Instructions for hippocampal subfield analysis and QC hints for ENIGMA projects

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Designed for the Subfield module of the developmental version of FS6.0 or FS7.1.1
Philipp Sämann, Neda Janashad,
Laura van Velzen, Derrek Hibar, Theo van Erp,
Christopher Whelan, Lianne Schmaal

If you have any questions or run into problems, please feel free to contact saemann@psych.mpg.de.

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Blueish parts in Courier Font can be directly used by copy & paste, with *reddish parts* such as *<your_individual_path>* requiring editing according to your local directory and file name structure. This manual covers the procedure for FS6.0 and FS7.1.1, yet the scripts come in two separate packages:

FS6.0.0

FS7.1.1

Scripts for FS v6.0.0 stable

Name of the file	File description	Function
loop_all.sh	Shell script	start multiple segmentations
extract_subfields_v10.sh	Shell script	Extract hippocampal subfields
HippoColors_v6.0.txt	Plan text file	Color code table for subfields QC
outliers_and_QC_support_v10.R	R script	Histograms and QC support
QC_subfields_step_1_prepare_v2.sh	Shell script	Collect files needed for QC gallery
QC_subfields_step_2_make_pngs_v2.m	Matlab script	Produce images for QC galery
QC_subfields_step_3_webpage_v2.sh	Shell script	Arrange images in an *html file
SUBFIELD_ANALYSIS_v1.6.R	R script	Perform actual statistical analysis[1]

Scripts for FS v7.1.1 release

Name of the file	File description	Function
loop_all.sh	Shell script	Start multiple segmentations
extract_subfields_hippamy_v21.sh	Shell script	Extract hippocampal subfields
HippoColors_v7.0.txt	Plan text file	Color code table for subfields QC
outliers_and_QC_support_v21.R	R script	Histograms and QC support
QC_subfields_step_1_prepare_v3.sh	Shell script	Collect files needed for QC gallery
QC_subfields_step_2_make_pngs_v3.m	Matlab script	Produce images for QC galery
QC_subfields_step_3_webpage_v2.sh	Shell script	Arrange images in an *html file
SUBFIELD_ANALYSIS_v1.6.R	R script	Perform actual statistical analysis[2]

* Possibly useful for amygdala analyses. Not needed for hippocampal QC.

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[1] Specific to the requirements of the ENIGMA-MDD hippocampal subfield project.
[2] Specific to the requirements of the ENIGMA-MDD hippocampal subfield project.

Step 1: Run hippocampal subfields segmentations in FreeSurfer v6.0 or v7.1.1

For the general download of FreeSurfer, we refer to:
https://surfer.nmr.mgh.harvard.edu/fswiki/DownloadAndInstall
where links to both version 6 and version 7 can be found.

For both versions, the general segmentation needs to run before the hippocampal subfield segmentation (in FS stable v6.0.0) or the combined segmentation of hippocampal subfields and nuclei of the amygdala (FS version 7.1.1 release). We have not tested the scripts for FS7.1.2.

As soon as downloaded, the FreeSurfer environment needs to be defined (possibly, these basic settings are automatically called within the .cshrc file that is started when a new shell is opened, or any other automated startup script – in this case, skip these step):

```
csh
setenv FREESURFER_HOME <directory_containing_FS_installation>/freesurfer
source $FREESURFER_HOME/SetUpFreeSurfer.csh
```

In addition, the path to the subject folders needs to be defined:
```
setenv SUBJECTS_DIR <full_path_to_subject_folders>
```

The command `recon-all` should be automatically linked. Calling it without parameters will prompt an explanation of the function. Typing `which recon-all` should give you the location of the script in case you have several versions of FreeSurfer installed.

The basic FreeSurfer segmentation (cortex and subcortical areas, duration 10-14 hours depending on the hardware configuration) is started by:
```
recon-all –s <subject_ID> -all
```

Hippocampal subfield segmentation in FS v6.0.0 (stable version)

Based on the general segmentation, the hippocampal subfield segmentation <u>for a single subject</u> and a T1 input is started by:

```
recon-all –s <subject_ID> -hippocampal-subfields-T1
```

Note that in FS6.0.0 (not in FS7.x) both the general segmentation and a subsequent hippocampal subfield segmentation can also be started by a single call:

```
recon-all –s <subject_ID> -all -hippocampal-subfields-T1
```

Respective variations of the flag exist for T2-input (-hippocampal-subfields-T2) or a combined input (-hippocampal-subfields-T1T2).

