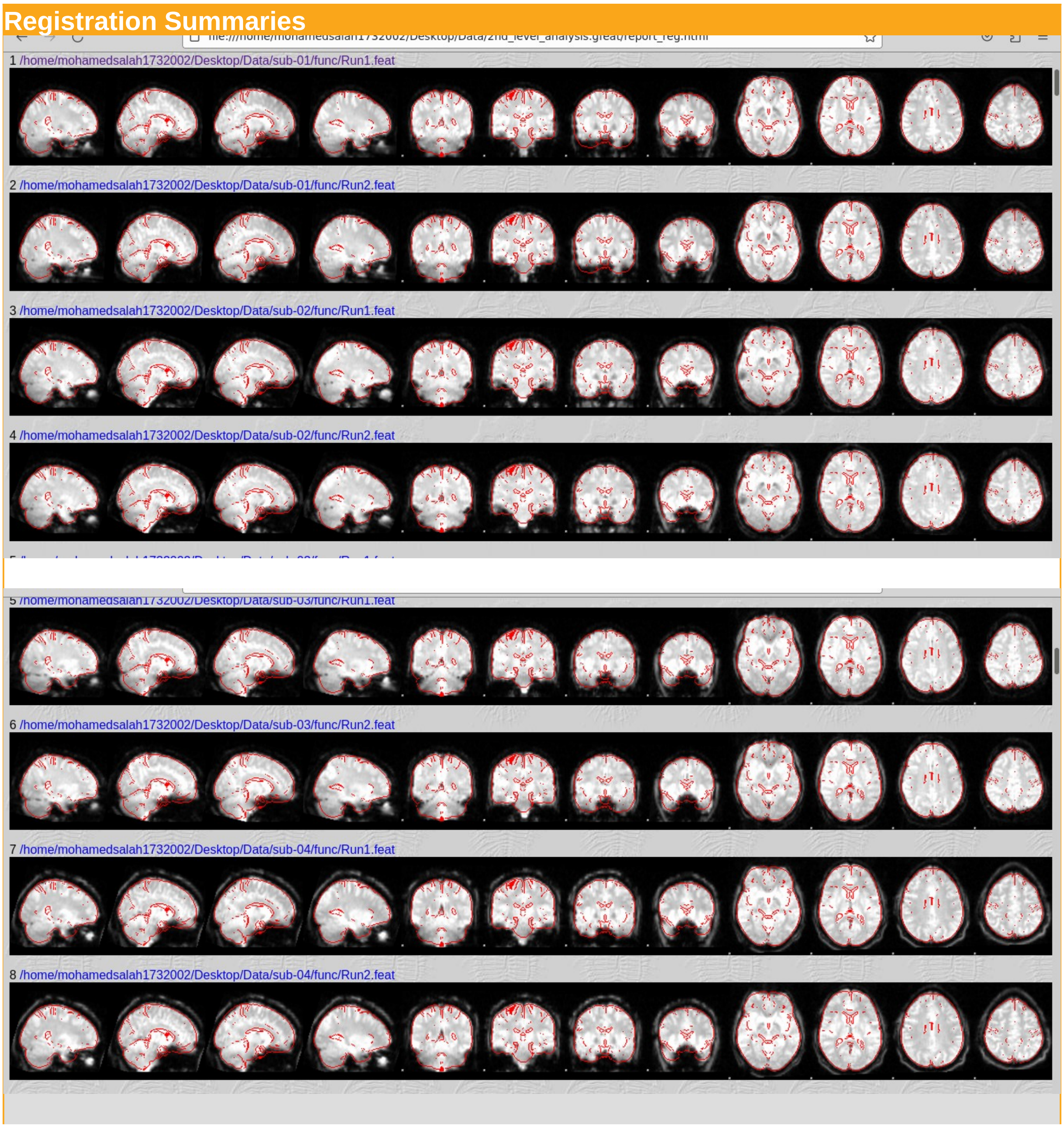
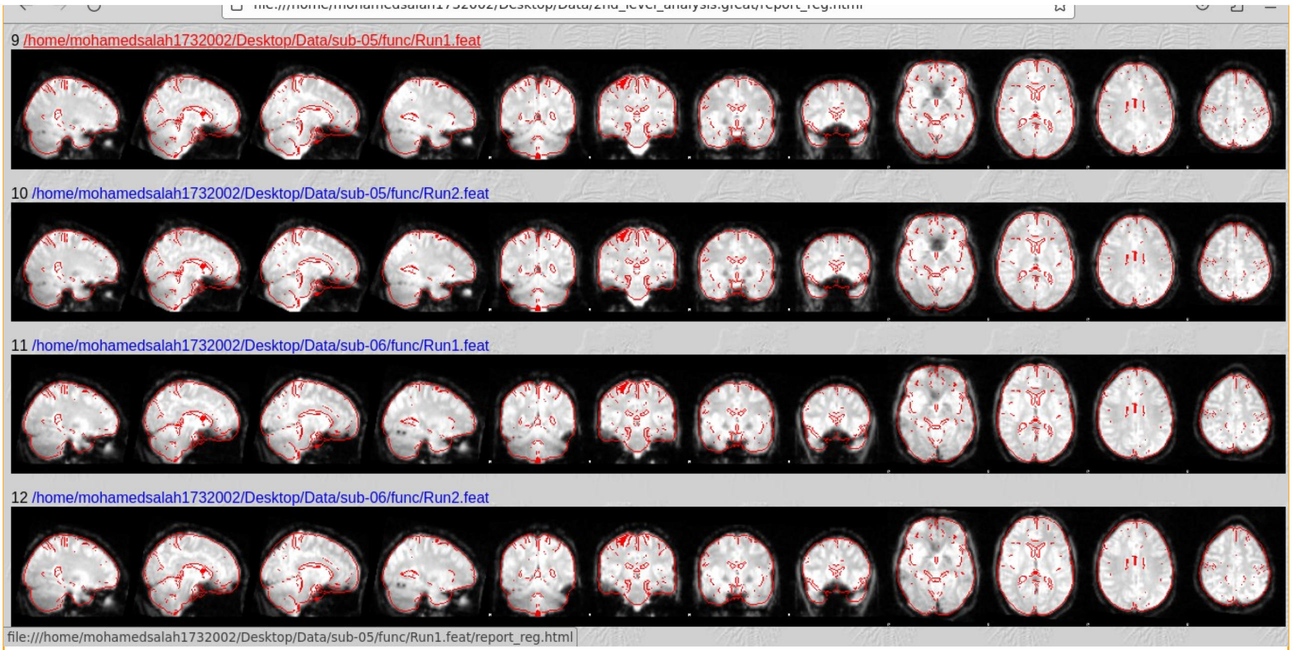
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C1 | group mean | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C2 | | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 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 |
| C4 | | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 | 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 |

