Voxelwise statistic, two independent groups

CONTENTS

Scenario

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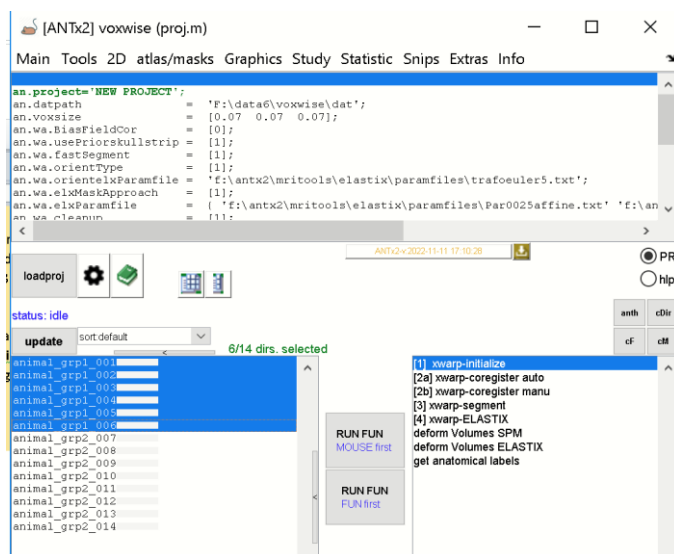
The Figure shows the list of 14 animals (left listbox). Animals of group-1 is selected (just for displaying purpose). Note that for this animals artificial data were created.
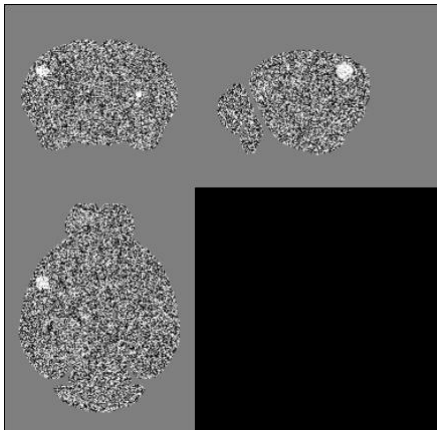

The figure shows the simulated image 'vimg.nii' (image is in standard-space) of one animal from the 2nd group with increased intensities centered at a) the left Primary somatosensory area mouth layer 4 and b) the right CaudoPutamen.

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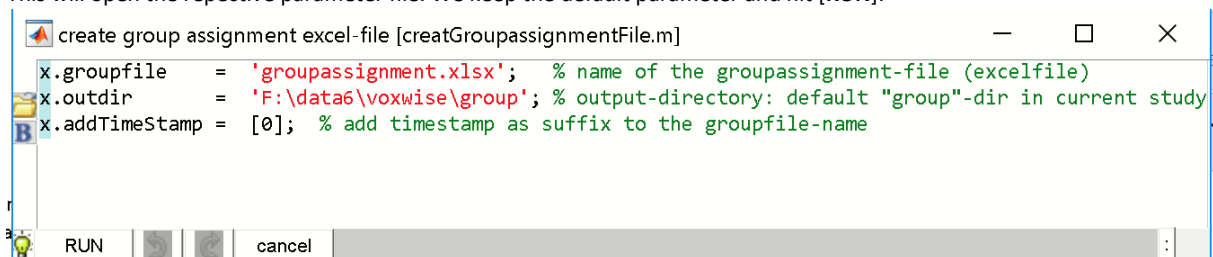
To this end, the Excelfile "groupassignment.xlsx" is created, located in the "group"-folder in the current study directory ("F:\data6\voxwise\group").

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 xstat.m):