Calling the hippocampal subfield segmentation <u>for your complete sample</u> requires a loop as follows:

1. Go to the FreeSurfer subject directory:
   ```
   bash
   cd <full_path_to_subject_folders>
   ```

2. Produce a list that contains the subjects for which you want to run the subfield segmentation tool, e. g. by:
   ```
   ls -d1 <your_subject_folder_root>* > subjects.txt
   ```
   <u>Example:</u> Your folders are labelled subject_001, subject_002 etc., then the command would be:
   ```
   ls -d1 subject_* > subjects.txt
   ```
   Double-check the list (no spaces, no other strings than subject folders etc.) by a standard text editor such as *jmacs* or *emacs* or *vi*.

3. Once checked, save the following lines as a script named *loop_all.sh* or use the respective script sent by mail:

```
#!/bin/bash
exec <subjects.txt>
while read x; do
recon-all -s $x –hippocampal-subfields-T1
done
```

To make this script executable, type: chmod a+x loop_all.sh
Eventually, start the script by typing: ./loop_all.sh

This starts the subfield segmentation script that usually takes about 30 min per case. The results are written into the already existing subfolder *mri*. There are 3 files produced per hemisphere, for the left hemisphere these file are:

(a) The volumetric results listed in a file *lh.hippoSfVolumes-T1.v10.txt*, and
(b) two images in mgz-format ref. as *lh.hippoSfLabels-T1.v10.mgz* and *lh.hippoSfLabels-T1.v10.FSvoxelSpace.mgz*.

The parcellation into 12 subfields per hemisphere as achieved in FS6.0.0 is the version **"FS60"** as represented in detail in the middle column in:
https://surfer.nmr.mgh.harvard.edu/fswiki/HippocampalSubfieldsAndNucleiOfAmygdala

It is important to understand that it is this parcellation that underlies all QC recommendations.

Hippocampal subfield segmentation in FS v7.1.1

In FS7.1.1, the hippocampal subfield segmentation can only one started jointly with the segmentation of the nuclei of the amygdala (duration: about 45-60 minutes):

`segmentHA_T1.sh <subject_ID>`

Note: Special versions exist for additional input images (segmentT2_HA.sh) or longitudinal data (segmentHA_T1l_long.sh). For details on the call syntax and requirements such as coregistration we refer to: https://surfer.nmr.mgh.harvard.edu/fswiki/HippocampalSubfieldsAndNucleiOfAmygdala

The results are written into the already existing subfolder *mri*. There are 10 files produced per hemisphere; for the left hemisphere, these files are:

File name	Explanation
`lh.hippoSfVolumes-T1.v21.txt`	Plain text file with volumes of 19 hippocampal subregions and 3 derived aggregations (body, head, whole)
`lh.amygNucVolumes-T1.v21.txt`	Plain text file with volumes of 9 amygdala subnuclei and 1 derived aggregation (whole)
`lh.hippoAmygLabels-T1.v21.FSvoxelSpace.mgz`	All hippocampal subregions and amygdala subnuclei ([1 mm]^3 resolution) – *no additional re-aggregation*
`lh.hippoAmygLabels-T1.v21.mgz`	All hippocampal subregions and amygdala subnuclei (high ([0.333 mm]^3 resolution) – *no additional re-aggregation*
`lh.hippoAmygLabels-T1.v21.CA.FSvoxelSpace.mgz`	Hippocampal subregions and amygdala subnuclei, merged according to the **"CA"**-aggregation ([1 mm]^3 resolution)
`lh.hippoAmygLabels-T1.v21.CA.mgz`	Hippocampal subregions and amygdala subnuclei, mered merged to the **"CA"**-aggregation (high ([0.333 mm]^3 resolution)
`lh.hippoAmygLabels-T1.v21.FS60.FSvoxelSpace.mgz`	Hippocampal subregions and amygdala subnuclei, merged according to the **"FS60"**-aggregation ([1 mm]^3 resolution)
`lh.hippoAmygLabels-T1.v21.FS60.mgz`[3]	Hippocampal subregions and amygdala subnuclei, merged according to the **"FS60"**-aggregation (high ([0.333 mm]^3 resolution)
`lh.hippoAmygLabels-T1.v21.HBT.FSvoxelSpace.mgz`	Hippocampal subregions and amygdala subnuclei, merged according to the **"HBT"**-aggregation ([1 mm]^3 resolution)
`lh.hippoAmygLabels-T1.v21.HBT.mgz`	Hippocampal subregions and amygdala subnuclei, merged according to the **"HBT"**-aggregation (high ([0.333 mm]^3 resolution)

