

# Basic fMRI analysis - Assignment

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June 15, 2023

## 1 Assignment description

This paper deals with study 4 of the suggested studies, where the purpose is to explore the Penfield map. It includes four tasks: moving feet, moving the left hand, moving the right hand, and moving the tongue. In their seminal paper published in 1937, Penfield and Boldrey [PB37] describe the somatic motor and sensory representation in the human cerebral cortex, thus establishing a precise topography of cortical localization. This was done by electrically stimulating the cortex and linking a discrete part of the brain with motor and sensory phenomena affecting a particular part of the body [Sch93]. A visual representation of the cortex and the associated body parts is shown in figure 1, as was further developed and published in 1950 by Penfield and Rasmussen [PR50], where the right side shows the primary motor cortex (M1) and the left side shows the primary sensory cortex (S1).

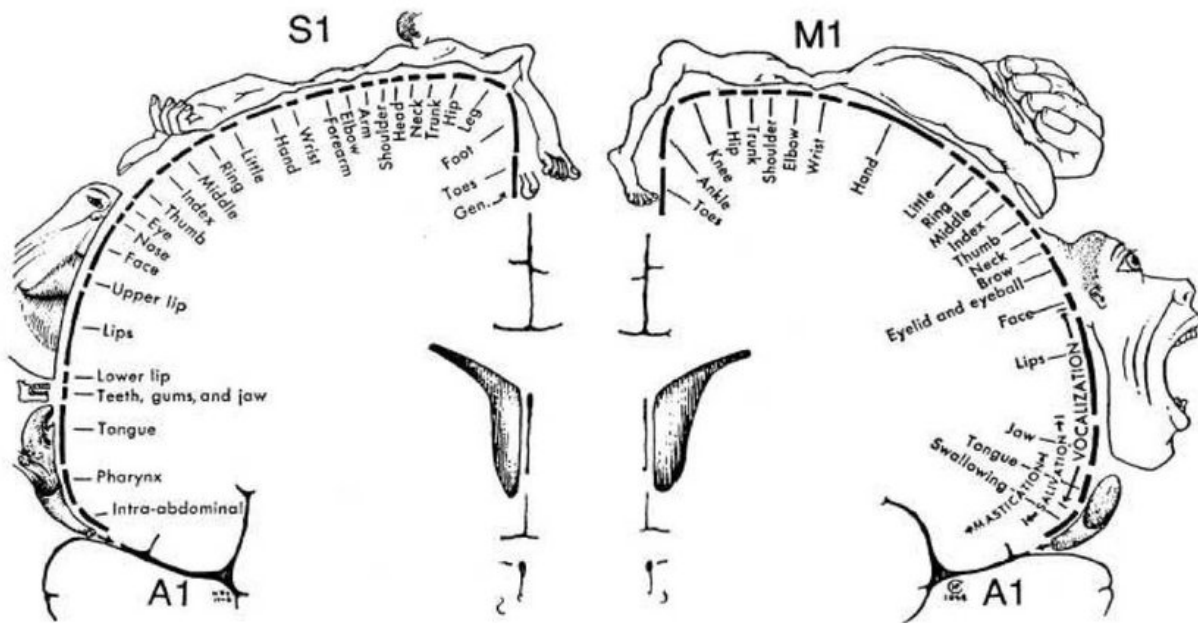


Figure 1: Penfield's homunculus

It becomes clear that there is a distinct cortical region dedicated to each of the four movements examined in the tasks. The feet correspond to the medial part of the primary motor cortex, the hands to a slightly more lateral part, while the tongue corresponds to a more lateral and inferior area.

## 1.1 Research question

Therefore, the research question of this paper is whether our subjects show such a distinct pattern of activation when performing the corresponding movements. Particularly: Does the feet BOLD signal differ from that of the hands and the tongue? Does the left hand BOLD signal differ from that of the right hand, the feet and the tongue? Does the right hand BOLD signal differ from that of the left hand, the feet and the tongue? And does the tongue BOLD signal differ from that of the feet and the hands? Because the interest of this study is in the movement of specific body parts, the focus is only on the primary motor cortex, the pre-motor cortex, and the supplementary motor area.

