Voxelwise statistic, two independent groups

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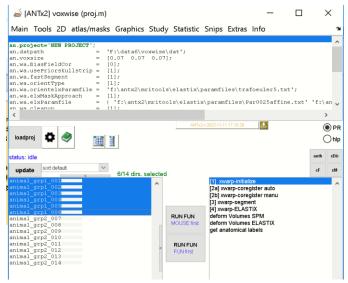
Scenario

-<u>Artificial data</u> for 14 animals were created, with spherical differences <u>centered</u> in the a) **left Primary** somatosensory area mouth layer 4 and b) the right CaudoPutamen

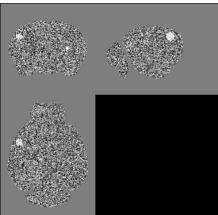
Here we have two groups with 6 and 8 animals in each group. The artificially created files are stored as "vimg.nii"-image in the respective animal-folders. Note that the animal-folders are empty, except of the "vimg.nii"-image. This image is in register to the Allen mouse brain atlas (i..e already in standard space).

Prerequisites

- -a project with project file was created ...when doing this analysis with your own data, the image that should be analyzed using voxelwise statistic must be in the standard/template space (here: Allen mouse brain space). For instance when analyzing the gray matter image("c1t2.nii"), the image has to be transformed to standard space to make voxelwise statistic feasable.
- -Templates: here ,the **mouse_Allen2017HikishimaLR**′-template is already imported in the study's templates-folder
- start ANT-gui and set current working directory to project-folder path
- -load project (m-file, here proj.m)



The Figure shows the list of 14 animals (left listbox). Animals of group-1 is selected (just for displaying purpose). Note that for this animals artificial data were created.



The figure shows the simulated image 'vimg.nii' (image is in standard-space) of one animal from the 2nd group with increased intensities centered at a) the left Primary somatosensory area mouth layer 4 and b) the right CaudoPutamen.

1) Create a group-assignment (Excel-file)

We now need a group-assignment file (Excel-file) with definitions of animals and group-assignment.

For this select: Statistic/create group assignment file from the ANTx-MENU.

This will open the repective parameter file. We keep the default parameter and hit [RUN].

To this end, the Excelfile "groupassignment.xlsx" is created, located in the "group"-folder in the current study directory ("F:\data6\voxwise\group").

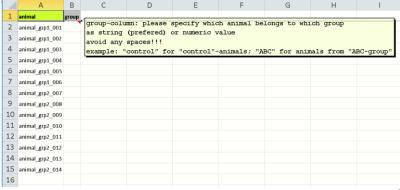
```
.. create group-assignment file ...wait

new group-file [groupassignment.xlsx]: Explorer or open

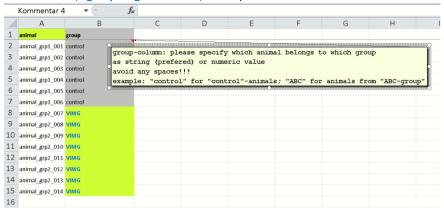
Done!

fx>>
```

You can select the 'open'-hyperlink in the command-window to open the Excel-file. The 1st sheet of the excelfile looks as follows:



The first column contains the animal-IDs of all animals of the current study. The 2nd column has to be filled with the respective group-assignment. Here the 1st 6 animals belong to the control-group, thus the 'control'-string is inserted for those animals, while the other animals belong to the "VIMG"-group, thus the "VIMG"-string is inserted in the 2nd column (see Fig. below). Note that the string-names are arbitrary. Try to avoid special characters! Save this modifications (here the same filename, "groupassignment.xlsx", is used).