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:
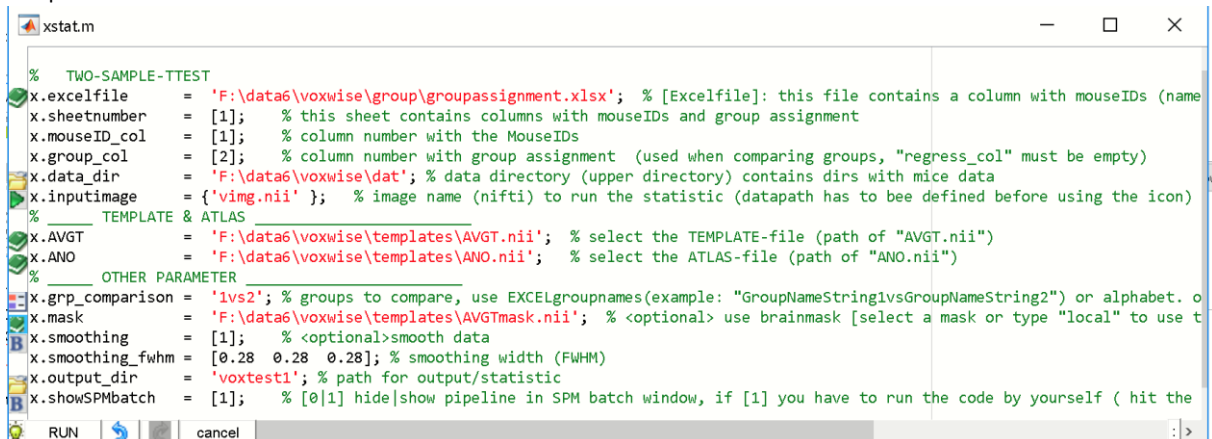
```
xstat.m                                                                          —    □    ×

%    TWO-SAMPLE-TTEST
x.excelfile      = ''; % [Excelfile]: this file contains a column with mouseIDs (names) and a column assigning the group
x.sheetnumber    = [1];    % this sheet contains columns with mouseIDs and group assignment
x.mouseID_col    = [1];    % column number with the MouseIDs
x.group_col      = [2];    % column number with group assignment  (used when comparing groups, "regress_col" must be empty)
x.data_dir       = 'F:\data6\voxwise\dat'; % data directory (upper directory) contains dirs with mice data
x.inputimage     = ''; % image name (nifti) to run the statistic (datapath has to bee defined before using the icon)
%  _____ TEMPLATE & ATLAS _____
x.AVGT           = 'F:\data6\voxwise\templates\AVGT.nii'; % select the TEMPLATE-file (path of "AVGT.nii")
x.ANO            = 'F:\data6\voxwise\templates\ANO.nii';  % select the ATLAS-file (path of "ANO.nii")
%  _____ OTHER PARAMETER _____
x.grp_comparison = '1vs2'; % groups to compare, use EXCELgroupnames(example: "GroupNameString1vsGroupNameString2") or alphabet. or
x.mask           = '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  0.28]; % smoothing width (FWHM)
x.output_dir     = 'voxtest1'; % path for output/statistic
x.showSPMbatch   = [1];    % [0|1] hide|show pipeline in SPM batch window, if [1] you have to run the code by yourself ( hit the g

   RUN                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].
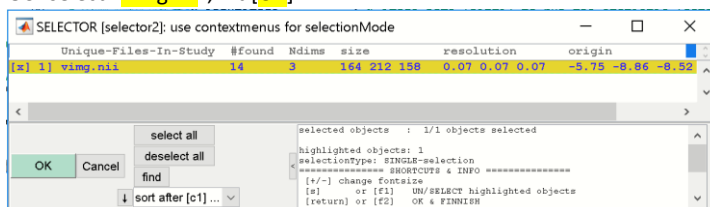

- "**x. AVGT**": click the left icon and select the AVGT-image ("AVGT.nii") from the study's templates-folder.

- "**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.

- "**x.output_dir**": define the output-folder for the voxelwise statistic, here "**voxtest1**".

The parameter-Gui should now looks as follows:


```
xstat.m                                                                          —    □    ×

%    TWO-SAMPLE-TTEST
x.excelfile      = 'F:\data6\voxwise\group\groupassignment.xlsx'; % [Excelfile]: this file contains a column with mouseIDs (name
x.sheetnumber    = [1];    % this sheet contains columns with mouseIDs and group assignment
x.mouseID_col    = [1];    % column number with the MouseIDs
x.group_col      = [2];    % column number with group assignment  (used when comparing groups, "regress_col" must be empty)
x.data_dir       = 'F:\data6\voxwise\dat'; % data directory (upper directory) contains dirs with mice data
x.inputimage     = {'vimg.nii' };    % image name (nifti) to run the statistic (datapath has to bee defined before using the icon)
%  _____ TEMPLATE & ATLAS _____
x.AVGT           = 'F:\data6\voxwise\templates\AVGT.nii'; % select the TEMPLATE-file (path of "AVGT.nii")
x.ANO            = 'F:\data6\voxwise\templates\ANO.nii';  % select the ATLAS-file (path of "ANO.nii")
%  _____ OTHER PARAMETER _____
x.grp_comparison = '1vs2'; % groups to compare, use EXCELgroupnames(example: "GroupNameString1vsGroupNameString2") or alphabet. o
x.mask           = 'F:\data6\voxwise\templates\AVGTmask.nii'; % <optional> use brainmask [select a mask or type "local" to use t
x.smoothing      = [1];    % <optional>smooth data
x.smoothing_fwhm = [0.28  0.28  0.28]; % smoothing width (FWHM)
x.output_dir     = 'voxtest1'; % path for output/statistic
x.showSPMbatch   = [1];    % [0|1] hide|show pipeline in SPM batch window, if [1] you have to run the code by yourself ( hit the

   RUN                cancel
```

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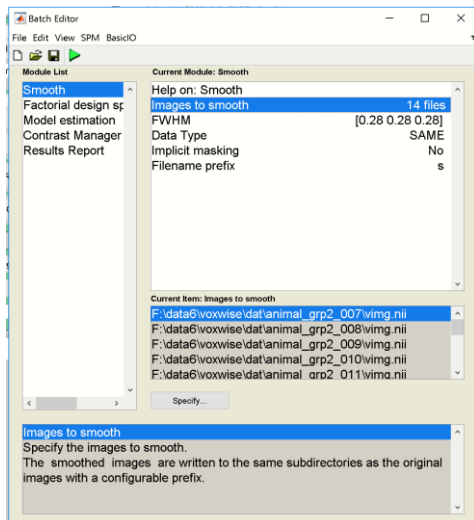
Here, everything seems to be ok and no further changes will be made. Hit [RUN BATCH]-button (green triangle) to execute this batch. The process will take a couple of minutes.

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**:

```
% % ====================================================
% % #g FUNCTION:        [xstat.m]
% % #b info :           xstat: voxelwise statistic [SPM-statistic] for normalized data (nifti volumes)
% % ====================================================
z=[];
z.stattype      = 'twosamplettest';                    % % STATISTICAL TEST
z.excelfile     = 'F:\data6\voxwise\group\groupassignment.xlsx'; % % [Excelfile]: this file contains a column with mouseIDs (names) and a column
                                                        % % assigning the group
z.sheetnumber   = [1];                                 % % this sheet contains columns with mouseIDs and group assignment
z.mouseID_col   = [1];                                 % % column number with the MouseIDs
z.group_col     = [2];                                 % % column number with group assignment(used when comparing groups, "regress_col"
                                                        % % must be empty)
z.data_dir      = 'F:\data6\voxwise\dat';              % % data directory (upper directory) contains dirs with mice data
z.inputimage    = 'vimg.nii';                          % % image name (nifti) to run the statistic (datapath has to bee defined before
                                                        % % using the icon)
z.AVGT          = 'F:\data6\voxwise\templates\AVGT.nii'; % % select the TEMPLATE-file (path of "AVGT.nii")
z.ANO           = 'F:\data6\voxwise\templates\ANO.nii'; % % select the ATLAS-file (path of "ANO.nii")
z.grp_comparison = '1vs2';                             % % groups to compare, use EXCELgroupnames(example: "GroupName1vsGroupName2")
                                                        % % or alphabet. order (example: "1vs2"), or
z.mask          = 'F:\data6\voxwise\templates\AVGTmask.nii'; % % <optional> use brainmask [select a mask or type "local" to use the AVGTmask.nii
                                                        % % from the templates folder]
z.smoothing     = [1];                                 % % <optional>smooth data
z.smoothing_fwhm = [0.28  0.28  0.28];                 % % smoothing width (FWHM)
z.output_dir    = 'voxtest1';                          % % path for output/statistic
z.showSPMbatch  = [1];                                 % % [0|1] hide|show pipeline in SPM batch window, if [1] you have to run the code
                                                        % % by yourself ( hit the green driangle), [0] piples runs automatically
xstat(1,z); % % RUN
```

To re-run the analysis just execute the code above.
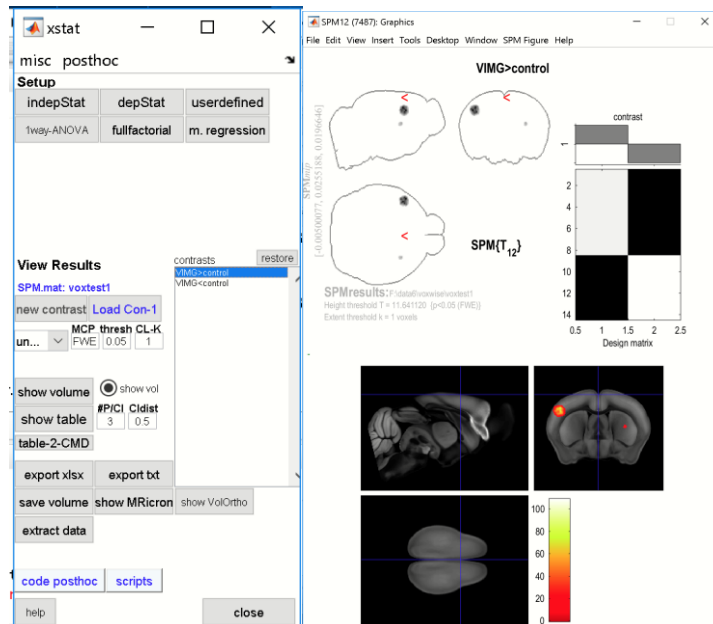
3) Examine Results

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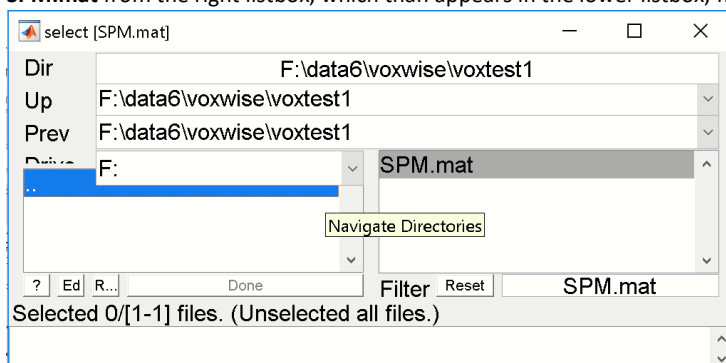
Basically, for the independent t-statistic two contrasts will be calculated by default ('A>B' and 'A<B'). Executing the [load CON1]-button displays the 1st contrast ('VIMG>control').

The SPM-graphics window will show the voxelwise results for the 1st 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.
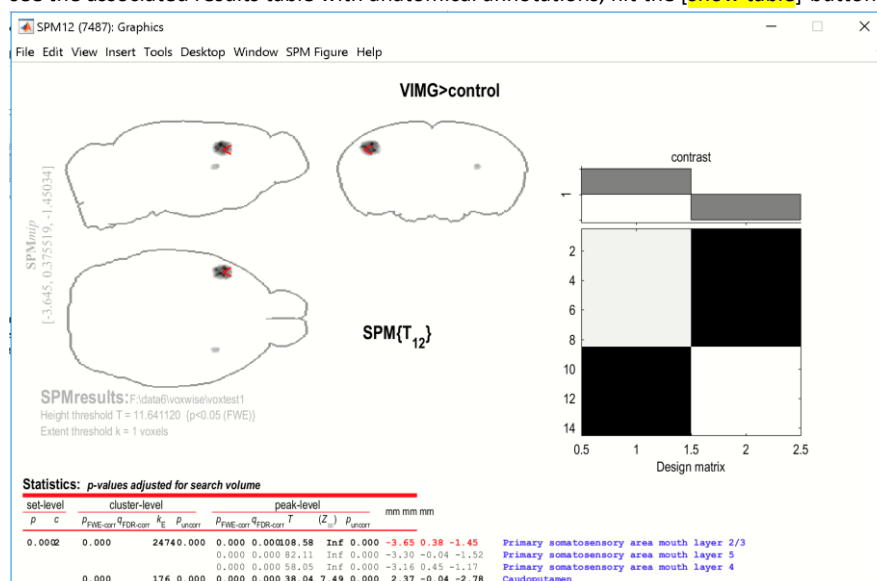
Because, the number of peaks per cluster **(#p/Cl)** is set to 3 and the cluster distance (**CLdist**) is set to 0.5 mm we see three peak clusters within the large left-hemispheric blob. You can change the respective settings for "#p/Cl"and "CLdist"in the xstat-GUI and **update** the graphic window by 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".
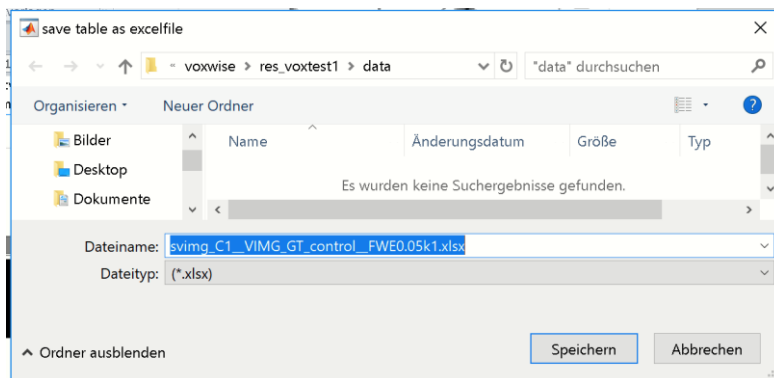
Without changing the path and filename, just hit the [save]-button.