## 1.2 Hypotheses

It is hypothesized that subjects will show similar activation patterns as predicted by Penfield’s map, as measured from the BOLD signal during the fMRI tasks. Particularly, it is expected that each body area, namely, feet, left hand, right hand, and tongue, will show different BOLD signals than the rest, which match the spatial ordering described by Penfield.

# 2 Materials and methods

## 2.1 fMRI acquisition

The data during all four tasks were acquired with the same fMRI scanning sequence, a TR (repetition time / time) of 2.5 seconds, and interleaved ascending slice ordering. Each task movement was performed with a visually paced cue alternated with rest, while for the feet task, participants were instructed to move both feet simultaneously. Additionally, an anatomical T1 weighted image was acquired for each subject.

## 2.2 Participants

A total of 18 subjects were included in this study, namely: 1, 3, 7, 8, 11, 14, 15, 20, 25, 26, 33, 40, 48, 50, 52, 54, 59, 60. Even though it was slightly time consuming to perform the pre-processing and the first level statistical analysis for 18 subjects, the results of the second level statistical analysis are more robust when including more subjects. Notably, subject 25 seemed to have confused the instructions and moved the left hand in the right hand task and vice versa. As a result, subject 25 was excluded from the analysis when examining the left and right hand activation.

## 2.3 Pre-processing

There were several pre-processing steps that needed to be performed before doing any statistical analyses. All steps were performed with the 12th version of SPM software, using dependencies between the steps. A batch with dependencies was created that was applied with the same configurations to all 18 subjects mentioned above. The batch included all steps mentioned in this section, as well as the first-level statistical analysis mentioned in section 2.4.

### 2.3.1 Realignment

Firstly, realignment essentially performs motion correction to remove noisy movement that is not of interest to the task at hand. This is done by rotating and translating the scan matrices of two images over the three axes until the sum of squares of the difference between them is minimal. This is also referred to as six-body rigid transformation, where the six-body aspect refers to a transformation with six degrees of freedom, which includes three translational (x, y, z) and three rotational (roll, pitch, yaw) components [Gro21]. These parameters describe how the fMRI data should be translated and rotated to align with a reference image or a standardized coordinate system. The rigid aspect implies that the transformation preserves the shape and size of the data without any deformation. This was combined with reslicing to change both the transformation matrix in the header, as well as the actual data. Using SPM’s *Realign : Estimate&Reslice* option, all parameters were left at default, using 0.9 estimation quality, estimation interpolation of 2nd degree B-Spline, and reslice interpolation of 4th degree B-Spline. The results of the realignment step are shown in figure 2.

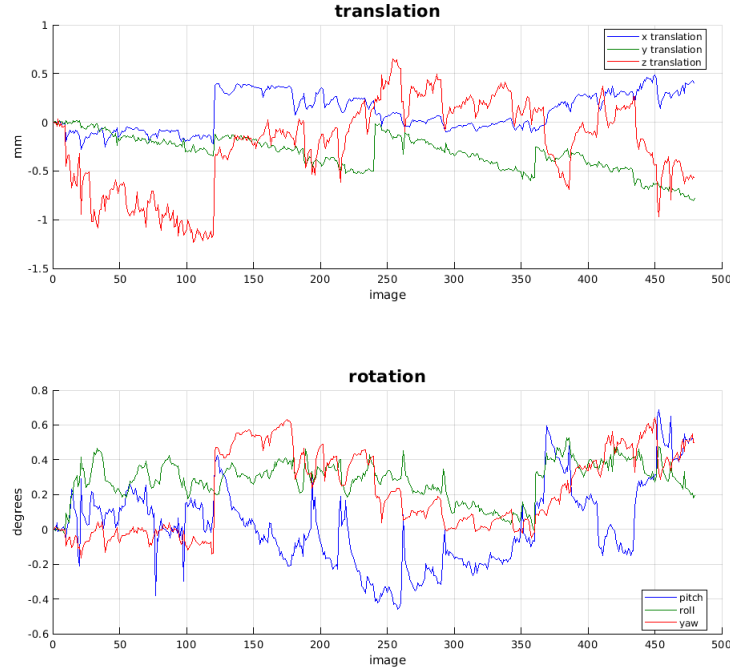


Figure 2: Realignment results

### 2.3.2 Slice timing correction

Slice timing correction is used to correct for the slightly different moments in time with which an fMRI sequence acquires the scans. Statistical modelling assumes that the data come from the same point in time, so this step makes sure this assumption is met. In this study, all tasks were block-designs, so slice timing correction is not necessary. However, it was applied since it does not decrease the quality of the analysis and it is a good learning experience.

