**Use this protocol for the analysis of longitudinal global brain measures, subcortical structures, cortical thickness and surface area data within FreeSurfer ROI's for the ENIGMA plasticity working group**

**If you have any questions or run into problems, please feel free to contact Rachel Brouwer (****r.m.brouwer-4@umcutrecht.nl****)**

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**Step 1: Extract and Organize Volumes (FreeSurfer)**

This script assumes that you have run the FreeSurfer pipeline on your baseline and follow-up data. If possible, please run the longitudinal FreeSurfer pipeline. This will generally improve the quality of data. Please use Freesurfer version 5.1 or 5.3.

Highlighted portions of the instructions may require you to make changes so that the commands work on your system and data.

Get the volume of each structure for each subject from FreeSurfer

The following script will extract and organize each of the volumes measures of the subcortical structures. The script assumes that your FreeSurfer output are organized in a standard way:

{Parent Folder baseline} 🡪 {Subject1} 🡪 {FreeSurfer Output Folders}

 🡪 {Subject2} 🡪 {FreeSurfer Output Folders}

 …

{Parent Folder follow-up} 🡪 {Subject1} 🡪 {FreeSurfer Output Folders}

 🡪 {Subject2} 🡪 {FreeSurfer Output Folders}

 …

The parent folders can be the same; and will probably be the same when you have run the longitudinal pipeline, in which case the output should be organized as:

{Parent Folder} 🡪 {Subject1\_baseline.long.Subject1\_template} 🡪 {FreeSurfer Output Folders}

 🡪 {Subject1\_followup.long.Subject1\_template} 🡪 {FreeSurfer Output Folders}

🡪 {Subject2\_baseline.long.Subject2\_template} 🡪 {FreeSurfer Output Folders}

 🡪 {Subject2\_followup.long.Subject2\_template} 🡪 {FreeSurfer Output Folders}

 …

* Prepare a .txt file called **longitudinal\_ENIGMA\_subjects.txt** and make sure the first two columns are the subjects-ids for baseline and follow-up. In preparation for the outlier detection step: The third column should contain the follow-up duration these subjects. Other columns (e.g. sex, age\_at\_baseline, age\_at\_follow\_up, diagnosis) are allowed but not necessary at this point. Please note: This file should contain the names of the Freesurfer directories in which the data are stored.
* **NOTE: baseline and follow up IDs should be different!**

For example:

*SubjectID\_baseline SubjectID\_followup Fup duration (yrs)*

Subject1\_baseline Subject1\_followup 3.8

Subject2\_baseline Subject2\_followup 4.6

Subject3\_baseline Subject3\_followup 2.0

…

Or in the longitudinal setup:

*SubjectID\_baseline SubjectID\_followup Fup duration (yrs)*

Subject1\_baseline.long.Subject1\_template Subject1\_followup.long.Subject1\_template 3.8

Subject2\_baseline.long.Subject2\_template Subject2\_followup.long.Subject2\_template 4.6

…

**FOR FREESURFER V5.1 USERS ONLY:**

If you have used Freesurfer version 5.1 the output file will not contain values for total brain volume. If you have Freesurfer 5.3 installed, you do not need to rerun the whole pipeline. You can extract new values separately by going into the Parent Folder for baseline and run:

for subject in `ls –d \*`

do

cd ${subject}

cp /crossectional\_Fsoutput\_subject/surf/?h.orig.nofix ${subject}/surf

mri\_segstats --seg mri/aseg.mgz --sum stats/aseg.stats --pv

mri/norm.mgz --empty --brainmask mri/brainmask.mgz --brain-vol-from-seg --excludeid 0 --excl-ctxgmwm --supratent --subcortgray --in mri/norm.mgz --in-intensity-name norm --in-intensity-units MR --etiv --surf-wm-vol --surf-ctx-vol --totalgray --euler --ctab

/usr/local/freesurfer/stable5/ASegStatsLUT.txt --subject $subject

cd -

done

Repeat for the Parent folder for follow up. The value for BrainSegNotVent will now be in the new stats file (aseg.stats). Please note that this will overwrite your previous aseg.stats files. Values for other brain measures also differ slightly between versions.

* The next step is to extract longitudinal global brain measures, subcortical structures, surface area and thickness values per ROI. Open the script **longitudinal\_extract.sh** and edit the paths to the Freesurfer main directory containing base and follow-up data, and the path to the file **longitudinal\_ENIGMA\_subjects.txt** created above (lines 3-5).

Save the extract\_longitudinal.sh script after editing. On the command line, you can run the script directly by running:

source longitudinal\_extract.sh

This script will typically run for a few minutes, depending on the number of subjects. The result of this step will be a comma-separated (CSV) file that can be opened in your favorite spreadsheet application (i.e. Excel). The first row is a header describing the extracted regions and names for each column. Each row after the first gives the brain measures for each subject at baseline and follow-up that are found in your **longitudinal\_ENIGMA\_subjects.txt** file. In the next step, you will do a QC of the segmentation quality. See ENIGMA\_QC\_LONG\_FS.docx

**Note 1:** This script checks for the existence of output files for both measurements (i.e. both subjectIDs and produces warnings when they are not present. These subjects will not be included in the .csv file.

**Note 2:** When you edit the files in Excel, be sure to keep them in CSV format when you save!