2) Perform the voxelwise-T-test (independent group)

We now want to run the independent voxelwise t-test comparing the two groups for the "vimg.nii"-image. For this select: **Statistic/SPM-statistic** from the **ANTx -MENU**. This will call the main GUI (from the function <u>xstat.m</u>):

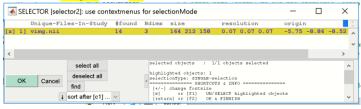


Next, select the [indepStat]-button to open the parameter-GUI for the voxelwise independent t-test (TWO-SAMPLE-TTEST). The default parameter-GUI looks as follows:

```
📣 xstat.m
                                                                                                                                                                                         TWO-SAMPLE-TTEST
x.excelfile
                                    : % [Excelfile]: this file contains a column with mouseIDs (names) and a column assigning the group
                                 [1];
[1];
                                             % this sheet contains columns with mouseIDs and group assignment % column number with the MouseIDs
   x.mouseID col
                                 [2]; % column number with group assignment (used when comparing groups, "regress_col" must be 
'F:\data6\voxwise\dat'; % data directory (upper directory) contains dirs with mice data 
''; % image name (nifti) to run the statistic (datapath has to bee defined before using the icon)
                                                                                                                                                      "regress_col" must be empty)
  🗱 x.data dir
x.inputimage
                             = 'F:\data6\voxwise\templates\AVGT.nii'; % select the TEMPLATE-file (path of "AVGT.nii")
= 'F:\data6\voxwise\templates\ANO.nii'; % select the ATLAS-file (path of "ANO.nii")
x.AVGT
x.ANO
               OTHER PARAMETER
                                 'Ivs2'; % groups to compare, use EXCELgroupnames(example: "GroupNameString1vsGroupNameString2") or alphabet. or 'F:\data6\voxwise\templates\AVGTmask.nii'; % <optional> use brainmask [select a mask or type "local" to use th
x.smoothing
                             = [1]:
                                            % <optional>smooth data
   x.smoothing_fwhm = [0.28 0.28]; % smoothing width (FWHM) x.output_dir = 'voxtest1'; % path for output/statistic
   x.output dir
                                             % [0|1] hide|show pipeline in SPM batch window, if [1] you have to run the code by yourself ( hit the
      RUN S cancel
```

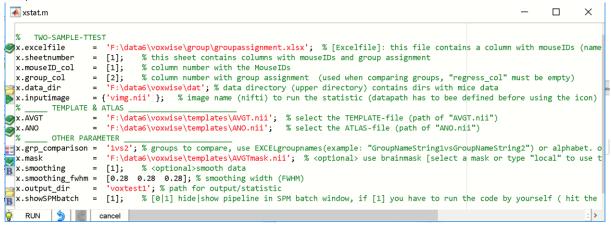
We now need to modify (or check) some parameters:

- "x.excelfile": click the left icon and select the group-assignment-file ("groupassignment.xlsx") located in the group-folder.
- "x. data_dir": click the left icon and select the study's dat-folder (This folder contain the animals-data and the image that should be statistically tested).
- "x. inputimage": click the left icon and select the image that should be statistically tested: Specifically in the file-selector GUI select "vimg.nii", hit [OK].



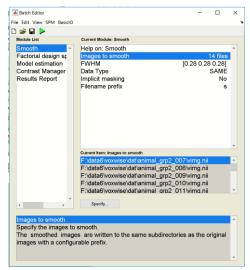
- "x. AVGT": click the left icon and select the AVGT-image ("AVGT.nii") from the study's templates-folder.
- "x. ANO": click the left icon and select the ANO-image ("ANO.nii", i.e. the Atlas) from the study's templates-folder.
- "x. mask": click the left icon and select the AVGTmask-image ("AVGTmask.nii") from the study's templates-folder. Note that the animal-folders only contain the 'vimg.nii'-image. Thus we have to assign the brain-mask ("AVGTmask.nii") from the templates-folder.
- " $\mathbf{x.output_dir}$ ": define the output-folder for the voxelwise statistic, here " $\mathbf{voxtest1}$ ".

The parameter-Gui should now looks as follows:



Note that the image 'vimg.nii' will be smoothed ("x.smoothing" is set to [1]) by the triple of the voxel-resolution ("x.smoothing_fwhm" is [0.28 0.28 0.28]). Note also, that "x.showSPMbatch" is set to [1]. This parameter will open the SPM-batch-GUI, when hitting the [RUN]-button, allowing for final post-parameter configurations.

Hit [RUN]-button to run the voxelwise statistic. Because the parameter "x.showSPMbatch" was set to [1], the SPM-batch-GUI will pop-up, allowing for specific post-parameter configurations and final checks (see Fig. below).



Here, everything seems to be ok and no further changes will be made. Hit [RUN BATCH]-button (green triangle) to execute this batch. The process will take a couple of minutes.

