SP2 Human Brain Organisation – Results for SGA2 Year 1 (D2.7.1 – SGA2)

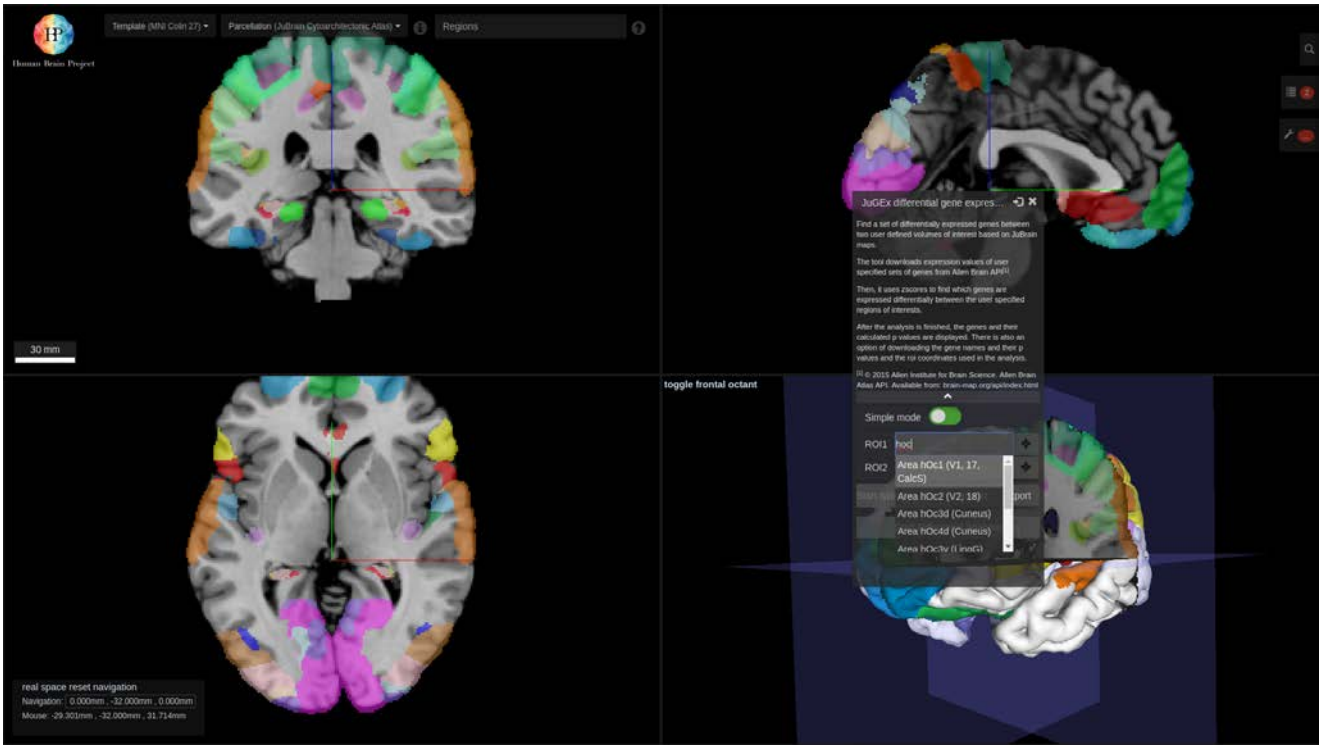


Figure 1: HBP interactive atlas viewer with the new version of the JuGEx plugin

Screenshot of the HBP interactive atlas viewer with the new version of the JuGEx plugin, which uses the new RESTful service hosted on the Neuroinformatics Platform.

Project Number:	785907	Project Title:	Human Brain Project SGA2

Document Title:	SP2 Human Brain Organisation – Results for SGA2 Year 1
Document Filename:	D2.7.1 (D14.1 D16) SGA2 M12 ACCEPTED 200730.docx
Deliverable Number:	SGA2 D2.7.1 (D14.1, D16)
Deliverable Type:	Report
Work Package(s):	WP2.1, WP2.2, WP2.3, WP2.4, WP2.5, WP2.6, WP2.7
Dissemination Level:	PU = Public
Planned Delivery Date:	SGA2 M12 / 31 Mar 2019, Request for revision: 22 Jul 2019
Actual Delivery Date:	Submitted: SGA2 M13 / 26 Apr 2019, Resubmitted: SGA2 M22 / 15 Jan 2020, Accepted: 30 Jul 2020

Authors:	Katrin AMUNTS, JUELICH (P20) Jean-Francois MANGIN, CEA (P11) Francesco PAVONE, LENS (P40) Pieter ROELFSEMA, KNAW (P91) Sabine BRADLER, JUELICH (P20)
Compiling Editors:	Sabine BRADLER, JUELICH (P20)
Contributors:	Timo DICKSCHEID, JUELICH (P20) Nicola PALOMERO-GALLAGHER, JUELICH (P20) Sarah GENON, JUELICH (P20) Irene COSTANTINI, LENS (P40) Huib MANSVELDER, VU (P113) Wim VANDUFFEL, KUL (P111) Cyril POUPON, CEA (P11) Markus AXER, JUELICH (P20) Dirk FELDMEYER, JUELICH (P20) Claus HILGETAG, UKE (P75)
SciTechCoord Review:	Martin TELEFONT, EPFL (P1)
Editorial Review:	Annemieke MICHELS, EPFL (P1)

