

# fMRat

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# 1.INTRO

fMRat is an extension to the popular SPM software (*Statistical Parametric Mapping*, The Wellcome Trust Centre for Neuroimaging <http://www.fil.ion.ucl.ac.uk/spm/>). fMRat is an automatic tool for preclinical scientists to enable full processing of fMRI rat time-series through a user-friendly interface.

fMRat makes use of several functions from the [SPM](#) package - `spm_realign.m`, `spm_coreg.m`, `spm_smooth`, `spm_run_fmri_design.m`, `spm_spm` among others-, as well as from the CBMGmosaic (Northwestern Cognitive Brain Mapping Group) -the ortho viewer code- and SPMMouse toolboxes (Wolfson Brain Imaging Centre, University of Cambridge <http://www.spmmouse.org/>) -the preset loading function-. However, SPMMouse is only available for SPM5, so it was slightly modified to be compatible with SPM5 and SPM8.

Since this tool follows the typical SPM pipeline, please refer to SPM books, website, documentation and papers for further technical details on fMRI processing.

## 2.PRE-REQUIREMENTS

- Matlab
- SPM in the Matlab path

## 3.INSTALLATION AND LAUNCH

- 1) Just add the fMRat folder to the Matlab path and use it. Execute "fMRat" in the Matlab command line to start the tool.
- 2) Testing images and provided atlases can be found [here](#).  
(IMPORTANT: If you are using the provided atlases for Wistar and Sprague-Dawley strains, please place the folders "Atlas\_SD" and "Atlas\_Wistar" in the same folder where fMRat code -and fmri.m function- are)

## 4.BEFORE YOU START

- **DATA CONCERNS**
- . Functional and structural images must be in native Bruker format or Nifti.

- Regions of Interest (ROIs) to be quantified must be in Nifti format only. (you may convert raw ROIs to Nifti with SPM functions `spm_vol.m`, `spm_write_vol.m`).
- Do not use blank spaces in the folders names where your datasets are.
- You may create a specific preset for your images (adapted to your images resolution) with SPMMouse tool (Wolfson Brain Imaging Centre, University of Cambridge), slightly modified here to allow its use with spm8. You only need to ensure the folder "spmmouse\_modified" is earlier than the SPM functions in the Matlab path, execute "spmmouse" in the Matlab command window and follow instructions. The output file **MUST** be named `preset.mat` and **MUST** be placed inside your atlas directory or inside the fMRat folder itself. This preset will contain some registration/visualization etc. parameters adapted to your dataset; but if you prefer to skip this step, fMRat will use the default provided by SPMMouse, which was built for C57 mice. This preset will affect the registration accuracy in the "Realign" step and the "Normalization to atlas" step.

### • **WORKFLOW**

- Use the default workflow whenever possible
- Some steps are compulsory, others are optional, and you can take isolated steps and continue the processing later on, but the steps must always be performed in the default order. Consistency won't be ensured otherwise.
- Compulsory steps: Realign, Design, Estimate, Display
- Optional steps: Normalization to atlas, Smooth



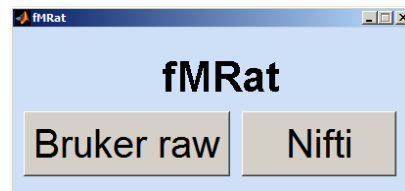
**IMPORTANT:** Whenever you continue a processing that was not finished don't forget to check the "Preserve previous processing steps" tickbox. Otherwise you will get an error or inconsistent results.

- **ERRORS**

- When an error happens the execution will continue with other image acquisitions and subjects, since this tool was thought to work in batch mode. Errors are written to a .txt log file in the fMRat code folder, make sure you have writing permissions in that folder.

## 5.USING FMRAT

The initial GUI prompts for the image format, Bruker raw or Nifti; in the case of Nifti, it will also ask for acquisition details not available in the Nifti header, such as the correspondence between structural, functional, and masking images.



Bruker format option will perform an automatic analysis of all subjects/studies found under the selected folder. It will assume the same blocks design, the same acquisitions method for the functional images, the same acquisition method for the structural images, and the same atlas (same strain) for all the acquisitions and studies found.

And the options selected will apply to all of them, so if you wish to analyze some cases with some options and some other cases in a different way or you have data acquired in different ways, you should place those studies under different folders and perform the analyses separately.

Nifti format option will perform an automatic analysis of all subjects/studies found under the selected folder. It will assume the same blocks design and the same atlas (same strain) for all of them, but the acquisition won't be an issue here since they are already in Nifti format. Therefore different datasets should be analyzed separately in different runs.