When done, you can obtain the **xstat-batch**, by typing: "char(anth)" in Matlab-cmd window or clicking the [anth]-button from the ANTx-main GUI:



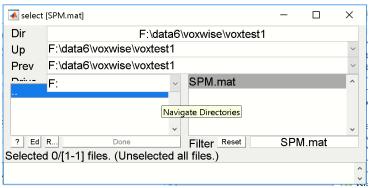
To rerun the analysis just re-run the code above.

3) Examine Results

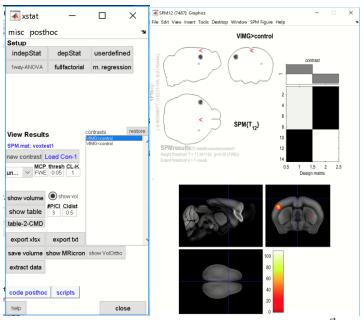
If the xstat-GUI is closed, type 'xstat' in the cmd-window or select: Statistic/SPM-statistic from the ANTx-MENU.

Next, hit the [load CON1]-button (below the xstat-"View Results" section) to load the 1st contrast from the analysis.

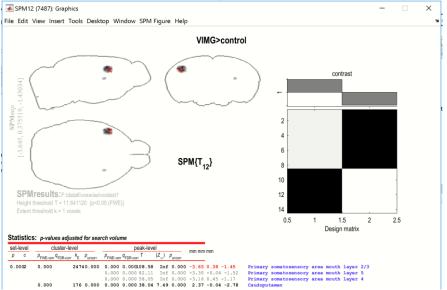
Next, select the path of the voxelwise analysis-folder ('voxtest1') and the respective SPM.mat-file (In the below Fig: Select SPM.mat from the right listbox, which than appears in the lower listbox, finally hit [Done]).



Basically, for the independent t-statistic two contrasts will be calculated by default ('A>B' and 'A<B'). Executing the [load CON1]-button displays the 1st contrast ('VIMG>control').



The SPM-graphics window will show the voxelwise results for the 1^{st} contrast 'VIMG>control' with the parameters, FWE at p=0.05, with min. survival spatial cluster (aka clustersize / sl-k) = 1 voxel. As expected, there are two significant 'blobs'. To see the associated results table with anatomical annotations, hit the [show table]-button from the xstat-GUI.



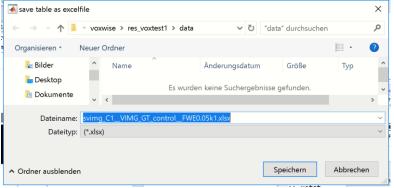
Because, the number of peaks per cluster (#p/Cl) is set to 3 and the cluster distance (CLdist) is set to 0.5 mm we see three peak clusters within the large left-hemispheric blob. You can change the respective settings for "#p/Cl" and "CLdist" in the xstat-GUI and update the graphic window by hitting the 'VIMG>control'-contrast from the xstat-listbox.

Again, to update any changes in the parameters just select the respective contrast from the xstat contrast listbox.

To show the volume again, hit the [show volume]-button.

3.1) EXPORT RESULTS-TABLE AS EXCELFILE

To export the results as Excel table, hit the [export xlsx]-button. The GUI will prompt you for a path and a proper filename of the Excel file. Note that per default, the output-path is predefined (new folder: 'res_'+name of the analysis-folder), here: "res_voxtest1" is the output-folder, which again contains a subfolder "data" that will contain the output. Also, the filename is predefined, based on the image, contrast-number, contrast-name, method, p-value and clustersize. Thus the filename is: "svimg C1 VIMG GT control FWE0.05k1.xlsx".

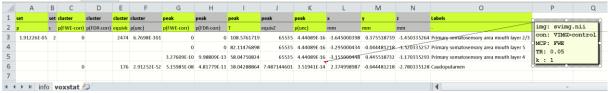


Without changing the path and filename, just hit the [save]-button.

```
...export table..wait..
voxSTAT-excelfile [svimg_C1__VIMG_GT_control__FWE0.05k1.xlsx]: Explorer or open
DONE!
fx >>
```

When done click the "open"-hyperlink to open the Excel file. The Excel file contains two sheets.