Description in GA:	WPs involved: WP2.1, WP2.2., WP2.3, WP2.4, WP2.5, WP2.6 & WP2.7. Summary of SP Results for M1-M12
Abstract:	This Deliverable describes the first results of SP2 in the first year of SGA2.
Keywords:	Human Brain Atlas, Receptor densities, fibres, JuGEx, cell morphology and physiology, comparative studies,
Target Users/Readers:	Neuroscientists

Table of Contents

Table of Tables

Table of Figures

History of Changes made to this Deliverable (post Submission)

Date	Change Requested / Change Made / Other Action
26 Apr 2019	Deliverable submitted to EC
22 Jul 2019	Resubmission with specified changes requested in Review Report Main changes requested: • Change 1: More precise information on the generated data, including how these are or going to be used and precisely how informative generated datasets are for the HBP infrastructure. This has to be particularly improved for O3, O5, O6, O10, O14. • Change 2: Their current state of integration into the HBP frameworks (Collaboratory and other databases) must be clarified. • Change 3: An appendix should be included explaining how, when and where the information about fibre bundles and connectivity will be instructive for other SPs in Modelling and Simulation achievements. (p.4-5)
10 Jan 2020	Revised draft sent by SP/CDP to PCO. Main changes made, with indication where each change was made: • Change 1: section 3.2.1 is updated for outputs O3, O6, section 3.2.2 is updated for outputs O5, O10, O14, section 4.1.4 is updated for output O3. More information on how the generated data are informative for the HBP infrastructure was provided for O3, O5, O6, O10, O14. • Change 2: Annex A has been updated with new links to datasets/software components, related to an HBP repository. Also, information about their status of integration into the HBP frameworks have been added. • Change 3: an Appendix has been included that describes the different connectivity datasets and their application in Modelling and Simulation.
15 Jan 2020	Revised version resubmitted to EC by PCO via SyGMa

1. Overview

SP2 – Human Brain Organisation generates neuroscientific concepts, tools and data which are key to achieve a comprehensive understanding of the multilevel and multi-scale organisation of the human brain. SP2 researchers use a large set of methodologies to investigate the different levels of brain organisation, ranging e.g. from receptors, single cell analysis, cytoarchitecture, fibre architecture to functional networks and connectivity. One of the greatest goals of SP2 is to develop the HBP Human Brain Atlas, which can be used by neuroscientists all over the world, in neurosurgery, and as a basis to understand the differences between the healthy and diseased brain. SP2 researchers provide morphological and physiological data used for brain modelling and simulation. Results from functional imaging studies are used for applications in neurorobotics. Furthermore, SP2 provides ground-breaking insights into the micro-to-macro organisation of the complex human brain fibre architecture in cortical, subcortical and white matter regions, by combining different imaging techniques (high-field and strong gradient diffusion MRI, 3D-Polarised Light Imaging, and Two-Photon-Fluorescence Microscopy) to the same brain tissue.

2. Introduction

SP2 – Human Brain Organisation generates neuroscientific concepts, tools and data which are key to achieve a comprehensive understanding of the multilevel and multi-scale organisation of the human brain.

SP2 delivers results contributing to two SP Key Results:

Key Result KR2.1: Multi-model and multi-scale human brain atlas - Concepts, methods, maps and data. In the HBP consortium, especially in SP2, we have expertise in a wide range of methods/technologies to generate data on different levels and scales. A first set has already been generated in SGA1 and has been further developed in SGA2. These data are and will be continuously curated and integrated into the HBP Human Brain Atlas. The HBP Human Brain Atlas is a powerful and unified framework for multi-modal and multi-scale analyses addressing the brain's complexity, which cannot be found elsewhere, and is available to users in the HBP and to the general scientific community.

Key Result KR2.2: High-resolution reconstruction of nerve fibre architecture applying 3 different imaging techniques in the same brain sample. We have identified three complementary imaging techniques that make it possible to obtain high-resolution fibre reconstructions with an unprecedented level of complexity: (i) high-field and strong gradient diffusion MRI (dMRI), (ii) 3D-Polarised Light Imaging (3D-PLI), and (iii) Two-Photon-Fluorescence Microscopy (TPFM). Combining the different modalities opens up new vistas to interpret the measurements in terms of the underlying, scale-dependent morphological entities. We provide ground-breaking insights into the micro-to-macro organisation of the complex human brain fibre architecture in cortical, subcortical and white matter regions.

This Deliverable describes only the first set of results generated by SP2 in the first year of SGA2.

Most datasets, tools etc. will only be finalised in SGA2 M24 and described in the M24 Deliverable D2.7.2, since data generation and development of tools takes more than one year and will be only finalised by then. Results described under Key Result KR2.1 are the JuGEx tool, microscale alignment of histological sections, receptor distributions for hippocampal regions and layers, an interactive tool for cytoarchitectonic brain mapping, a machine learning algorithm for 3D segmentation and morphochemical classification of neurons, functional connectivity maps of hippocampus, results on figure-ground modulation, physiological recordings from human temporal neocortex interneurons, a protocol to stain SST, VIP and PV interneurons on SWITCH/TDE clearing samples, a pipeline for a cellular specific 3D-reconstruction of the human hippocampus, a procedure for labelling excitatory and inhibitory neurons, an ultra-fast large-area light-sheet microscope, results on cortical plasticity and perceptual learning induced by VTA stimulation, neural activity in the early visual system of mouse, monkey and human, attentional modulation of sensory processing, layer- and Cell Type-Specific Modulation of Excitatory Neuronal Activity in the Neocortex and an online version of the Economo and Koskinas Atlas. Results described under Key Result KR2.2 include a long and U-fibre bundle atlas, an HPC-based simulation of white matter tissue and a global tractography tool ready and usable for high resolution dMRI.

