fMRI analysis of Brain Activations induced by the Flanker Task

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***Abstract*—This study aims to compare the brain activations between congruent and non-congruent cases when trying to solve the flanker task using fMRI data analysis techniques.**

I. Introduction

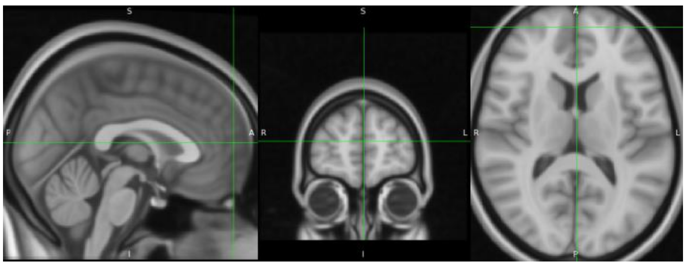
The Eriksen flanker task is a set of tests to assess selective attention and inhibitory function. In this task, the target is positioned in the center and is flanked by nontarget stimuli. The individual is requested to press the left or right arrow key according to the target's direction. There are three types of nontarget stimuli in the Eriksen flanker task, we will focus on the congruent stimulus, in which the direction of the nontarget stimuli is the same as the target, and the incongruent stimulus ,in which the direction of the nontarget stimuli is the opposite of the target. Therefore, choosing a correct response is more challenging than the compatible mode. We will explore the brain activations corresponding to each mode and compare them using structural and fMRI data collected from 26 subjects to gain more insight into the different brain functions.

II. Brain Anatomy

A. *Telencephalon*

1) *Brain Lobes*

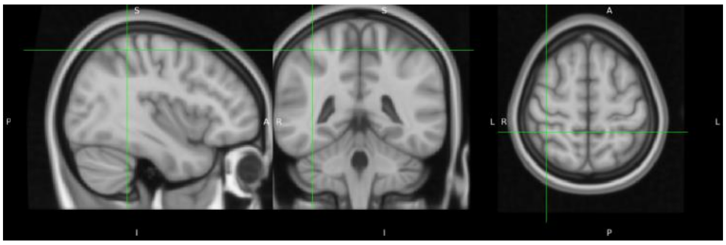
a) *Frontal Lobe*



Main Functions:

a) Controls higher execute functions as planning, problem solving, reasoning and emotional regulation.
b) Has the primary frontal cortex which initiates voluntary movements of a specific body part.
c) Is involved in smell recognition.

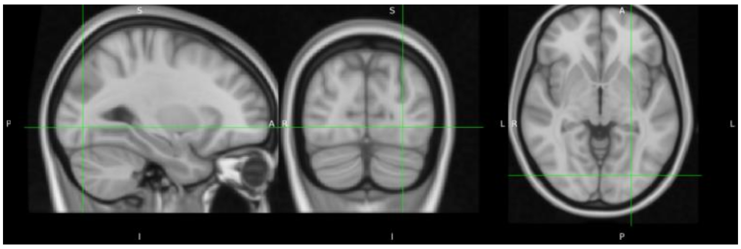
b) *Parietal Lobe*



Main Functions:

a) Feelings interpretation.
b) Processing of sensory inputs as touch, taste, temperature, texture, pressure and pain.
c) Comprehending spatial relationships.
d) Includes the Wernicke's area that assists the brain in understanding spoken language.

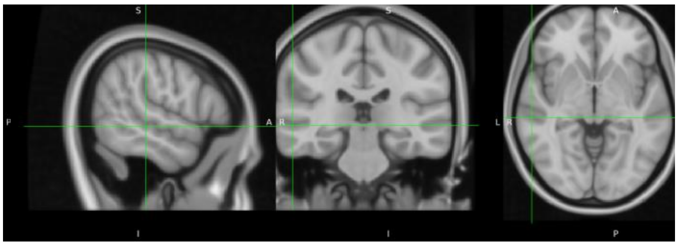
c) *Occipital Lobe*



Main Functions:

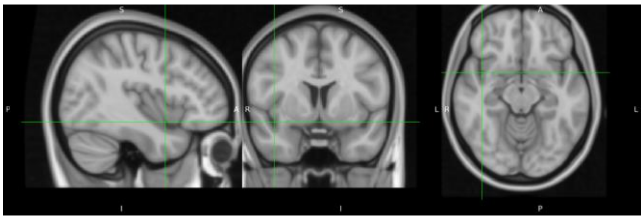
e) Visual processing, it contains the primary visual cortex, which is responsible for receiving visual input from the eyes. Subsequently, this data is transmitted to various secondary visual processing regions, which are tasked with analyzing aspects such as depth perception, spatial location, and object recognition.
f) Responsible for image and object recognition.

d) ***Temporal Lobe***



Main Functions:
1) Takes part in memory storage.
2) Contains the primary auditory cortex, which receives auditory information from the ears and interprets it.
3) Certain areas in the temporal lobe make sense of complex visual information including faces and scenes.
4) Contains the hippocampus, responsible for memory, learning and emotions.

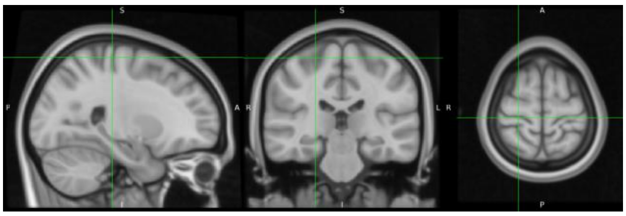
e) ***Insula***



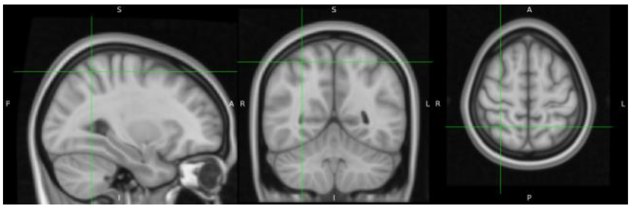
Main Functions:
1) Important for taste and sensorimotor processing, auditory risk-reward behavior and vestibular functioning that helps maintain balance and stability.
2) Responsible for the awareness of our bodies and emotions collectively, creating our perception of the present moment, conscious realization and awareness of our bodily states.
3) Partly responsible for the perception of pain.

2) ***Fissures/sulci separating the lobes***

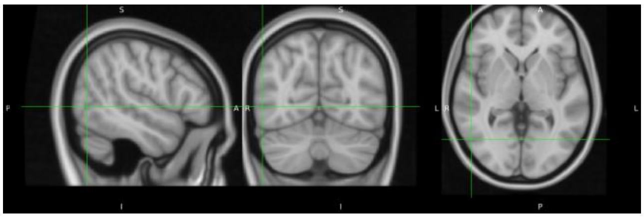
1) The central sulcus: **Separates the frontal and parietal lobes.**



2) The parieto-occipital fissure: **Separates the parietal and occipital lobes.**



3) The lateral sulcus: Separates the frontal and temporal lobes.

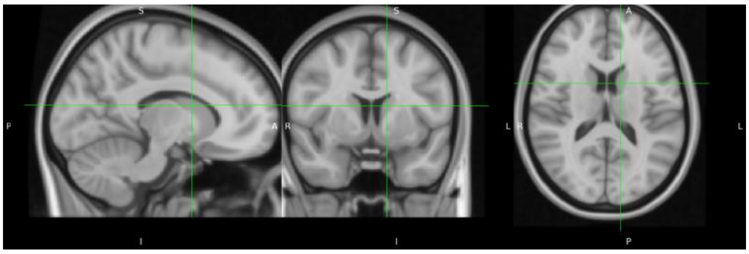


3) ***Basal Ganglia***

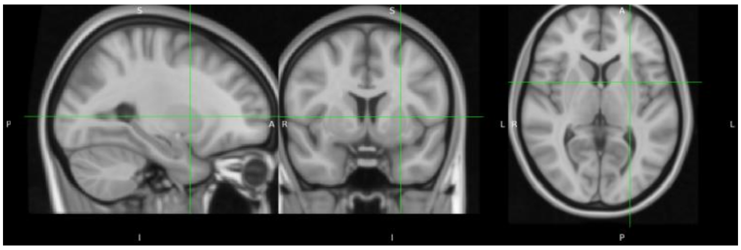
a) Striatum

Main Functions:
1) Processing sensory information related to the body's spatial orientation, helping to fine-tune motor responses by relaying instructions to the thalamus, which influences motor activity. This process ensures movements are appropriately coordinated with the body's current position.
2) Maintaining body and limb posture, enhancing the speed and accuracy of movements directed towards specific targets, producing a smooth execution of both voluntary and involuntary movements.
3) Cognitive and emotional processes such as memory, learning and emotions.

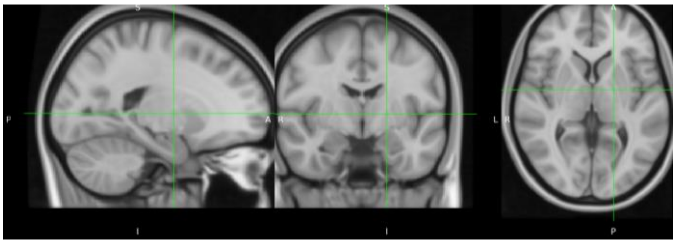
1) Caudate



2) Putamen

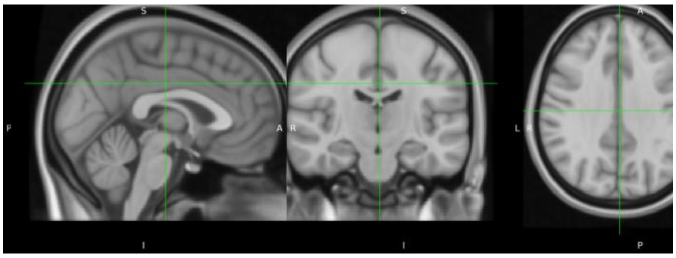


b) Globus Pallidus



4) Additional Structures

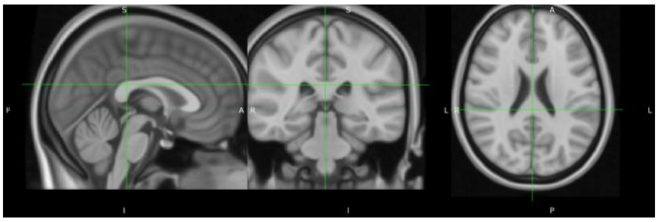
a) Cingulate Cortex



Main Functions:

1) Areas of the anterior cingulate cortex that are densely interconnected with limbic system structures like the amygdala and hypothalamus is thought to be involved with a number of functions related to emotional regulation and the expression of states or desires.
2) Contributes to the regulation of autonomic and endocrine responses, pain perception, and the selection and initiation of motor movements.
3) Involved in various aspects of cognition ranging from decision-making to the management of social behavior.
4) Is a part of the default mode network.
5) Regulates the balance between internally and externally-focused attention, making it a crucial structure in awareness and attentional focus.

b) Corpus Callosum

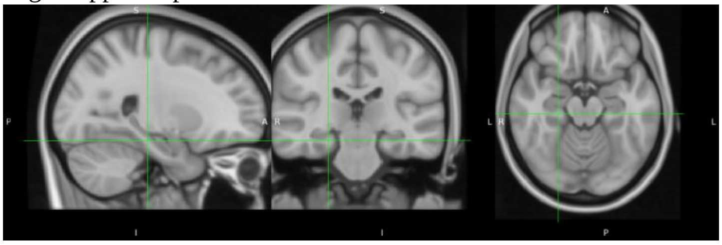


Main Function:

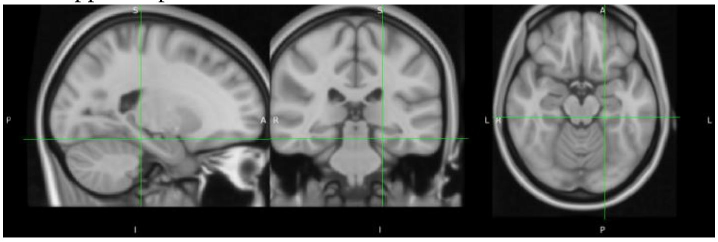
1) The connection of the two hemispheres, allowing for the transfer of signals as sensory, motor and cognitive information.

c) Hippocampus

Right hippocampus



Left hippocampus

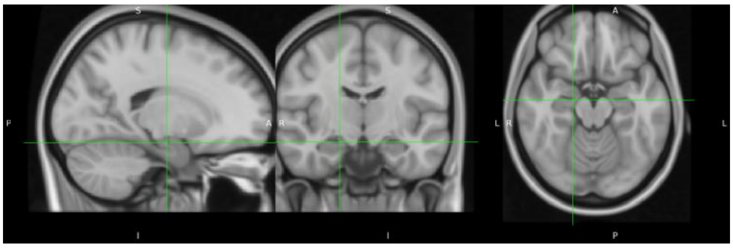


Main Function:

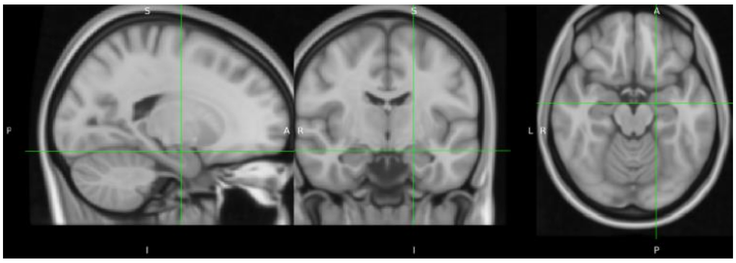
1) Spatial navigation and the formation of a cognitive map which is a type of mental representation related to acquisition, storing, recalling, and decoding of information on relative locations.
2) Emotional Behavior and behavioral inhibition.
3) Regulation of hypothalamic functions.
4) Transferring short-term memory to long-term.

d) Amygdala

Right amygdala



Left amygdala

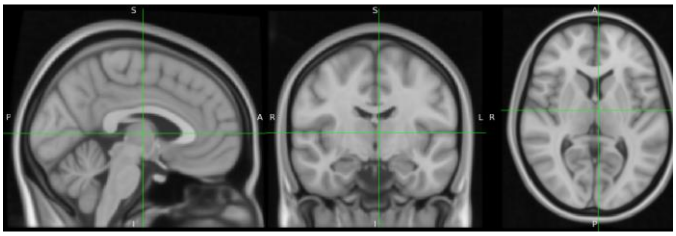


Main Function:

1) Responsible for emotional processing, learning and regulation.
2) The main emotion it controls is fear, but also aggression, learning through rewards and punishment, emotions that relate to parenting, caregiving, memories and learned behaviors related to addiction.
3) Involved in the fight-or-flight response.
4) Social communication and understanding, including behavior interpretation.