- **BRUKER FORMAT**

Suppose a data structure like this one:

```

└─\subject1
    └─subject
        └─AdjStatePerStudy
            └─AdjResult
                └─Fieldmap
                    └─\1
                    └─\2
                    └─\3

```

(AXIAL functional raw file)

Each folder “1”, “2”, “3”, etc. is a different acquisition and the 2dseq files are the reconstructed data in raw Bruker format. The “acqp”, “method” and “reco” files are text parameter files that will be read at runtime in order to associate the functional images and their corresponding structural images in a fully automated way. DO NOT CHANGE this folder structure nor rename the files/folders. Results (.tiff maps and .txt files) will be printed 4 levels higher than the images folder, i.e. inside “subject1” and “subject2” folders.

And suppose that both subjects were acquired under the same conditions (same block paradigm, same structural acquisition method, same functional acquisition method, and animals of the same strain).

Steps to run the tool for the automatic analysis of both subjects are the following:

1. Choose your dataset upper folder (E:\bruker\_test in our example):

**REQUIRED**

Choose your fMRI directory:

**Paradigm**

Block design must begin and end with resting blocks (or use Advanced design)

Advanced

Enter your design pars here -> (All in number of images)  
 N\_rest:  1  
 N\_stim:  0.5  
 NR\_total:  0

**Bruker acq methods:**  
 Anatomical images method:  RARE  
 Functional images method:  EPI

**Advanced options (Do NOT change if you are not sure):**  
 Options (strongly recommended to check all):  
☐ Preserve previous processing steps (check if you are processing isolated steps in stead of "all")  
☒ Format conversion & data structure  
☒ Realign  
 -----(If registration to atlas went wrong, manually reorient realigned images to atlas and start from here)-----  
☒ Normalize to atlas  
 In case of studies with different functional images resolutions, set final resampling resolution to:  
 Mode of resolutions:    
☒ Smooth X=Y=Z=  1.2 mm  
☒ Design  
☒ Estimate  
☒ Display  
 Inference ☐ Uncorrected p threshold  0.05 and cluster size  4  
☒ FWE corrected p threshold  
 ROIs (to quantify signal change) folder  
 E:\testAtlas\_SD

Choose atlas type:  
 (atlas are strain-dependent)  
☒ Handed atlas  
 Sprague-Dawley  
  
☐ Custom atlas  
 Atlas dir

2. Specify your blocks design in "scans" units:

- “N\_rest” = number of images acquired at rest blocks
- “N\_stim” = number of images acquired at stimulation blocks
- “NR\_total” = total number of volumes acquired per fMRI series

(A schematic paradigm will be drawn in the plot on the right)

OR use the Advanced Design, see Advanced options.

**REQUIRED**

Choose your fMRI directory:

**Paradigm**

Block design must begin and end with resting blocks (or use Advanced design)

Advanced  
Paradigm  
Regressors

Enter your design pars here ->  
(All in number of images)

N\_rest  1  
N\_stim  0  
NR\_total  0

**Bruker acq methods:**

Anatomical images method:  RARE

Functional images method:  EPI

**Advanced options (Do NOT change if you are not sure):**

Options (strongly recommended to check all):

- ☐ Preserve previous processing steps  
(check if you are processing isolated steps in stead of "all")
- ☒ Format conversion & data structure
- ☒ Realign  
----- (If registration to atlas went wrong, manually reorient realigned images to atlas and start from here) -----
- ☒ Normalize to atlas ----->  
In case of studies with different functional images resolutions,  
set final resampling resolution to:  
 Mode of resolutions
- ☒ Smooth X=Y=Z=  1.2 mm
- ☒ Design
- ☒ Estimate
- ☒ Display  
Inference ☐ Uncorrected p threshold  0.05 and cluster size  4  
☒ FWE corrected p threshold
- ROIs (to quantify signal change) folder  
 E:\tst\Atlas\_SD

**Choose atlas type:**  
(atlas are strain-dependent)

☒ Handed atlas  
 Sprague-Dawley

☐ Custom atlas  
Atlas dir

3. Specify the names of the acquisition methods for the structural and functional images. Acquisitions performed with this methods will be automatically detected and related to each other:

**REQUIRED**

Choose your fMRI directory:

**Paradigm**

Enter your design pars here -> (All in number of images)

N\_rest  1  
N\_stim  0.5  
NR\_total  0

**Brucker acq methods:**

Anatomical images method:    
Functional images method:

**Advanced options (Do NOT change if you are not sure):**

Options (strongly recommended to check all):

☐ Preserve previous processing steps  
(check if you are processing isolated steps in stead of "all")

☒ Format conversion & data structure

☒ Realign  
----- (If registration to atlas went wrong, manually reorient realigned images to atlas and start from here) -----

☒ Normalize to atlas  
In case of studies with different functional images resolutions, set final resampling resolution to:

☒ Smooth X=Y=Z=  mm

☒ Design

☒ Estimate

☒ Display

Inference ☐ Uncorrected p threshold ☐ FWE corrected p threshold  and cluster size

ROIs (to quantify signal change) folder

Choose atlas type:  
(atlas are strain-dependent)

☒ Handed atlas

☐ Custom atlas  
Atlas dir

- Specify your atlas dir ("**Custom atlas**" button, it must be in Nifti format) or select one of the provided atlas ("**Handed atlas**"):



**REQUIRED**

Choose your fMRI directory:

**Paradigm**

Enter your design pars here -> (All in number of images)

N\_rest:  1  
N\_stim:  0.5  
NR\_total:  0

**Brucker acq methods:**

Anatomical images method:  RARE Functional images method:  EPI

**Advanced options (Do NOT change if you are not sure):**

Options (strongly recommended to check all):

☐ Preserve previous processing steps  
(check if you are processing isolated steps in stead of "all")

☒ Format conversion & data structure

☒ Realign  
----- (If registration to atlas went wrong, manually reorient realigned images to atlas and start from here) -----

☒ Normalize to atlas  
In case of studies with different functional images resolutions, set final resampling resolution to:

Mode of resolutions:

☒ Smooth X=Y=Z=  1.2 mm

☒ Design

☒ Estimate

☒ Display

Inference: ☐ Uncorrected p threshold ☒ FWE corrected p threshold  0.05 and cluster size  4

ROIs (to quantify signal change) folder:  E:\tst\Atlas\_SD

**Choose atlas type:**  
(atlas are strain-dependent)

☒ Handed atlas

Sprague-Dawley

☐ Custom atlas

Atlas dir:



At this point, we recommend the user to save the parameters selected before running with the use of the bottom left button "**Save Config**". This allows them to be loaded later with the "**Load Config**" button.

1. Press "**START**" button and enjoy 😊

- ***NIFTI FORMAT***

Suppose a data structure like this one:

```
E:\nifti_test
└─\subject1
    └─functional1
        └─Image_d_5_0001.nii
        └─Image_d_5_0002.nii
        └─Image_d_5_0003.nii
        ...
        └─Image_d_5_0115.nii
    └─structural
        └─2dseq.nii

└─subject2
    └─functional1
        └─Image_d18_25_0001.nii
        └─Image_d18_25_0002.nii
        └─Image_d18_25_0003.nii
        ...
        └─Image_d18_25_0115.nii
    └─structural
        └─2dseq.nii
```

Each folder “functional1” is a different acquisition (you might have “functional2”, “functional3”, etc) and the 2dseq.nii files are the structural images but this time they are already in Nifti format. This is useful for users with a different scanner manufacturer other than Bruker.

Suppose that both subjects were acquired under the same conditions (same block paradigm, and animals of the same strain). Results (.tiff maps and .txt files) will be printed 2 levels higher than the images folder, i.e. inside “subject1” and “subject2” folders.

Steps to run the tool for the automatic analysis of both subjects are the following:

1. Choose your dataset upper folder (E:\nifti\_test in our example):

**REQUIRED**  
Choose your fMRI directory:

Browse

**Paradigm**

Block design must begin and end with resting blocks (or use Advanced design)

Advanced  
Paradigm  
Regressors

ACQ

STIM

NR

1 CYCLE

TR(ms)

Enter your paradigm here: (in number of images)

N<sub>rest</sub>

N<sub>stim</sub>

NR<sub>total</sub>

0.5

0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1

**Advanced options (Do NOT change if you are not sure):**

☐ Preserve previous processing steps  
(check if you are processing isolated steps in stead of "all")

Options (strongly recommended to check all):

☒ Realign  
----- (If registration to atlas went wrong, manually reorient realigned images to atlas and start from here) -----

☒ Normalize to atlas  
In case of studies with different functional images resolutions, set final resampling resolution to:

Mode of resolutions

X mm Y mm Z mm

X=Y=Z= 1.2 mm

☒ Smooth

☒ Design

☒ Estimate

☒ Display

Uncorrected p threshold

FWE corrected p threshold 0.05 and cluster size 4

ROIs (to quantify signal change) folder:

E:\stAtlas\_SD

Browse

Choose atlas type (atlas are strain-dependent):

☒ Handed atlas

Sprague-Dawley

☐ Custom atlas

Atlas dir

Browse

Load Config

Save Config

START

Now a new “Nifti\_selector” window will appear:

**Nifti\_selector**

Please select the first of each your functional series, its anatomical reference image and one mask.

e:\ e:\CODE

...  
.svn  
Atlas\_SD  
Atlas\_Wistar  
Bruker2nifti.m  
Manual

Drop to functional ListBox

Drop to anatomical ListBox

Drop to masks ListBox

FUNCTIONAL IMS

ANATOMICAL IMS

MASKS

0 Image(s) selected Remove Clear all

0 Image(s) selected Remove Clear all

0 Image(s) selected Remove Clear all

x Repeat

Load struct Store struct

OK

As the tool does not have any info here from the manufacturer regarding the acquisition methods and their order of acquisition, it is the user’s duty to establish the relation between each functional image, its corresponding structural/anatomical image and the mask to be used. In this case, each functional acquisition can be masked with a different mask if needed.



If you need to replicate the same masking file for several functional images, please use the “**Repeat**” button below each ListBox.



At this point, we recommend the user to save the structure created before running with the use of the bottom left button “**Save Config**”. This allows the same structure to be loaded later with the “**Load Config**” button.

Once you finished, press “OK” button in the right bottom corner.

2. Specify your blocks design in "scans" units:

“**N\_rest**” = number of images acquired at rest blocks  
“**N\_stim**” = number of images acquired at stimulation blocks  
“**NR\_total**” = total number of volumes acquired per fMRI series

(A schematic paradigm will be drawn in the plot on the right)

“**TR**” = Time to acquire each volume (Repetition time of the MRI acquisition in case of EPI, time per volume for other conventional sequences)

**REQUIRED**  
Choose your fMRI directory:

**Paradigm**

Block design must begin and end with resting blocks (or use Advanced design)

TR(ms)  Enter your paradigm here: (in number of images)  N<sub>rest</sub>  0.5  
N<sub>stim</sub>  0  
NR<sub>total</sub>  1

**Advanced options (Do NOT change if you are not sure):**

☐ Preserve previous processing steps (check if you are processing isolated steps in stead of "all")

Options (strongly recommended to check all):

☒ Realign -----(If registration to atlas went wrong, manually reorient realigned images to atlas and start from here)-----

☒ Normalize to atlas ----->

In case of studies with different functional images resolutions, set final resampling resolution to:

Mode of resolutions:  X  mm Y  mm Z  mm

☒ Smooth X=Y=Z=  1.2 mm

☒ Design

☒ Estimate

☒ Display

Inference: ☐ Uncorrected p threshold  0.05 and cluster size  4

☒ FWE corrected p threshold

ROIs (to quantify signal change) folder:  E:\stAtlas\_SD

**Choose atlas type (atlas are strain-dependent):**

☒ Handed atlas

Sprague-Dawley

☐ Custom atlas

Atlas dir

3. Specify your atlas dir (“**Custom atlas**” button, it must be in Nifti format) or select one of the provided atlas (“**Handed atlas**”):

**REQUIRED**  
Choose your fMRI directory:

**Paradigm**

Block design must begin and end with resting blocks (or use Advanced design)

TR(ms)  Enter your paradigm here: (in number of images)  N<sub>rest</sub>  0.5  
N<sub>stim</sub>  0  
NR<sub>total</sub>  1

**Advanced options (Do NOT change if you are not sure):**

☐ Preserve previous processing steps (check if you are processing isolated steps in stead of "all")

Options (strongly recommended to check all):

☒ Realign -----(If registration to atlas went wrong, manually reorient realigned images to atlas and start from here)-----

☒ Normalize to atlas ----->

In case of studies with different functional images resolutions, set final resampling resolution to:

Mode of resolutions:  X  mm Y  mm Z  mm

☒ Smooth X=Y=Z=  1.2 mm

☒ Design

☒ Estimate

☒ Display

Inference: ☐ Uncorrected p threshold  0.05 and cluster size  4

☒ FWE corrected p threshold

ROIs (to quantify signal change) folder:  E:\stAtlas\_SD

**Choose atlas type (atlas are strain-dependent):**

☒ Handed atlas

Sprague-Dawley

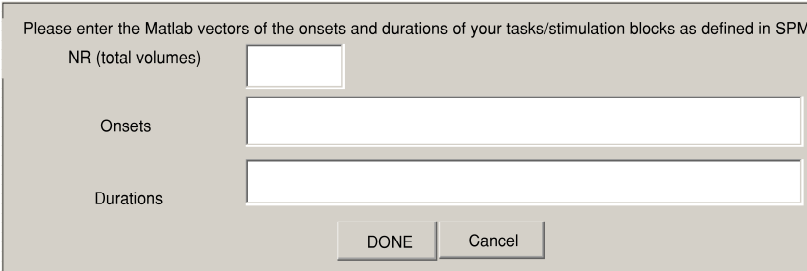
☐ Custom atlas

Atlas dir

- **ADVANCED OPTIONS**

The different steps included in the default pipeline can also be executed independently, but always in the same order as they appear in the GUI. Please read WORKFLOW concerns in the BEFORE YOU START section.

- Normalization step: the user can select the final resolution for the warped images. The default is the mode of all resolutions found for each analysis (for the subjects and acquisitions included in the “**Nifti\_selector**” window), but it can be customized in the 3 axes.
- The smoothing kernel size (FWHM) can also be selected. An isotropic filter will be applied.
- Inference details are also editable. The user can select without “multiple comparisons” correction or with family-wise error correction (FWE), the statistical p threshold and the clustering threshold.
- ROIs folder: This is optional and can be left empty. If the user wants the percentage signal change to be quantified in some specific regions of interest (ROIs), the binary masks for those ROIs should be placed in the same folder and they should be named “ROI\*\*\*.nii”. The folder name must be specified in this edit box.
- Advanced design  
The user may specify the onsets and durations of heterogeneous blocks through the "Paradigm" button. If duration is a scalar, it will be assumed to be the same for every block/onset. Total number of repetitions (**NR**) will also be required here:



Please enter the Matlab vectors of the onsets and durations of your tasks/stimulation blocks as defined in SPM

NR (total volumes)

Onsets

Durations

- Covariates  
Covariates can be added to the design matrix by pressing "Regressors" button. The user may specify a covariate matrix through a text file or pasting the matrix inside the Edit box, the same way as in SPM:



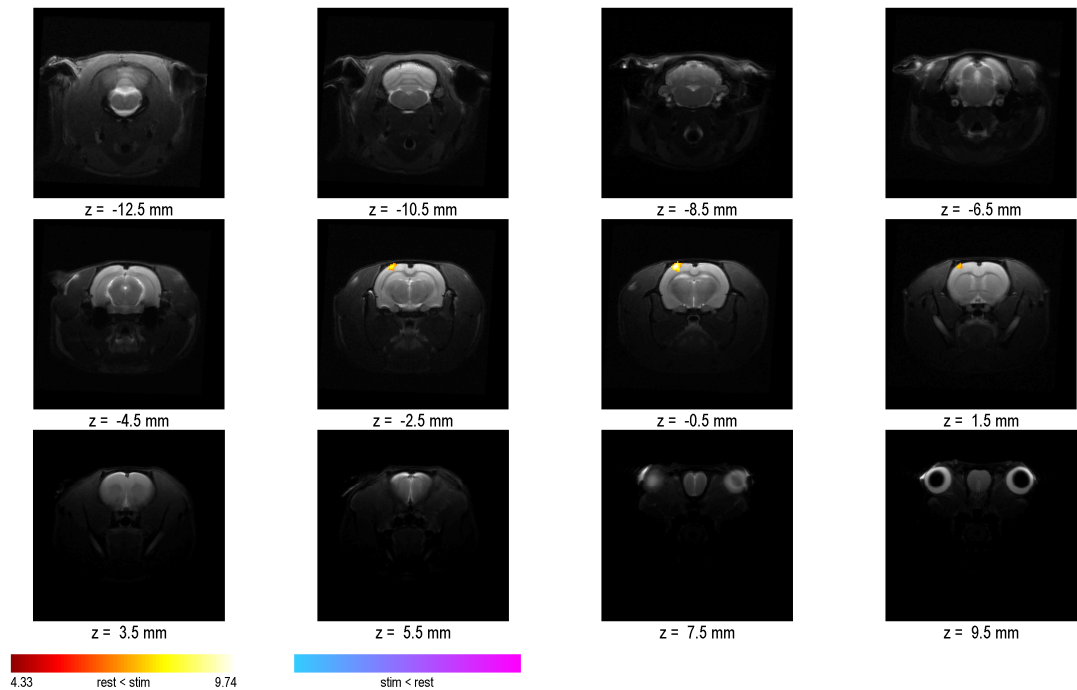
(The matrix read will be printed under the edit box)