Specifically, slice timing correction was performed using SPM's *Slice Timing* function. It essentially interpolates between scans to create a new image with time-corrected values, as if all slices were acquired simultaneously. As such, it is necessary to pick a reference point representing the acquisition timepoint of the entire timeseries, and this was chosen to be halfway in the acquisition. Since the acquisition protocol was interleaved, starting from number 1, the selected scan used as a reference point was number 2. The total number of slices was 40, TR was 2.5 seconds, and TA (defined as  $TR - (TR/nslices)$ ) was 2.4375 seconds. Even though performing time slice correction after realignment entails the risk of incorrect time differences on the statistical model, this was preferred over the interpolation issues that might come up when doing slice timing correction on the non-realigned data and then realigning the data.

### 2.3.3 Coregistration

Thirdly, coregistration was performed to align and register the functional brain images acquired during the fMRI tasks with structural brain images, obtained from a high-resolution anatomical MRI scan. This ensures that the functional and structural images are in the same spatial reference frame, enabling accurate localization of functional brain activity within the anatomical context. Using SPM's *Coregister : Estimate* function, the reference image was defined as the mean image that was generated when applying realignment, while the source image was the subject's anatomical MRI scan using T1 contrast. Placing the mean image as reference means that it remains static, while the anatomical image is the one that is being adjusted. The alignment of the scans of these different modalities is done using the mutual information criterion  $I(u, v)$ , shown in formula 1, which essentially measures the information that one random variable  $u$  conveys about another random variable  $v$ .

$$I(u, v) = h(v) - h(v|u) = h(u) - h(u|v) \quad (1)$$

Formula 1 uses the entropy of the random variable  $v$ ,  $h(v)$ , which is a measure of the average uncertainty in  $v$ , and the conditional entropy  $h(v|u)$ , which quantifies the uncertainty of  $v$  conditioned on observations of  $u$ . These

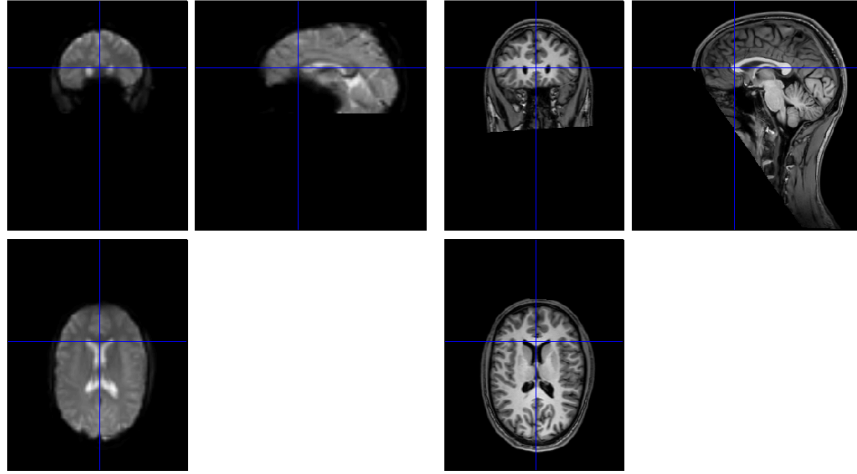
terms are described in formulas 2 and 3.

$$h(v) = -\mathbb{E}_v[\log_2(P(v))] \quad (2)$$

$$h(u|v) = -\mathbb{E}_u[\mathbb{E}_v[\log_2(P(v|u))]] \quad \text{where } P \text{ denotes probability density} \quad (3)$$

In the context of fMRI coregistration, the mutual information criterion measures the statistical dependence between the intensities of corresponding voxels in the functional and structural images [TFW<sup>+</sup>99]. It quantifies the amount of information that one image provides about the other, indicating how well the two images are aligned. By evaluating the mutual information between the voxel intensities, the coregistration algorithm uses six-parameter rigid body transformation (translation, rotation, scaling, etc.) to find the parameters that maximize the mutual information. This alignment ensures that functionally relevant brain regions are accurately matched with their corresponding anatomical structures, enhancing the accuracy of subsequent analyses and interpretations of fMRI data.