B. ***Diencephalon***

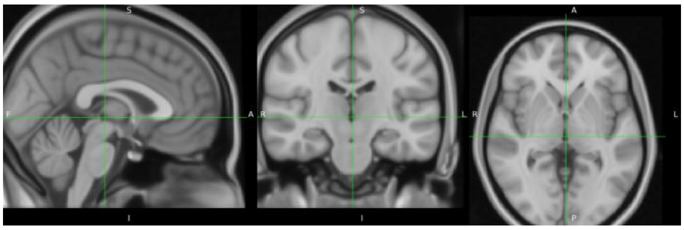
1) ***Thalamus***



Main Functions:

1) Relaying sensory and motor information.
2) Prioritizing attention.
3) Has an important role in consciousness, preserving alertness.
4) Thinking (cognition) and memory, it is connected with structures of the limbic system, which is involved in processing and regulating emotions, formation and storage of memories, sexual arousal and learning.

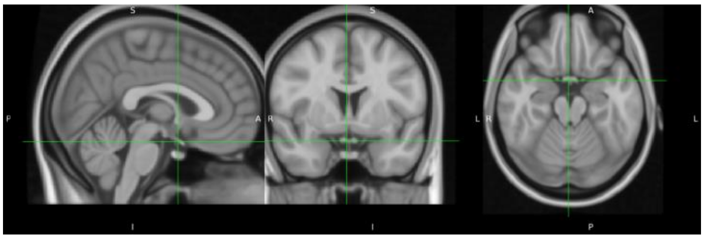
2) ***Hypothalamus***



Main Functions:

1) Maintaining the body's homeostasis by stimulating or inhibiting many of the body's activities as regulating body temperature, appetite and body weight, heart rate and blood pressure, etc.
2) Connects the endocrine and the nervous system.
3) Is involved in many essential functions of the body, including: childbirth, emotions, sleep cycles, balancing body fluids, appetite and thirst control.

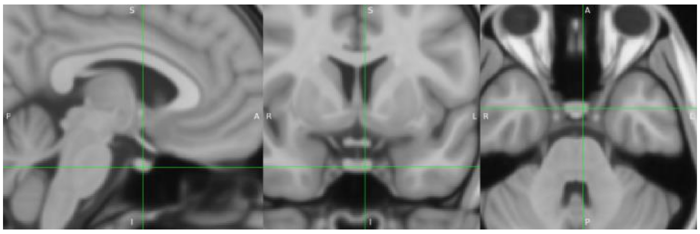
3) ***Optic Chiasm***



Main Function:

1) Allows the crossing of fibers from the nasal retina to the optic tract on the opposite side, enabling the right visual field information to travel to the visual cortex in the left hemisphere of the brain through the left optic tract and vice versa. This causes visual information from the eye on one side to be processed by the occipital cortex of the opposite hemisphere.

4) ***Pituitary Gland***

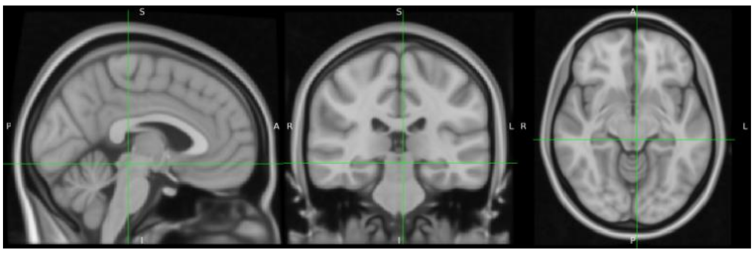


Main Functions:

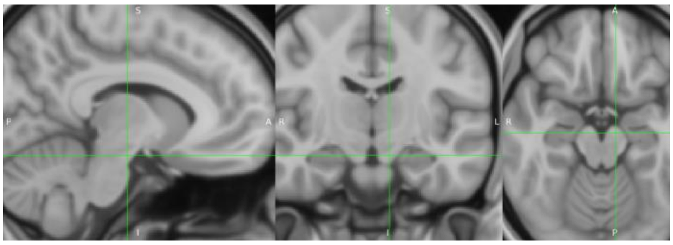
1) Controls the function of other glands in the body, regulating the flow of hormones from the thyroid, adrenals, ovaries and testicles. Note that it receives chemical signals from the hypothalamus through its stalk and blood supply.
2) Hormones it produces include: Follicle-stimulating hormone (FSH), Growth hormone (GH), Luteinizing hormone (LH), Prolactin and Thyroid-stimulating hormone (TSH).
3) Hormones stored and released by the pituitary gland, but made by the hypothalamus include: Oxytocin and Antidiuretic hormone (ADH).
4) Partly responsible for the perception of pain.
5) Temperature Regulation

C. ***Brainstem***

1) ***Midbrain***



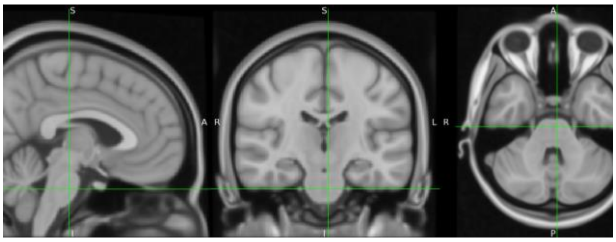
a) ***Cerebral Peduncles***



Main Functions:

1) Represent the paired connections between the cerebellum and the brainstem, relaying information to the cerebellum to coordinate complex movements and maintains the body's balance and posture.
2) Responsible for pain processing, arousal and alertness.

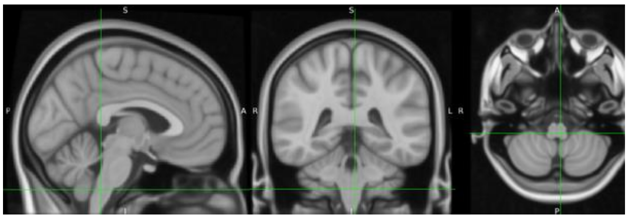
2) ***Pons***



Main Functions:

1) Pain signal management.
2) Controls unconscious processes like the sleep cycle and breathing in cooperation with the other regions of the brainstem.
3) Connects with the cerebellum to handle balance and movement and with many junction points for nerves that control muscles and carry sensory information from the head and face.

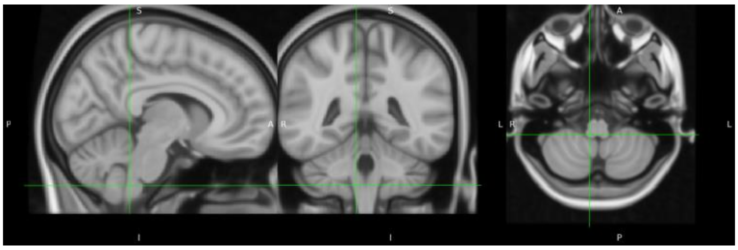
3) ***Medulla***



Main Functions:

1) Most of the major nerves converge at the spine, carrying signals to and from the brain through the medulla. These nerves include four of the 12 cranial nerves (connecting areas as the tongue and throat to the brain).
2) Controlling automatic vital processes.
3) Connects the cardiovascular and respiratory systems link together into a united system to control the heart rate, breathing, blood pressure and more.

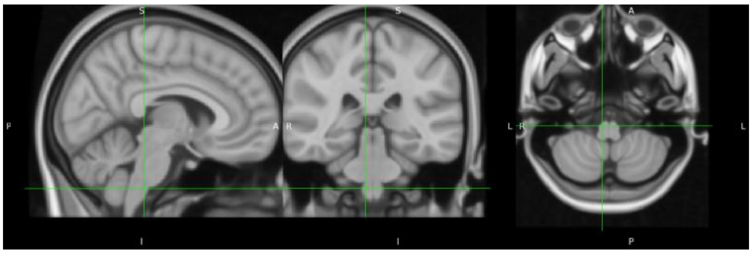
a) ***Inferior Olives***



Main Functions:

1) Learning and timing of movements, and comparing intended with achieved movements.
2) Functions as a relay station between the spine and cerebellum, integrating motor and sensory information to provide feedback and training to cerebellar neurons.

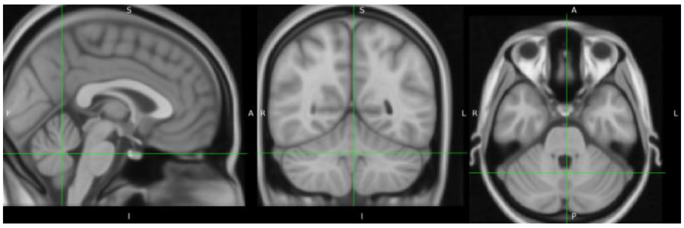
b) ***Pyramids***



Main Functions:

1) Acts as a Crossover point where the crossing of motor fibers occurs so that the left hemisphere controls the right side of the body and vice versa. Therefore, it is responsible for the control and coordination of voluntary actions on both sides of the body.

D. ***Cerebellum***



Main Functions:

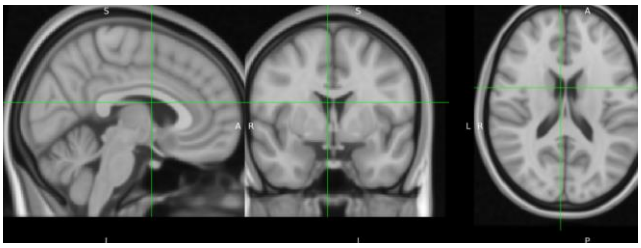
1) Maintaining balance: The cerebellum detects disruptions in balance and movement and sends a feedback signal to the body to counteract imbalance.
2) Responsible for motion coordination especially for movements that necessitates the coordination of many muscle groups, it times muscle activities to successfully execute this movement.
3) Motor Learning, adapting motor programs to make accurate motions as in in learning to ride a bicycle or play a musical instrument.
4) Controls muscle tone, posture and voluntary muscle activity

E. The Ventricular System

Main Functions:

1) Secretion of CSF and maintaining its circulation through the ventricular system.
2) The CSF allows for the transport of nutrients and waste in and out of the neural tissue.

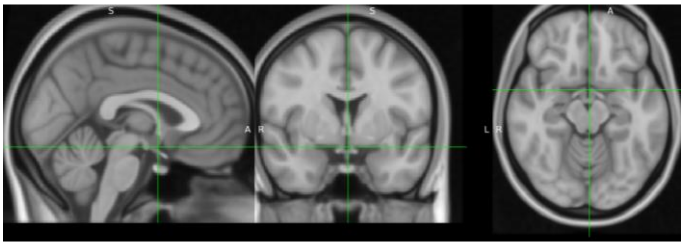
1) The Lateral Ventricles



Additional Function

1) Contains the choroid plexus which is responsible for the formation of the blood-CSF barrier, which restricts free passage of solutes between the blood and CSF.