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[3] Aggregation used for QC

Go to the FreeSurfer subject directory:

```bash
cd <full_path_to_subject_folders>
```

1. Produce a list that contains the subjects for which you want to run the subfield segmentation tool, e. g. by:

```
ls -d1 <your_subject_folder_root> > subjects.txt
```

Example: Your folders are labelled subject_001, subject_002 etc., then the command would be:

```
ls -d1 subject_* > subjects.txt
```

Double-check the list (no spaces, no other strings than subject folders etc.) by a standard text editor such as *jmacs* or *emacs* or *vi*.

2. Once checked, save the following lines as a script named *loop_all.sh* or use the respective script sent by mail:

```
#!/bin/bash
exec <subjects.txt
while read x; do
segmentHA_T1.sh $x
done
```

To make this script executable, type: chmod a+x loop_all.sh
Eventually, start the script by typing: ./loop_all.sh

Step 2: Collect the subfield volumes into a file *hippo_subfields.csv*

For this, two scripts *extract_subfields_v10.sh* (for FS6.0.0) and *extract_subfields_v21.sh* (for FS7.1.1) exist that collect all subregional volumes and the recon-all total hippocampal volumes along with three global volumes (eTIV, brain volume, total GM) into a file labelled *hippo_subfields.csv.*

Make sure the respective script is executable:

For FS6.0.0: `chmod a+x extract_subfields_v10.sh`
For FS7.1.1: `chmod a+x extract_subfields_hippamy _v21.sh`

Copy the respective script to your FreeSurfer subject directory. To make it loop through the right subject folders, there are two ways to customize the script:

<u>Way A</u>: Change the line
       for subj_id in $(ls -d WG_*); do
       to a name fitting your subject folders, e. g. subject_001, subject_002, would result in:
       for subj_id in $(ls -d subject_*); do
<u>Way B</u>: Use a subject list from a file, e. g. */home/user/somefolder/subjects.txt*
       Then, in the script the line
       for subj_id in $(ls -d WG_*); do
       would then need to be changed to:
       for subj_id in $(cat /home/user/somefolder/subjects.txt); do

       (Ideally you use the same *subjects.txt* file already defined during step 1.)

Eventually, run the script:

For FS6.0.0: `./extract_subfields_v10.sh`
For FS7.1.1: `./extract_subfields_hippamy _v21.sh`

The result is a comma-separated (CSV) file that can be opened in your favourite spreadsheet application (e. g. Excel) or a text editor (e. g. Emacs). The first row is a header describing the extracted regions and names for each column. Each row after the first gives the volume measures for each subject found in your FreeSurfer directory. In the next step, you will do a QC of the segmentation quality.

    <u>Note 1:</u> if you get error messages such as:
    extract_subfields.sh: line 13: printf: 491.769324: invalid number
    run the following command before running the sh extract_subfields.sh command:
    export LC_NUMERIC="en_US.UTF-8"

Note 2: When you edit the files in Excel, be sure to keep them in CSV format when you save and check whether the columns are still separated by commas and text strings are *not* enclosed by inverted commas.

Step 3: Outlier detection, histogram generation and QC support

Again, a case distinction needs to be made between FS6.0.0 and FS7.1.1. Generally, this step requires that you have R installed.