Figure 3, shows the results of the coregistration procedure. Specifically, figure 3a shows the anterior part of the corpus callosum, with the functional images on the left, and the anatomical on the right, while figure 3b shows the histograms of the voxel signal intensities.



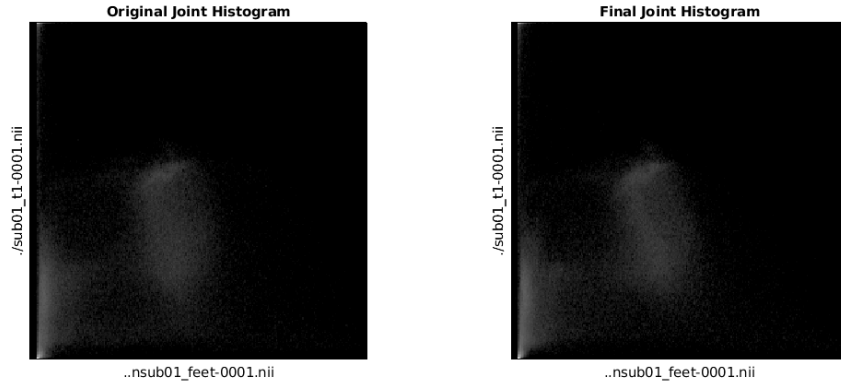
(a) Anterior part of the corpus callosum

#### Normalised Mutual Information Coregistration

$$X1 = -0.000*X - 0.000*Y - 0.340*Z + 69.944$$

$$Y1 = -0.340*X - 0.011*Y + 0.000*Z + 103.169$$

$$Z1 = -0.011*X + 0.333*Y - 0.000*Z - 46.511$$



(b) Coregistration histogram

Figure 3: Coregistration results for subject 1, feet task

Figure 3a, shows quite a good match between the functional and the anatomical images, while the final joint histogram is sharper than the original one. These results indicate that coregistration was done successfully.

### 2.3.4 Normalization

Normalization is a necessary step when analyzing fMRI data from several subjects for group-level analyses. There is an inherent anatomical and functional variability among individuals, since each brain has a unique size, shape, and orientation, thus making direct voxel-wise comparisons between individuals problematic. Normalization maps the fMRI data to a common coordinate space, typically a standardized brain template or an atlas. Using, SPM's *Normalize : Estimate* function, the mapping was done to the ICBM space template for European brains (there is an option for East Asian brains as well), taking as input the coregistered images using dependencies. Firstly, affine transformations, like scaling, rotation, shearing, and translation perform spatial adjustments while preserving the overall shape and structure of the scans. Secondly, basis functions are used to model and represent the temporal and spatial characteristics of the fMRI signal, resulting in a deformation field that can be applied to the anatomical scan. Additionally, a regularization parameter of 0.0001 was included to reduce the noise captured by the normalization procedure.

Having estimated the normalization parameters based on the coregistered anatomical image, these parameters need to be applied to the functional images to move them to MNI space. This is done in two separate steps, using SPM's *Normalise : Write* function twice, since there are multiple sessions in this study. Firstly, for each of the four tasks, the deformation field is specified by a dependency to the deformation field described above, and the images to write are specified by a dependency to the slice timing corrected images of the corresponding tasks. Voxel size was set to 3 in all three dimensions, while all other parameters were kept at default. Secondly, the images to write were specified by a dependency to the coregistered images, while the deformation field was the same, and the voxel size was set to 1 in all three dimensions.

### 2.3.5 Smoothing

The last pre-processing step is smoothing, which becomes necessary because normalization is inherently imperfect, thus limiting the spatial overlap in activity between subjects. Therefore, smoothing is used to account for these spatial inaccuracies. Specifically, smoothing is a spatial filter applied to the fMRI data, which effectively reduces noise and spatially blurs the signal. SPM's *Smooth* function by default uses a Gaussian filter for spatial smoothing, assigning higher weights to voxels closer to the center of the kernel, thus creating a bell-shaped distribution of weights. This weighting scheme results in a smooth blurring effect that preserves the underlying spatial structure while reducing noise. The strength of smoothing is determined by the width of the Gaussian distribution, often referred to as the full width at half-maximum (FWHM), where a larger FWHM corresponds to stronger smoothing with more pronounced blurring of the data. This was left at default at 8 in all three dimensions, while the images to smooth were specified by a dependency to the normalized images of each task.