# Registration summary

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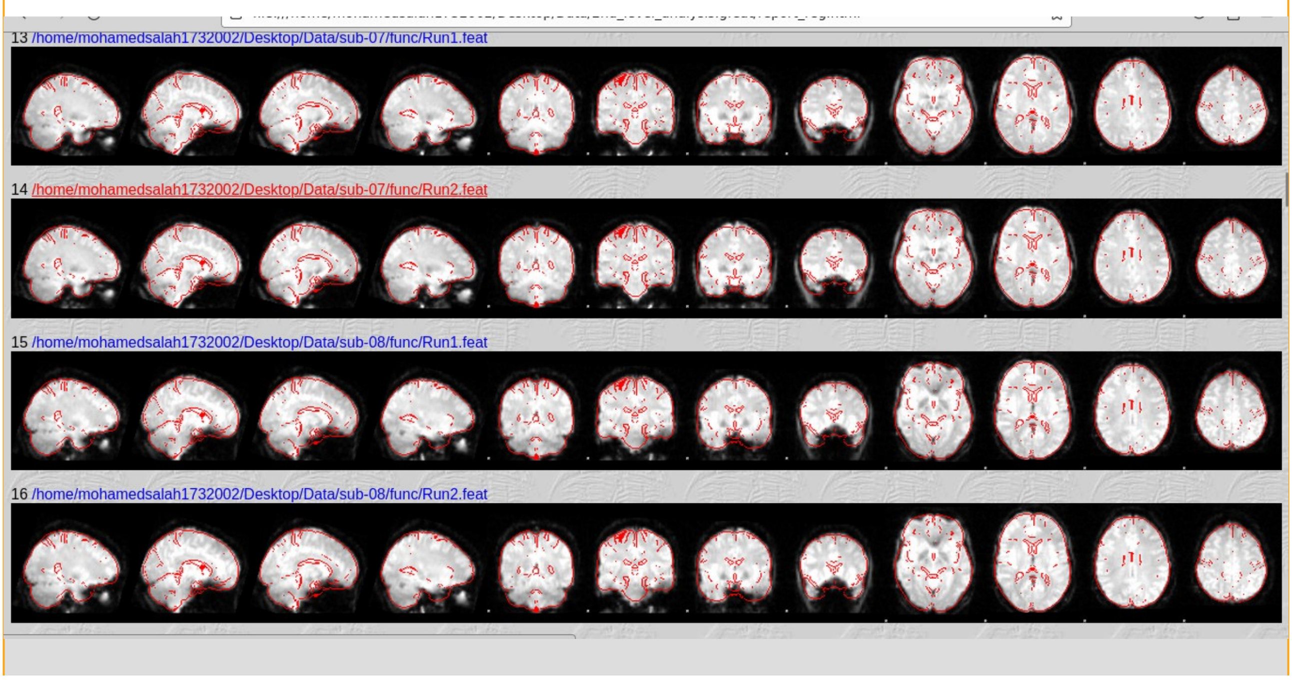
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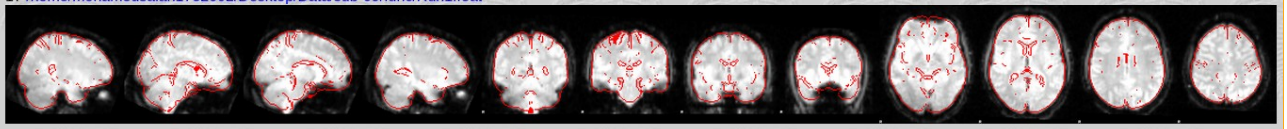

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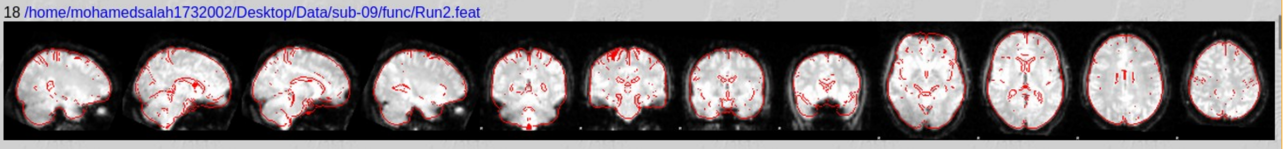

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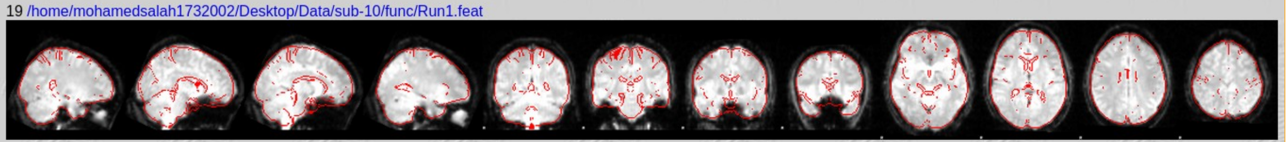

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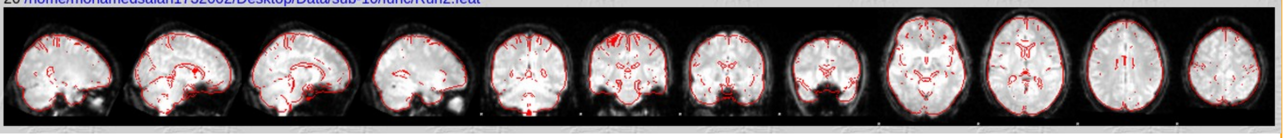

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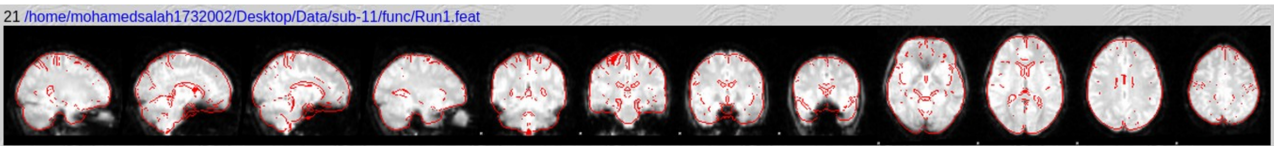

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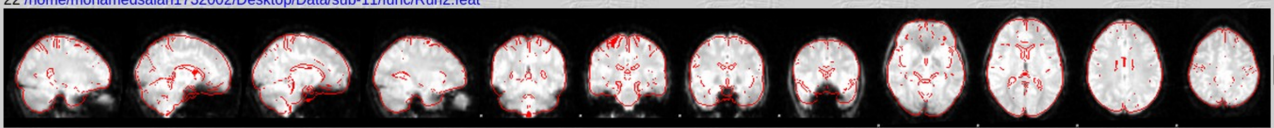

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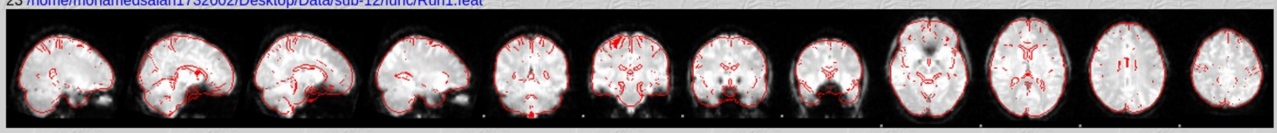

