SAMIT

Small Animal Molecular Imaging Toolbox

University of Groningen, University Medical Center Groningen

Molecular Imaging Center, Department of Nuclear Medicine and Molecular Imaging

October 2017

The aim of this toolbox is to facilitate voxel-based and/or volume-based analysis of small animal PET and SPECT brain images.

It also provides an automatized procedure for the construction of new tracer-specific templates for the spatial registration of small animal PET and SPECT brain images.

Contents:

Before to start

Required Software

Matlab 7.1 or above (http://www.mathworks.nl/products/matlab/)

SPM12 (http://www.fil.ion.ucl.ac.uk/spm/)

SAMIT (http://mic-umcg.github.io/samit/)

Installation of SPM and SAMIT toolbox

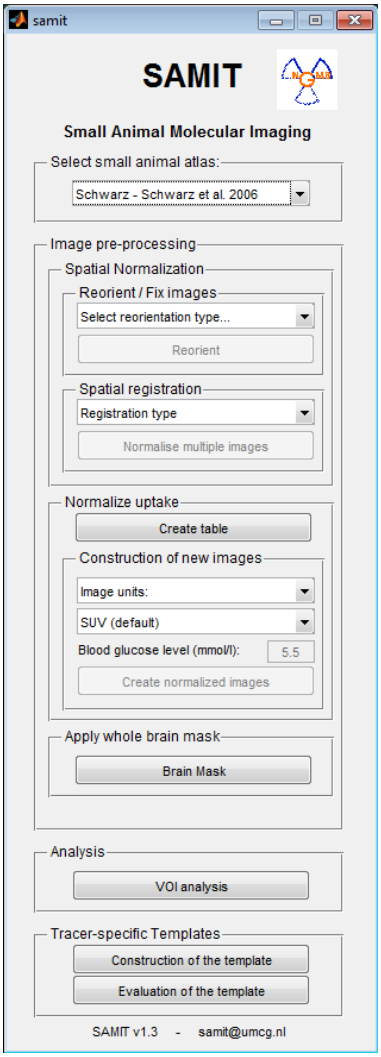
1. Install SPM (http://en.wikibooks.org/wiki/SPM/Download)
2. (Recommended) Update SPM to its latest version. Type the following command in Matlab: *spm_update('upgrade')*
3. Extract the content of the downloaded SAMIT file, and rename the obtained folder as *samit* (e.g. from 'samit-1.3' to 'samit')
4. Move this *samit* folder inside the 'toolbox' directory located in your SPM installation.

After this installation, the *SAMIT* toolbox will be available from the 'toolbox' pull-down menu in the main SPM window.

Notes

This manual and the current version of SAMIT is designed to work only with 'static' (single frame) images

SAMIT: Small Animal Imaging Toolbox



It also provides an automatized procedure for the construction of new tracer-specific templates for the spatial registration of small animal PET and SPECT brain images.

The toolbox is intended to work in combination with Statistical Parametric Mapping (SPM) software, and most of the SAMIT functions require the presence of this software in the Matlab environment.

Select Atlas

The very first step to use SAMIT is to select the desired animal atlas. This step is used to populate some default values and to allow the interaction with the rest of the options.

Image pre-processing

Spatial normalization

1. **Reorient.** This option allows reorienting images that were previously registered using other software packages, e.g. PMOD or VINCI. It allows basic operations such as to relocate the coordinates system of the image or reorient the image in the direction expected by SPM.
    a. *PMOD2SPM*: This option allows to reorient the images created in PMOD to the orientation expected in SPM.
    b. *SPM2PMOD*: In this case, the images in SPM space are reoriented into PMOD space.
    c. *VINCI*: The NIfTI files created in VINCI present an error in the stored transformation matrix, which can cause problems with the voxel size and the orientation in SPM. This simple fix solves the issue.
    d. *Bregma*: The location of the coordinates system in the images are a crucial step while handling the images. The zero or "origin" of the coordinates system is located in frequently located in bregma in the small animals, but not all the animal template follow this recommendation.
    e. *Center*: If bregma is not properly defined in the atlas, the center of the image is a good alternative for coordinates system.
2. **Spatial registration.** This section of the toolbox use the normalization process implemented in SPM8, and allows the selection of multiple images at once.
    a. Registration type (for further details see 'spm_affreg' from SPM)
    b. Normalize multiple images

Notes:

This step is not necessary if the images were previously aligned to the template in another software package (e.g. PMOD or VINCI).