Further SP2 outputs can be found in Deliverables D2.1.1, D2.7.3 (CDP3 M12 compound Deliverable), D2.2.1 (CDP4 M12 compound Deliverable).

3. Key Result KR2.1 Multi-model and multi-scale human brain atlas - Concepts, methods, maps and data

3.1 Outputs

3.1.1 Overview of Outputs

Output 1: JuBrain Gene Expression (JuGEx) ID: C2254

Output 2: microscale alignment of histological sections ID: C2271

Output 3: receptor distributions for hippocampal regions and layers ID: C2315

Output 4: machine learning algorithm for 3D segmentation and morphochemical classification of neurons ID: C2354

Output 5: functional connectivity maps of hippocampus ID: C773

Output 6: figure-ground modulation ID: C2287

Output 7: physiological recordings from human temporal neocortex interneurons ID: C2352

Output 8: protocol to stain SST, VIP and PV interneurons on SWITCH/TDE clearing samples ID: C2353

Output 9: SWITCH immunohistochemistry technique integrated with the TDE clearing method ID: C2293

Output 10: labelling excitatory and inhibitory neurons ID: C2347

Output 11: ultra-fast large area light sheet microscope ID: C2349

Output 12: cortical plasticity and perceptual learning induced by VTA stimulation ID: C2469

Output 13: neural activity in the early visual system of mouse, monkey and human ID: C2470

Output 14: attentional modulation of sensory processing ID: C2471

Output 15: layer- and cell type-specific modulation of excitatory neuronal activity in the neocortex ID: C2345

Output 16: online version of the Economo and Koskinas Atlas ID: C3026

Output 17: manifolds of the cortical folding patterns ID: 2362

Output 18: multimodal alignment toolbox ID: C2259

Output 19: using manifold structure for functional connectome matrices estimation ID: C2291

3.1.2 Output 1 - JuBrain Gene Expression (JuGEx)

Software component: SGA2 T2.6.1 Query and analysis tool for Allen human gene expressions grouped by atlas regions, ID:C2254

Leader: Timo DICKSCHEID

Extension and deeper atlas integration of the JuGEX tool for differential gene expression analysis: We continued the work on integrating the JuGEx (JuBrain Gene Expression) method for differential gene expression analysis in different brain regions with the HBP atlas services. See http://www.fz-juelich.de/inm/inm-1/DE/Forschung/_docs/JuGex/JuGex_node.html (Figure 2). After rewriting the original Matlab Code into a Python library, this tool has now been extended and incorporated into the Neuroinformatics Platform as a RESTful service. To access this service, an interactive plugin for

the interactive atlas viewer (link to component C2802 in Task T5.4.3) has been developed. HBP users now have access to this method via jupyter notebooks, or directly from the interactive viewer.

A major new feature is that users now have much more flexibility to configure the brain regions used for the analysis. In particular, they can choose a threshold on the probability of each cytoarchitectonic area to specify an input region. To retrieve the thresholded area masks from the Neuroinformatics Platform, a new prototype back end service has been developed ("pmap service"). The pmap service acts as a specific image provider, which thresholds existing images on request, and allows merging multiple thresholded images into one to create a binary mask. In the case of JuGEx, this allows to select a probability threshold, and to use multi-area selections as an input.

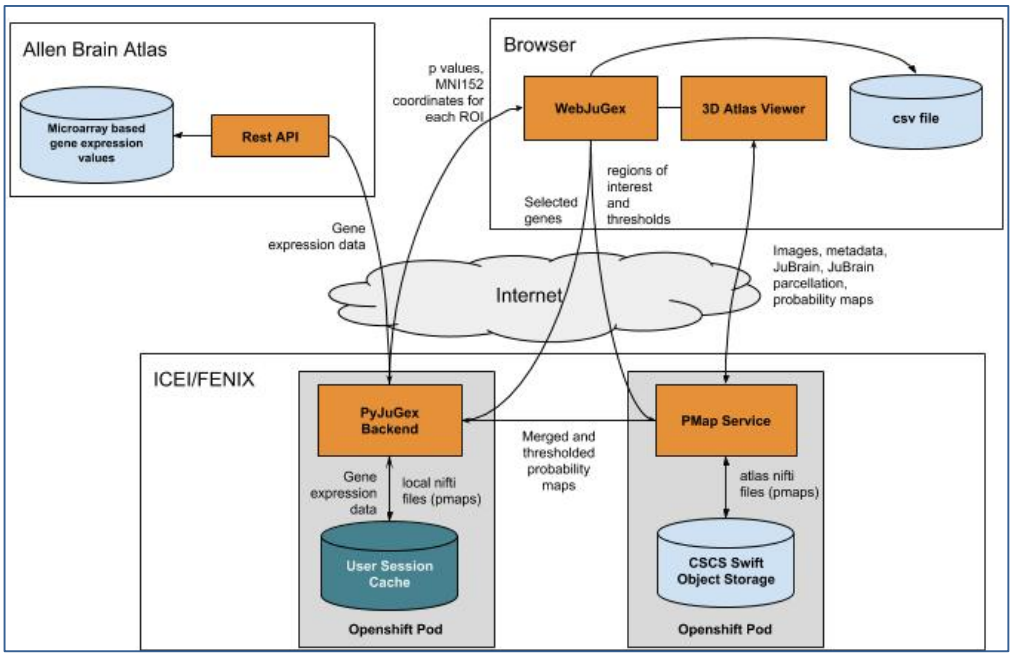


Figure 2: Software architecture of Web-JuGEx integrated in the HBP ecosystem.