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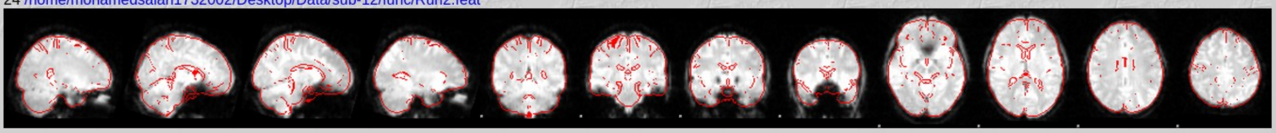

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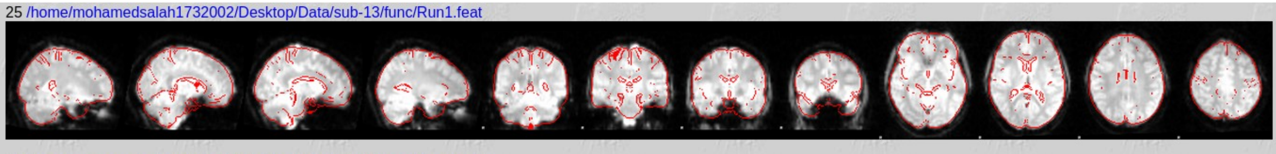

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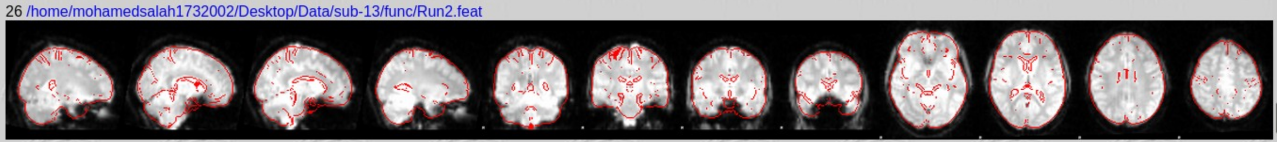

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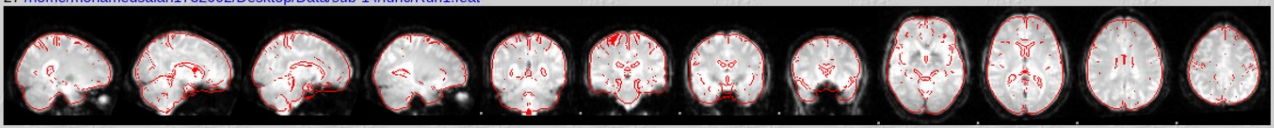

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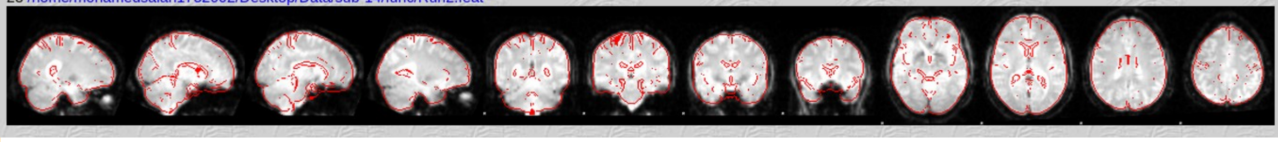

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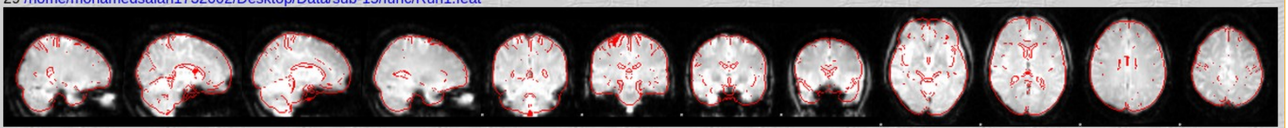

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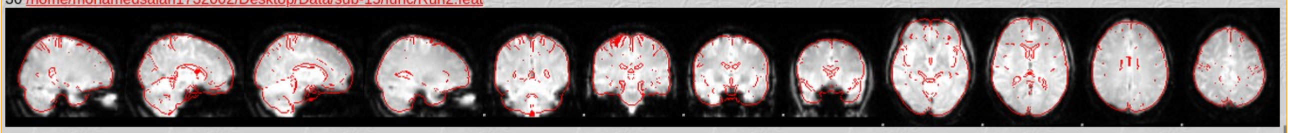

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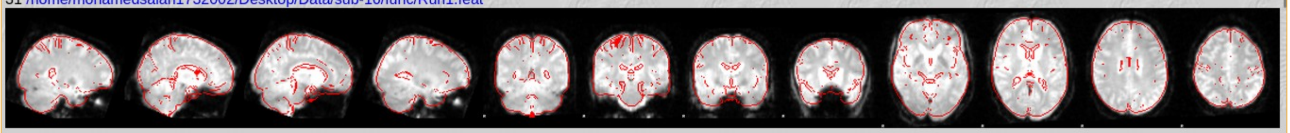

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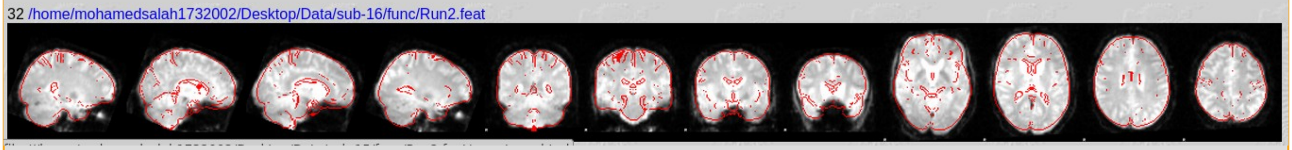

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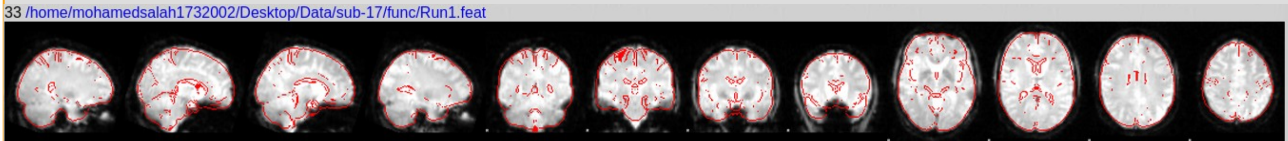

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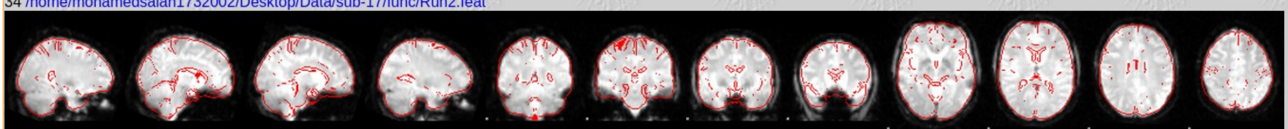

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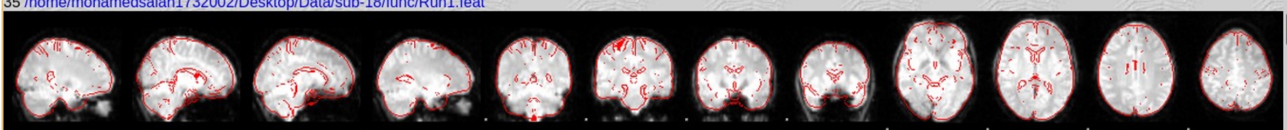