The "voxstat"-sheet contains the results-table:

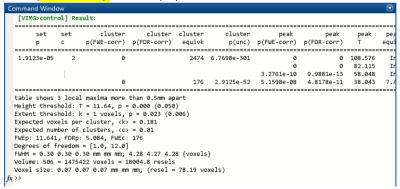


The "info"-sheet contains information to backtrack the statistical results:

1	Field	value		
2	Image	svimg.nii		
3	contrast	VIMG>control		
4	MCP	FWE		
5	Threshold	p<0.05		
6	CLustersize	k=1		
7	contrastName	VIMG>control		
8	contrastNo	No-1		
9	statstic	T		
10	contrastDir	[1 -1]		
11	dir :	F:\data6\voxwise\voxtest1		
12	INPUT-DATA***			
13	ANIMAL/Dir	Path		
14	animal_grp2_007	F:\data6\voxwise\dat\animal_grp2_007\svimg.nii	1	0
15	animal_grp2_008	F:\data6\voxwise\dat\animal_grp2_008\svimg.nii	1	0
16	animal_grp2_009	F:\data6\voxwise\dat\animal_grp2_009\svimg.nii	1	0
17	animal_grp2_010	F:\data6\voxwise\dat\animal_grp2_010\svimg.nii	1	0
18	animal_grp2_011	F:\data6\voxwise\dat\animal_grp2_011\svimg.nii	1	0
19	animal_grp2_012	F:\data6\voxwise\dat\animal_grp2_012\svimg.nii	1	0
20	animal_grp2_013	F:\data6\voxwise\dat\animal_grp2_013\svimg.nii	1	0
21	animal_grp2_014	F:\data6\voxwise\dat\animal_grp2_014\svimg.nii	1	0
22	animal_grp1_001	F:\data6\voxwise\dat\animal_grp1_001\svimg.nii	0	1
23	animal_grp1_002	F:\data6\voxwise\dat\animal_grp1_002\svimg.nii	0	1
24	animal_grp1_003	F:\data6\voxwise\dat\animal_grp1_003\svimg.nii	0	1
25	animal_grp1_004	F:\data6\voxwise\dat\animal_grp1_004\svimg.nii	0	1
26	animal_grp1_005	F:\data6\voxwise\dat\animal_grp1_005\svimg.nii	0	1
27	animal_grp1_006	F:\data6\voxwise\dat\animal_grp1_006\svimg.nii	0	1
28	ADDITIONAL INFO***			
29		table shows 3 local maxima more than 0.5mm apart		
30		Height threshold: T = 11.64, p = 0.000 (0.050)		
31		Extent threshold: k = 1 voxels, p = 0.023 (0.006)		
າ 4	info /vox	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		

3.2) DISPLAY RESULT TABLE IN COMMAND WINDOW

Select [table-2-CMD]-button display the results-table in the command window:



3.3) EXPORT RESULTS-TABLE AS TXT-FILE

To export the results as text-file, hit the [export txt]-button. When path/file is not changed this will save the results-table as "svimg_C1_VIMG_GT_control_FWE0.05k1.txt" in the "res_voxtest1\data"-folder:

```
| Swing_C1_VIMO_GT_control_FWE0.05k1.bt | Swin
```

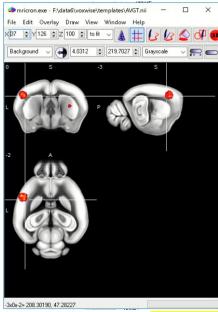
3.4) SAVE THREHOLDED IMAGE AS NIFTI-FILE

To save the thresholded (surviving) t-map as NIFTI-file hit the [save volume]-button. The GUI will prompt you for a path and a proper filename. Note that per default, the output-path is predefined (new folder: 'res_'+analysis-folder), here: "res_voxtest1" in the subfolder "data". Also, the filename is predefined, based on the image, contrast-number, contrast-name, method, p-value and clustersize. Thus, the filename is: "thresh svimg C1 VIMG GT control FWE0.05k1.nii".