The best results are obtained when the images and the reference template have similar dimensions and a good initial overlap.

Normalize uptake

This section allows the normalization of the uptake values in multiple images. The procedure has two steps:

1. **Create table.** The input file can be created manually or with the help of *'Create table'* button. It will ask for the images that will be normalized, and it will generate a table. The content of this table can be filled with any text or spreadsheet editor. When completed, the table can be saved as a tabulated file (*.txt) or Excel file (*.xls / *.xlsx).
   Columns:
   a. First column: Full path and name of the image
   b. Second column: Activity of the injected tracer (MBq)
   c. Third column: Animal body weight (gr)
   d. Fourth column: glucose level in the blood (mmol/l).

	A	B	C	D
1	File Name	Dose (MBq)	Weight (gr)	Glucose (mmol/L)
2	C:\Tests\samit\11C-PK11195 Rat 01.nii	50	250	6.5
3	C:\Tests\samit\11C-PK11195 Rat 02.nii	60	280	8

Note: *If the glucose was not measured, or it is not going to be used for the normalization, the whole column can be removed.*

2. **Construction of new images.**
   a. *Image units.* Select the radioactivity units used when the image was created.
   Note: By default, PMOD software store the images as kBq/cc
   b. Normalization type.
      i. SUV (Standardized Uptake Values). The new image will have the suffix '*–SUV*'
      ii. SUVglc (SUV corrected for blood glucose). The new image will have the suffix '*–SUVglc*'
      iii. SUV whole brain. The SUV will be corrected for the mean uptake value of the whole brain. The new image will have the suffix '*–SUVw*'
      iv. IDg (Percentage of injected dose per gram). The new image will have the suffix '*–IDg*'
   c. *Basal Glucose Plasma.* The reference value for the glucose level (only available when SUVglc is selected).
   d. *Create normalized images.* The new images will be created according to the parameters selected and the information probided in the file (table).

$$\mathrm{SUV} = \frac{\text{Uptake in the Image}}{\text{Injected dose}/\text{body weight}}$$

$$\mathrm{IDg} = \frac{\text{Uptake in the Image}}{\text{Injected dose}} \times 100$$

$$\mathrm{SUVglc} = \mathrm{SUV} \times \frac{\text{Blood glucose level}}{\text{Reference glucose level}}$$

$$\mathrm{SUVw} = \frac{\mathrm{SUV}}{\text{Whole brain uptake}}$$

Apply whole brain mask

A new image will be created removing the signal from the outside of the brain. The brain mask will be selected automatically according to the default parameters of the atlas. The new constructed images will have the prefix *'m'*.

Analysis

The purpose of this section is to facilitate the extraction of the descriptive values from the images, which can be further used for statistics analysis. The program will ask you for the image containing the Volumes of Interest (VOIs), and then will ask for the image(s) from where the values want to be calculated. The results will be saved in a tabulated text file (*.txt) and in a Matlab file (*.mat).

Important: *The extraction of the results will proceed even if the files had different orientation or dimensions than the VOIs. Its recommend to use only images already aligned and with the same dimensions than the desired SAMIT template. The results obtained with images with different orientations and/or dimensions might not be correct!!*

Templates

The construction process to obtain tracer-specific PET and SPECT templates have been automatized as described in Vallez Garcia et al. 2015 (doi). The program assumes that the images are already aligned between them in space and uptake (e.g. in PMOD software and then reoriented to SPM). The first image selected in the list will be used as the reference image, and all the other images will be aligned to this one in the first step of the template construction.