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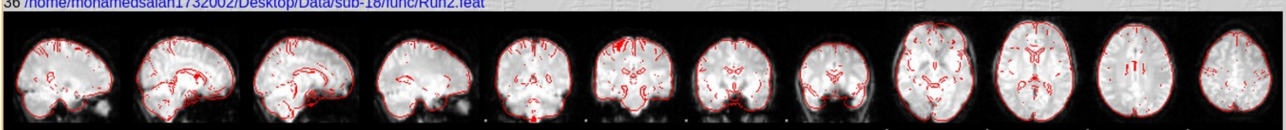

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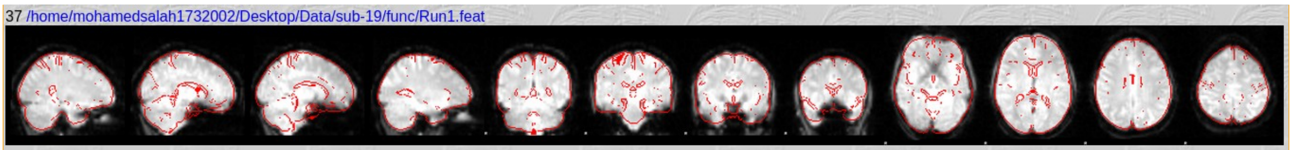

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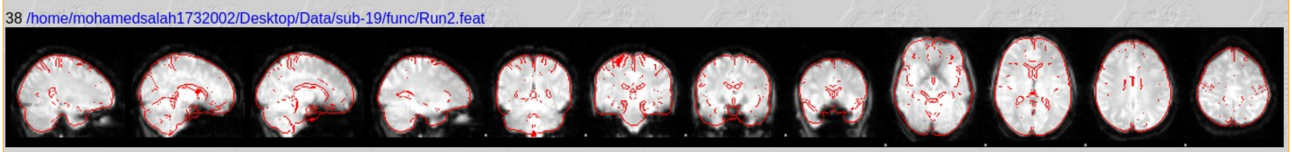

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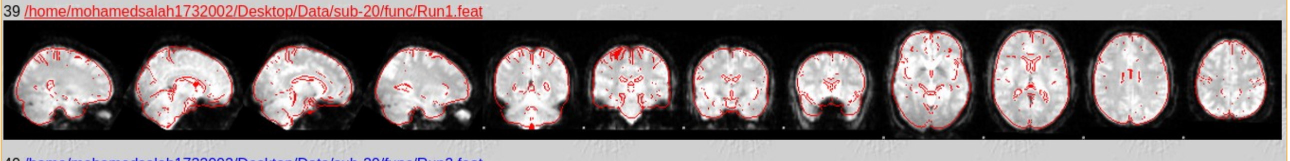

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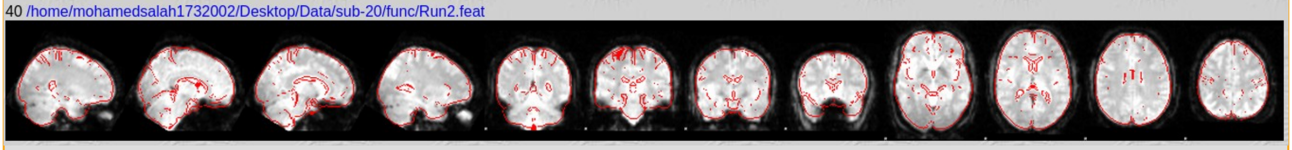

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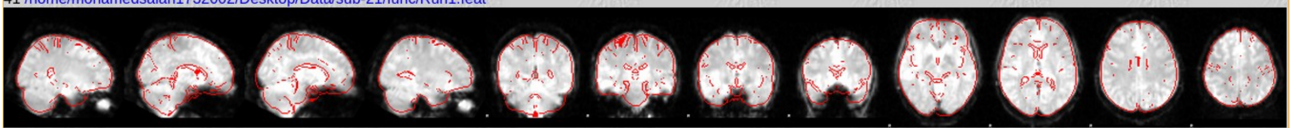

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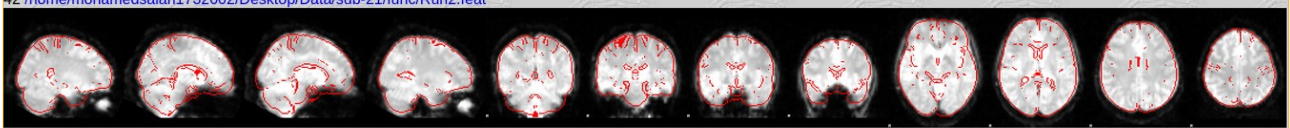

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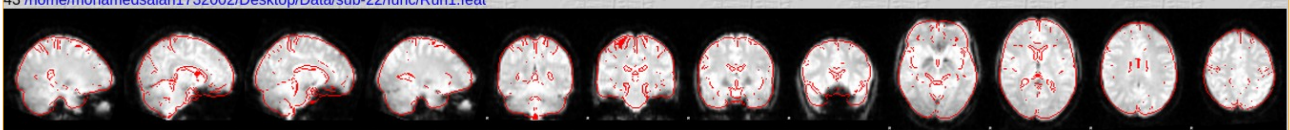

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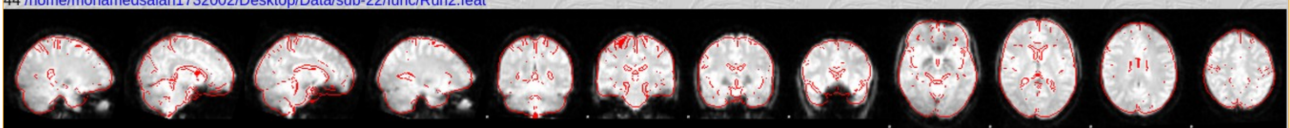

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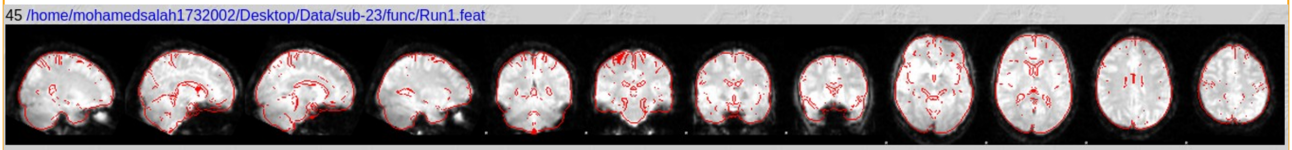