3.1.3 Output 2 - microscale alignment of histological sections

Dataset component: SGA2 T2.6.2 Layer-specific 3D cell densities in human visual cortex, ID:C2271

Leader: Timo DICKSCHEID

Improved micro-scale alignment of histological sections: We investigated whether the precision of a 3D-reconstruction from cell-stained serial sections, imaged at 1 micron, can reach the level of individual cells. While previous work has exploited trajectories of vessel structures for cross-section alignment (extended manuscript currently under revision], we have now developed an algorithm for identifying large numbers of pairs of bisected cell bodies in neighbouring tissue sections. This is challenging as the shape and appearance of cell bodies is highly repetitive, and the fraction of bisected cells is in the order of 20-40% at the typical section thickness of 20 micrometres. Based on the novel detection and matching algorithm, we have successfully computed the first reconstructions of local regions of interest (ROI) that resolve microstructures at the level of individual cells. These reconstructions use depth focusing to image more than 20 virtual sections at different levels of depth inside each physical tissue section. A manuscript for the method is now in preparation, and the first of these cellular resolution ROIs will go into the Human Brain Atlas in the second half of 2019.

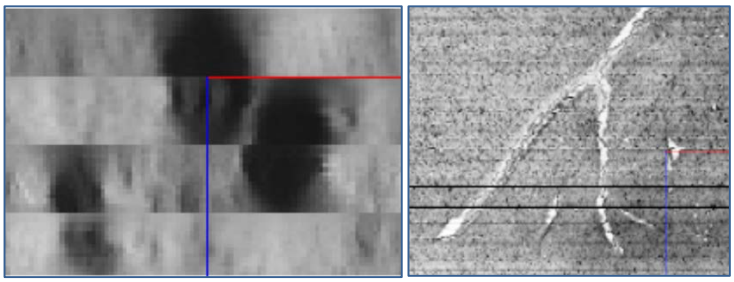


Figure 3: Reconstructed cells and vessels from histological sections

Left: Detail of a cross-sectional view into 4 physical tissue sections, each imaged at 20 different focal depths, leading to a total of 80 optical sections that have been aligned by explicitly matching pairs of bisected cells using our novel algorithm. Right: Larger area of a similar cross-sectional view in a region of interest, depicting a vessel bifurcation that has been reconstructed with high precision.

3.1.4 Output 3 - receptor distributions for hippocampal regions and layers

Dataset component: SGA2 T2.3.5 Quantification of multiple receptor distributions for hippocampal regions and layers, ID:C2315

Leader: Nicola PALOMERO-GALLAGHER

We provided densities in fmol/mg protein of receptors for GABA and glutamate in the dentate gyrus (DG) and Cornu ammonis 1-3 (CA1-CA3) regions of the rat and human hippocampus. For the rat hippocampus, the dataset also provides densities measured in the oriens, pyramidal, radiatum and lacunosum-molecular layers of the CA1-CA3 regions, in the lucidum layer of the CA3 region, as well as in the molecular and granular layers and in the hilus of the DG.

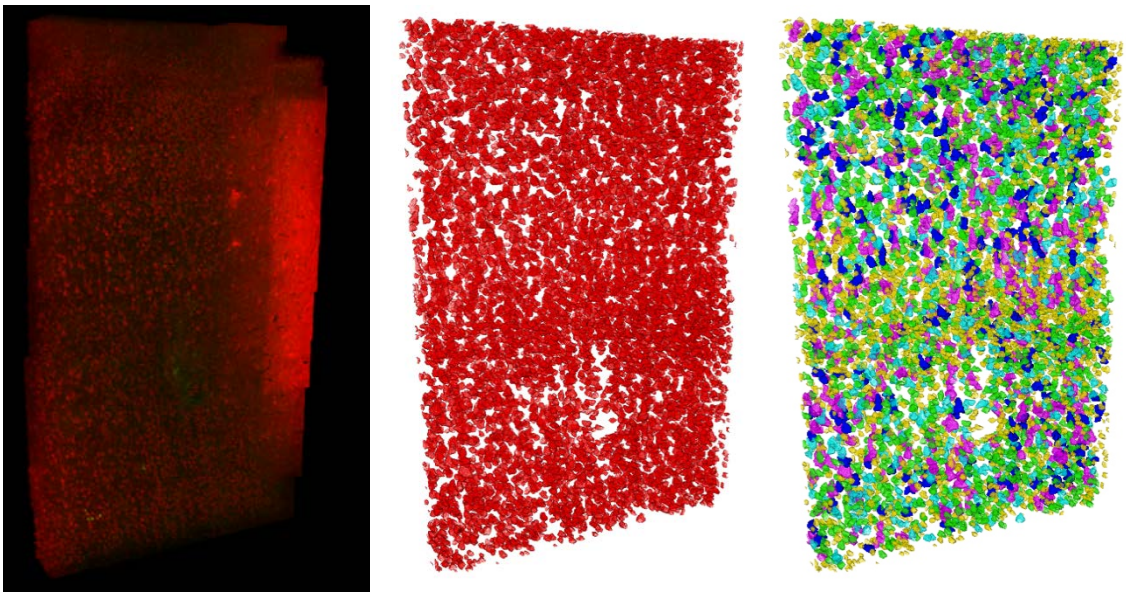
In the rat hippocampus, we quantified the density of the GABAergic GABAA receptor, in the human hippocampus the densities of the GABAergic GABAA and GABAB receptors, as well as of the GABAA associated benzodiazepine binding sites. In both species, we quantified the densities of glutamatergic AMPA, NMDA and kainate receptors. This dataset characterises the molecular basis of excitatory and inhibitory neurotransmission in the dentate gyrus (DG) and Cornu ammonis 1-3 (CA1-CA3) regions of the hippocampus of both species.