Note: *It is recommended to check the images that will be used for the construction of the template with Check Image Registration (Check Reg) in SPM, to select the most appropriate reference image and to confirm that the images are correctly aligned between them.*

The steps performed by the program are:

1. Spatial normalization of the selected images to the first one
2. Construction of a mean symmetrical image
3. Co-registration of the symmetrical image to the reference MRI template
4. Co-registration of the images used for the construction of the template with the parameters obtained in the previous step. This new images are saved with a prefix *'r'* and can be used for the evaluation of the template
5. The final version of the template and its registration with the MRI is displayed

Several files will be created when the construction of the template is completed:

- *Name_coreg.mat* The co-registration matrix obtained in step 3
- *Name_MRI_Size.nii.* The new template with the same dimensions and voxel size as the reference MRI
- *Name_Original_Size.nii* A version of the template but that preserves the original dimensions of the images. The co-registration matrix is stored in the file, but the image is not resliced. This image can be used for the construction of other versions of the template with different dimension size.

The evaluation of the registration accuracy of the images to the template can be performed by selecting 'Evaluation of the template'. The previously constructed template must be selected (*_MRI_Size.nii) followed by the new version of the images used for the construction of the template.

Voxel-based Analysis in SPM

The voxel-based analysis is performed in a similar way as it is done in humans. The following steps are recommended:

1. Select the appropriate small animal atlas
2. Perform spatial normalization of the images to the reference template
3. Normalize the uptake signal
4. Apply the brain mask to all the images
5. Smooth the images (Implicit masking: Yes)
6. Define the statistical model
7. Estimate the model
8. Display the results

Example

In this section, we will go through all the steps needed to perform the data analysis using a sample data set.

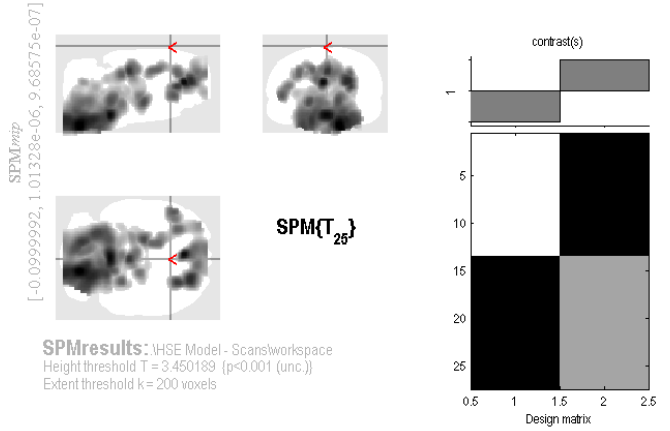
1. Download the data set an extract its content into a new folder. The dataset consists of [^{11}C]PK11195 PET brain scans from male Wistar rats, including a healthy group (n=13) and an intervention group with Herpes Encephalitis (HSE).
2. In Matlab, go to the folder where the dataset has been extracted. It is always recommended to define a working folder, since several files may be generated in SPM and located by default in the working directory.
3. Start SPM by typing in Matlab: *spm pet*
4. From the Toolbox menu in the SPM interface select 'samit'. The SAMIT interface must remain open during the whole procedure otherwise the default human parameters will be used.
5. Select the small animal atlas to be used. In this example select *Schwarz (Schwarz et al. 2006).*
6. Define the location of the 'origin'. In this data set, the current location of the origin is in the center of the image. This information can be checked using the '*Display*' option in SPM. In SAMIT interface, select '*Bregma*' option in the *'Reorient / Fix images'* section, and click '*Reorient*' button. Select all the images located in the folders 'HSE' and 'Healthy'.
   This process will relocate the origin in a position of the image close to where bregma is expected to be. The new images will be located in the same folder than the original ones but with the prefix 'r'
7. An initial registration of the images was performed during its pre-processing. Nevertheless, we are going to perform again the automatic registration process on the images in SAMIT, for didactical purposes. The accuracy of the alignment between the images can be observed by selecting some images with *'Check Reg'* in SPM interface.
   a. Select the *'almost rigid body'* option in the *'Spatial Registration'* section
   b. Click in *'Normalise multiple images'*
   c. Select all the reoriented images (i.e. prefix 'r') from the healthy and HSE folders
   d. Select the tracer-specific template located inside the SAMIT folder, in this example the [^{11}C]PK11195 template.