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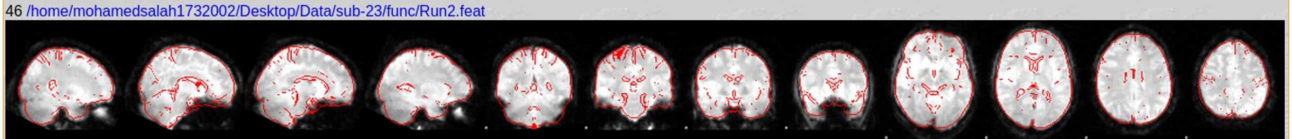
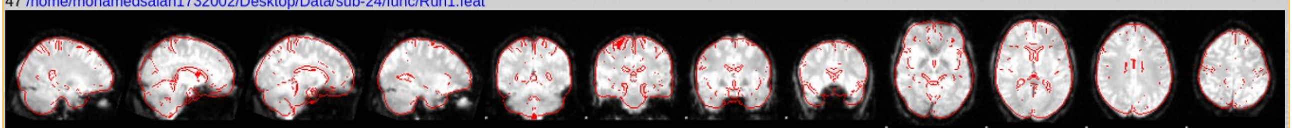
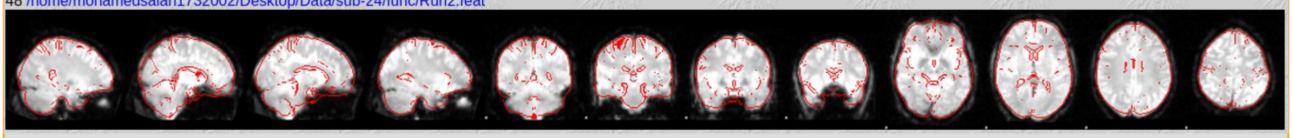
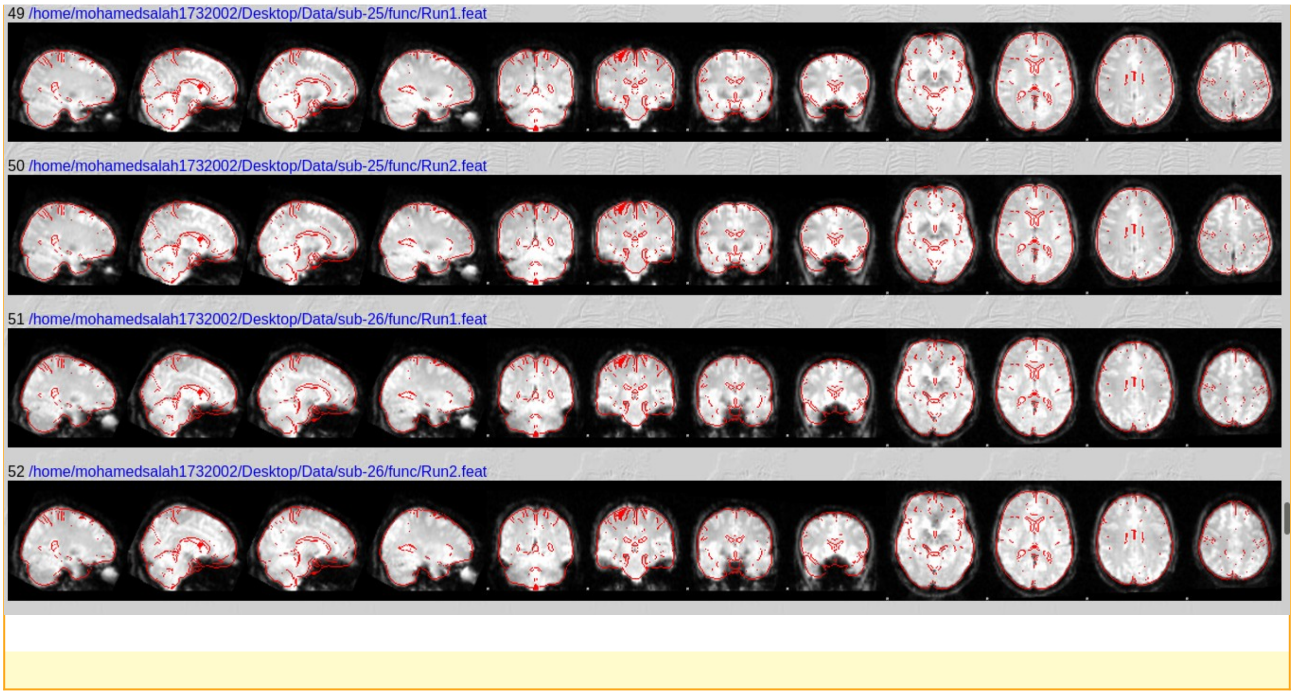