## 2.4 First-level statistical analysis (individual level)

The first level statistical analysis aims at estimating the task related brain activity per voxel for each subject. As described earlier, each task contains a cue for the corresponding body movement alternated with rest. Therefore, based on the cue onset for each task, a model can be estimated for when a voxel in the brain may be correlated with the task at hand. Firstly, the model was specified using SPM's *fMRI model specification* function, where the interscan interval was set to 2.5 seconds, the microtime resolutions was set to 40, and the microtime onset was set to 21. There were four sessions, one for each task, where the input scans were specified by a dependency on the smoothed images of the last pre-processing step. The cue onsets and the durations were specified for each session as given in the assignment details, while no extra regressors were included, and the masking threshold was set to 0.5.

Next, the parameters of the model were estimated using SPM's *Model estimation* function, where the input model was specified by a dependency on the model specification *SPM.mat* file that was generated from the previous step. The method used for parameter estimation was the restricted maximum likelihood method, also referred to as "classical" in SPM.

Lastly, SPM's *Contrast Manager* function was used to specify the t-contrast for each session, using the *SPM.mat* file with the estimated parameters of the previous step by a dependency. Specifically, according to the research questions stated in section 1.1, the BOLD signal of each task was compared to that of all other conditions, using

a weight vector like  $[3 - 1 - 1 - 1]$ , where in this example 3 corresponds to the signal from the feet task (the first session), and each  $-1$  corresponds to each of the remaining tasks, namely, left hand, right hand, and tongue.

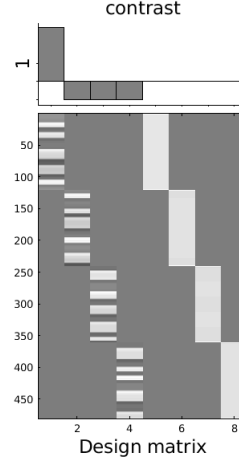


Figure 4: Design matrix showing contrast 1 (feet vs other tasks)

Figure 4 shows the design matrix, with the contrast for the first task as an example.

## 2.5 Second-level statistical analysis (group level)

The second-level statistical analysis is necessary for group-wise inference. So, although the first-level analysis can identify the statistically significant voxels in one individual, the second-level analysis allows to compare all individuals and identify statistically significant voxels group-wise. This was done with one-sample t-tests, comparing each task with the remaining three. The output directory and the *con* files corresponding to each contrast were specified using SPM's *Factorial design specification function*. Then, the model parameters were estimated using SPM's *Model estimation* function, using the restricted maximum likelihood algorithm, and a dependency on the *SPM.mat* file of the previous step. Finally, SPM's *Contrast Manager* function was used to specify the *T* contrasts and the contrast sessions, using a dependency to the *SPM.mat* file of the previous step, while the weight vector was set to 1. This procedure was used four times, one for each task with the corresponding *con* files.

Additionally, masking was used to specify the regions of interest (ROI), to only look at the voxels in the specified areas. This essentially decreases the number of statistical comparisons, thus enhancing statistical power. Using SPM's *result* to open the final *SPM.mat* file of the second-level analysis, masking was specified using an atlas with the Brodmann areas. The areas selected were area 4 corresponding to the primary motor cortex and area 6 corresponding to the premotor cortex and the supplementary motor area [DNP17].

## 3 Results

### 3.1 First-level statistical analysis

Figure 5 shows the results of the first-level analysis for subject 1, as an example. The voxels with a p-value smaller than the p-value shown at *set-level*, after performing the family wise error (FWE) correction, are statistically significant, so they are associated with the task at hand for the specific subject. However, as we are interested in group activity, the results of the second-level statistical analysis are more relevant.