## • **OUTPUT FILES**

1. Uncompressed .tiff files will be printed for each fMRI series with the resulting t-map and Z-score map overlaid onto the structural image. Example:

### Z MAP

Functional: E:\tst\_fMRI\fnifti\subject1\functional\SPM.mat  
Background: E:\tst\_fMRI\fnifti\subject1\functional\w2dseq.nii



2. One “signal\_pos.txt” file and one “signal\_neg.txt” file will be printed inside each study folder. These files contain the parameters used for the whole pipeline as well as the percentage signal change calculated for the whole image, for the masked area (usually the brain without the background) and for the optional user ROIs if any. Mean value, standard deviation, maximum value and the voxel localization for the maximum value are listed here. Example:



```

=====
July 31, 2014 6:07:57.177 PM
DEFAULTS:
d      W:\Proyectos\fmri\FMRI_RATA\
t      E\nifti_test
im_name Image
anat_seq RARE
func_seq ePI_FMRI
Nrest  15
Nstim  5
NR      115
preprocess 0
realign 1
coreg 1
design 1
estimate 1
display 1
mode_reg 2
custom_atlas 0
atlas_dir
sp      1
custom_resol 0
rx      0.3
ry      0.3
rz      2
atlas   E:\CODE\Atlas_SD\atlas.nii
emask   0
rois_dir E:\CODE\Atlas_SD
smooth  1
kernel  1.2  1.2  1.2
an_mode 1
fwe     1
p       0.05
k       4
inifti  1
preserve 0
-----
Acq      Area(pixels) All_voxels:mean All_voxels:std.dev. MAX_all_voxels I J K Mask(brain):mean
Mask(brain):std.dev. MAX_Only_masked I J K
functional 77 0.002 0.048 1.956 34 66 11 0.027 0.185 1.956 34
66 11

```

In this example, the mean %signal change of the whole image, including background, was 0.002%, with a standard deviation of 0.048%. The maximum %signal change was 1.956%, and was located at [34, 66, 11] coordinates (in voxels). The maximum for the brain mask is the same, as expected, whereas the mean %signal change of the masked brain is higher than for “All\_voxels” since it does not include the background.

- **ATLASES PROVIDED AND ROIS QUANTIFICATION**

Two in-vivo T2 atlases are provided [here](#), for Sprague-Dawley and for Wistar strains respectively, to make the tool testing easier. ROIs for the primary somatosensorial cortex of the forelimbs and hindlimbs are also provided for both atlases. But the authors would recommend to use some custom atlas with better contrast and resolution, like the ex-vivo atlases previously reported (i.e. G. Allan Johnson, Evan Calabrese, Alexandra Badea, George Paxinos, Charles Watson. *A multidimensional magnetic resonance histology atlas of the Wistar rat brain*. NeuroImage Volume 62, Issue 3, September 2012, Pages 1848–1856).

- **TESTING IMAGES**

Two datasets are provided [here](#), one in raw Bruker format, and another one in Nifti format.

Bruker format: data scheme is the same as in USING FMRAT / BRUKER FORMAT section (what a nice coincidence ;) ). Details needed:

Subject1:

"N_rest"	=	15
"N_stim"	=	5
"NR_total"	=	115
"TR"	=	3000
"Anatomic images method"	=	RARE
"Functional images method"	=	EPI_fMRI
Species	=	Wistar

Subject2:

"N_rest"	=	15
"N_stim"	=	5
"NR_total"	=	115
"TR"	=	3000
"Anatomic images method"	=	RARE
"Functional images method"	=	EPI_fMRI
Species	=	Sprague-Dawley

Nifti format: data scheme is the same as in USING FMRAT / NIFTI FORMAT section (upss...another coincidence)

Subject1:

"N_rest"	=	15
"N_stim"	=	5
"NR_total"	=	115
"TR"	=	3000
Species	=	Wistar

Subject2:

"N_rest"	=	15
"N_stim"	=	5
"NR_total"	=	115
"TR"	=	3000
Species	=	Sprague-Dawley

Wistar and Sprague-Dawley subjects should be analyzed independently because they require different atlases, but you can try

to analyze them all together as Wistar only to check the tool multisubject functionality.