47 /home/mohamedsalah1732002/Desktop/Data/sub-24/func/Run1.feat



48 /home/mohamedsalah1732002/Desktop/Data/sub-24/func/Run2.feat



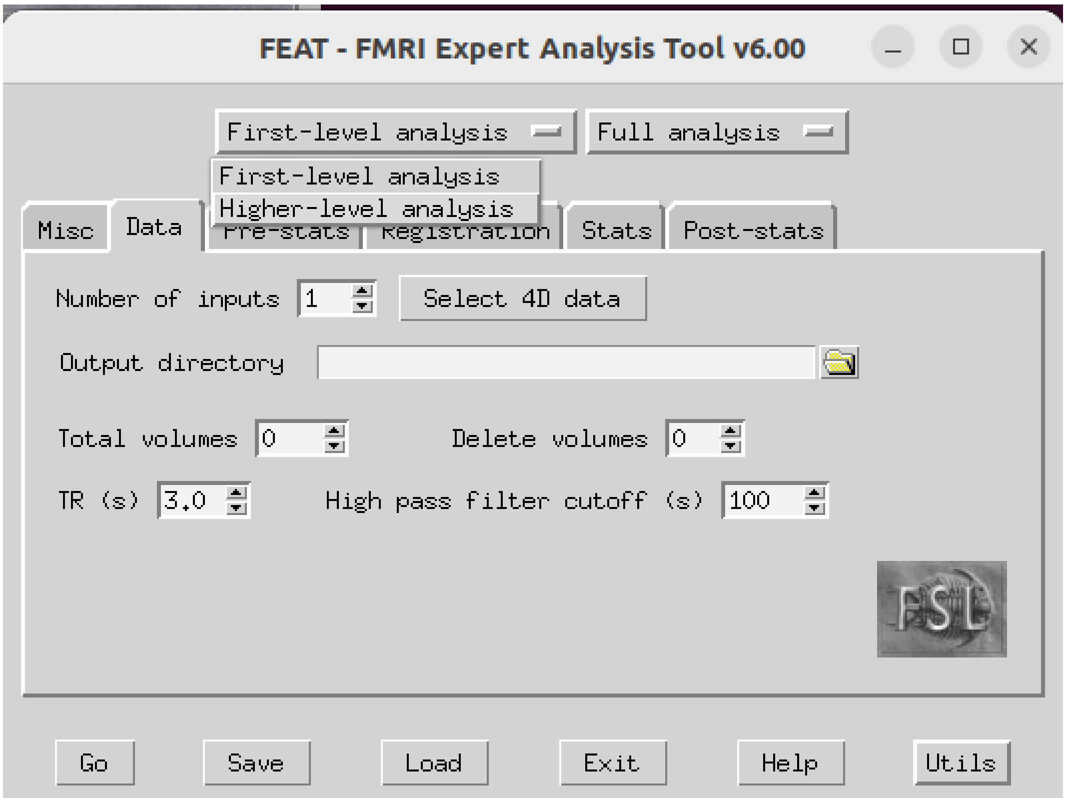



**Note that all images are attached with the report for a better quality.**

# 3rd Level

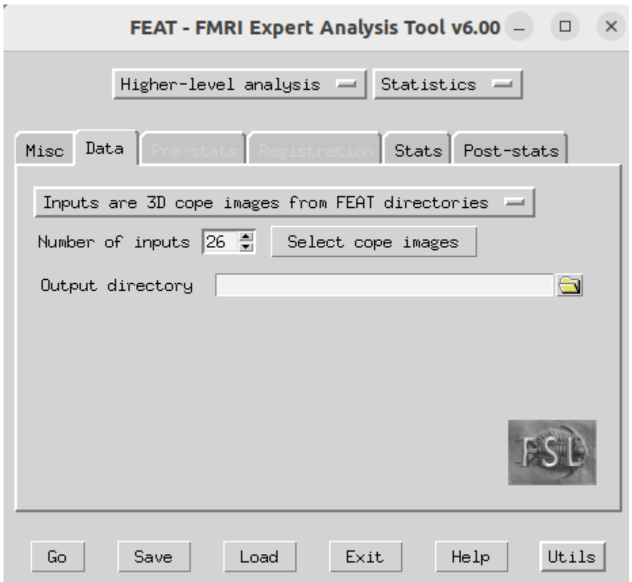
## Steps *for Cope3*

1)open Fet gui and instead of choosing first level analysis , choose higher level analysis



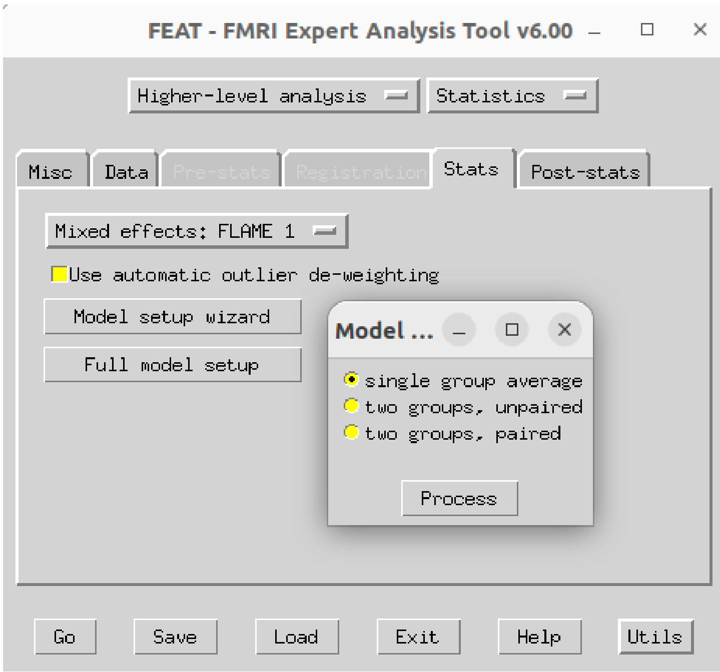

2)choose your 3D cope images as an input here instead of manually selecting each directory , we will use wildcard selection to select all 26 gfet at once by running the following command in the terminal after navigating to cope3.feat/stats

**ls $PWD/cope* | sort -v**




3)select your ouput directory

4) from the stats choose mixed effects Flame 1 and Model setup wizard and choose

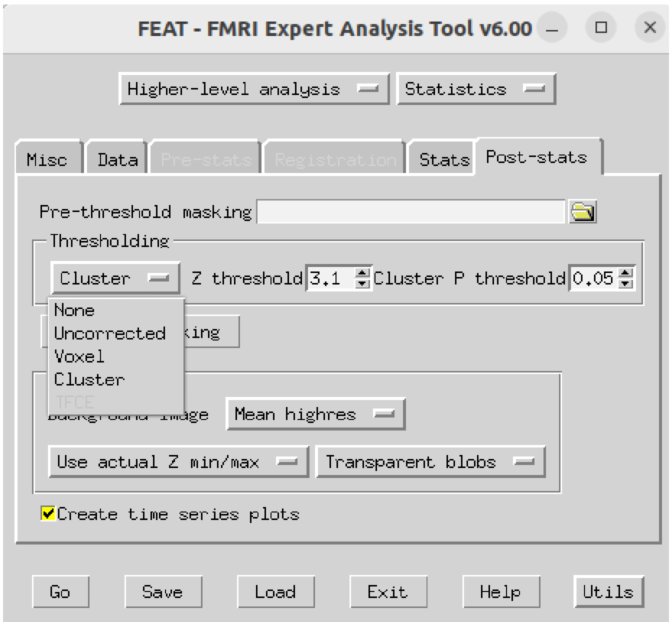



single group average as we already took the contrast in 2nd level and click process

5) go to post-stats tab and make sure it uses cluster based thresholding with:

- Z-threshold of 3.1
- P threshold of 0.05

Note that the difference between the thresholding configurations is that:



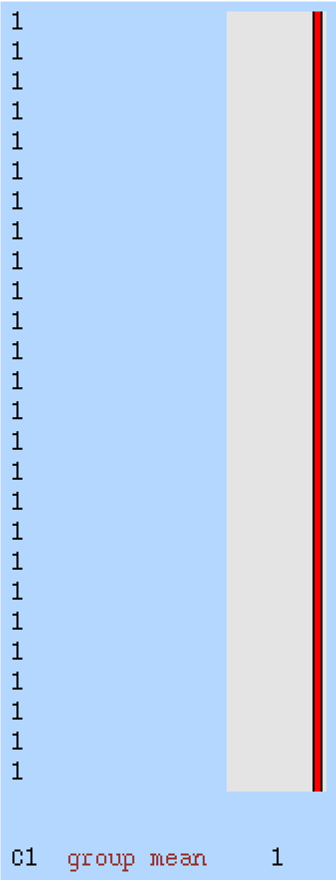

Cluster thresholding => search for segnificant clusters
Voxel thresholding => search for segnificant voxels
Uncorrected thresholding => Don’t correct for multiple comparsions
None => no thresholding/segnificance

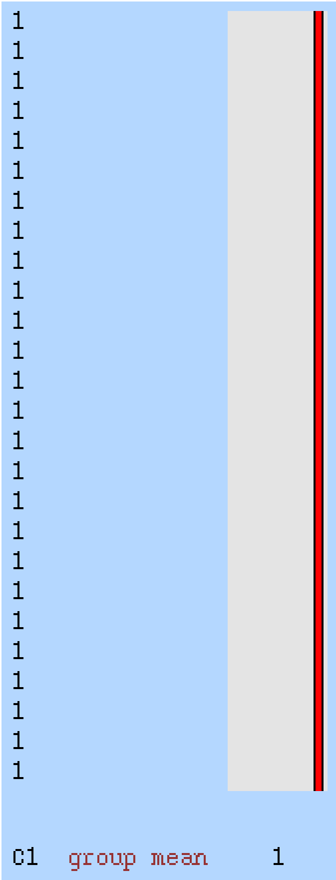
6) Click go

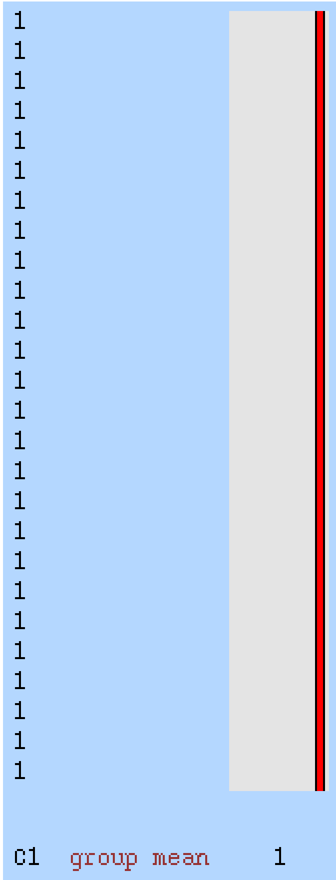
**After you do all the steps , Repeat for Cope1 & Cope2**