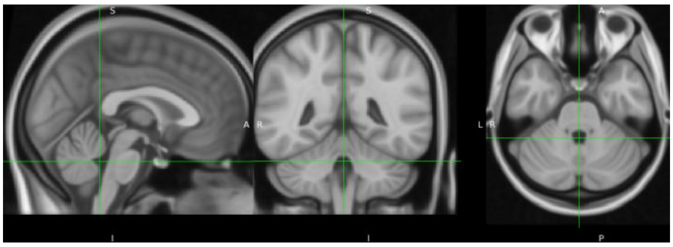
2) The third ventricle



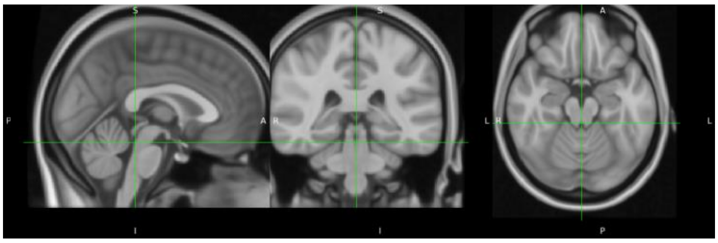
Main Functions:

1) Secretion of CSF and maintaining its circulation through the ventricular system.
2) The CSF allows for the transport of nutrients and waste in and out of the neural tissue.

3) The fourth ventricle



4) Cerebral Acqueduct

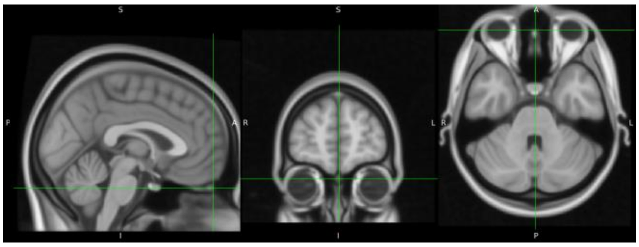


Main Functions:

1) Allows for the flow of CSF between the third and fourth ventricles, maintaining its circulation around the brain. CSF is produced in the ventricles and circulates through the ventricular system, providing mechanical support and cushioning for the brain and spinal cord.

F. Additional Structures

1) The olfactory bulb



Main Functions:

1) Receives the olfactory input from the olfactory neurons in the nasal olfactory epithelium.
2) Relays this information to the brain through the olfactory tracts.

III. QUALITY CONTROL

A. Anatomical Artifacts

The T1 structural images were checked for artifacts as the chemical shift artifacts and the zipper artifact but none were found.

B. Motion Artifacts in the fMRI data

The functional data were visually checked and categorized into three categories; no visible motion artifact, slight motion, excessive motion. Out of the 52 runs (2 for each subject), 34 runs had no visible motion, 11 had slight motion and only 7 had excessive motion. The motion in some runs was very apparent as head rotations and in others was more subtle and could be seen when comparing the first and last volumes of the fMRI data.

IV. PREPROCESSING

Preprocessing steps were applied on the data using the FSL library for medical image data analysis. These include skull stripping from both the functional and structural data to remove the skull and extra structures, followed by the FSL FEAT analysis, motion correction using the MCFLIRT algorithm provided by FSL using 12 DOFs, followed by smoothing using a 5mm Gaussian kernel and registration of the functional data on the MNI152 2mm template using the full search as a starting point. Following are the experiments done in each of the preprocessing steps and a sample of the results.

A. Skull Stripping

Starting with the anatomical data, the skull and extra structures of no interest have to be removed. Several thresholds were explored starting with 0.5 as a default value and adjusting it as seen fit. 0.5 was found to be too conservative in most subjects and the most common threshold used was 0.2.

- *Sample Subject (sub-02):*

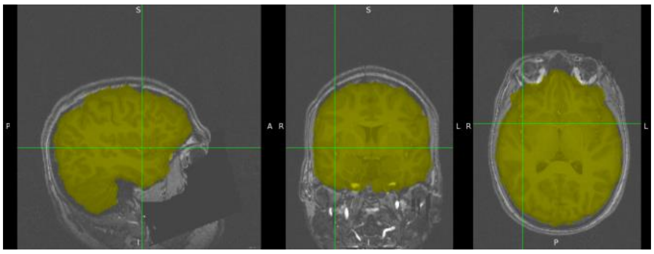


Figure 1: Using a 0.1 Threshold

A large portion of the skull is still left, it is most obvious on the axial slice but can also seen on the other 2 viewing planes

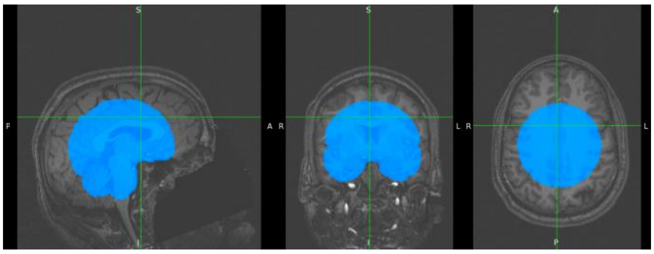


Figure 2: Using a 0.9 Threshold

Too conservative, about 60% of the brain itself was removed.

(Exercise) Comparing only these 2 threshold options, this output is as expected, when we increase the threshold the skull stripping becomes more conservative and removes more of the brain tissue, this is apparent in the 0.9 image, if I were to choose, the 0.1 image will be much more preferred, it would cost more computation but losing brain tissue that can not be retrieved can lead to the failure of the experiment all along and loss of information, being less conservative is better in this case.

Now checking other intermediate thresholds that are not too conservative nor too liberal.

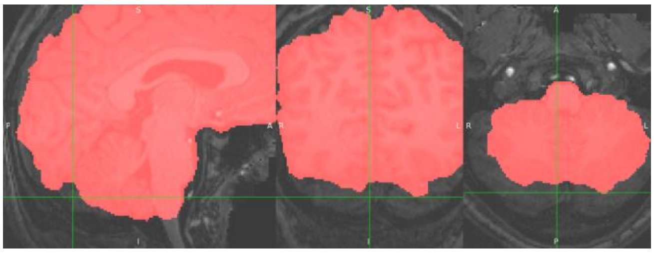


Figure 3: Using a threshold of 0.5

We can see it is still a little too conservative, we can see parts of the posterior brain region were removed (zoomed on them in the figure).

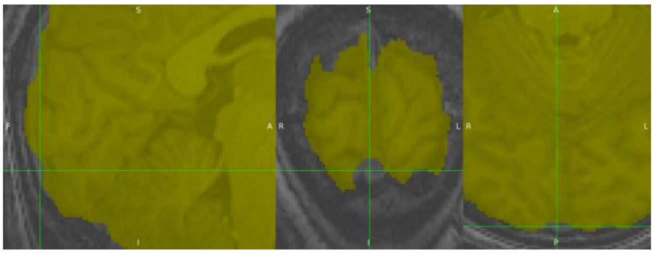


Figure 4: Using a threshold of 0.3

Using that of 0.3 was much better than 0.5, but we can still see that some brain tissue was removed at the posterior side as well.

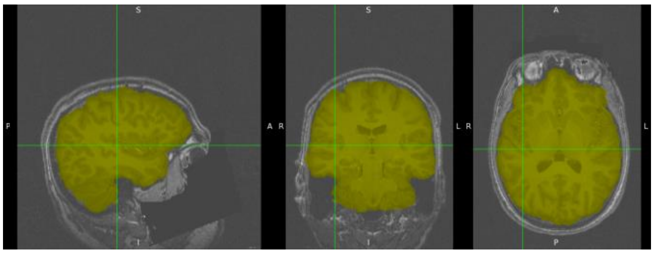


Figure 5: Using a threshold of 0.2

We can see that overall, the 0.2 produced a very good balanced extraction.

- *(Exercise) Overlay Color options:*
  Using the yellow color option for the overlay worked best for visual inspection, it was not too opaque allowing to see the structural image without the need to hide the layout-over and was vibrant enough to show on the greyscale T1 image.

B. Feat Analysis

The preprocessing default parameters unless stated otherwise are a threshold of 0.2 for skull stripping, MCFLIRT algorithm for motion correction, registration using full search and 12 DOFs, smoothing using a 5mm gaussian kernel and no slice-timing correction technique applied. The analysis sample results will be shown on subject 8’s (sub-08) run 2.

1. Motion Inspection

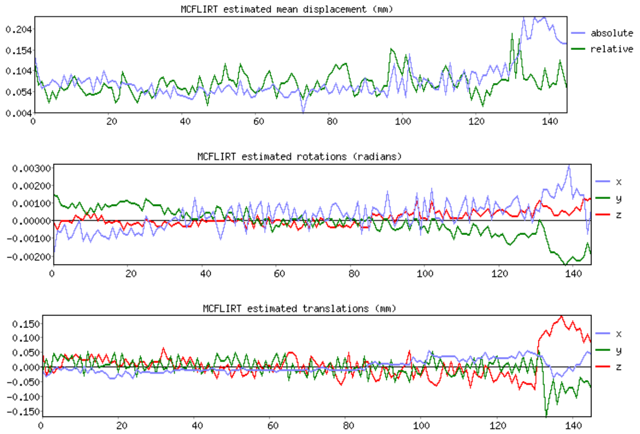


Figure 6: Motion Profiles for run 2

Checking the motion profiles for run 2, it is noticed that there is marked motion, both displacement and rotation represented in the previous figure, between volume 130 and volume 146. This can also be seen in the fMRI data as tis part is less defined in comparison to the other runs. Since the value is larger than half a voxel in our case, there is a need for motion correction, which is carried out using MCFLIRT. We also note that we can deduce from the motion profiles that the reference volume was taken to be the middle volume (around volume 73), since the y-axis of all profiles shows values of zero at this particular volume.

2. Smoothing

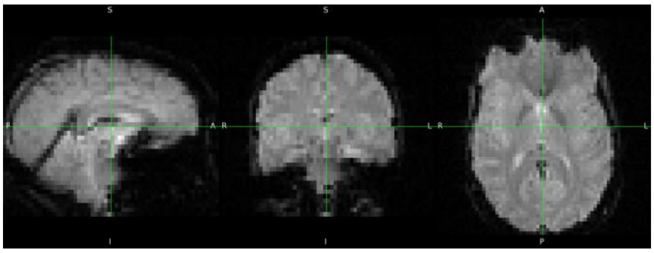


Figure 7: Sample Data before Preprocessing

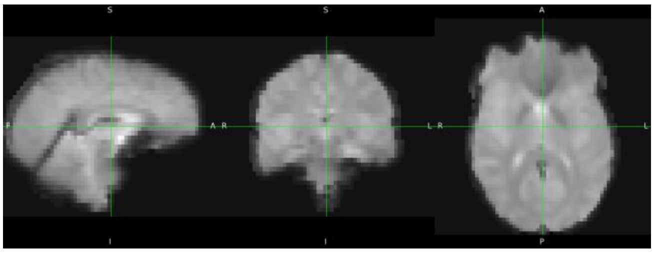


Figure 8: After smoothing with a 5mm gaussian kernel

We notice that after smoothing, a lot of the noise in the fMRI data is removed. On the other hand, details are also removed and spatial resolution is decreased. Even though this is the case, the advantages of smoothing usually outweigh its disadvantages. In some cases where we are studying a very minute structure, we might not apply smoothing at all to preserve the distinction between different structures and areas of the brain.

3. Registration

Final Result (fMRI on template)

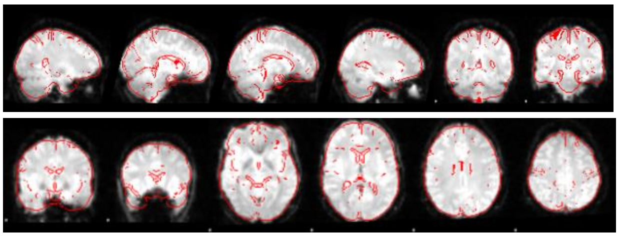


Figure 9: fMRI data on MNI152 2mm (adult)

The alignment looks satisfactory other than some slight differences in the inferior part of the brain. To be expected since human brains are different. More advanced nonlinear algorithms like BBR can be used for more accurate results.

The final transformation matrix used to map the fMRI data to the template:

```
 1.09798816      -0.0073204017  0.04946010758  -16.00802592
 0.03379193329    0.9888634772  0.3006137817   -3.369811711
-0.04990630854  -0.2496161064  1.148919243     11.81370574
 0               0              0               1
```

Intermediate steps

Aligning the functional data to the T1 high resolution structural data. We can see that they are hard to align, due to the large difference in resolution.

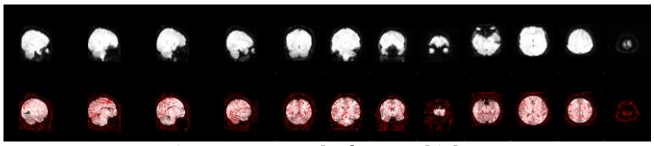


Figure 10: example_func_to_highres

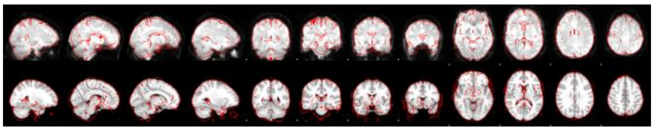


Figure 11: example_func_to_standard_space

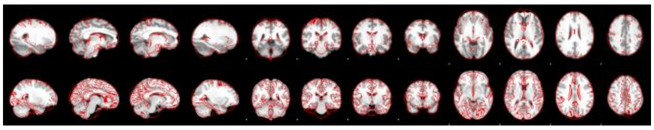


Figure 12: highres_to_standard_space

The last 2 processes produce much better results than the first, going from the T1 image to the standard space is easy since they are both of high resolution, producing excellent results. Mapping the fMRI to the standard space is also easier than to the T1, producing good alignment.