For FS6.0.0: Have the script *outliers_and_QC_support_hipp_v21.R* ready in the same folder as your subfield volume file *hippo_subfields.csv.*

1. Change directories to the location of your hippo_subfields.csv file generated in step 2.

   `cd <full_path_to_hippo_subfields.csv_file>`

2. Run the script:

   `R --no-save --slave < outliers_and_QC_support_`**`hipp_v21`**`.R > QCsupport.log`

For FS7.1.1: Have the script *outliers_and_QC_support_hippamy.R* ready in the same folder as your combined hippocampal subfield and amygdala nuclei volume file *hippoamy_subfields.csv.*

1. Change directories to the location of your hippoamy_subfields.csv file generated in step 2.

   `cd <full_path_to_hippoamy_subfields.csv_file>`

2. Run the script:

   `R --no-save --slave < outliers_and_QC_support_`**`hippamy`**`.R > QCsupport.log`

This will generate a file *QCsupport.log* that contains the result of two type of 'abnormality' checks:
- **Outliers** across the group (larger then 2 standard deviations): both the subject and the affected subfield or global variable are listed.
- **'Rank violations'**: Exploration of several large datasets demonstrated that subfield volumes show a rather stable ranking within a subjects:
  - Hippocampal tail volumes below rank #3 occur rarely.
  - The subiculum not being rank #4 occurs rarely.

NB: Just because a subject is an outlier or because it deviates from the mentioned rank orders does not necessarily mean they should be excluded from the analysis. The outlier detection should merely turn your attention to such cases, as extreme values may be a hint towards technical failure of the segmentation.

All filters may lead to overlapping cases, so the script eventually merges these and outputs a list of subjects that should be inspected. *Example:*

```
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%  Resulting minimum list of cases from steps 1 & 2 for visual QC  %
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
Perform QC on WG_050
Perform QC on WG_056
Perform QC on WG_075
Perform QC on WG_078
[...]
```

See **steps 4a-c** how to prepare the **visual QC** on these cases.
- For short hints towards the hippocampal QC, please see step 4c and page 10.
- For more details see our paper: https://onlinelibrary.wiley.com/doi/full/10.1002/hbm.25326

In addition, the **histograms** of each measure should be inspected. The **summary statistics** may be useful for raw value tables in publications.

In the original project these files were packed together and sent to the central analysis site.

```
tar -cf <YourSampleName>_SF_QC.tar *_hist_volumes.png SummaryStats_hippo_subfields.txt
```

Step 4: Preparing the visual QC

Visual Q/C means that images with an overlay of the hippocampal subfields on the T1 are viewed and assessed. As the subfield segmentation is based on the general delineation of the hippocampus during the general subcortical segmentation as part of the volume-based processing stream called by *recon-all*, the subfields usually (>98%) lie correctly in the hippocampal area.

Three scripts (Steps 4a to 4c) have to be run to prepare the actual visual Q/C.

Note: In case you have outliers in the distribution of one of the 3 global variables (eTIV, Brain, TotalGM), this requires going back to the Q/C of the segmentation of the cortical surfaces and cortical ribbons using ENIGMA scripts (https://enigma.ini.usc.edu/protocols/imaging-protocols/).

Step 4a: Preparation step

For FS6.0.0: Shell script **QC_subfields_step_1_prepare_v2.sh**
For FS7.1.1: Shell script **QC_subfields_step_1_prepare_v2_HA.sh**

This step creates a folder *assembly* within the already existing FreeSurfer folder *mri*. Copies of a background image (nu.mgz), the general segmentation result file (aparc+aseg.mgz) and the hippocampal subfields ([r/l]h.hippoSfLabels-T1.v10.mgz)[4] are put into this folder, images are transformed to NIFTI format and sampled to a resolution suitable for overlay. Before running the script, three adaptations within the *QC_subfields_step_1_prepare_v2.sh* need to be made:
- Adaptation 1: Define the variable **subject_folder** as needed.
- Adaptation 2: Define the location of FreeSurfer/bin (i. e. variable **FS_bin**).
- Adaptation 3: Define **the list if subjects**, i. e. change the line
    for subject_id in $(cat /home/philipp/listsubjects.txt); do
    to match the location and name of your subject list (ideally, again *subjects.txt* as defined).

Make sure the script is executable

For FS6.0.0:   chmod a+x QC_subfields_step_1_prepare_v2.sh

For FS7.1.1:   chmod a+x QC_subfields_step_1_prepare_v2_HA.sh

and run it (from any location) by calling:

For FS6.0.0:   ./QC_subfields_step_1_prepare_v2.sh

For FS7.1.1:   ./QC_subfields_step_1_prepare_v2_HA.sh

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[4] For FS7.1.1. the *combined* hippocamus/amygdala output is used: [l/r]h.hippoAmygLabels-T1.v21.FS60.mgz.