```
...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:

	Field	value		
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)		

3.2) DISPLAY RESULT TABLE IN COMMAND WINDOW

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

```
Command Window
  [VIMG>control] Result:
  ===============================================================================
     set    set     cluster    cluster cluster    cluster     peak      peak     peak   pea
      p      c    p(FWE-corr) p(FDR-corr) equivk    p(unc) p(FWE-corr) p(FDR-corr)   T    equi
  ===============================================================================
  1.9123e-05   2        0              2474 6.7698e-301      0          0  108.576   Ir
                                                  0          0   82.115   Ir
                                          3.2761e-10 9.9881e-13   58.048   Ir
               0              176 2.9125e-52 5.1598e-08 4.8178e-11   38.043   7.4
  ===============================================================================
  table shows 3 local maxima more than 0.5mm apart
  Height threshold: T = 11.64, p = 0.000 (0.050)
  Extent threshold: k = 1 voxels, p = 0.023 (0.006)
  Expected voxels per cluster, <k> = 0.181
  Expected number of clusters, <c> = 0.01
  FWEp: 11.641, FDRp: 5.084, FWEc: 176
  Degrees of freedom = [1.0, 12.0]
  FWHM = 0.30 0.30 0.30 mm mm mm; 4.28 4.27 4.28 {voxels}
  Volume: 506 = 1475422 voxels = 18004.8 resels
  Voxel size: 0.07 0.07 0.07 mm mm mm; (resel = 78.19 voxels)
fx >>
```

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:


```
  svimg_C1__VIMG_GT_control__FWE0.05k1.txt
2  ===============================================================================
     set    set     cluster    cluster cluster    cluster     peak      peak     peak   peak      peak   x     y    z                                                        Labels
3     p      c    p(FWE-corr) p(FDR-corr) equivk    p(unc) p(FWE-corr) p(FDR-corr)   T    equivZ   p(unc) mm    mm   mm                                                         -
4  ===============================================================================
5  1.9123e-05   2        0              2474 6.7698e-301      0          0  108.576   Inf  4.4409e-16 -3.65  0.38 -1.45  Primary somatosensory area mouth layer 2/3
6                                                  0          0   82.115   Inf  4.4409e-16 -3.30 -0.04 -1.52  Primary somatosensory area mouth layer 5
7                                          3.2761e-10 9.9881e-13   58.048   Inf  4.4409e-16 -3.16  0.45 -1.17  Primary somatosensory area mouth layer 4
8               0              176 2.9125e-52 5.1598e-08 4.8178e-11   38.043   7.487 3.5194e-14  2.37 -0.04 -2.78  Caudoputamen
9  ===============================================================================
10 table shows 3 local maxima more than 0.5mm apart
11 Height threshold: T = 11.64, p = 0.000 (0.050)
12 Extent threshold: k = 1 voxels, p = 0.023 (0.006)
13 Expected voxels per cluster, <k> = 0.181
14 Expected number of clusters, <c> = 0.01
15 FWEp: 11.641, FDRp: 5.084, FWEc: 176
16 Degrees of freedom = [1.0, 12.0]
17 FWHM = 0.30 0.30 0.30 mm mm mm; 4.28 4.27 4.28 {voxels}
18 Volume: 506 = 1475422 voxels = 18004.8 resels
19 Voxel size: 0.07 0.07 0.07 mm mm mm; (resel = 78.19 voxels)
20
```