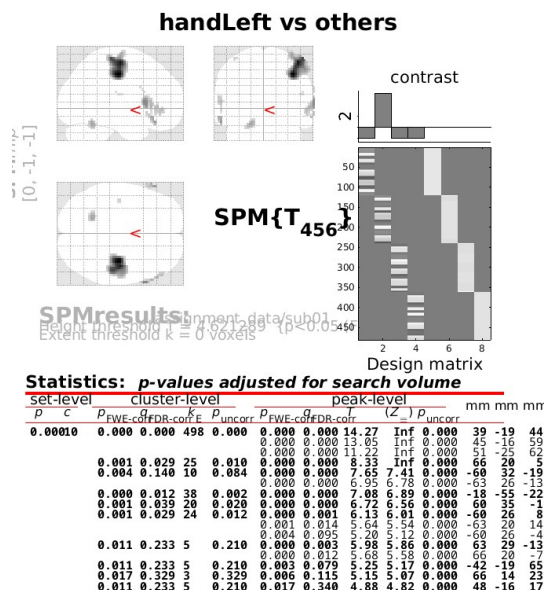
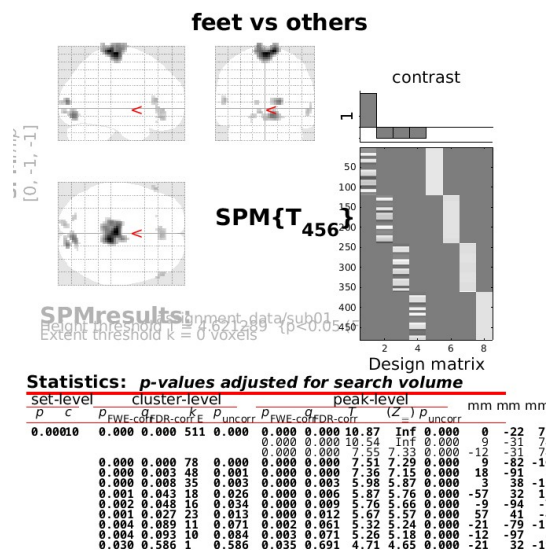
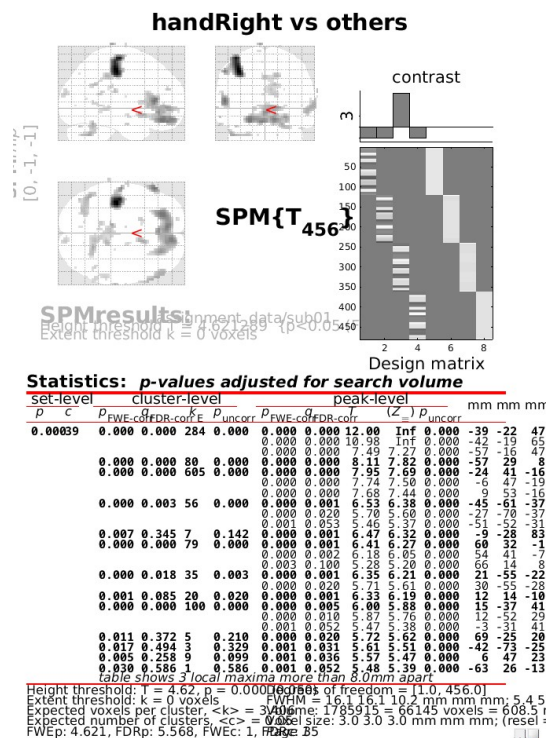


table shows 3 local maxima more than 8.0mm apart  
Height threshold:  $t = 4.62$ ,  $p = 0.0001$  (df = 11.0, 456.0)  
Extent threshold:  $k = 0$  voxels  
FWHM = 16.1 16.1 10.2 mm mm mm; 5.4 5  
Expected voxels per cluster,  $<k> = 340$  voxels; 1785015 = 66145 voxels = 608.5  
Expected number of clusters,  $<c> = 0$  voxel size: 3.0 3.0 3.0 mm mm mm; (resel):  
FWEp: 4.621, FDRp: 5.672, FWec: 1, FDRc: 16