Step 4b: Create overlay images

For FS6.0.0:   Matlab script *QC_subfields_step_2_make_pngs_v2.m*
For FS7.1.1:   Matlab script *QC_subfields_step_2_make_pngs_v2_HA.m*

For this step you need Matlab and the (free) set of help functions to handle NIFTI images:
http://www.mathworks.com/matlabcentral/fileexchange/8797-tools-for-nifti-and-analyze-image

Make sure that the Matlab command *load_nii* is linked and can be called.

Then open the distributed script *QC_subfields_step_2_make_pngs_v2.m* (for FS6.0.0) or *QC_subfields_step_2_make_pngs_v2_HA.m* (for FS7.1.1) in the Matlab editor and change the 6 variables in the area "ADAPT AS REQUIRED" as follows:

Variable	Comment
subject_folder	Full link to your FS subject folder
dirOUT_general	Link to an (empty) folder into which the Q/C images will be written out.
HIPPOmap	Link to the distributed color-code table *HippoColors_v6.0.txt* (or *HippoColors_v7.0.txt* in FS7.1.1 that includes the amygdala label colors)
fid	Change this line so it matches the link to your subject file.
columnwidth	This is for a case that your subject list contains folder names of different length (e. g. "subject_sample_AGHG_01" next "to subject_X1"). If unsure, make a guess of the longest strong and add +10...
transparency	This defines, how transparent the subfields are when overlaid onto the T1-image [0=transparent, 1=opaque]. A value of 0.8 is a good starting point.
transparency_aseg	Transparency of overlay of recon-all based hippocampal mask; default is set to 0.15.

After these adaptations, run the Matlab script. It will take about 20-40 seconds per case, yet, note, that all cases are processed twice (first pass: classical output with colored subfields; second pass: T1WI plus overlay with aseg-result and hippocampal fissure, and T1-WI only). The result should be several *png files in the respective subject subfolders.

Step 4c: Collect all *png images into a browsable html-file

The step pulls together all *png files into a html file that can then be loaded into any standard browser. Please adapt the 'WG_' in the respective line in the *QC_subfields_step_3_webpage_v2.sh* script: subject_list=`ls -d1 WG_*` to match your subject folders' names.

If you want to reduce the amount of cases merged in the html-file (as some browsers crash in the case of too many subjects), there are two simple solutions:

(1) Move some cases temporarily away into a subfolder,

(2) restrict the selection for the subject_list,

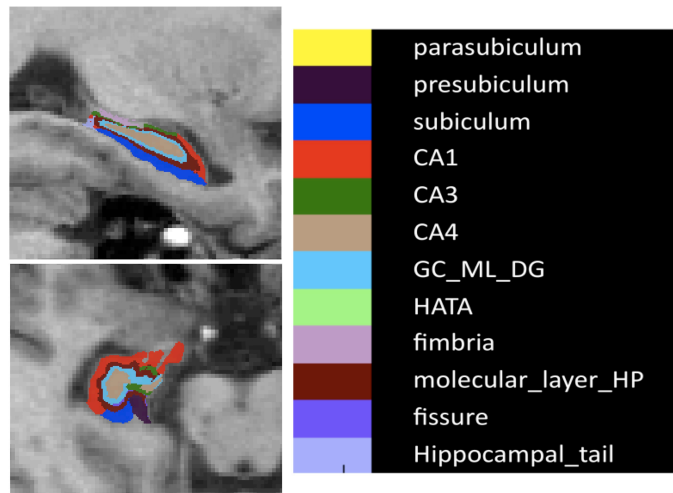When you call the script, the Q/C folder must be given as argument:

`./QC_subfields_step_3_webpage_v2.sh` *<full_path_to_QC_folder>*

Note: The folder likely corresponds to the variable *dirOUT_general* in the previous Matlab script.