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".
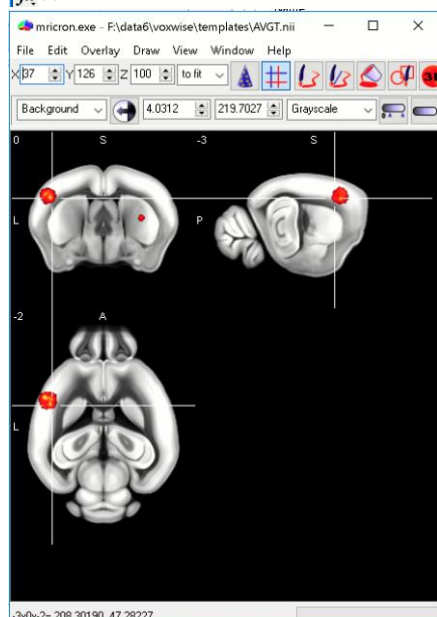

When done you can select the hyperlink 'MRicron' from the command-window to open the saved statistical t-map overlaid onto the template ('AVGT.nii') via MRicron.

```
   ...saving volume...wait..
   voxSTAT-volume [AVGT.nii - thresh_svimg_C1__VIMG_GT_control__FWE0.05k1.nii]: Explorer or MRicron
   Done!
fx >>
```


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:

	A	B	C	D	E	F
	A1		fx	animal		
1	animal	group	Primary somatosensory area mouth layer 2/3	Primary somatosensory area mouth layer 5	Primary somatosensory area mouth layer 4	Caudoputamen
2			[-3.645,0.37552,-1.4503]	[-3.295,-0.044481,-1.5203]	[-3.155,0.44552,-1.1703]	[2.375,-0.044481,-2.7803]
3	animal_grp2_007	VIMG	1.726580307	1.583499897	1.03667339	0.586593763
4	animal_grp2_008	VIMG	1.726013138	1.523868497	1.047932843	0.680032164
5	animal_grp2_009	VIMG	1.732084074	1.623216213	1.031859421	0.640895512
6	animal_grp2_010	VIMG	1.756582342	1.609011189	0.995671776	0.647707161
7	animal_grp2_011	VIMG	1.661035182	1.618879633	0.984121443	0.572897365
8	animal_grp2_012	VIMG	1.689820455	1.649107412	1.045689146	0.604441618
9	animal_grp2_013	control	1.65941989	1.512732024	1.113033319	0.562852522
10	animal_grp2_014	control	1.645523203	1.575806608	1.1022894	0.600093372
11	animal_grp1_001	control	-0.437822931	-0.412956494	-0.254568327	-0.132901531
12	animal_grp1_002	control	-0.364898759	-0.367373546	-0.250484564	-0.180948705
13	animal_grp1_003	control	-0.433699804	-0.320627867	-0.334920369	-0.104890912
14	animal_grp1_004	control	-0.383939185	-0.434580515	-0.232331362	-0.188000394
15	animal_grp1_005	VIMG	-0.403700956	-0.37832031	-0.239073993	-0.168422552
16	animal_grp1_006	VIMG	-0.40903667	-0.393954919	-0.268529537	-0.13337737
17						
18						

extractdata

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-l, "#p/Cl"and "CLdist") are used.

```
  summary saved  [sum__svimg_con1__VIMG_GT_control__FWE0.05k1.pptx]: Explorer or open
fx >>
```
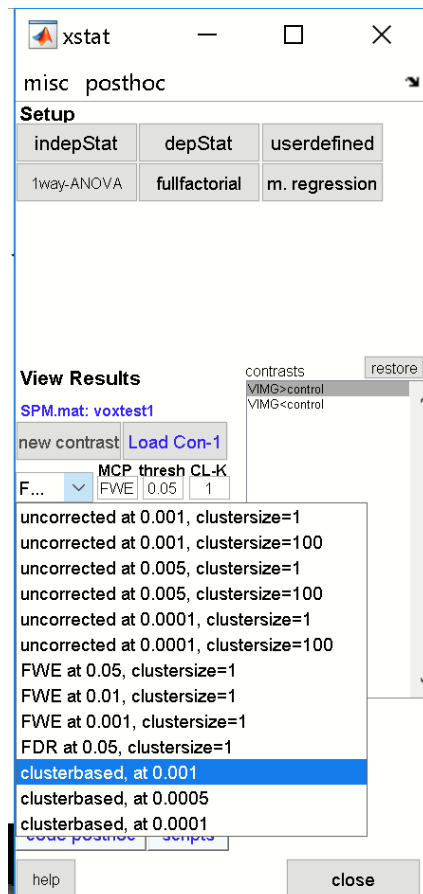
When done, hit the "open"-hyperlink from the cmd-window to open the PPT-file:
Note that the PPT-file is directly stored in the "res_voxtest1"-folder ('res_'+analysis-folder).
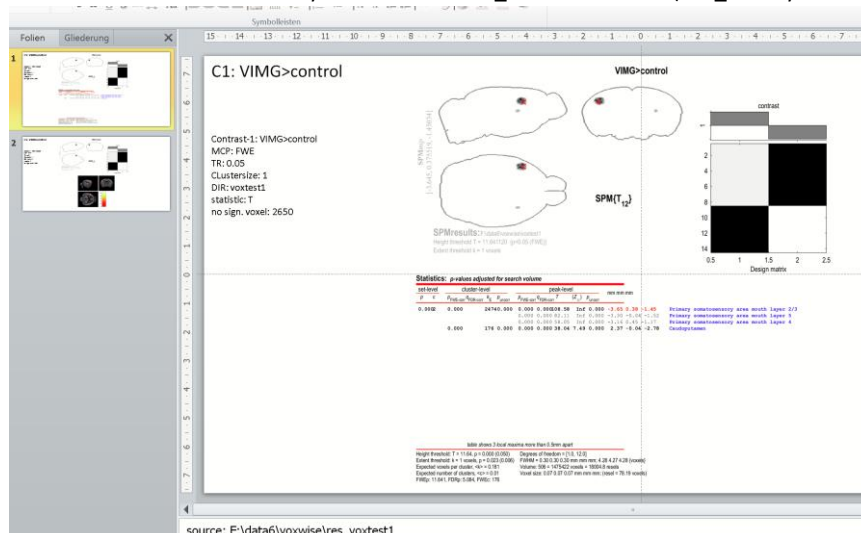
3.7) Change to Cluster-based approach