# Matrix Design

| Cope-1 | Cope-2 | Cope-3 |
|---|---|---|



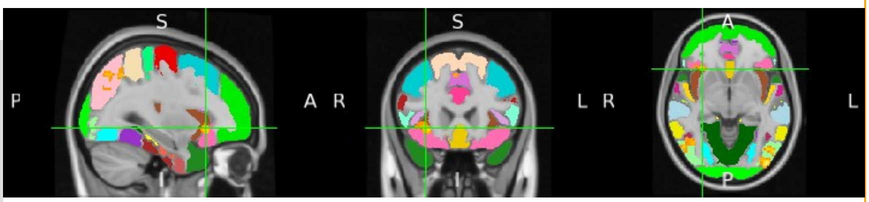
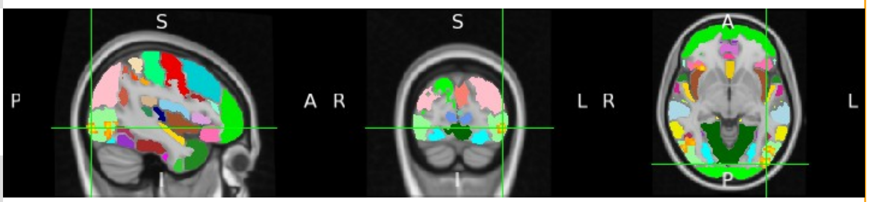
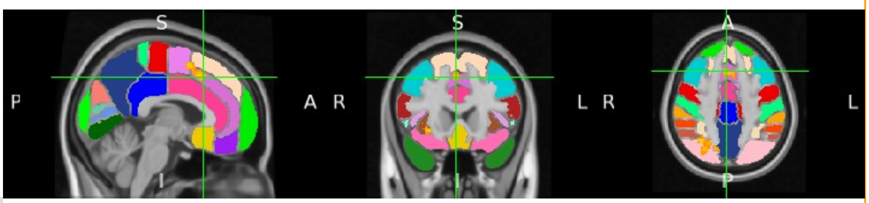



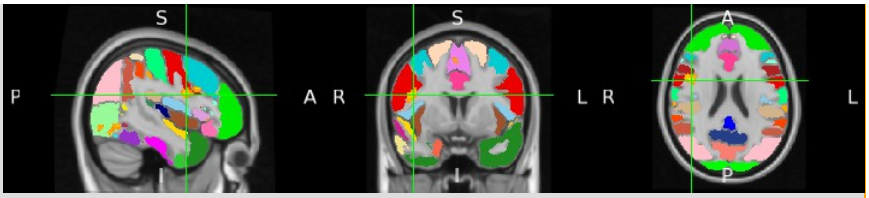
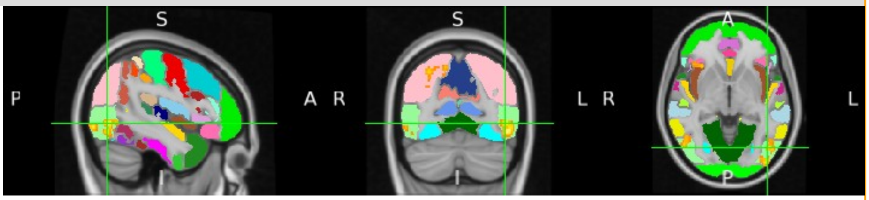
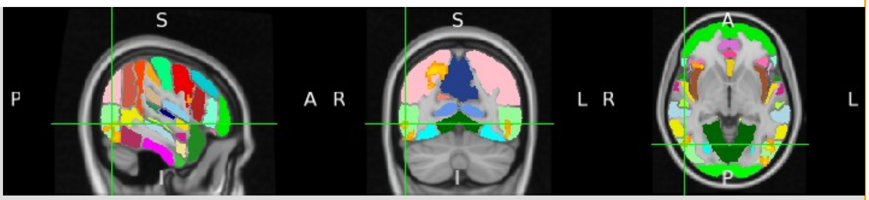
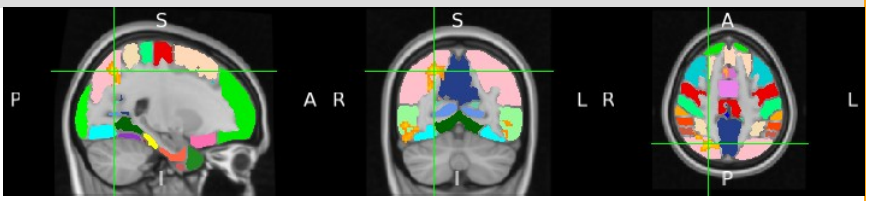
# Segnificant Clusters

To view the segnificant clusters:

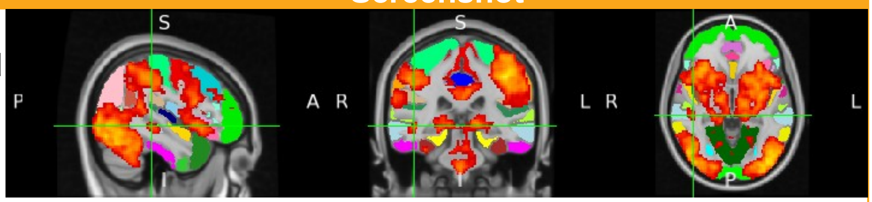
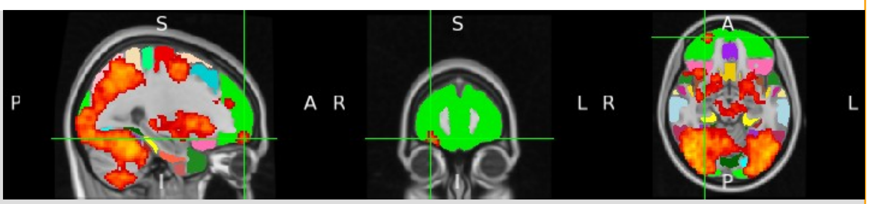
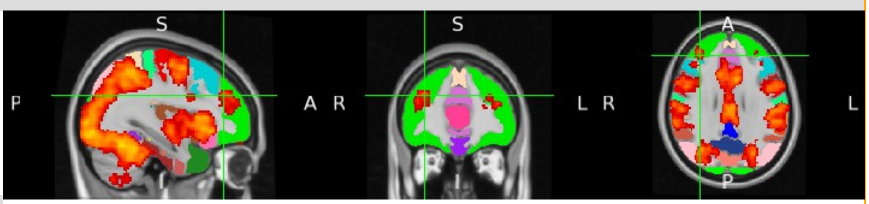
-Open fsleyes
-Open the MNI template
-overlay the harvard-oxford ATLAS to check segnificant clusters in different brain regions.
-load the thresh_zstat.nii.gz
-change the contrast to see the segnificant clusters Ex: Red-yellow
-click on the gear icon and change the interpolation configuration to be linear
-from view,layouts choose FEAT mode