1) Kernel Size Parameter

To compare 3mm and 12mm output images

Using a Gaussian kernel to smooth the fMRI data in order to remove noise that might be present.

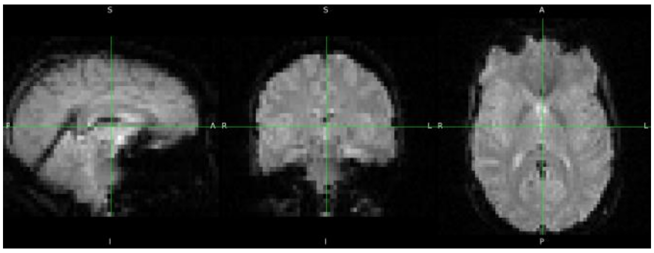


Figure 13: Run 1, No preprocessing

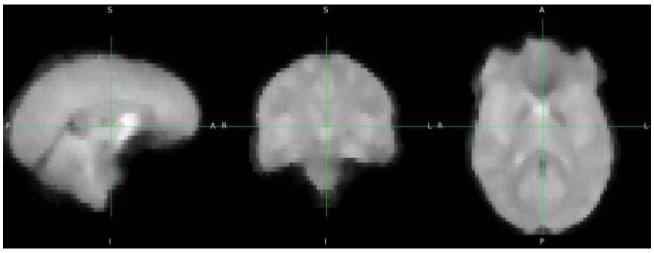


Figure 14: Run1, Kernel Size 3mm

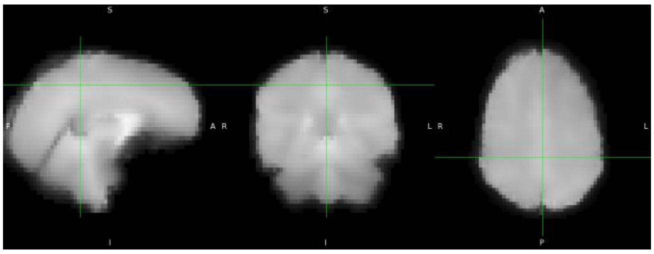


Figure 8: Run 1, Kernel Size 12mm

We can see that that increasing the kernel size means increasing the smoothing done and increasing the spatial resolution as we use more pixels of the original image to produce one pixel in the final output. The 12mm kernel erased most details we had in the original data. This would cause ambiguity in mapping our results (brain activation) to the corresponding area of the brain. A 3mm kernel left a lot of detail, but since it is very small, it might not be able to remove a lot of the noise since it takes a much smaller number of pixels. Therefore, it is always a tradeoff.

2) Registration DOFs Parameter

This parameter represents the type and number of transformations allowed to be done during the registration process in order to overlay the fMRI data on the MNI152 2mm template to be able to carry group analysis and generalize our results.

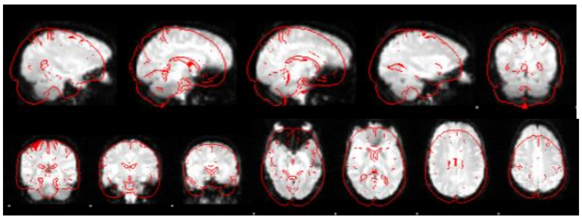


Figure 15: Registration using 3 DOFs

This is the final result of registration of the functional data on the MNI template.

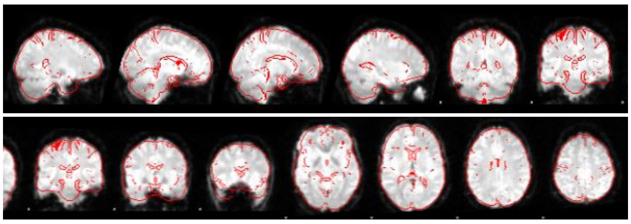


Figure 16: Registration using 12 DOFs

Comparing the 3 and 12 DOFs, we can see the 3 DOFs (translation only) did a really bad job aligning the functional data on the template, this is because the shape and size of the subject's brain is different than the template. Therefore, only trying to align them by translations without zooming to match the size, rotation to match the orientation and shearing to adjust the shape is not sufficient. On the other hand, 12 DOFs produced a much desirable result in comparison, it had more operations in hand to match the two.

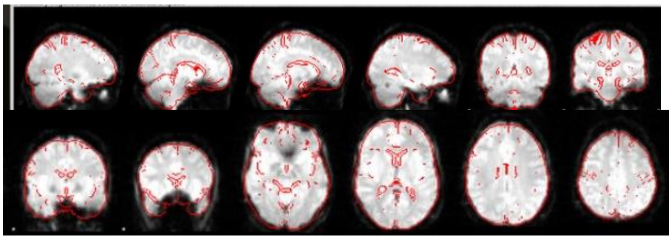


Figure 17: Run 1, BBR

A vast difference can not be seen visually between the 12 DOFs and BRB images in this case, in some views the 12 DOFs has done a better job and in others the BBR, maybe the difference would be more apparent with 6 or 9 DOFs and is very apparent with the 3 DOFs image. Though the BRB took a markedly longer time. Therefore, for a time-dependent analysis, 12 DOFs can be used, for a detail-oriented study, maybe one that studies very minute structures of the brain where a small error in registration would cause confusion of the brain regions, the non-linear BBR algorithm could be used.

V. MODELLING AND STATISTICS

Let us examine the time series data of sub-01, run 2, voxel of coordinates (-0.72, 24.94, 20.94):

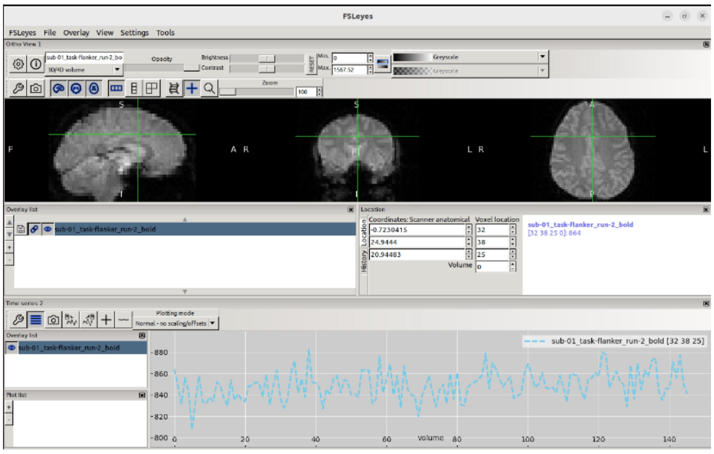
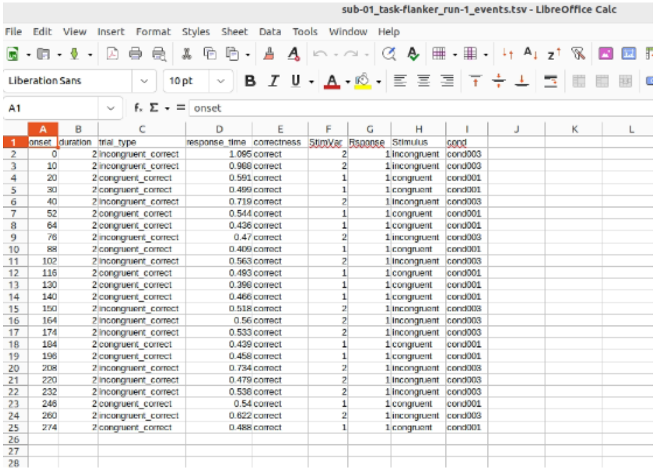


Figure 17: Time series of one vovel

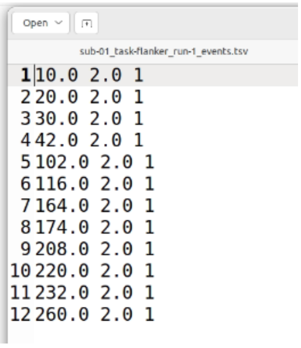
We want to test whether the intensity of each voxel is correlated to our tasks (our design matrices).
This is our paradigm from the _events.tsv timing file



onset	duration	trial_type	response_time	correctness	StimVar	Rsponse	Stimulus	cond
0	2	incongruent_correct	1.095	correct	2	1	incongruent	cond003
10	2	incongruent_correct	0.988	correct	2	1	incongruent	cond003
20	2	congruent_correct	0.591	correct	1	1	congruent	cond001
30	2	congruent_correct	0.499	correct	1	1	congruent	cond001
40	2	incongruent_correct	0.719	correct	2	1	incongruent	cond003
52	2	congruent_correct	0.544	correct	1	1	congruent	cond001
64	2	congruent_correct	0.436	correct	1	1	congruent	cond001
76	2	incongruent_correct	0.47	correct	2	1	incongruent	cond003
88	2	congruent_correct	0.409	correct	1	1	congruent	cond001
102	2	incongruent_correct	0.563	correct	2	1	incongruent	cond003
116	2	congruent_correct	0.493	correct	1	1	congruent	cond001
130	2	congruent_correct	0.398	correct	1	1	congruent	cond001
140	2	congruent_correct	0.466	correct	1	1	congruent	cond001
150	2	incongruent_correct	0.518	correct	2	1	incongruent	cond003
164	2	incongruent_correct	0.56	correct	2	1	incongruent	cond003
174	2	incongruent_correct	0.533	correct	2	1	incongruent	cond003
184	2	congruent_correct	0.439	correct	1	1	congruent	cond001
196	2	congruent_correct	0.458	correct	1	1	congruent	cond001
208	2	incongruent_correct	0.734	correct	2	1	incongruent	cond003
220	2	incongruent_correct	0.479	correct	2	1	incongruent	cond003
232	2	incongruent_correct	0.538	correct	2	1	incongruent	cond003
246	2	congruent_correct	0.54	correct	1	1	congruent	cond001
260	2	incongruent_correct	0.622	correct	2	1	incongruent	cond003
274	2	congruent_correct	0.488	correct	1	1	congruent	cond001

Figure 18: Our paradigm

Here we can see the time of onset, duration and type off our stimuli. We use the run.sh file to separate the data for our regressors of interest, the produced file is in the three-column format required by FSL. This is the congruent stimuli data separated.



```
10.0 2.0 1
20.0 2.0 1
30.0 2.0 1
42.0 2.0 1
102.0 2.0 1
116.0 2.0 1
164.0 2.0 1
174.0 2.0 1
208.0 2.0 1
220.0 2.0 1
232.0 2.0 1
260.0 2.0 1
```

Figure 19: Congruent stimuli data

Using the GLM from FSL FEAT statstics with the following model setup: 2 regressors: named “incongruent” and “congruent”. Three COPEs, “incongruent” which equals the value of our first EV, “congruent” which carries the value of the second EV and “difference” which is the difference between the first and the second EVs. Z_threshold of 3.1. Note that the following analysis is done on run 1 of sub-05.

Here we can visualize the design matrix and contrasts we will be correlating the time series of the voxels with, this is produced by convoluting the HRF with our stimuli timing to produce shifted values of the HRF in time of stimulation. The leftmost column is the **high-pass filter** that removes any low frequency noise, such as breathing.

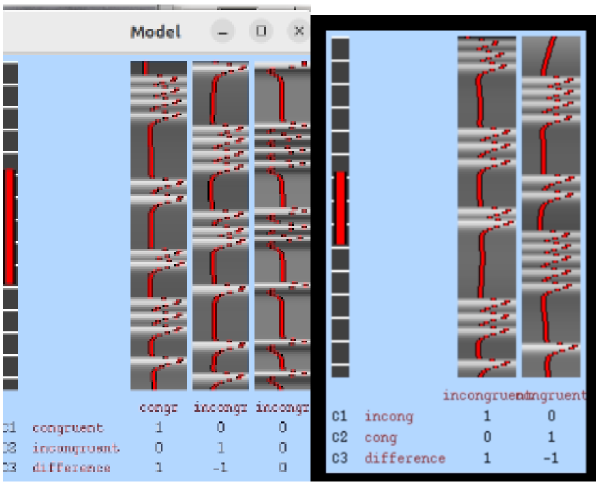


Figure 20: Design matrices and contrasts

We can now check the activations that exceeded the given thresholds for our 2 tasks.

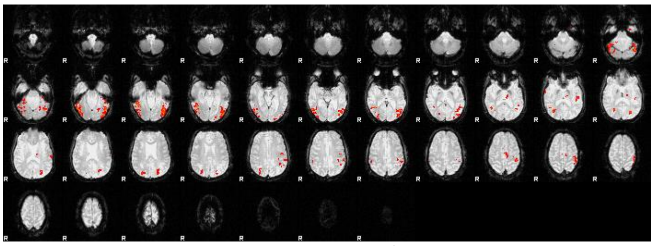


Figure 21: Thresholded activations for the congruent case

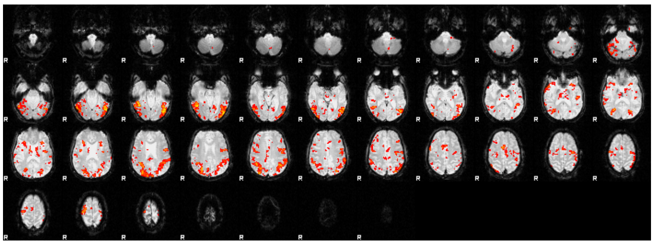


Figure 22: Thresholded activations for the incongruent case

Comparing the activations, we can see that more brain areaas were activated during the incongruent task, this is to be expected as in the congruent task, the subject should make a conscious effort to discard the extra distraction faced because of the conflicting answer and noise.