   The new images will be located in the same folder with the prefix 'w'

8. Normalize uptake: Information about the injected dose and the weight of the animals is provided in the file 'SUV table.txt'
    a. Click on *'Create table'*
    b. Select all the files created previously (i.e. with the prefix 'wr')
    c. Select a name for the new file that will be created
    d. Open the file with a text or spreadsheet editor. Fill the information according to the one provided in 'SUV table.txt'. No data was collected for the glucose level, so there is no need for the last column and it can be removed. Save the file as tabulated text file or Excel file
    e. Select the image units. In this study, the original images were acquired in *Bq/cc*
    f. Select the normalization procedure. For this example, we will use the *SUV* option
    g. Click on *'Create normalized images'*. The new images will be located in the same folder than the previous ones but including the suffix '-SUV'
9. Apply a mask to the whole brain. This step will avoid the influence of uptake outside the brain during the smoothing procedure.
    a. Click on the *'Brain Mask'* button
    b. Select the images to which apply the mask (i.e. those with the suffix –SUV)
    c. The new images will be located in the same folder than the previous ones with the prefix 'm'
10. Apply smoothing to the image
    a. Click in *'Smooth'* button from the SPM interface
    b. Select the images to smooth (i.e. those with the prefix 'm')
    c. Select the desired FWHM. For this example, we can leave the default value (*Note:* recommend values are between 0.8 and 0.12 mm)
    d. Select 'Implicit masking' to 'Yes'. This will avoid the smoothing to go outside the masked region
    e. Run the batch: Click on the icon of a green arrow or select *File > Run Batch*
    f. The new images will be located in the same folder than the previous ones with the prefix 's'. These are the final images to be used in the voxel-based analysis
11. Statistical model. In this section, we will take care of the statistical model, the estimation of the values and display the results
    a. Basic model. First, we have to define the model. In the present example, it is a simple two-sample t-test design (for further details, please refer to the SPM manual)
        i. Click on *'Basic Models'* button in the main SPM window
        ii. *Directory*: choose the directory were data will be written (e.g. '*workspace*')
        iii. Select the *'Design'* as 'Two-sample t-test'
        iv. Select the Healthy group as 'Group 1 scans'
        v. Select the HSE group as 'Group 2 scans'
        vi. Select 'Explicit Mask', using the whole brain mask located in SAMIT folder (i.e. Schwarz_intracranialMask.nii)
    b. Estimate the model
        i. In the menu of the Batch Editor select *SPM > Stats > Model estimation*
        ii. Select the new module 'Model estimation' from the left section of the Batch Editor
        iii. Click on *'Dependency'* button, located in the lower right section
        iv. Select the Factorial design option
        v. Save the batch for future modifications
    c. Run the batch: Click on the icon of a green arrow or select *File > Run Batch*