The result is a browsable file *ENIGMA_HippoSubField_QC.html* that can be loaded into standard browser such as *Firefox* or *Explorer* or *Chrome* (in addition, working memory overload can be avoided by closing all other applications before). The scheme is as follows: 2 x 12 axial, 2 x 12 coronal and 2 x 12 sagittal images. The images can be enlarged by clicking on them.
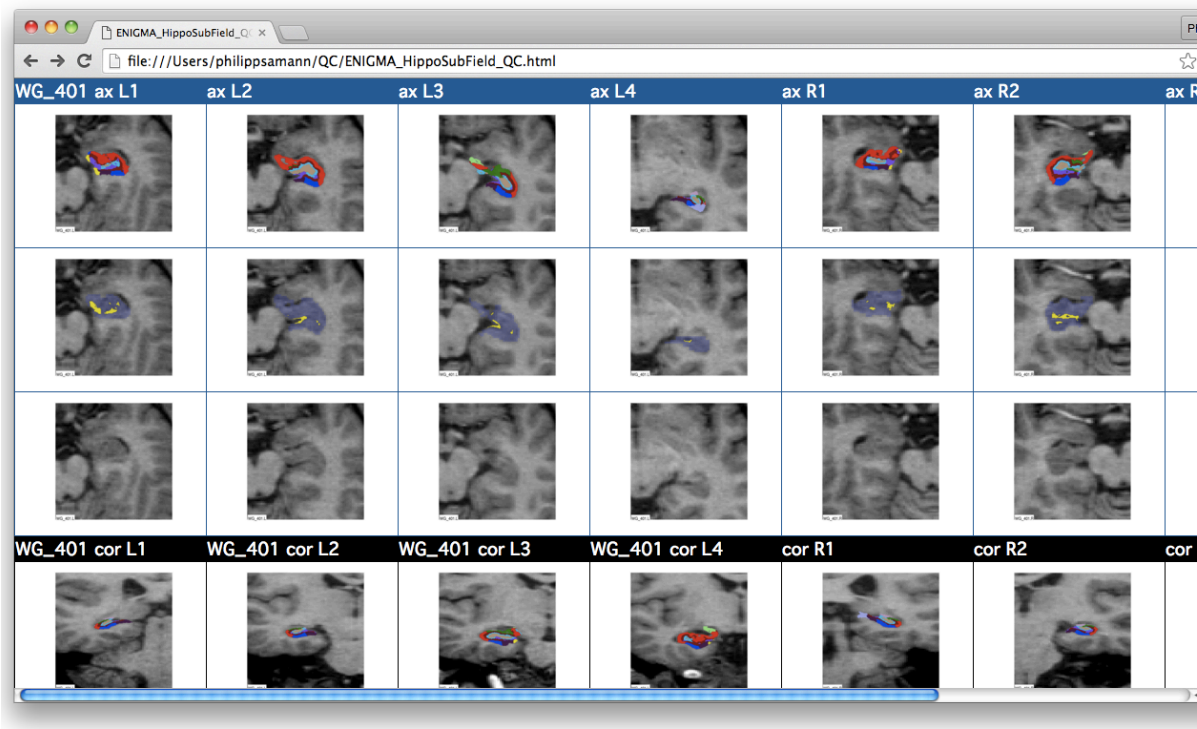
The **first** row shows the subfields with the color codes as listed on the right. Note that the hippocampal fissure is also included (purple). In the case of FS7.1.1 output the amygdala subnuclei will also be displayed.

Color	Subfield
yellow	parasubiculum
dark purple	presubiculum
blue	subiculum
red	CA1
green	CA3
tan	CA4
light blue	GC_ML_DG
light green	HATA
light purple	fimbria
dark red	molecular_layer_HP
purple	fissure
light blue/lavender	Hippocampal_tail

The **second row** shows an overlay of the T1 with
(a) the **whole hippocampus** (<u>plus the **amygdala** after combined segmentation in FS7.1.1</u>) as returned from the general subcortical segmentation (aparc+aseg.mgz file), depicted in blueish/transparent,
(b) the **hippocampal fissure** depicted in yellow.
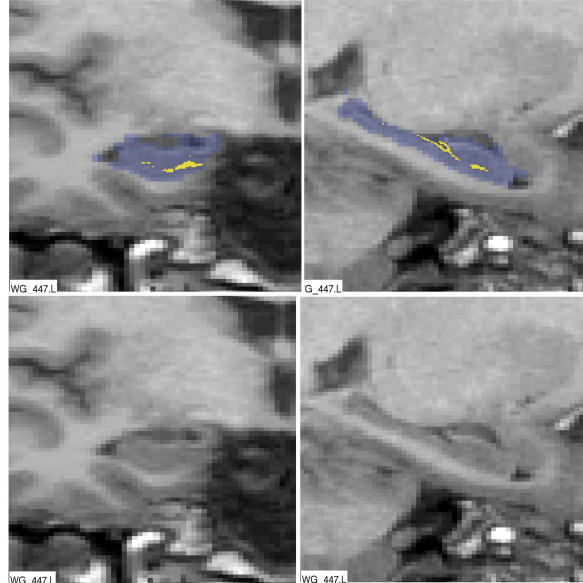
The **third row** shows the (bias-corrected) T1 without any overlay.