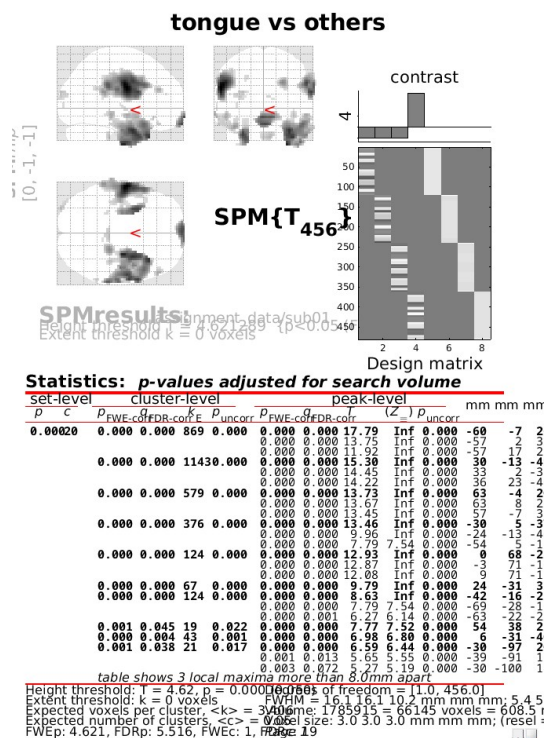
table shows 3 local maxima more than 8.0mm apart  
Height threshold:  $t = 4.62$ ,  $p = 0.0001$  (df = 11.0, 456.0)  
Extent threshold:  $k = 0$  voxels  
FWHM = 16.1 16.1 10.2 mm mm mm; 5.4 5  
Expected voxels per cluster,  $<k> = 340$  voxels; 1785015 = 66145 voxels = 608.5  
Expected number of clusters,  $<c> = 0$  voxel size: 3.0 3.0 3.0 mm mm mm; (resel):  
FWEp: 4.621, FDRp: 5.639, FWec: 3, FDRc: 20

(a) Feet task

(b) Left hand task



(c) Right hand task



(d) Tongue task

Figure 5: Example first-level statistics from subject 1

### 3.2 Second-level statistical analysis

As was mentioned in section 2.5, only the Brodmann areas 4 and 6 were included, so the results are limited to these brain areas. Moreover, family wise error correction was performed, so for a group of voxels to show statistically significant BOLD responses, the FWE-corrected p-value needs to be larger than the set-level p-value.

Figure 6 shows the results of the group wise analysis. Contrary to the hypothesis, for the feet task it seems that there are no voxels that show a statistically significant BOLD response compared to other tasks ( $p > 0.0012$ ,  $Z = 4.69$  and  $Z = 4.59$ ). However, the location of the shown clustered voxels corresponds to the location of the feet as originally proposed by Penfield.