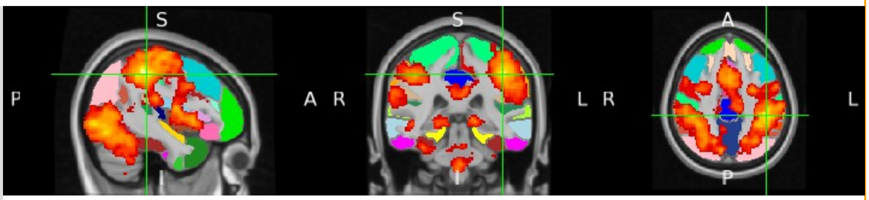
## Cope-3

| | Region | Function | Screenshot |
|---|---|---|---|
| 1 | 52% inasular cortex | self-awareness, cognitive control, and social cognition. |  |
| | 29% Frontal Orbital Cortex | executive functions, decision-making, social behavior, and emotional regulation. | |
| 2 | 66% Lateral Occipital Cortex, inferior division | visual processing and object recognition |  |
| | 10% Occipital Pole | High resolution visual processing | |
| 3 | 81% Paracingulate Gyrus | Emotional processing,cognitive control,Decision making and Social cognition |  |
| | 7% Cingulate Gyrus, anterior division | emotional processing and cognitive control. | |
| 4 | 22% Precentral Gyrus | motor control. | |
| | | | |

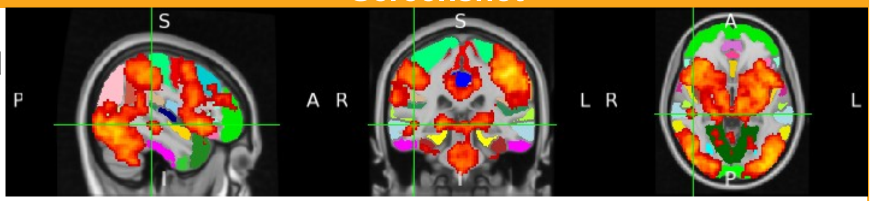
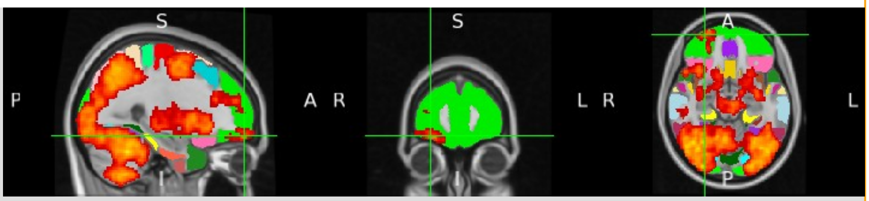
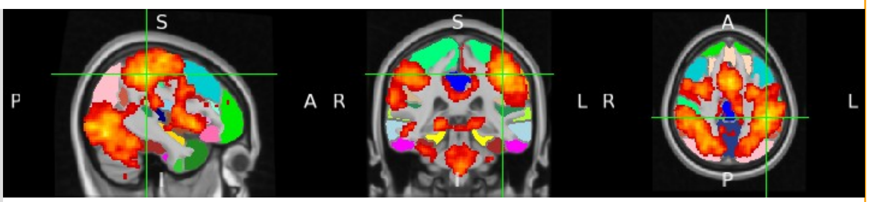
| | | | |
|---|---|---|---|
| | 12% Inferior Frontal Gyrus, pars opercularis | speech production and language processing. |  |
| 5 | 79% Lateral Occipital Cortex, inferior division | visual object recognition |  |
| 6 | 87% Lateral Occipital Cortex, inferior division | visual processing |  |
| 7 | 49% Lateral Occipital Cortex, superior division | visual object recognition |  |

## Cope-2

| | Region | Function | Screenshot |
|---|---|---|---|
| 1 | 45% Middle Temporal Gyrus, posterior division | visual motion processing and perception. |  |
| 2 | 73% Frontal Pole | Cognitive control,Decision making and Social cognition |  |
| 3 | 52% Frontal Pole | Cognitive control,Decision making and Social cognition |  |
| | 16% Middle Frontal Gyrus | Memory related functions | |

| | | | |
|---|---|---|---|
| 4 | 32% Postcentral Gyrus | somatosensory processing. | |
| | 20% Supramarginal Gyrus, anterior division | language processing and phonological awareness. |  |

# Cope-1

| | Region | Function | Screenshot |
|---|---|---|---|
| 1 | 45% Middle Temporal Gyrus, posterior division | visual motion processing and perception. |  |
| 2 | 73% Frontal Pole | Cognitive control,Decision making and Social cognition |  |
| 3 | 32% Postcentral Gyrus | somatosensory processing. | |
| | 17% Superior Parietal Lobule | spatial processing , visuospatial perception, attention, and working memory |  |

**Observations:**

- Most  segnificant clusters are in brain regions that their main function is associated with visual processing , decision making and memory related tasks with vaildates the FLANKER task

-The following regions are activated equally in both cope1 – incongruent– and cope2 – congruent– :

1)45% Middle Temporal Gyrus, posterior division
2)73% Frontal Pole
3)32% Postcentral Gyrus
4)17% Superior Parietal Lobule

- in the contrast cope – Cope3–  , 4 out of the 7 segnificant clusters can be found in two different brain regions.

# Exercises

## Exercise 1:

In the Post-stats tab, set the Thresholding to None, and re-run the analysis (changing the output directory to something that indicates that no threshold is being used). Examine the results in fsleyes. How do they compare to the cluster-corrected results?

Answer => changing the thresholding configuration from cluster based to none will result in the following:

- The results would include false-positive regions.

- All activation values will be seen in different brain regions , both segnificant and non-segnificant.

- Results won't be reliable as when working with cluster based thresholding.

## Exercise 2:

Do the same procedure in the previous exercise, this time using an Uncorrected threshold. Then, repeat the procedure with a Voxel threshold. Note any differences between these results and what you generated with the clustercorrected results. In your own words, describe why the results are different

| Thresholding approach | Main differences |
|---|---|
| **Cluster based** | **Activation areas are segnificant clusters.** |
| | **Good way to reduse false positives.** |
| **Voxel based** | **Search for each segnificant voxel individually.** |
| | **Localize the activation areas more secifically.** |
| | **Not a vaild approach to reduce false positives.** |
| **Uncorrected** | **Evaluates each voxel individually in a statistical approach.** |
| | **No correction which means hugh risk for false positives.** |
| | |

That's it for the 2nd & 3rd level analysis , for high resolution images, check the attached files.