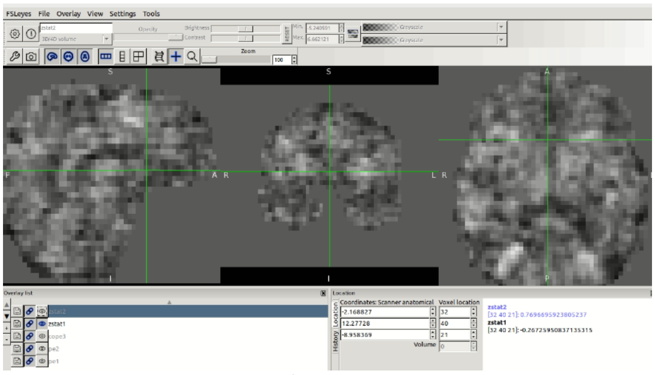


Figure 23: The z statistic of principal estimate 1 (incongruent)

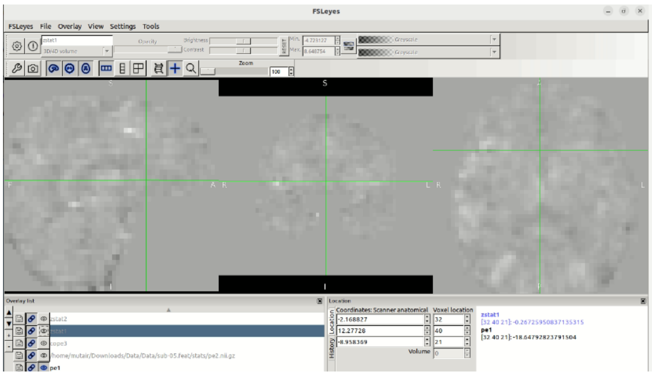


Figure 24: The principal estimate 1 (incongruent)

The pixels of brighter intensity are the ones more active with our task. Now we will threshold the z_stat1 at 3.1 (the same threshold used in the feat analysis) and layover PE 1. We get the corresponding brain areas that were active during the incongruent task. We could have also used COPE 1 instead.

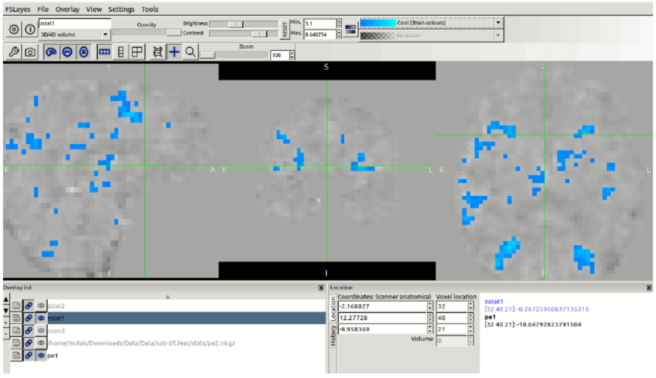


Figure 25: pe1 + z_stat1 (incongruent)

Visualizing COPE 3, we can see the areas of the brain which had a different response for the congruent and incongruent tasks. COPE 3 was defined as the difference between the incongruent and congruent PEs.

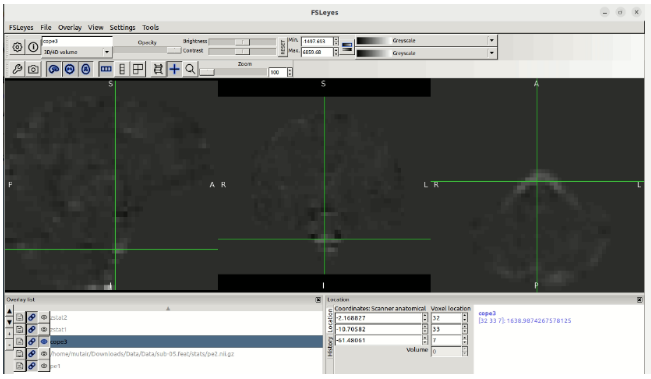


Figure 26: COPE 3 (difference)

Looking at the time series data and our fitted model at the voxel (40, 30, 30) and the thresholded activations, we can see that this voxel was activated during the incongruent task and we note the peak allignment between the blue and red curves in the time series, indicating this correlation.

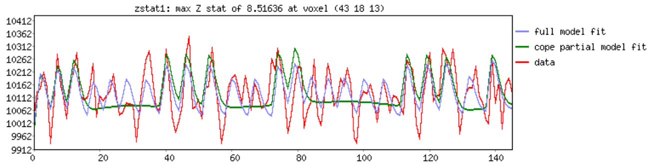


Figure 27: The time series of the original data vs the fitted model

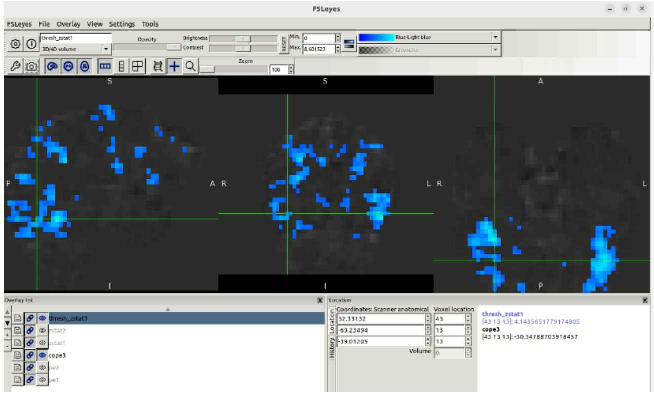


Figure 28: The thresholded activations at the voxel in the time series

VI. SCRIPTING AND AUTOMATION

Our previous analysis was all done manually which is time-consuming and subject to human error. This is why the automation of these analyses is a must.

A. *Feat Analysis Automation*

a) Skull Stripping Check

First, we prepare the anatomical image input to the feat analysis. This should be the skull-stripped image. The following script checks the existence of this image, marked by the "_brain" ending, if not available, it uses the bet command of fsl to produce it using a threshold of 0.2 which we found to be the mode of the thresholds tested manually.

```
for id in `seq -w 1 26`
do
    subj="sub-$id"
    echo "===> Starting processing of $subj"
    echo
    cd $subj

      # If the brain mask doesn't exist, create it
      if [ ! -f anat/${subj}_T1w_brain_f02.nii.gz ]
      then
              bet2 anat/${subj}_T1w.nii.gz anat/${subj}_T1w_brain_f02.nii.gz -f 0.2
          # echo "Skull-stripped brain not found, using bet with a fractional intensity threshold of 0.2"
      fi
    cd ..
done
```

i) Script breakdown

```
for id in `seq -w 1 26`
```

Loops on the sequence of numbers from 0 to 26, preserving the format used in the flanker data where all numbering has a width of 2, the "-w" flag adds leading zeroes to equate the width.

```
subj="sub-$id"
echo "===> Starting processing of $subj"
echo
cd $subj
```

For every subject, subj carries the string of their directory name (ex: sub-09).

We then navigate to the directory of this subject

.

```
if [ ! -f anat/${subj}_T1w_brain_f02.nii.gz ]
then
        bet2 anat/${subj}_T1w.nii.gz anat/${subj}_T1w_brain_f02.nii.gz -f 0.2
  # echo "Skull-stripped brain not found, using bet with a fractional intensity threshold of 0.2"
 fi
```

Checks the existence of a file (using the -f flag) under the anat directory with the given name (This shows the importance of a consistency in naming). if not, create it using the bet2 command and a threshold of 0.2. If it exists, proceed to the next subject.

```
cd ..
```

We return to the outer directory containing all subjects' data.

Note: We preferrably should have named the files "_brain" not "_brain_f02" to preserve the other files whose threshold was found manually to have an optimal value other than 0.2.

b) Applying the Full Feat Analysis

```
# Generate the subject list to make modifying this script
# to run just a subset of subjects easier.

for id in `seq -w 1 26` ; do
    subj="sub-$id"
    echo "===> Starting processing of $subj"
    echo
    cd $subj

        # Copy the design files into the subject directory, and then
        # change "sub-01" to the current subject number
        cp ../design_run1.fsf .
        cp ../design_run2.fsf .

        # Note that we are using the | character to delimit the patterns
        # instead of the usual / character because there are / characters
        # in the pattern.
        sed -i.bak  "s|sub-01|${subj}|g" design_run1.fsf
        sed -i.bak  "s|sub-01|${subj}|g" design_run2.fsf

        # Now everything is set up to run feat
        echo "===> Starting feat for run 1"
        feat design_run1.fsf
        echo "===> Starting feat for run 2"
        feat design_run2.fsf
                echo

    # Go back to the directory containing all of the subjects, and repeat the loop
    cd ..
done
```

i) Script Breakdown

The outer loop is exactly the same as that of the skull stripping check.

```
# Copy the design files into the subject directory, and then
# change "sub-01" to the current subject number
cp ../design_run1.fsf .
cp ../design_run2.fsf .

# Note that we are using the | character to delimit the patterns
# instead of the usual / character because there are / characters
# in the pattern.
sed -i.bak  "s|sub-01|${subj}|g" design_run1.fsf
sed -i.bak  "s|sub-01|${subj}|g" design_run2.fsf

# Now everything is set up to run feat
echo "===> Starting feat for run 1"
feat design_run1.fsf
echo "===> Starting feat for run 2"
feat design_run2.fsf
        echo
```

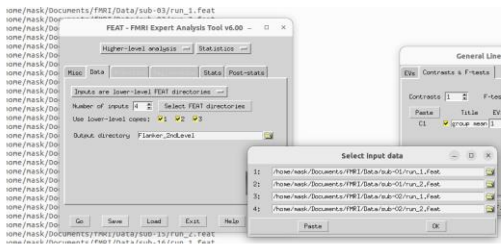
We first setup the full feat analysis using the FSL Gui as discussed before for both run 1 and run 2 of subject 1 and save the settings in the design.fsf files named design_run1.fsf and design_run2.fsf. We then copy these files into the directories of all subjects. The next step is to change the parameters specific to the subject in each file using the sed command, "-i.bak" flag creates a backup file with .bak extension before applying any changes. The "s" at the start indicates a substitution operation and the "g" at the end to replace all occurences. The first line replaces the string carrying the subject name in the design.fsf of run 1. and the second for run 2. At the end run the feat analysis using the modified design.fsf files for both runs. This is the design_run1.fsf file for subject 17. We can see the subject number has been successfully changed after running the script.

```
design_run1.fsf
~/Documents/fMRI/Data/sub-17

# Run Featwatcher
set fmri(featwatcher_yn) 1

# Cleanup first-level standard-space images
set fmri(sscleanup_yn) 0

# Output directory
set fmri(outputdir) "/home/mask/Documents/fMRI/Data/sub-17/run_1"

# TR(s)
set fmri(tr) 2.000000

# Total volumes
set fmri(npts) 146

# Delete volumes
set fmri(ndelete) 0

# Perfusion tag/control order
set fmri(tagfirst) 1

# Number of first-level analyses
set fmri(multiple) 1

# Higher-level input type
# 1 : Inputs are lower-level FEAT directories
# 2 : Inputs are cope images from FEAT directories
set fmri(inputtype) 2

# Carry out pre-stats processing?
set fmri(filtering_yn) 1

# Brain/background threshold, %
set fmri(brain_thresh) 10

# Critical z for design efficiency calculation
```

The output of the last 2 sections are the 52 feat directory (2 for the 2 runs of each subject) and the 26 skull stripped anatomical images for all subjects.

VII. 2ND LEVEL ANALYSIS

a) Procedure:

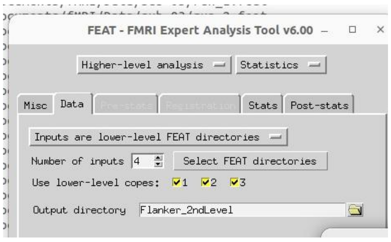


According to the nomenclature of FSL, in the 2nd level analysis, we combine the data of the COPEs for the runs of every subject to produce one set of COPEs representative for this subject to be used for the 3rd level analysis. We will carry out the averaging of the parameter estimates and contrast estimates from the 1st-level analyses for each subject.