3.1.5 Output 4 - machine learning algorithm for 3D segmentation and morphochemical classification of neurons

Software component: SGA2 T2.6.4 Morphological classification of neurons with immunohistological staining, ID:C2354

Leaders: Giacomo MAZZAMUTO, Timo DICKSCHEID

We have developed a machine-learning algorithm for 3D segmentation and morphochemical classification of neurons in 2-photon fluorescence microscopy, and successfully applied this for the first time to segment images acquired with two-photon fluorescence microscopy from various subjects, as illustrated in Figure 4.



Microscopic image | Segmentation | Morphochemical classification

Figure 4: neurons in 2-photon fluorescence microscopy

3D segmentation and morphochemical classification of neurons in 2-photon fluorescence microscopy.

3.1.6 Output 5 – functional connectivity maps of hippocampus

Dataset component: SP2 - Selected multi-modal regional maps with cognitive features, ID:C773

Leader: Simon EICKHOFF, Sarah GENON

By using brain parcellation applied to neuroimaging markers of functional connectivity, we generated robust maps of the functional organisation of the hippocampus in healthy adults. These maps feature a dominant differentiation across the anterior-posterior axis that was characterised with regards to behavioural functions with large datasets of activation studies. Applying the same CBP (connectivity based parcellation) approach to neuroimaging markers of co-plasticity at the macro-scale (structural covariance) generated complementary maps featuring a differentiation along the medial-lateral axis with close resemblance to the previously known cytoarchitectonic subdivisions (Figure 5). Our maps are openly available at http://anima.fz-juelich.de/studies/Plachti_Hippocampus_2019 and the conceptual ideas supported by this work are discussed in two reviews.

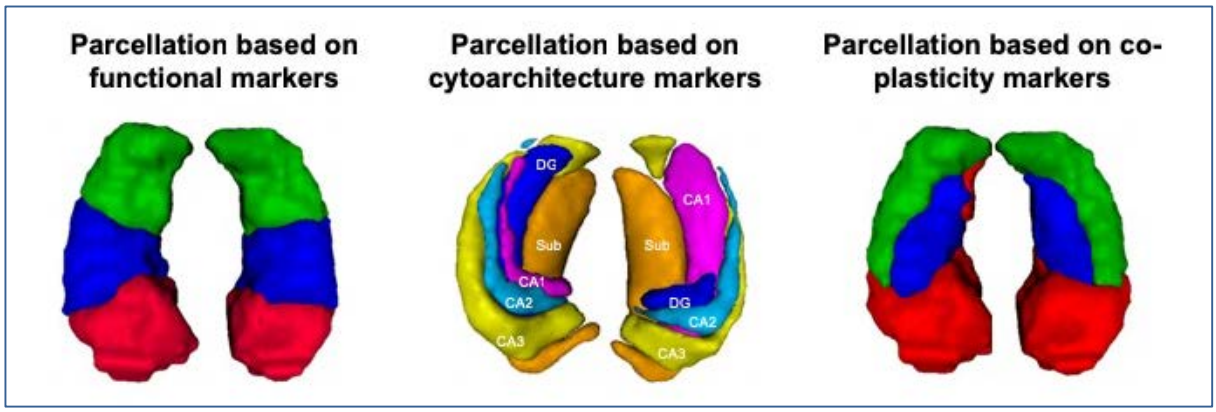


Figure 5: Parcellations of the Hippocampus

Maps of the functional organisation of the hippocampus in healthy adults using brain parcellation applied to neuroimaging markers of functional connectivity.

Most studies of the hippocampus in brain pathologies are based on hippocampus's model focusing on cytoarchitecture subfields. However, our review work on brain organisation and function suggests that complementary maps, in particular based on functional data, are relevant to study and understand dysfunction in pathologies such as dementia and psychiatric diseases.

Our parcellation of the hippocampus has been published. The manuscript has appeared online only very recently (2019) and accordingly, the maps were released online only in March 2019.