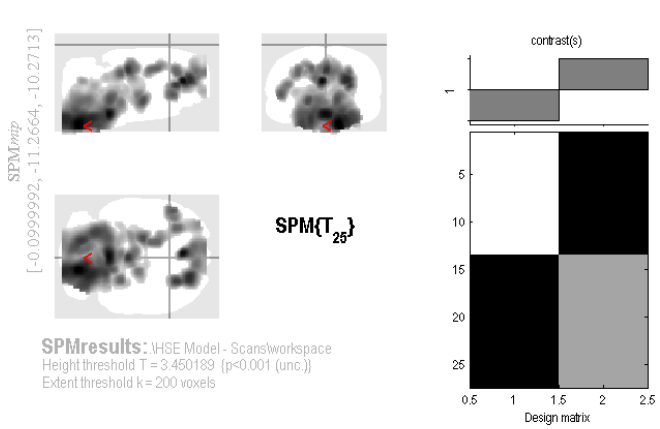
d. Show the results
    i. Click on *'Results'* button in the main SPM window
    ii. Select the *'SPM.mat'* file located in the directory selected previously during the specification of the model
    iii. Click on *'Define a new contrast'*
        1. In the name type "Healthy < HSE"
        2. Type: t-contrast
        3. Contrast: -1 1
        4. Click on *'OK'*
        5. Select the contrast and press *'Done'*
    iv. Apply masking: *none*
    v. Title for comparison: leave the current title and press enter
    vi. Choose the p-value adjustment (FWE or uncorrected). In this example select *none*
    vii. Choose the p-value. In this example type *0.001*
    viii. Extent threshold (voxels). In this example type *200*
e. Results are presented in a maximum intensity projection (MIP) display on a glass rat brain. Since the MRI rat template was spatially aligned with the Paxinos atlas the resulting coordinates are equivalent to those in the atlas
f. To visualize the results over the MRI go to *Display > Overlays… > Section* and choose the rat MRI template located in the SAMIT folder

Healthy < HSE



SPMresults: \HSE Model - Scans\workspace
Height threshold T = 3.450189 {p<0.001 (unc.)}
Extent threshold k = 200 voxels

Healthy < HSE



SPMresults: \HSE Model - Scans\workspace
Height threshold T = 3.450189 {p<0.001 (unc.)}
Extent threshold k = 200 voxels

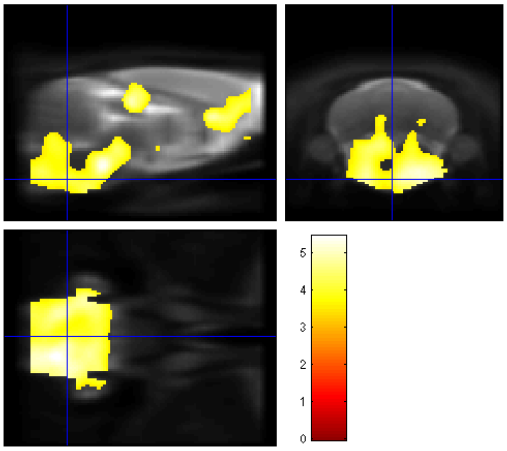
Statistics: ***p-values adjusted for search volume***

set-level		cluster-level				peak-level					mm mm mm		
p	c	$p_{FWE-corr}$	$q_{FDR-corr}$	k_E	p_{uncorr}	$p_{FWE-corr}$	$q_{FDR-corr}$	T	$(Z_\equiv)$	p_{uncorr}			
0.066	2	0.000	0.000	33600	0.000	0.017	0.314	5.45	4.39	0.000	1.7	-12.2	-10.
						0.052	0.314	4.90	4.06	0.000	0.1	-5.6	-3.
		0.000	0.000	8416	0.000	0.023	0.314	5.30	4.30	0.000	-0.5	1.8	-4.

table shows 3 local maxima more than 8.0mm apart

Height threshold: T = 3.45, p = 0.001 (0.583)
Extent threshold: k = 200 voxels, p = 0.477 (0.341)
Expected voxels per cluster, <k> = 417.119
Expected number of clusters, <c> = 0.42
FWEp: 4.914, FDRp: Inf, FWEc: 8416, FDRc: 8416

Degrees of freedom = [1.0, 25.0]
FWHM = 3.3 3.4 3.4 mm mm mm; 16.4 17.2 16.8 {voxels}
Volume: 1875 = 234415 voxels = 46.0 resels
Voxel size: 0.2 0.2 0.2 mm mm mm; (resel = 4751.62 voxels)