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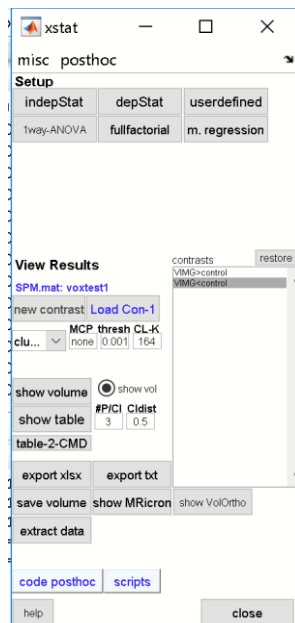
When selected the cluster-based approach will automatically run, and first set the p-value to 0.001, uncorrected and estimates the spatial threshold, i.e. the minimum number of neighboring voxels mandatory for a significant cluster. Next, this spatial threshold is used for the cluster-based approach.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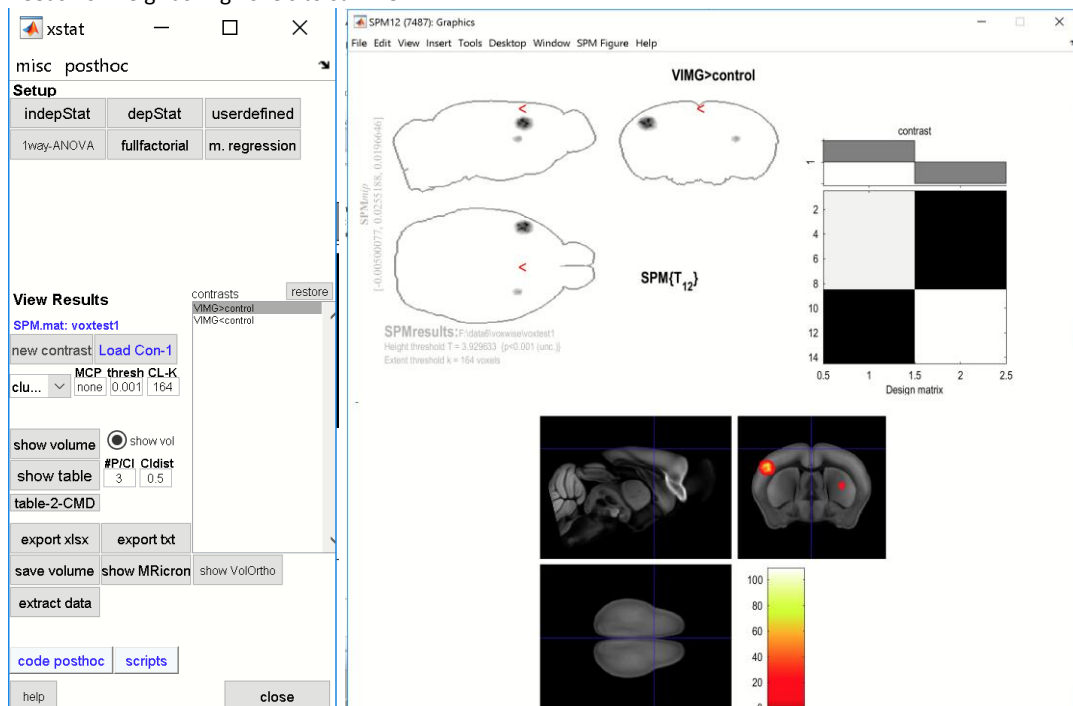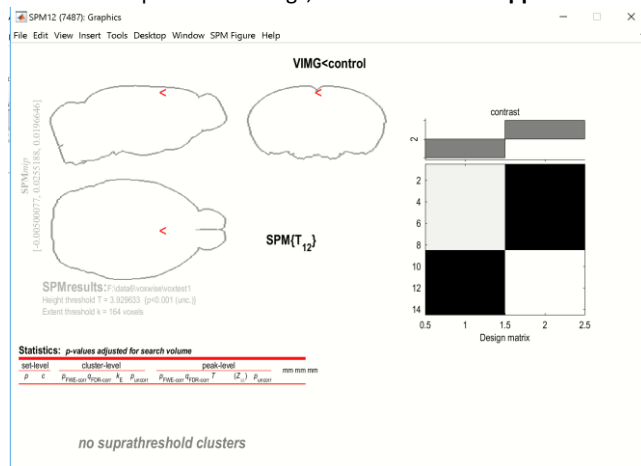

For the cluster-based approach, the results are quite similar compared to the FWE-approach.