3.1.7 Output 6 - figure-ground modulation

Dataset component: SGA2 - T2.2.2 Neuronal mechanism transforming a visual stimulus into an eye movement plan, ID:C2287

Leader: Pieter ROELFSEMA

We recorded from superior colliculus (SC) while monkeys made saccades towards figure-ground stimuli and compared SC figure-ground responses to those of visual cortex (V1 and V4) and frontal cortex (FEF). We found that SC shows figure-ground modulation (FGM), already on a single cell level. Interestingly, FGM is faster and occurs on a larger spatial scale than in V1. Furthermore, within SC itself, across the different SC layers, a transformation from visual information to a motor response occurred. FGM correlates with saccade onset and landing position. Moreover, FGM was found to be cue invariant and also present for more complex figure-ground displays. FGM also occurred in the absence of attention, an effect that is not seen in FEF and V1. Thus, SC is not only able to quickly process complex visual stimuli, but also uses the visual information to plan precise saccades towards these stimuli. This suggests that SC is part of an evolutionary preserved system that quickly detects salient stimuli and enables making fast saccades towards these stimuli. Later in time, SC probably interacts with the visual and frontal cortex to accomplish a more detailed representation of the stimulus and guide (more cognitive) behaviour according to this.

3.1.8 Output 7 - physiological recordings from human temporal neocortex interneurons

Dataset Component: SGA2 T2.5.2 Physiological data recording of interneurons from human and mouse temporal neocortex; ID:C2352

Leader: Huib MANSVELDER

Physiological data from human temporal neocortex interneurons were acquired from somatostatinergic, VIP, and parvalbuminergic interneurons. In this study, we find that cholinergic inputs selectively augment and speed up lateral inhibition between pyramidal neurons mediated by MCs, but not by BCs. Optogenetically activated cholinergic inputs depolarize MCs through activation of ß2 subunit-containing nicotinic AChRs, not muscarinic AChRs, without affecting glutamatergic inputs to MCs. We find that these mechanisms are conserved in human neocortex. Cholinergic inputs thus enable cortical pyramidal neurons to recruit more MCs, and can thereby dynamically highlight specific circuit motifs, favoring MC-mediated pathways over BC-mediated pathways. See Obermayer *et al*., Nature Commun. 4101 (2018).

3.1.9 Output 8 - protocol to stain SST, VIP and PV interneurons on SWITCH/TDE clearing samples

Dataset component: SGA2 T2.5.2 Integration of physiological data recording from human temporal neocortex and 3D morphological reconstruction of the same interneurons, ID:C2353

Leaders: Leonardo SACCONI, Irene COSTANTINI

Morphological characterisation of interneurons with two-photon fluorescence microscopy: A protocol to stain SST, VIP and PV interneurons on SWITCH/TDE clearing samples was developed. Imaging of samples are done with a custom-made two-photon fluorescence microscope that allows to obtain a resolution of 0.5 x 0.5 x 2 µm in slices of 500 µm of thickness.

3.1.10 Output 9 - SWITCH immunohistochemistry technique integrated with the TDE clearing method

Dataset Component: SGA2 - T2.3.1 Layer-specific excitatory and inhibitory neuronal maps of hippocampus. ID:C2293

Leaders: Francesco PAVONE, Irene COSTANTINI

Setting a pipeline for a cellular specific 3D reconstruction of a whole human hippocampus at microscopic resolution: The three-dimensional reconstruction of large volumes of the human neural networks at cellular resolution is one of the biggest challenges of our days. Commonly, fine slices of samples marked with colorimetric techniques are individually imaged. This approach, in addition to being time-consuming, does not consider spatial cell organisation, leading to information loss. To overcome these limitations, we develop a methodology that allows analysing the cytoarchitecture of the human brain in three dimensions at high resolution. In particular, we exploit the possibility of combining high-resolution large-areas 3D-imaging techniques with clearing methodologies. In particular, in this task, we successfully integrate the SWITCH immunohistochemistry technique (Murray *et al*. 2015) with the TDE clearing method (Costantini *et al*. 2015),Figure 6.

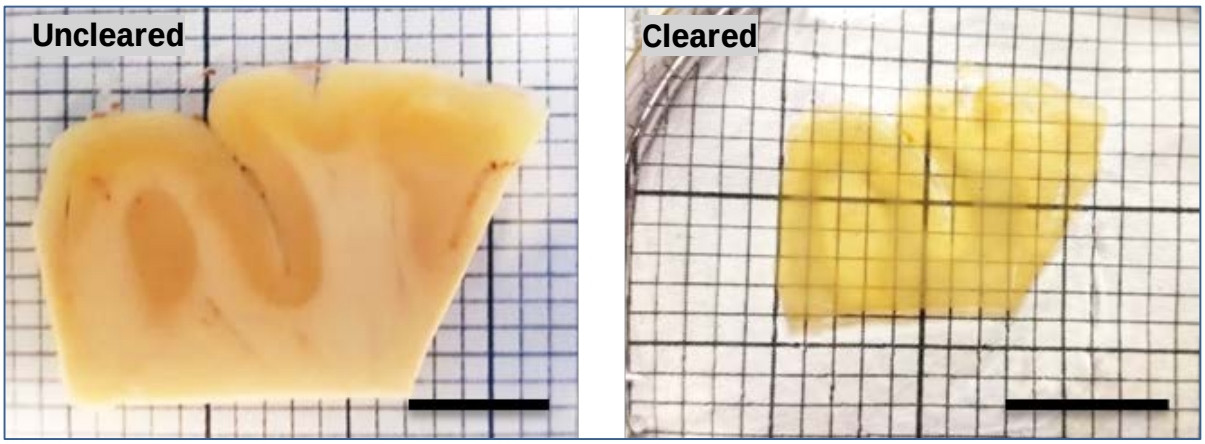


Figure 6: SWITCH/TDE cleared human cortex

A slice of human brain cortex before and after the SWITCH/TDE clearing. Scale bar = 1 cm