Flexible Factorial Design: simplified method to organize the data

Here it is described how to simplify the input of data in the flexible factorial design. For example, assuming we have:

- Factor 1: *subject* (4 animals)
- Factor 2: *group* (2 groups: 'Control' and 'Intervention')
- Factor 3: *condition* (3 scans performed at different time points)

1. Create a table with the following data (e.g. in Excel)
    a. First column: location of the image file
    b. Second column: Index number of the image
    c. Third column: Index number of the animal
    d. Fourth column: Index number of the group
    e. Fifth column: Index number of the condition

File	Index	Animal	Group	Condition
D:\Matlab\Test\Rat01 Control Scan1.nii	1	1	1	1
D:\Matlab\Test\Rat01 Control Scan2.nii	2	1	1	2
D:\Matlab\Test\Rat01 Control Scan3.nii	3	1	1	3
D:\Matlab\Test\Rat02 Control Scan1.nii	4	2	1	1
D:\Matlab\Test\Rat02 Control Scan2.nii	5	2	1	2
D:\Matlab\Test\Rat02 Control Scan3.nii	6	2	1	3
D:\Matlab\Test\Rat03 Intervention Scan1.nii	7	3	2	1
D:\Matlab\Test\Rat03 Intervention Scan2.nii	8	3	2	2
D:\Matlab\Test\Rat03 Intervention Scan3.nii	9	3	2	3
D:\Matlab\Test\Rat04 Intervention Scan1.nii	10	4	2	1
D:\Matlab\Test\Rat04 Intervention Scan2.nii	11	4	2	2
D:\Matlab\Test\Rat04 Intervention Scan3.nii	12	4	2	3

2. Then, in the Batch Editor of SPM, under the *Factorial design specification* select *"Design > Flexible Factorial > Specify Subjects or all Scans & Factors >* ***Specify all****"* (by default "Subjects" is used).
3. Double click in *Scans*. In the new window do click on the button located on the left side "**Ed**". Copy & Paste here the first column of the table, with the address of the image files. Accept & Done.
4. Double click in *Factor matrix*. Copy & Paste here the last four columns of the table.

References

Atlases

- Ma et al., 2005. A three-dimensional digital atlas database of the adult C57BL/6J mouse brain by magnetic resonance microscopy. Neuroscience, 135(4) doi: 10.1016/j.neuroscience.2005.07.014
- Ma, Y. et al., 2008. In Vivo 3D Digital Atlas Database of the Adult C57BL/6J Mouse Brain by Magnetic Resonance Microscopy. Frontiers in neuroanatomy, 2(April) doi: 10.3389/neuro.05.001.2008
- Schwarz et al., 2006 A stereotaxic MRI template set for the rat brain with tissue class distribution maps and co-registered anatomical atlas: application to pharmacological MRI. NeuroImage 32(2) doi: 10.1016/j.neuroimage.2006.04.214

SAMIT and tracer-specific templates

- Vállez Garcia et al., 2015. A Standardized Method for the Construction of Tracer Specific PET and SPECT Rat Brain Templates: Validation and Implementation of a Toolbox. PLoS One, 10(3) doi: 10.1371/journal.pone.0122363
- Casteels et al., 2013. Construction and evaluation of quantitative small-animal PET probabilistic atlases for [18F]FDG and [18F]FECT functional mapping of the mouse brain. PLoS One, 8(6) doi: 10.1371/journal.pone.0065286
- Casteels et al., 2006. Construction and evaluation of multitracer small-animal PET probabilistic atlases for voxel-based functional mapping of the rat brain. J Nucl Med, 47(11) (link)