When done you can select the hyperlink 'MRicron' from the command-window to open the saved statistical t-map overlaid onto the template ('AVGT.nii') via MRicron.

```
...saving volume...wait..
voxSTAT-volume [AVGT.nii - thresh_svimg_C1__VIMG_GT_control__FWE0.05k1.nii]: <a href="mailto:Explorer">Explorer</a> or <a href="mailto:MRicron">MRicron</a>
Done!
<a href="mailto:fix">fix</a>>>
```



Alternatively, you can hit the [show MRicron]-button from the xstat-GUI and select the respective NIFTI ("thresh_svimg_C1_VIMG_GT_control_FWE0.05k1.nii").

3.5) EXTRACT PEAK DATA

To extract the single data (for each animal) of the surviving peak-data of the results-table hit the [extract data] button. The GUI will prompt you for a path and a proper filename of the file. Note that per default, the output-path is predefined (new folder: 'res_'+analysis-folder), here: "res_voxtest1" in the subfolder "data". Also, the filename is predefined, based on the image, contrast-number, contrast-name, method, p-value and clustersize. Thus the filename is:

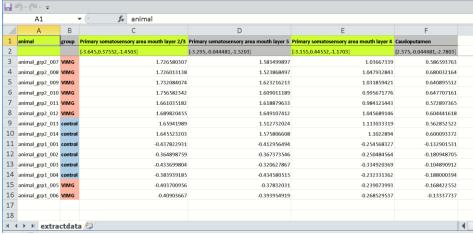
"svimg_C1__VIMG_GT_control__FWE0.05k1_NPC3_**SINGLEDATA**.xlsx". Thus, just hit [save].

```
..Extracting data ...wait...DONE!

data extracted [svimg_C1__VIMG_GT_control__FWE0.05k1_NPC3_SINGLEDATA.xlsx]: Explorer or open

fx >>
```

When done, hit the "open"-hyperlink from the cmd-window to open the Excelfile:



The file contains the single-values from each animal (with animal-id and group-assignment) for the 4 observed peaks.

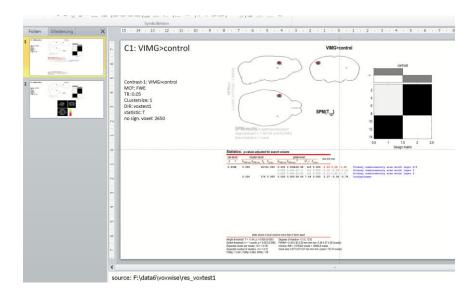
3.6) CREATE SUMMARY OF CURRENT CONTRAST

To obtain a fast summary of the current contrast, select from the **xstat-MENU**: **posthoc/create summary of current contrast**. This will create a Powerpoint-file with the summay for the 1st contrast. Note that the current settings from the xstat-GUI (MCP, thresh, CL-I, "#p/Cl"and "CLdist") are used.

```
summary saved [sum_svimg_con1_VIMG_GT_control_FWE0.05k1.pptx]: Explorer or open fx >>
```

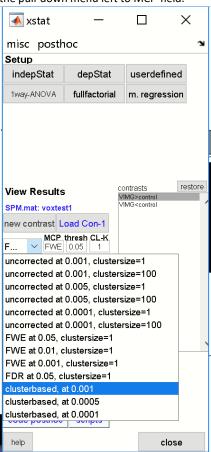
When done, hit the "open"-hyperlink from the cmd-window to open the PPT-file:

Note that the PPT-file is directly stored in the "res_voxtest1"-folder ('res_'+analysis-folder).

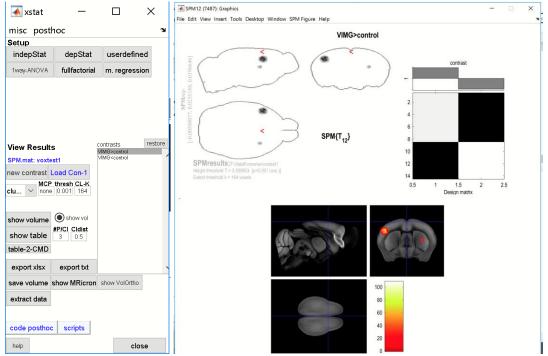


3.7) Change to Cluster-based approach

Now, we are switching from the FEW-method to the cluster-based approach. For this select 'clusterbased, at 0.001' from the pull-down menu left to MCP-field:



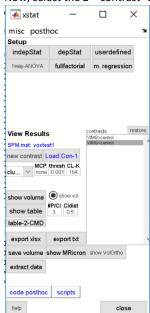
When selected the cluster-based approach will automatically run, and first set the p-value to 0.001, uncorrected and estimates the spatial threshold, i.e. the minimum number of neighboring voxels mandatory for a significant cluster. Next, this spatial threshold is used for the cluster-based approach. Here, a significant cluster-based height threshold at p=0.001 needs 164 neighboring voxels to survive.