3.1.11 Output 10 – labelling excitatory and inhibitory neurons

Report Component: SGA2 T2.3.2 Multiple staining for characterisation of different neuronal types, ID:C2347

Leader: Irene COSTANTINI

Implementation of a multi-staining on SWITCH/TDE treated samples: In order to perform a discrimination between excitatory and inhibitory neurons we developed a labelling procedure that co-stains all the neurons with an anti-NeuN antibody and the inhibitory neurons with an anti-GAD67-65 antibody. Using the segmentation tools implemented in Task T2.6.4 we are able to classify the neurons with the present of one or multiple staining. Using the co-staining, by subtraction, we can discriminate between the two neuronal types: if the cell presents both NeuN and GAD staining is inhibitory, while if presents only the NeuN staining is excitatory.

3.1.12 Output 11 - ultra-fast large area light sheet microscope

Report Component: SGA2 T2.3.2 Integration of ultra-fast large-area fluorescence microscope with Switch clearing techniques, ID:C2349

Leaders: Leonardo SACCONI, Irene COSTANTINI

Building the ultra-fast large area light sheet microscope: To image large volumes of human brain tissue at high resolution in short time we built an ultra-fast inverted light sheet microscope (Figure 7). The microscope has the following characteristics: isotropic 1 um resolution, up to 4 channels (markers), designed to image 0.5 mm thick samples, high data through-put (up to 3 GB/s), confocal line detection (high contrast), real-time autofocus (high sharpness), volumetric rate: 0.1 mm3/s. The implemented methodologies allow to reconstruct large volumes of human brain tissues in short time, giving the morphological characterisation of the sample at high resolution.

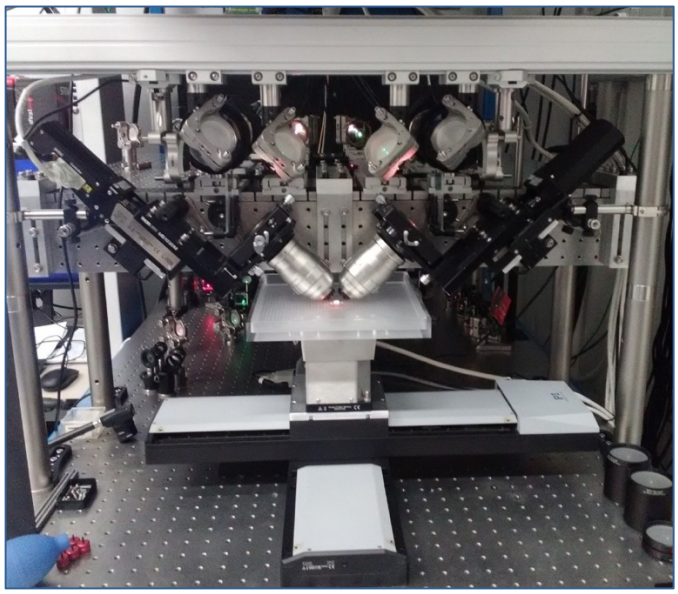


Figure 7: Photo of the inverted ultra-fast large-area light sheet microscope.

3.1.13 Output 12 - cortical plasticity and perceptual learning induced by VTA stimulation

Report component: SGA2 T2.5.5 Feedback interactions in monkey and human, ID:C2469

Leader: Wim VANDUFFEL

Paper on cortical plasticity and perceptual learning induced by VTA (ventral tegmental area) stimulation is resubmitted. We have finished an additional set of challenging experiments (both behavioural and fMRI) on monkeys, in which we fully control for selective spatial attention. From these experiments, we can conclude, that the plasticity effects can be induced while the monkeys are not consciously perceiving the stimuli that were paired with VTA stimulation (Figure 8). The data are almost entirely analysed and we are preparing a manuscript for publication.

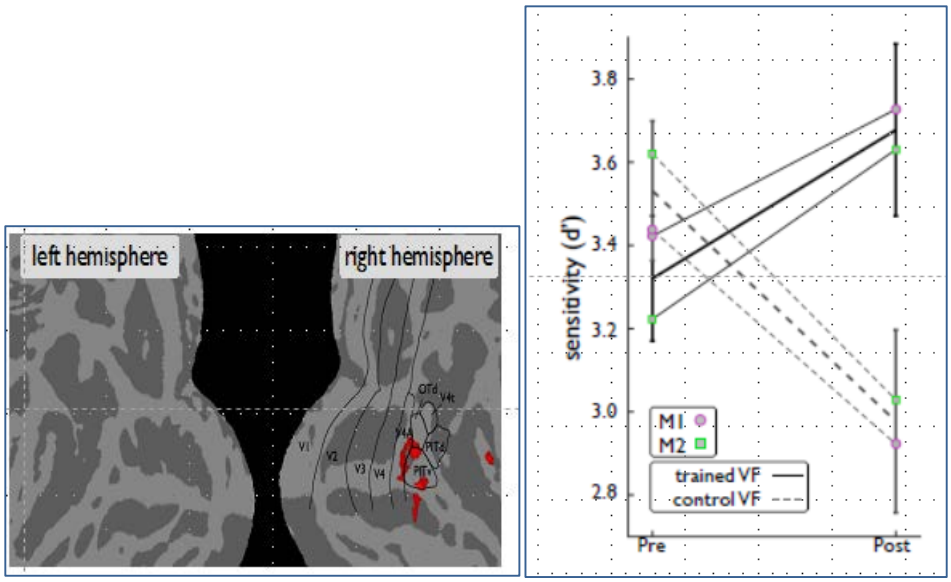


Figure 8: Cortical plasticity and perceptual learning induced by VTA stimulation.