4) Check the other contrast
Now, select the 2nd contrast **'VIMG<control'** form the xstat-contrast listbox.

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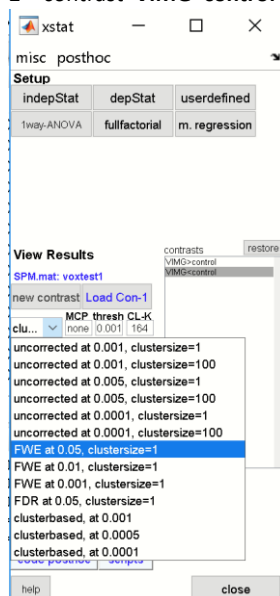
We see that for the 2nd contrast, **'VIMG<control',** nothing survives FWE-correction:
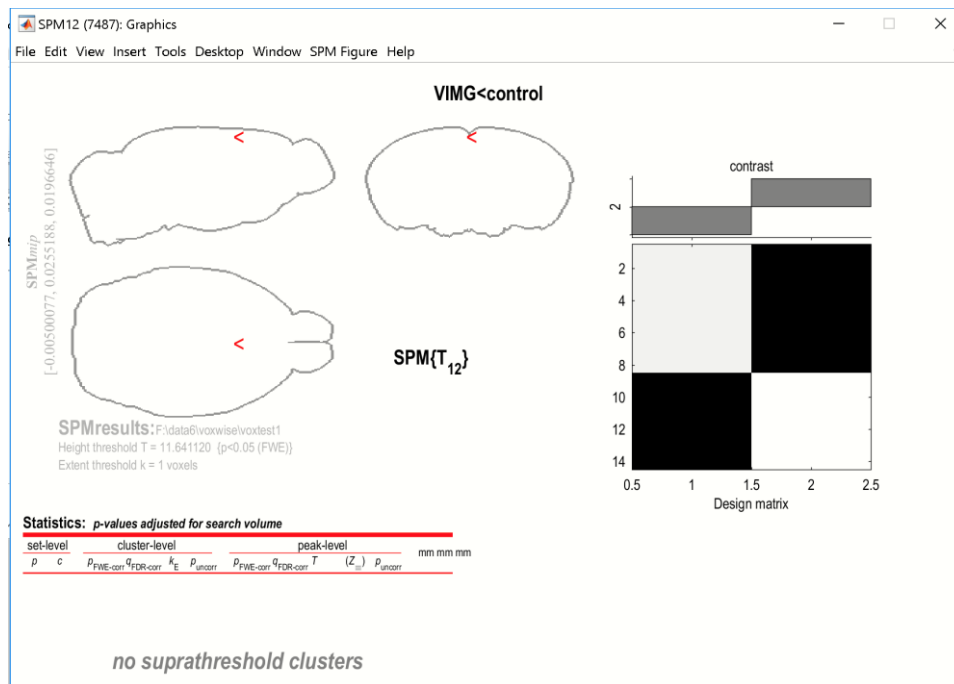
5) CREATE BIG-SUMMARY

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'   -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.
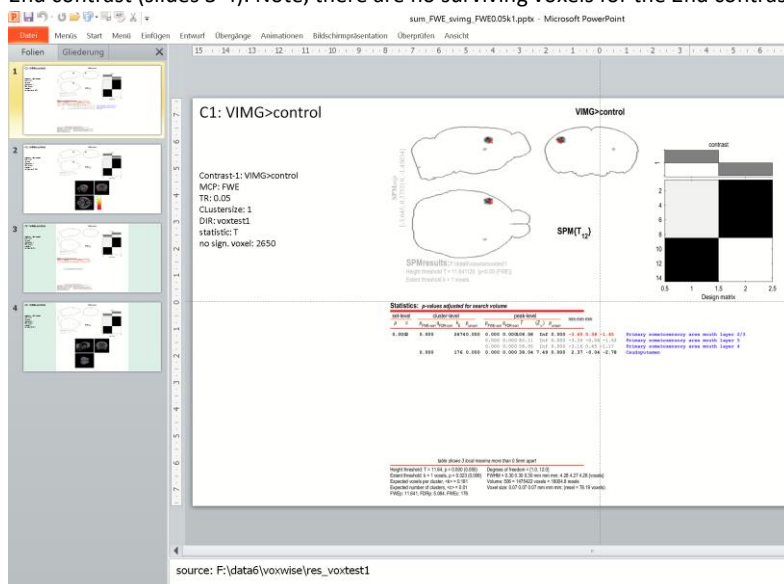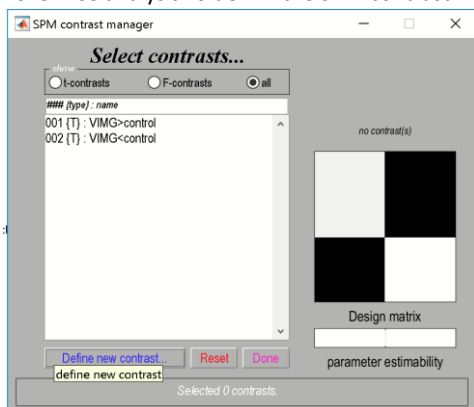