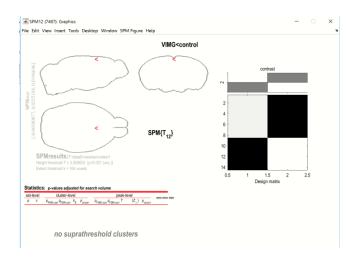
For the cluster-based approach, the results are quite similar compared to the FWE-approach.

4) Check the other contrast

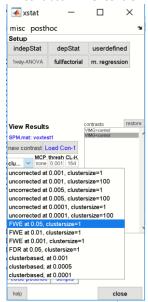
Now, select the 2nd contrast **'VIMG<control'** form the <u>xstat-contrast listbox</u>.



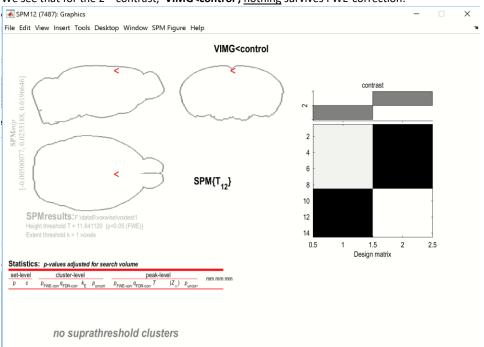
Based on the previous settings, the cluster-based approach shows no significant clusters.



Now, select 'FWE at 0.05, clustersize=1' from the xstat-pulldown MENU and update the graphics-window by selecting the 2nd contrast 'VIMG<control' from the contrast-listbox



We see that for the 2nd contrast, **'VIMG<control'**, nothing survives FWE-correction:



5) CREATE BIG-SUMMARY

Sometimes it is useful to obtain a summary for all existing contrasts and all methods (uncorrected, FEW and cluster-based approach). For this, select from the xstat-MENU: posthoc/all methods summary & export tables all contrasts. This will create three PowerPoint-files in the "res_voxtest1"-folder and also export the resulting tables as Excel-files and will store the respective t-maps as NIFTI-files.

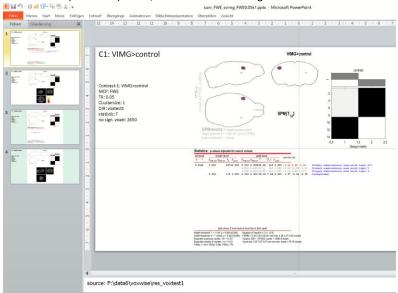
The procedure will create three PPT-files:

 $'sum_UNCOR_svimg_none0.001k1.pptx' \quad \ -uncorrected \ at \ p=0.001$

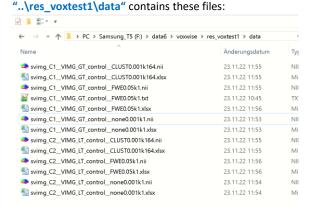
'sum_FWE_svimg_FWE0.05k1.pptx' -using the FWE approach

'sum CLUST svimg CLUST0.001k164.pptx' -using the cluster-based approach

The below figure displays the summary of the FWE approach. This Powerpoint-file contains the table (or first part of the table, depending on the table size) and displays the volume with surviving voxels for the 1st contrast (slides 1-2) and the 2nd contrast (slides 3-4). Note, there are no surviving voxels for the 2nd contrast.