Note: In addition to browsing the (static) html-file, for a single case, one should also view at least about 10 cases dynamically by calling the FreeSurfer viewer within the subfolder *assembly:*

`freeview nu.gz left_discreteLabels.mgz`   (for the left hemisphere)
`freeview nu.gz right_discreteLabels.mgz` (for the right hemisphere)

If you switch the Color map to *Lookup Table* and select *FreeSurferColorLUT*, the same colors as in the html-file should be displayed in FreeSurfer. It is recommended to scroll through the cases to get an impression of the variability of the subfields and their appearance at different slice positions:
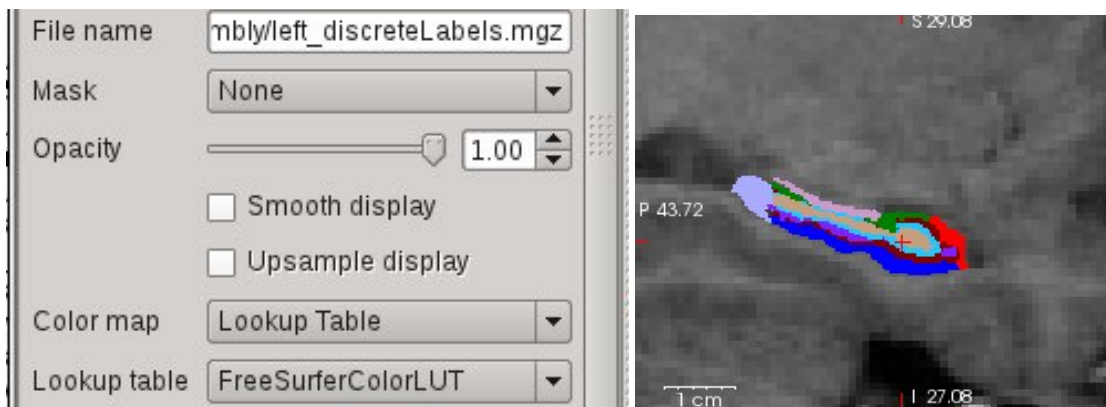
Note: While in the case of FS7.1.1 the field of view will automatically include the amygdala, no established rules exists for a QC of the amygdala so far.

Hints for the visual QC

The goal of the visual QC is to (i) get an overview of the segmentation quality of the whole sample, and (ii) to identify cases that should be excluded as a whole. We currently do not recommend to exclude single subfields of one case.

1. The general segmentation of the subcortical structures (the FreeSurfer volume processing pipeline) has probably undergone QC during previous projects, based on the standard ENIGMA protocols[5]. If not, the hippocampal segmentation result is now again displayed in the respective **2nd row** in the browser. Here, no larger portions of the hippocampus should be cut off, and the mask should not be shifted (by several millimeters) as sometimes seen in completely failed cases.

2. **Which cases should undergo visual QC?** Ideally, all cases are briefly checked for good positioning of the hippocampal mask as a whole, and fissure within the hippocampus. As minimum, cases list after executing the R script *outliers_and_QCsupport.R* should be reviewed.

3. The **hippocampal fissure** is generated by the segmentation algorithm, yet does not represent a proper tissue subfield but is a thin CSF space within in the complex hippocampal convolute. On the sagittal slices and most coronal slices, the fissure is typically positioned *within* the hippocampus mask. By assessing the fissure location one avoids to circularly use the subfields that are later used as variables of interest in the analysis. The corresponding **T1WI slices** (without overlay) are displayed in the 3rd row. Finding the fissure within the hippocampus (ideally in areas with corresponding low T1 signal) with its typical longish configuration on sagittal slices is a good indicator that the probability subfields atlas was properly coregistered with the T1WI.