Note that this routine also creates the NIFTI-files and result-tables (Excel-files) for all contrast. The folder **"..\res_voxtest1\data"** contains these files:

6) Define new Contrast

To define a new contrast hit [new contrast]-button from the **xstat-GUI**. When asked, select the specific SPM.mat 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.
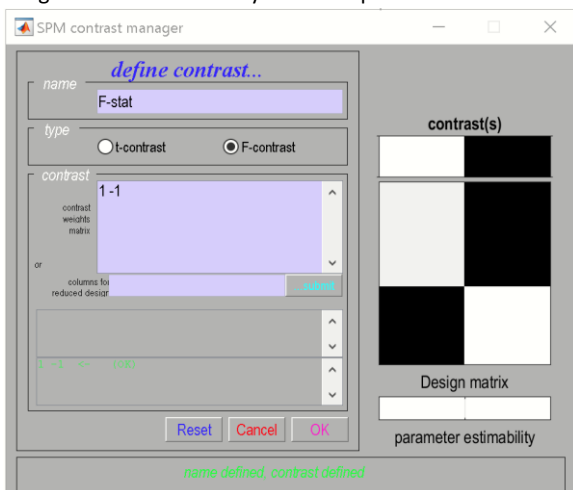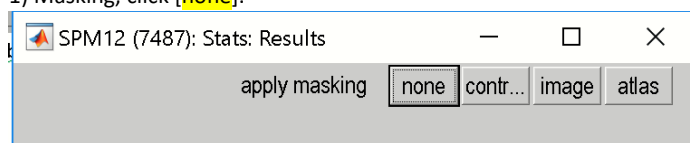

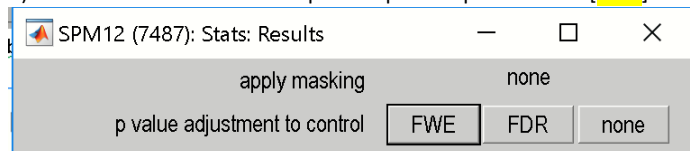Then, hit [submit]-button, and hit [OK]. When done, a 3rd contrast (F-contrast) with name **"F-stat"** is created.

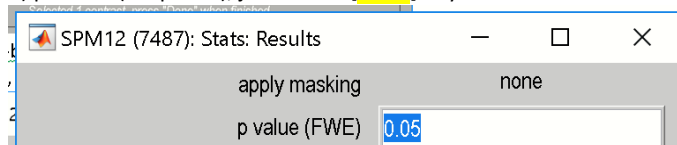Hit [Done]-button. Next, you have to enter some parameters:
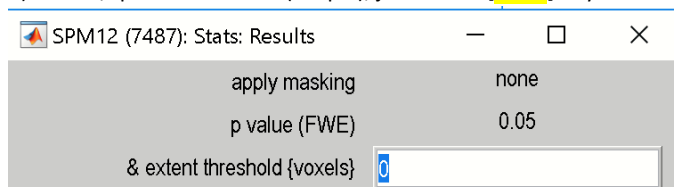
1) Masking, click [none].

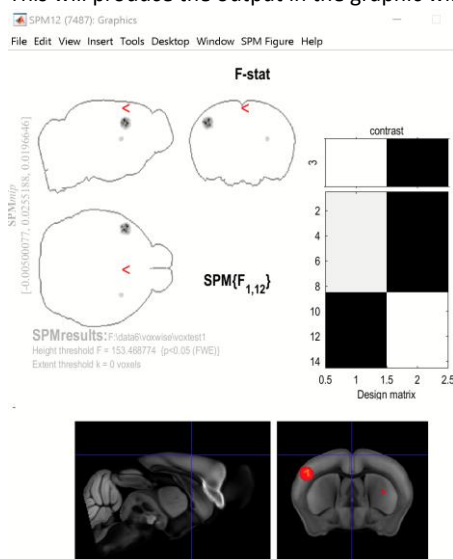2) Method to control the multiple comparison problem: hit [FWE].

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

4) Extend/spatial threshold: (keep 0), 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';                             %STATISTICAL TEST
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   = [1];                                          %column index with the MouseIDs
z.group_col     = [2];                                          %column index with group assignment
z.data_dir      = 'F:\data6\voxwise\dat';                       %data directory (upper directory)
z.inputimage    = 'vimg.nii';                                   %image name (nifti) to run the statistic
z.AVGT          = 'F:\data6\voxwise\templates\AVGT.nii';        % TEMPLATE-file (in templates-folder)
z.ANO           = 'F:\data6\voxwise\templates\ANO.nii';         % ATLAS-file (in templates-folder)
z.grp_comparison = '1vs2';                                      %groups to compare (do not modify)
z.mask          = 'F:\data6\voxwise\templates\AVGTmask.nii';    %<optional> "brainmask
z.smoothing     = [1];                                          %<optional>smooth data: [0]no,[1]yes
z.smoothing_fwhm = [0.28  0.28  0.28];                          %smoothing kernel (FWHM)
z.output_dir    = 'voxtest2';                                   %path for output-folder for statistic
z.showSPMbatch  = [0];                                          %hide|show pipeline in SPM-batch-manager
xstat(1,z);                                                     %RUN
```

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.