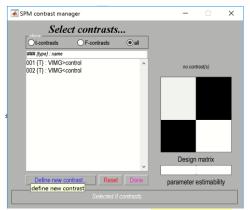


Note that this routine also creates the NIFTI-files and result-tables (Excel-files) for all contrast. The folder

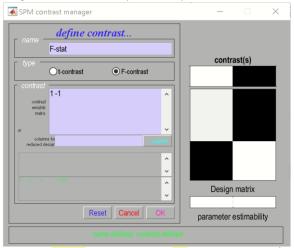


6) Define new Contrast

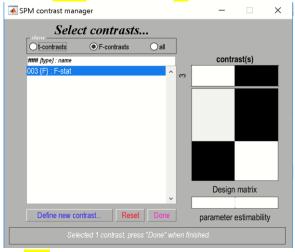
To define a new contrast hit [new contrast]-button from the xstat-GUI. When asked, select the specific <u>SPM.mat</u> from the voxelwise analysis-folder. In the SPM contrast-manager, select [Define new contrast]:



Here we select an F-contrast (radio: F-contrast). Enter 'F-stat' in the name-field and enter [1-1] in the field 'contrast weights matrix'. Please try to avoid special characters for the contrast-name.

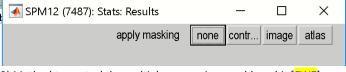


Then, hit [submit]-button, and hit [OK]. When done, a 3rd contrast (F-contrast) with name "F-stat" is created.

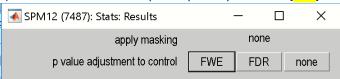


Hit [Done]-button. Next, you have to enter some parameters:

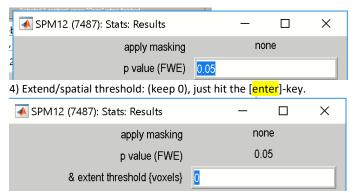
1) Masking, click [none].



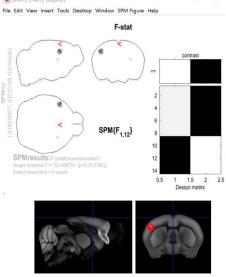
2) Method to control the multiple comparison problem: hit [FWE].



3) p-value: (keep 0.05), just hit the [enter]-key.



This will produce the output in the graphic window for the F-contrast:



Note also that the contrast list box in the xstat-GUI will be updated (now containing 3 contrasts):



From here you can modify the parameters and update the graphic window by selecting a contrast (such as the 3rd contrast) from the contrast-listbox.

7) Work from Command line

7.1) run voxelwise statistic from command line

```
z=[];
z.stattype
                 = 'twosamplettest';
z.excelfile
                 = 'F:\data6\voxwise\group\groupassignment.xlsx'; %[Excelfile] containing mouseIDs and group
z.sheetnumber
                 = [1];
                                                                   %sheet with columns mouseIDs and group
z.mouseID_col
                                                                   %column index with the MouseIDs
                 = [1];
z.group_col
                                                                  %column index with group assignment
                 = [2];
z.data_dir
                 = 'F:\data6\voxwise\dat';
                                                                  %data directory (upper directory)
```

```
z.inputimage = 'vimg.nii';
                                                                                %image name (nifti) to run the statistic
            = 'F:\data6\voxwise\templates\AVGT.nii';
= 'F:\data6\voxwise\templates\ANO.nii';
z.AVGT
                                                                                % TEMPLATE-file (in templates-folder)
z.ANO
                                                                                % ATLAS-file (in templates-folder)
z.grp_comparison = '1vs2';
z.mask = 'F:\data6\voxwise\templates\AVGTmask.nii';
z.smoothing = [1];
                                                                                %groups to compare (do not modify)
                                                                                %<optional> "brainmask
                                                                                %<optional>smooth data: [0]no,[1]yes
z.smoothing_fwhm = [0.28 0.28 0.28];
z.output_dir = 'voxtest2';
z.showSPMbatch = [0];
                                                                                %smoothing kernel (FWHM)
                                                                                %path for output-folder for statistic
                                                                                %hide|show pipeline in SPM-batch-manager
xstat(1,z);
```

execute this code to re-run the voxelwise statistic.

7.2) Create full report

```
xstat('loadspm','F:\data6\voxwise\voxtest2'): % open xstat-GUI and load SPM.mat-file xstat('fullreport'); % create full report, i.e. three PPTfiles (uncorrected, FWE, cluster-based) + export result tables and statistical maps as NIFTI-files
```

For more information, see scripts [scripts]-button and help of xstat.