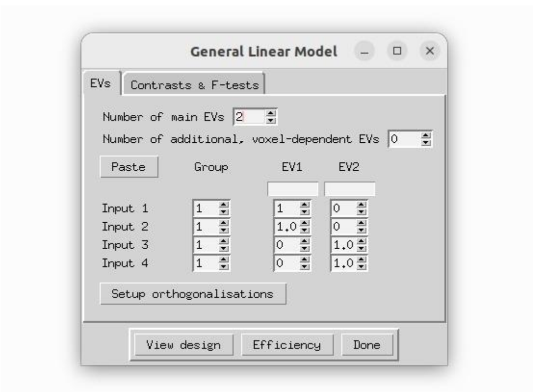
Using the feat_gui of FSL, we can select all 52 feat directories of the 2 runs for the 26 subjects, we can use the command: ls -d $PWD/sub-??/func/run*, which lists the directories only, included in the current working directory (the main directory containg all subjects), included in the func folder of a directory whose name follows the pattern (sub-??) as sub-08 and starts with run (as run_1). This way, we can get the paths of all directories without the need to manually select them. Theses were the output paths pasted into a text file.


```
1 /home/mask/Documents/fMRI/Data/sub-01/run_1.feat
2 /home/mask/Documents/fMRI/Data/sub-01/run_2.feat
3 /home/mask/Documents/fMRI/Data/sub-02/run_1.feat
4 /home/mask/Documents/fMRI/Data/sub-02/run_2.feat
5 /home/mask/Documents/fMRI/Data/sub-03/run_1.feat
6 /home/mask/Documents/fMRI/Data/sub-03/run_2.feat
7 /home/mask/Documents/fMRI/Data/sub-04/run_1.feat
8 /home/mask/Documents/fMRI/Data/sub-04/run_2.feat
9 /home/mask/Documents/fMRI/Data/sub-05/run_1.feat
10 /home/mask/Documents/fMRI/Data/sub-05/run_2.feat
11 /home/mask/Documents/fMRI/Data/sub-06/run_1.feat
12 /home/mask/Documents/fMRI/Data/sub-06/run_2.feat
13 /home/mask/Documents/fMRI/Data/sub-07/run_1.feat
14 /home/mask/Documents/fMRI/Data/sub-07/run_2.feat
15 /home/mask/Documents/fMRI/Data/sub-08/run_1.feat
16 /home/mask/Documents/fMRI/Data/sub-08/run_2.feat
17 /home/mask/Documents/fMRI/Data/sub-09/run_1.feat
18 /home/mask/Documents/fMRI/Data/sub-09/run_2.feat
19 /home/mask/Documents/fMRI/Data/sub-10/run_1.feat
20 /home/mask/Documents/fMRI/Data/sub-10/run_2.feat
21 /home/mask/Documents/fMRI/Data/sub-11/run_1.feat
22 /home/mask/Documents/fMRI/Data/sub-11/run_2.feat
23 /home/mask/Documents/fMRI/Data/sub-12/run_1.feat
24 /home/mask/Documents/fMRI/Data/sub-12/run_2.feat
25 /home/mask/Documents/fMRI/Data/sub-13/run_1.feat
26 /home/mask/Documents/fMRI/Data/sub-13/run_2.feat
27 /home/mask/Documents/fMRI/Data/sub-14/run_1.feat
28 /home/mask/Documents/fMRI/Data/sub-14/run_2.feat
29 /home/mask/Documents/fMRI/Data/sub-15/run_1.feat
30 /home/mask/Documents/fMRI/Data/sub-15/run_2.feat
31 /home/mask/Documents/fMRI/Data/sub-16/run_1.feat
32 /home/mask/Documents/fMRI/Data/sub-16/run_2.feat
33 /home/mask/Documents/fMRI/Data/sub-17/run_1.feat
34 /home/mask/Documents/fMRI/Data/sub-17/run_2.feat
35 /home/mask/Documents/fMRI/Data/sub-18/run_1.feat
36 /home/mask/Documents/fMRI/Data/sub-18/run_2.feat
37 /home/mask/Documents/fMRI/Data/sub-19/run_1.feat
38 /home/mask/Documents/fMRI/Data/sub-19/run_2.feat
39 /home/mask/Documents/fMRI/Data/sub-20/run_1.feat
40 /home/mask/Documents/fMRI/Data/sub-20/run_2.feat
41 /home/mask/Documents/fMRI/Data/sub-21/run_1.feat
42 /home/mask/Documents/fMRI/Data/sub-21/run_2.feat
43 /home/mask/Documents/fMRI/Data/sub-22/run_1.feat
44 /home/mask/Documents/fMRI/Data/sub-22/run_2.feat
45 /home/mask/Documents/fMRI/Data/sub-23/run_1.feat
```

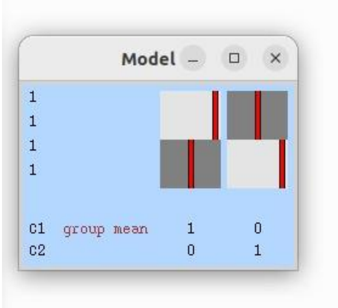
Here we show the process on 2 subjects (sub-01 and sub-02).



After choosing the input (feat) directories and specifying that we want the analysis on our 3 previously specified COPEs (incongruent, congruent and difference), we setup the options in the stats tab. Choosing the fixed effects option which will only average the inputs without any type of inference or statistical test just yet. Then in the full model setup, we choose the number of EVs which is equal to the number of subjects. We put ones in front of the inputs for each EV (subject) to average these inputs.



In the contrasts and f-tests tab, we choose 2 contrasts and 0 f-tests since we will not be doing it just yet. Changing all of the numbers on the diagonal to 1 creates a single contrast estimate for each subject which is the average of that subject's parameter estimates.



After clicking go, we will have our output. The output directory contains 3 folders (for the 3 COPEs we specified), each folder carries a number of COPE files, one for each subject.

We tried to compare the first COPEs from the feat analysis and the produced averaged first COPE (for sub-01), but since they are in different spaces, it is not that obvious.

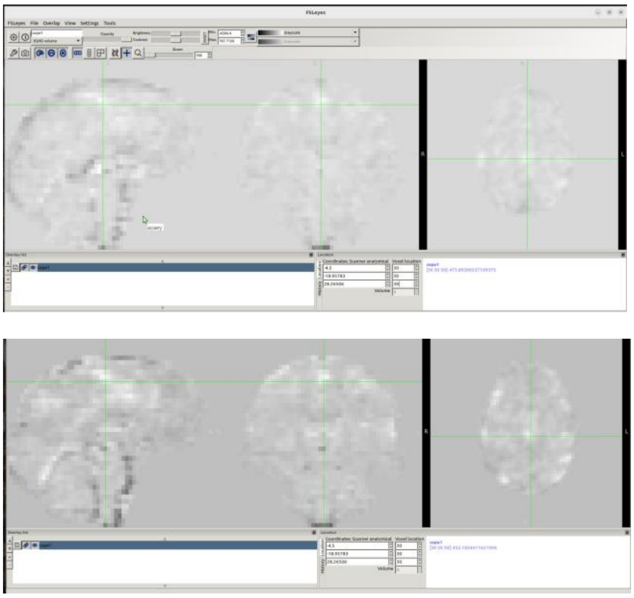


Figure 29: COPE 1 of the 2 runs of sub-01

As we can see written in the location widget, the COPEs of the two runs are in the Scanner Anatomical space. While the averaged COPE is in the MNI 152 space.

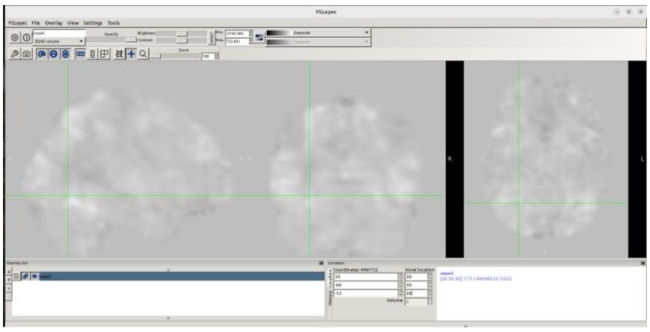


Figure 30: COPE1, combined runs of sub-01

b) Sample Results for the 2nd level analysis

The following are a sample of the thresh_zstat files of COPE3 (incongruent - congruent) for 2 subjects. These contain the average activations of the 2 runs after combination and is thresholded at a z-stat of 3.1 to show the significant brain areas that were activated in the incongruent task only.

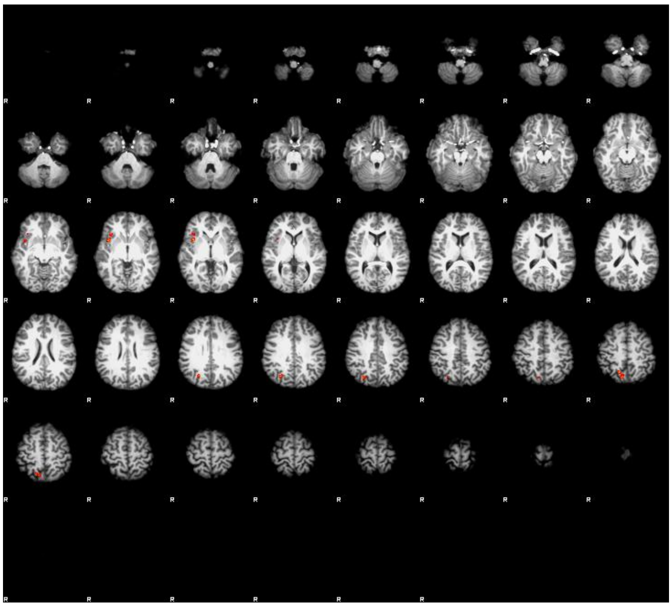


Figure 31: zstat8 – sub-08

We can also see the intersubject differences in activation. We can clearly notice that in subject 8 many more areas were active as compared to subject 7. In the 3rd level analysis, we are going to combine the results of these subjects and statistically test them to try to eliminate as much of these personal differences and generalize on the whole population.

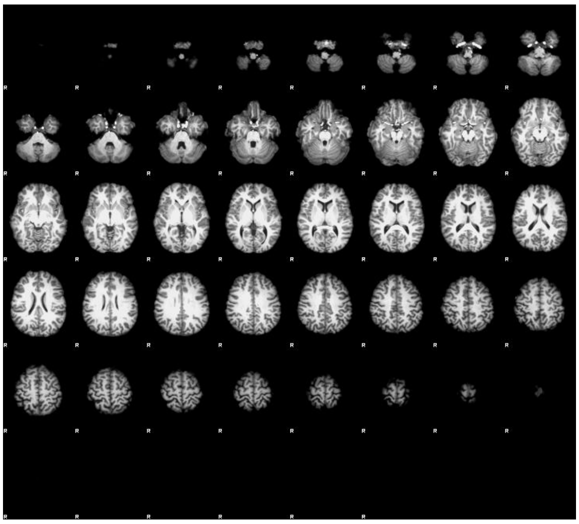
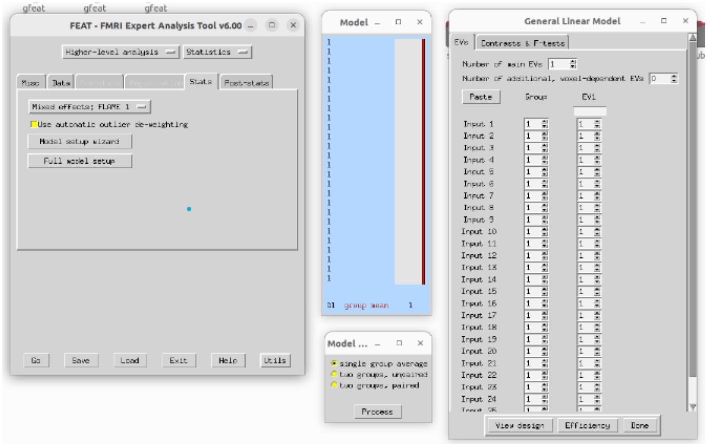


Figure 32: zstat8 – sub-07

VIII. 3RD LEVEL ANALYSIS

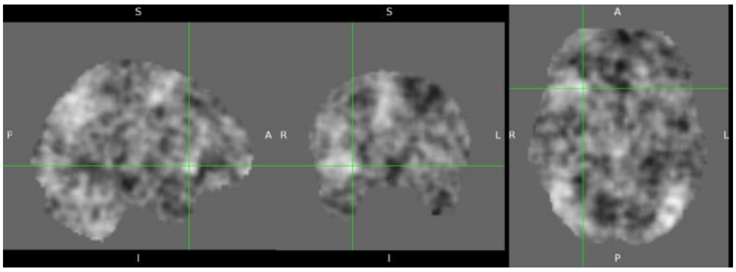
The 3rd level group analysis was carried with the following settings other than the post-stats tab:



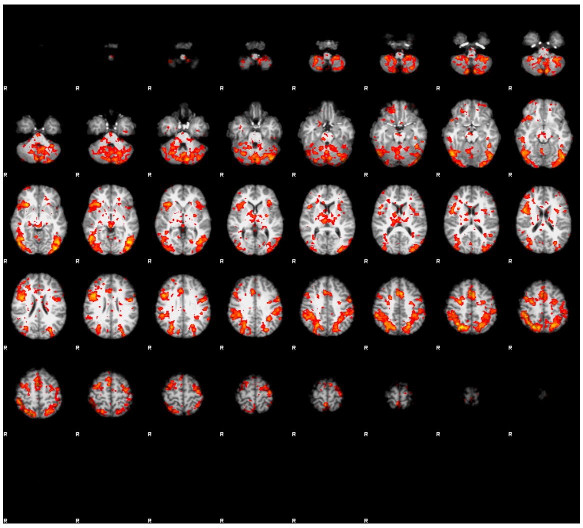
In the post-stats, many thresholding options were explored, let us first showcase the result, the averaged group map for every option, and then comment on them:

A) Thresholding options:

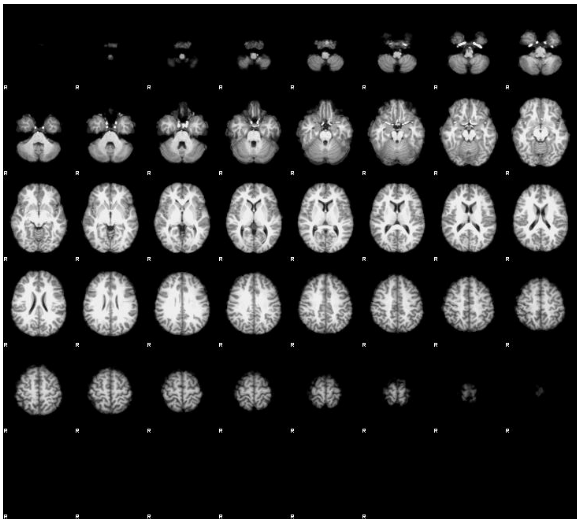
a) None



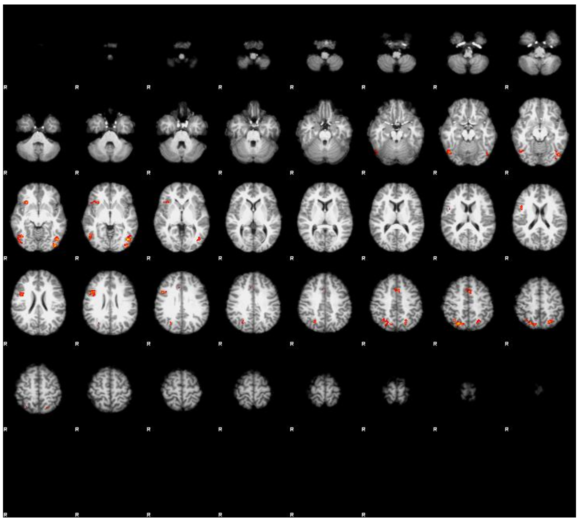
b) Uncorrected



c) Voxel



d) Cluster



B) Observations:

None does not apply thresholding at all and does not discard any pixels, it just outputs the COPE as it is, but as the combination of the 26 COPEs of all subjects.

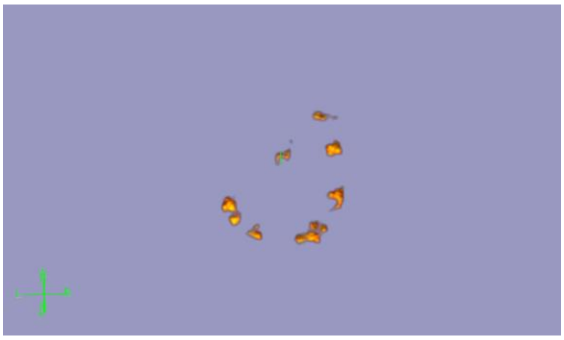
For Uncorrected, it does not correct for multiple hypothesis and thus is too liberal, producing too many activations as significant as we see in the figure, therefore it is not too helpful here as well.

For the voxel option, according to the fslwiki “GRF-theory-based maximum height thresholding is carried out, with thresholding at the level set, using one-tailed testing. This test is less overly-conservative than Bonferroni correction.” For Bonferroni correction, we would divide the threshold by the number of voxels which would yield a very small threshold that no voxel would satisfy. Although the voxel thresholding is less stringent. It is still so stringent as almost no voxel passed as we can see in its figure.

Finally, for the cluster option, it does not test separate voxels but divides the brain into a number of clusters, making the correction for this number more liberal than voxel-wise correction. As we see in the figure, it produced a number of activated areas less than the uncorrected thresholding and more than the voxel thresholding. This is the type we are going to proceed with. Next, we will analyze the clusters it produced.

C) Cluster Analysis:

The previous analysis produced 8 clusters. These can be displayed as discrete segment in FSLeyes 3D view.



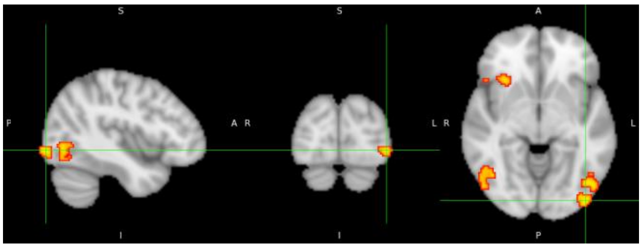
The eight clusters main info are contained in the following table, including its size, number of voxels in each cluster, p-value, notice how it is smaller for clusters of a larger number of voxels and vice versa, Z max, which is the maximum Z-threshold in this cluster which corresponds to the peak of activation, Max location, which is the coordinates of this peak and the COG location, which is the center of gravity for this cluster.

Cluster browser
cope1: filtered_func_data
Z statistics for COPE1 (grou | Add Z statistics | Add cluster mask

Cluster index	Size (voxels)	P	-log10(P)	Z Max	Z Max location	COG location
8.0	355.0	2.32e-06	5.63	4.27	[32.0 29.0 61.0]	[32.15 29.65 59.25]
7.0	252.0	6.72e-05	4.17	3.94	[21.0 29.0 29.0]	[20.3 29.4 30.85]
6.0	234.0	0.000127	3.9	4.55	[67.0 27.0 34.0]	[67.8 27.2999999999
5.0	217.0	0.000234	3.63	4.17	[21.0 69.0 51.0]	[22.25 67.44 48.8]
4.0	129.0	0.00764	2.12	3.97	[57.0 31.0 62.0]	[57.0 31.05 60.35]
3.0	111.0	0.017	1.77	3.84	[47.0 71.0 59.0]	[44.6565 71.3 58.5]
2.0	110.0	0.0177	1.75	4.06	[29.0 74.0 33.0]	[26.95 74.45 33.78]
1.0	96.0	0.0339	1.47	4.42	[66.0 19.0 33.0]	[65.25 19.549999999

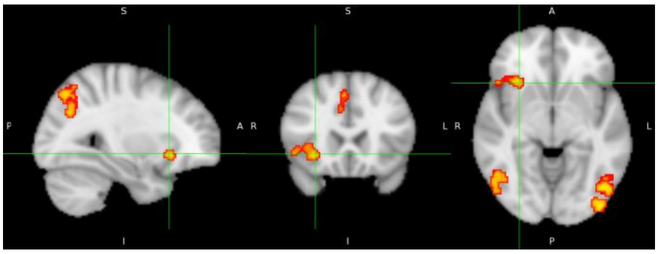
Next are the clusters and their corresponding region according to the Harvard-Oxford Cortical Atlas.

a) Cluster 1



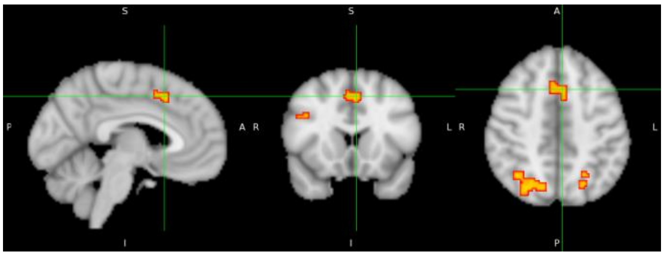
66% Lateral Occipital Cortex, inferior division

b) Cluster 2



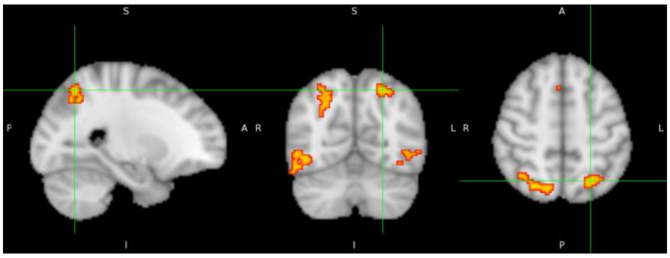
58% Insular Cortex
22% Frontal Orbital Cortex

c) Cluster 3



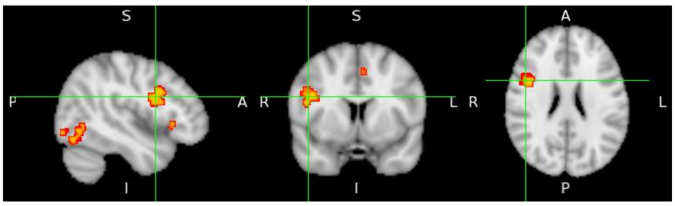
74% Paracingulate Gyrus

d) Cluster 4



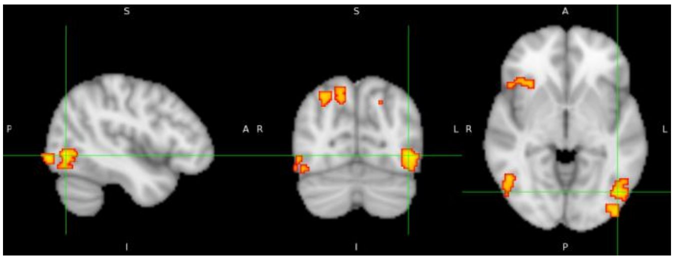
53% Lateral Occipital Cortex, superior division

e) Cluster 5



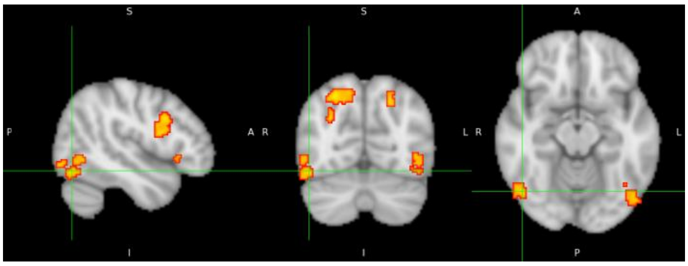
79% Lateral Occipital Cortex, inferior division

f) Cluster 6



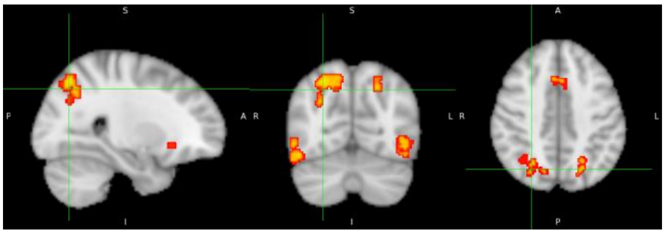
79% Lateral Occipital Cortex, inferior division

g) Cluster 7



71% Lateral Occipital Cortex, superior division

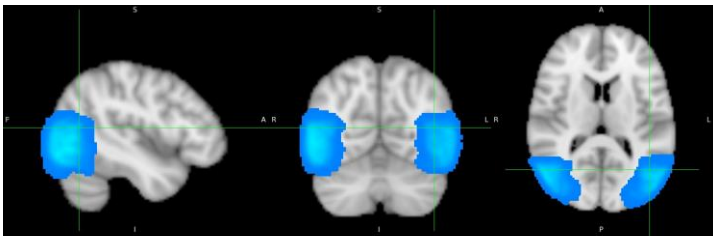
h) Cluster 8



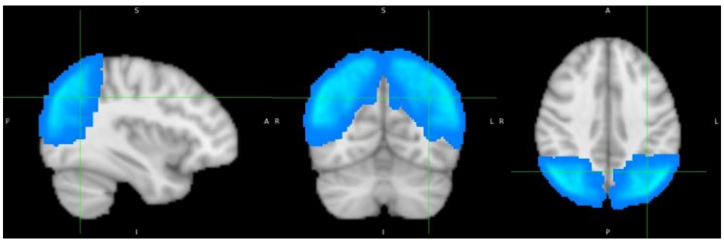
68% Lateral Occipital Cortex, inferior division

Therefore, we can list the possibly activated areas:

i) Lateral Occipital Cortex, inferior and superior divisions

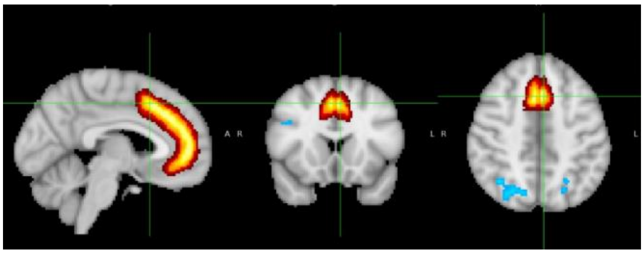


Inferior division of the LOC

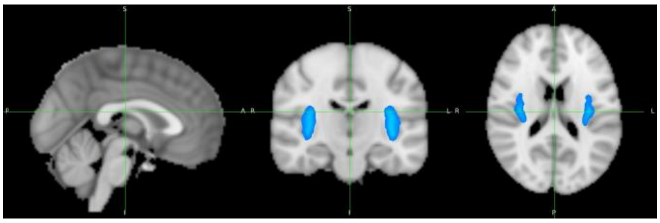


Superior division of the LOC

ii) Paracingulate Gyrus



iii) Insular Cortex



Using the regions, peaks and clusters produced from the whole-brain exploratory analysis, we will carry out the ROI analysis to further explore the significance and source of variation in response whether it comes from the congruent or incongruent activations in each case.