List of Publications that used SAMIT

- David Vállez García, Erik FJ de Vries, Jun Toyohara, Kiichi Ishiwata, Kentaro Hatano, Rudi AJO Dierckx, and Janine Doorduin. 2015. "Evaluation of [(11)C]CB184 for Imaging and Quantification of TSPO Overexpression in a Rat Model of Herpes Encephalitis." *European Journal of Nuclear Medicine and Molecular Imaging* 42 (7):1106–18. https://doi.org/10.1007/s00259-015-3021-x.
- Fiona E Parkinson, Soumen Paul, Dali Zhang, Shadreck Mzengeza, and Ji Hyun Ko. 2016. "The Effect of Endogenous Adenosine on Neuronal Activity in Rats: An FDG PET Study." *Journal of Neuroimaging* 26 (4):403–5. https://doi.org/10.1111/jon.12349.
- Jürgen Sijbesma, Xiaoyun Zhou, David Vállez García, Martin C. Houwertjes, Janine Doorduin, Chantal Kwizera, Bram Maas, et al. 2016. "Novel Approach to Repeated Arterial Blood Sampling in Small Animal PET: Application in a Test-Retest Study with the Adenosine A1 Receptor Ligand [11C]MPDX." *Molecular Imaging and Biology* 18 (5). Molecular Imaging and Biology:715–23. https://doi.org/10.1007/s11307-016-0954-9.
- David Vállez García, Andreas Otte, Rudi A.J.O. Dierckx, and Janine Doorduin. 2016. "Three Month Follow-Up of Rat Mild Traumatic Brain Injury: A Combined [18 F]FDG and [11 C]PK11195 Positron Emission Study." *Journal of Neurotrauma* 33 (20):1855–65. https://doi.org/10.1089/neu.2015.4230.
- Andrea Parente, Paula Kopschina Feltes, David Vállez García, Jürgen Sijbesma, Cristina M Moriguchi Jeckel, Rudi AJO Dierckx, Erik FJ de Vries, and Janine Doorduin. 2016. "Pharmacokinetic Analysis of 11C-PBR28 in the Rat Model of Herpes Encephalitis: Comparison with (R)-11C-PK11195." *Journal of Nuclear Medicine* 57 (5):785–91. https://doi.org/10.2967/jnumed.115.165019.

- Zhou, X., J. Doorduin, P. H. Elsinga, R. A.J.O. Dierckx, E. F.J. de Vries, and C. Casteels. 2017. "Altered Adenosine 2A and Dopamine D2 Receptor Availability in the 6-Hydroxydopamine-Treated Rats with and without Levodopa-Induced Dyskinesia." *NeuroImage* 157 (December 2016). Elsevier:209–18. https://doi.org/10.1016/j.neuroimage.2017.05.066.
- David Vállez García, Janine Doorduin, Daniele de Paula Faria, Rudi A J O Dierckx, and Erik F J de Vries. 2017. "Effect of Preventive and Curative Fingolimod Treatment Regimens on Microglia Activation and Disease Progression in a Rat Model of Multiple Sclerosis." *Journal of Neuroimmune Pharmacology® : The Official Journal of the Society on NeuroImmune Pharmacology* 12 (3). Journal of Neuroimmune Pharmacology:521–30. https://doi.org/10.1007/s11481-017-9741-x.
- Andrea Parente, David Vállez García, Alexandre Shoji, Isadora Lopes Alves, Bram Maas, Rolf Zijlma, Rudi AJO Dierckx, Carlos A Buchpiguel, Erik FJ de Vries, and Janine Doorduin. 2017. "Contribution of Neuroinflammation to Changes in [11 C]flumazenil Binding in the Rat Brain: Evaluation of the Inflamed Pons as Reference Tissue." *Nuclear Medicine and Biology* 49 (June). Elsevier Inc.:50–56. https://doi.org/10.1016/j.nucmedbio.2017.03.001.
- Isadora Lopes Alves, David Vállez García, Andrea Parente, Janine Doorduin, Rudi Dierckx, Ana Maria Marques da Silva, Michel Koole, Antoon Willemsen, and Ronald Boellaard. 2017. "Pharmacokinetic Modeling of [(11)C]flumazenil Kinetics in the Rat Brain." *EJNMMI Research* 7 (1). EJNMMI Research:17. https://doi.org/10.1186/s13550-017-0265-4.