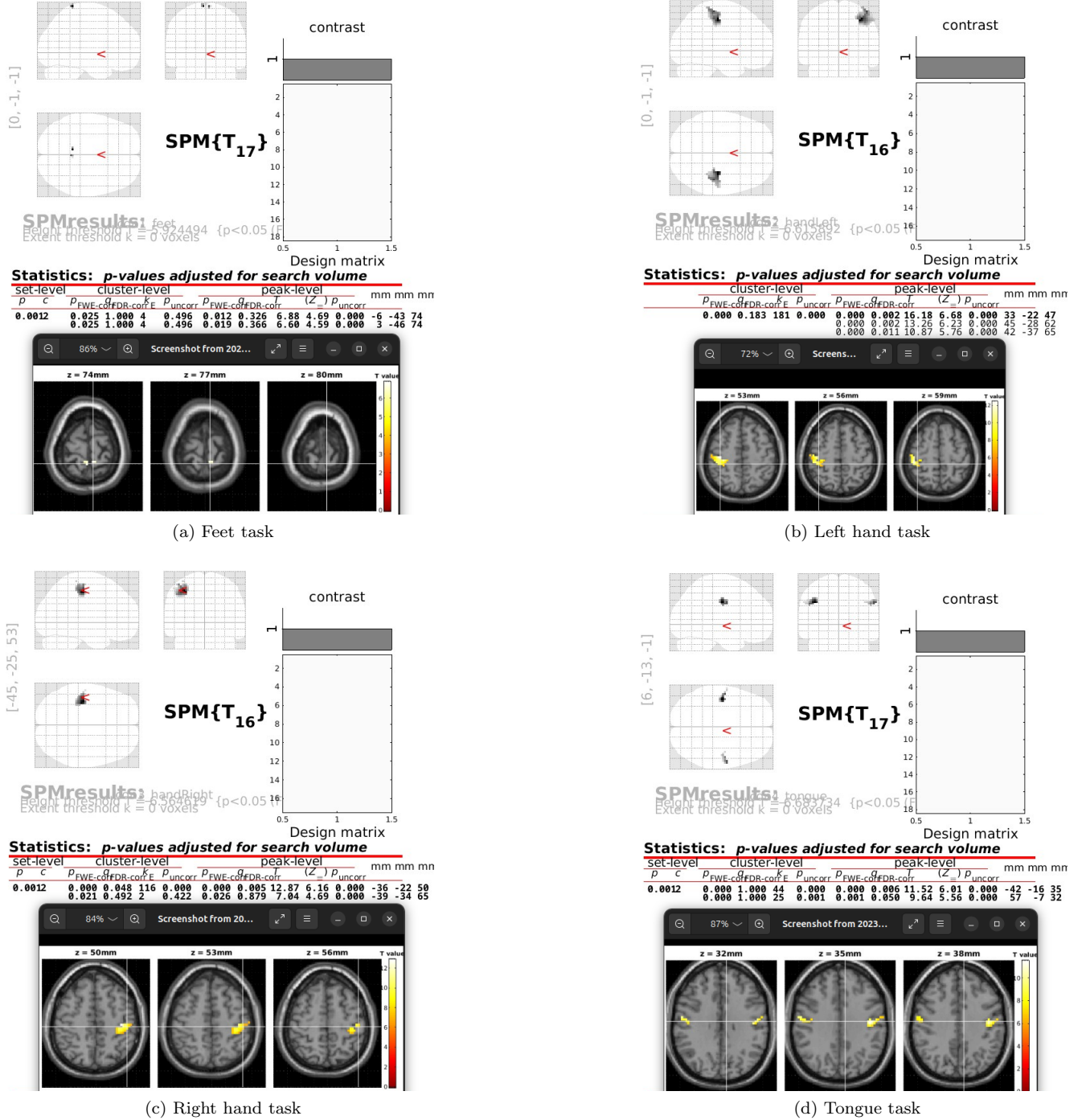


Figure 6: Second-level statistics



Results from the remaining tasks seem to conform to our hypotheses. Specifically, for the left hand task there is one cluster of voxels in the right hemisphere that shows a statistically significant BOLD response compared to the other tasks ( $p < 0.001, Z = 6.68$ ). Similarly, for the right hand task there is one cluster of voxels in the left hemisphere that reaches statistical significance ( $p < 0.001, Z = 6.16$ ). For the tongue task, there are two cluster of voxels that reach statistical significance, one in the left hemisphere ( $p < 0.001, Z = 6.01$ ) and one in the right ( $p < 0.001, Z = 5.56$ ). Additionally, visual inspection of the statistically significant voxels show that the brain areas in all tasks corresponds to the brain areas proposed by Penfield.

## 4 Limitations

There were a few things that could have been done differently to increase the power of this study and the robustness of the results. Specifically, the use of DARTEL normalization, instead of the affine normalization that was performed as described in section 2.3.4, could have produced better overlap between subjects. This is because DARTEL normalization would create a group-wise template by iteratively aligning and averaging the individuals' anatomical images, while affine normalization employs a single pre-existing template (in the current case it was the european ICBM). Additionally, DARTEL uses nonlinear transformation by employing diffeomorphic registration by modelling the flow of the moving image into the fixed image using a pair of vector fields, thus allowing for more accurate alignment of anatomical structures than linear transformations [Gro21].

Additionally, the inclusion of motion parameters as nuisance regressors could have further improved the results. This could mitigate the confounding effects of motion related artifacts on the BOLD signal that is a naturally occurring phenomenon when subjects lay for a long time in the scanner. The motion regressors would be included in the general linear model as nuisance parameters alongside the other variables, thus minimizing their effect on the fMRI signal. Although realignment controls for motion correction as a pre-processing technique, it is not equivalent to including motion regressors in the model, but for the current study, including nuisance regressors was omitted because it would complicate the analysis.

Therefore, performing DARTEL normalization and including nuisance regressors could improve the results in this study. This could potentially allow to find a statistically significant result for the first task, thus confirming the Penfield map for feet movement as well.

## 5 Conclusion

Conclusively, the hypotheses of this research are supported for the tasks regarding left hand movement, right hand movement, and tongue movement. However, the hypothesis regarding feet movement is not supported, as there were no voxels that showed a statistically significant BOLD signal compared to the remaining three tasks. Nevertheless, the BOLD signal from all tasks confirm the spatial ordering of the Penfield map, in the sense that brain activation during these tasks corresponds to the brain areas proposed by Penfield.

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