4. **Should single subfields be excluded?** No, rather the whole subject is excluded in the case of severe segmentation problems. Technically, it is sufficent to set *one* subfield in the *hippo_subfields.csv* table to NA; the scripts will interpret this as complete exclusion. Below are four examples from typically varying samples that need not necessarily be removed (from left to right): (1) "Hole" in CA1 (red), (2) "hole" in CA3 (green) (caused by partial volume effects in relation with the alveus, a white matter structure not written out); (3) extension of the subiculum (blue) with neglectable volume effect), and (4) bulky CA.
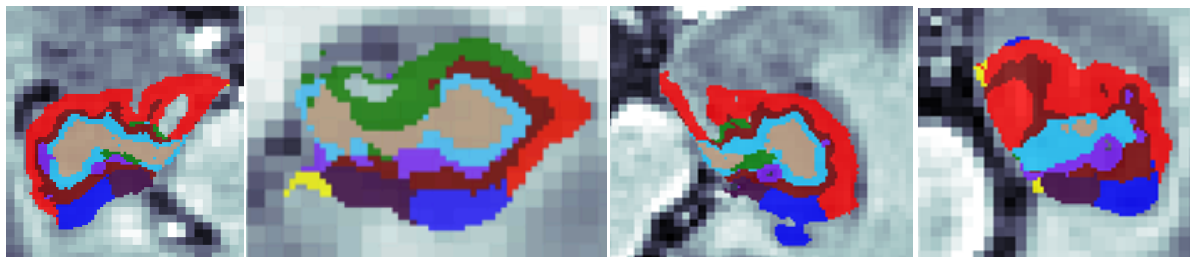
If you would like to discuss individual cases, the QC folder can be compressed and uploaded onto the MPIP ftp-server – access is provided after a short mail to `saemann@psych.mpg.de`

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[5] http://enigma.ini.usc.edu/protocols/imaging-protocols/quality-checking-subcortical-structures

Step 5: Exclude poor segmentations and pack files

Based on visual QC, you may have identified cases with poor segmentations or starting locations. You can try to re-run

```
recon-all -s <SubjID_to_be_rerun> -hippocampal-subfields-T1
```

for those cases and go through step 2–4 again (however, usually, there is high reproducibility in that the anew result will be very similar). If the result remains suspicious, exclude these volumes from *hippo_subfields.csv* by marking **any subfield with *NA***. Any values replaced by NA in any of the subfields (or the global volumes) will lead to exclusion of the whole case from histogram generation, the summary stats and the actual later main analysis. Therefore, it is important to re-run step 3 after marking cases with NA.

Step 6: Main analysis

*The following instructions provide you with a wide set of first level results useful for a subsequent meta-analysis. The prerequisite is a covariates file constructed exactly as specified in **syntax.pdf.***

The actual statistical analysis needs two input files:

1. hippo_subfields.csv

2. Covariates.csv

These input files need to be located in the folder from which you start R.

Then, call R and then check that the sure ppcor package is installed. Check by the command `search()` – if the ppcor library is not listed, install it by:

`install.packages("ppcor")`

and activate the library by

`library("ppcor")`

Eventually, run the analysis script by:

`source("SUBFIELD_ANALYSIS_v1.6.R")`

Three variables can flexibly be altered by the user, yet, it should first be run with the default settings:

whole	defines the global correction variable; default is '**eTIV**'; theoretical alternatives are 'Brain' or 'TotalGM'
write_logfile	**default = 1**: 0 = report messages on the fly; 1 = write logfile
AgeSq_and_interactions	**default = 1**, so age^2, gender-by-age and gender-by-age^2 are added to (mostly) all models; 0 = the three terms are excluded from the models

The output will by many *Rdata files and the *log-file (if requested). Please pack these files together using

`tar -cf *Rdata *log <YourSampleName>_SF_ANALYSIS.tar`

`gzip <YourSampleName>_SF_ANALYSIS.tar`

These files were then collected from the different sites and used for the ENIGMA-MDD Hippocampal Subfields meta-analysis. In case you are interested in such analysis and you would like to use existing R scripts for this, please contact Dr. med. P. G. Sämann (saemann@psych.mpg.de) or Dr. L. Schmaal (lianne.schmaal@unimelb.ed.au) for details.