IX. ROI Analysis

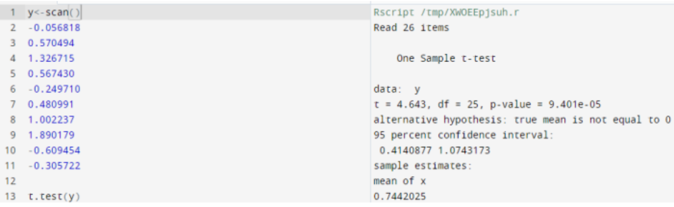
The first step was to combine (stack) all the z-stat files for all subjects for the 3 COPEs. We then use the previous anatomical masks or the spherical masks that will be discussed next to analyze only a specific region instead of the whole brain. This is also called the confirmatory analysis.

A. Anatomical Regions Mask Method

Further examining the regions of the brain containing the peaks of activation as mentioned earlier. The mask is applied to the z-stat data of each subject, output from the 2nd level analysis, and then this ROI is averaged producing a number per subject and thus a vector of 26 numbers for all subjects.

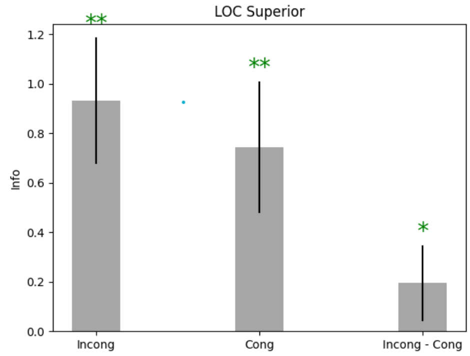
Sample example from COPE1 of the Paracingulate Gyrus:

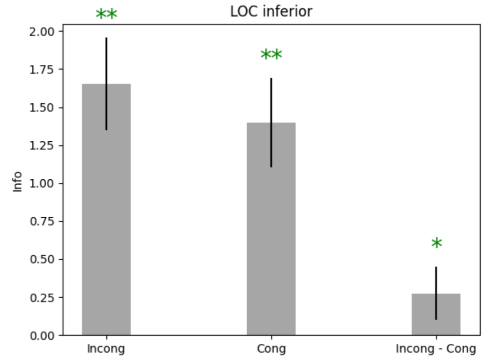
0.707189, 1.177148, 0.337843, 0.597983, 1.378953, -0.276876, 0.284848, 0.937029, 0.621375, 0.880175, 0.985725, 1.167820, 0.496908, -0.737823, 1.346500, -0.330900, 0.637811, -1.254036, 0.788787, 0.217306, 1.066148, 0.169204, 0.492851, 0.466009, -0.047969, 0.536686

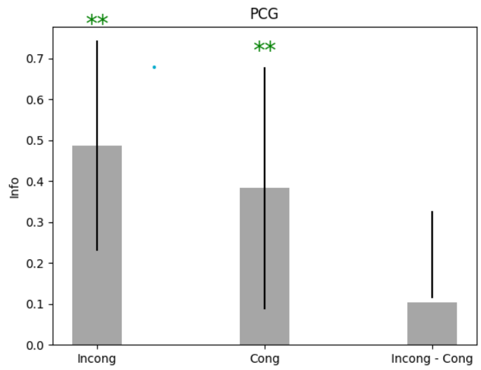


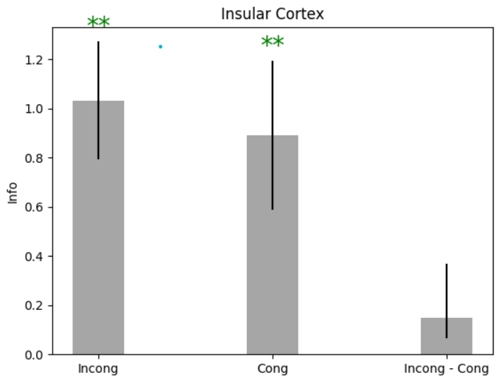
The R code and output used to produce the graphs

The following graphs represent the mean (represented by the height of the bar), range including the 95% confidence interval (represented by the black straight line) and the significance of the averaged stats of the incongruent, congruent and the difference between incongruent and congruent included in the mask region (less than 0.05 represented by one star and less than 0.001 by two stars).









This is the code used to produce these graphs:

```
import matplotlib.pyplot as plt
```

```python
# Define the data for the three sets
data = [
    {'mean': 2.294417 , 'range': (
1.801369, 2.787465),
'significance':7.503e-10},
    {'mean': 1.594459  , 'range':
(  1.176826 ,2.012092),
'significance':  3.215e-08},
    {'mean': 0.7442025, 'range': (
0.4140877, 1.0743173), 'significance':
9.401e-05},
    ]

# Create the figure and axis
fig, ax = plt.subplots()

# Set the positions for the bars
positions = [1, 2, 3]

# Draw the bars, lines, and stars
for i, datum in enumerate(data):
    pos = positions[i]
    mean = abs(datum['mean'])
    y_min, y_max = datum['range']
    significance = datum['significance']

    # Draw the bar
    ax.bar(pos, mean, color='gray',
width=0.3, alpha=0.7)

    # Draw the line
    ax.plot([pos, pos], [y_min, y_max],
color='black', linewidth=1.5)

    # Determine the number of stars
    if significance <= 0.001:
        stars = '**'
    elif significance <= 0.05:
        stars = '*'
    else:
        stars = ''

    # Draw the stars if any
    if stars:
        ax.text(pos, y_max + 0.01, stars,
ha='center', va='bottom', color='green',
fontsize=20)

ax.set_title("Insular Cortex using
Spherical Mask")
```

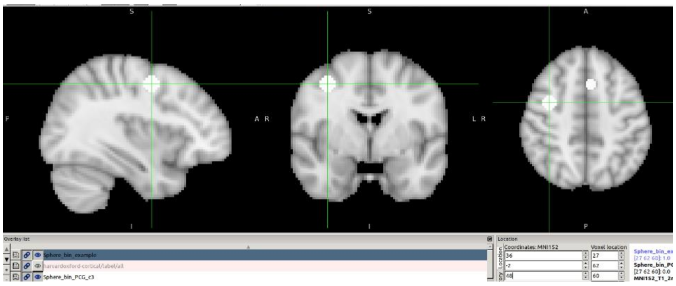
```python
# Set the x-ticks to the positions
ax.set_xticks(positions)

# Set the labels for the x-ticks
ax.set_xticklabels(['incong', 'cong',
'incong - cong'])

# Set the labels for the axes
ax.set_ylabel('Info')

# Display the plot
plt.show()
```

Carrying the one-sample t-test, with the null hypothesis that the mean of the difference of incongruent and congruent equals zero, on the averaged statistics of the whole anatomical regions, the p-values of both LOC regions were found to be below 0.05 and thus the difference is significant and the null hypothesis is rejected. Assuring what we found previously about the peaks of activations in these regions being genuine. The PCG and insular cortex were insignificant when taking the whole region. We will further check the regions using the ROI as spheres around the peaks of activation. The whole anatomical region might be too large to judge the statistical significance. Another note is that we can see that this difference comes from a larger value in the incongruent statistics. This could be seen earlier when looking at the activation maps for the incongruent and congruent as the incongruent maps contained more active areas which could be explained as the incongruent task needing more effort and control to not be flanked by the noise of the arrows being in opposite direction to the correct answer.

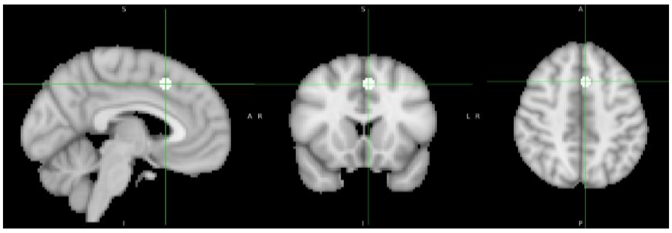
B. *Spherical Mask Method*

An alternative method by using a binarized sphere with a specific radius, here we used a 5mm-radius sphere, to extract the region around the activation peak and therefore is more localized compared to the previous method. This is an example on the spherical extraction with a center at MNI coordinates (36, -2, 48) to voxel coordinates using FSLeyes produces (27, 62, 60), and the following commands:

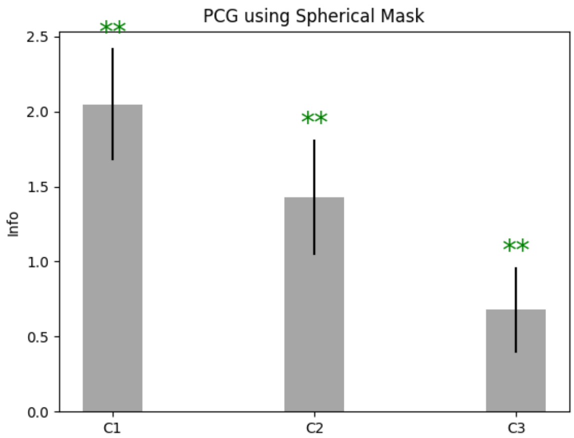
```
motair@motair-virtual-machine:~/Downloads/Data$ fslmaths $FSLDIR/data/standard/MNI152_T1_2mm.nii.gz -mul 0 -add 1 -roi 27 1 62 1 60 1 0 1 exercise.nii.gz -odt float
[redacted]
motair@motair-virtual-machine:~/Downloads/Data$ fslmaths exercise.nii.gz -kernel sphere 7 -fmean Sphere_example.nii.gz -odt float
motair@motair-virtual-machine:~/Downloads/Data$ fslmaths Sphere_example.nii.gz -bin Sphere_bin_example.nii.gz
```

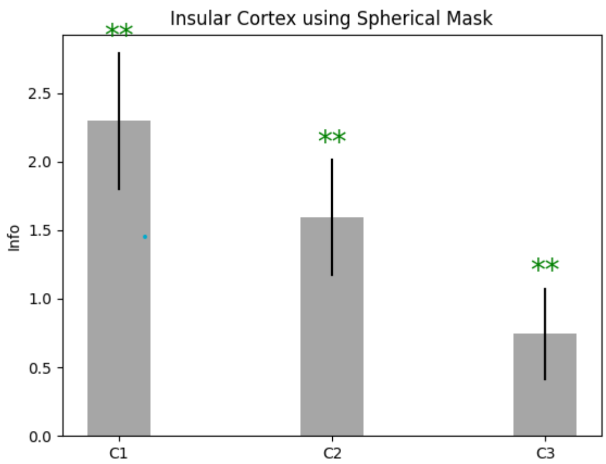


Back to our data, let us check the produced graphs for the 2 insignificant regions in the previous analysis.



The sphere mask around the Paracingulate Gyrus





We can notice that by taking a smaller area, the activation was found to be significantly different between our two tasks. And similar to the previous method, the incongruent case (C1) has a higher mean.

X. INTERPRETATION

The Paracingulate gyrus as a part the cingulate cortex deals with many cognitive functions. Most memorably in our case is **conflict management** (especially in the anterior cingulate cortex). This goes hand in hand with our results. In the congruent case, the middle arrow and the arrows it is being flanked by are in the same direction, causing no conflict. While in the incongruent task, the middle arrow is being overshadowed by the other arrows in the opposite direction causing a conflict in the subject's thinking causing the activation of this brain area.

The lateral occipital cortex as a whole is a visual area that takes part in object recognition is said to be dynamically coupled with each of two distributed patterns of neural activity depending upon the percept (default or alternative) elicited by a bistable figure. (doi: 10.1089/brain.2012.0119) This bistable figure can mean that the visual sight has too possible interpretations. This can be considered similar to our task in that there is a confusion between the arrows in the incongruent case due to the opposite directions seen causing a similar effect to the bistable image which would not occur in the case of the congruent.

The Insula is very relevant in cognitive neuroscience. It is involved in the attention and salience processing. Insula activation, along with dorsal anterior cingulate (dACC), is observed in response to "oddball" stimuli interspersed among a series of the same item. Together, the insula and dACC, amygdala, and other subcortical structures are often referred to as the "salience network", the function of which is to identify the most homeostatically relevant among multiple competing internal and external stimuli. (https://doi.org/10.1016/S0042-6989(01)00073-6). This is by definition the Flanker's task. In the incongruent case, there is an "oddball" stimulus which is the middle arrow that we are trying to determine the direction of. Thus it makes sense for the insula to have a significant difference in activation between the incongruent and congruent cases.