Left: Cortical plasticity in monkey induced by pairing a visual stimulus with electrical stimulation of the ventral tegmental area. Right: Perceptual improvement (d-prime) in discriminating a visual stimulus indicated by stimulating the VTA in primates.

3.1.14 Output 13 - neural activity in the early visual system of mouse, monkey and human

Report Component: SGA2 T2.5.6 Comparative mapping of visuo-motor cortex in monkey and human, ID:C2470

Leader: Wim VANDUFFEL

In Task T2.5.6. we are comparing neural activity in the early visual system of three species (mouse / monkey / human) while the subject perceptually segregates the scene into figures and background. We have found remarkable similarities between the three species in the time course of spiking activity (Figure 9), suggesting that the mechanisms of figure-ground segregation may be conserved across species. The work has produced two novel data-sets: 1) monkey laminar electrophysiological data from V1 showing the laminar distribution of figure-ground modulation and 2) exceptionally rare electrophysiological data from human V3 showing that spike rates are modulated by figure-ground assignment in the human visual cortex. Mouse data-sets and fMRI data-sets in human and monkey are currently nearing completion.

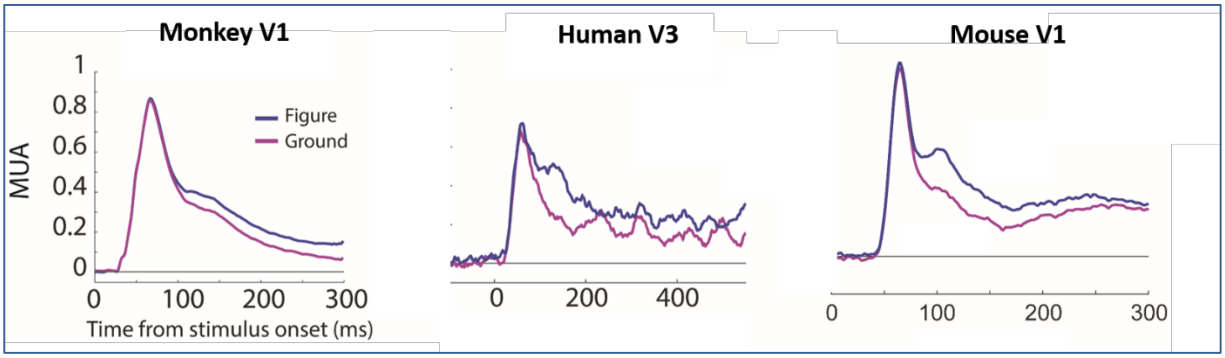


Figure 9: Spiking activity in monkey V1, human V1 and mouse V1

Multi-unit spiking activity recorded from macaque monkey V1, human V3 and mouse V1 showing that spike rates are increased on figures (blue lines) compared to backgrounds (purple lines). In all three species, the modulation begins after a delay at around 100ms.

Two other experiments directly compared the functional architecture in human and non-human primates (NHP). In the first experiment, we aimed to identify the cortical regions involved in fusing two depth cues (disparity and motion parallax). Several previous studies indicated the involvement of human area V3B/KO in this process. Functional imaging showed that monkey MT is a key player in this cue integration process, indicating that although the dorsal stream in both species is important for integration of depth cues, this happens in slightly different areas. Thus, although human and monkey MT share many functions, this is not the case for all functionalities. (paper *in press* in Plos Biol, Figure 10).

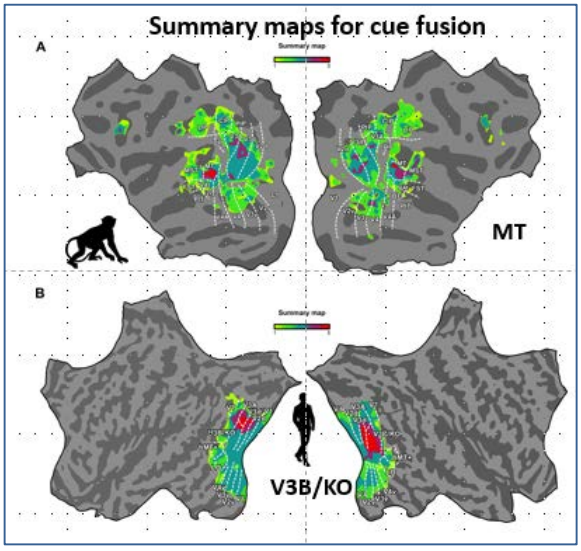


Figure 10: Depth cue fusion in human and monkey.

In the second experiment, we performed a sub-mm resolution mapping experiment in rhesus monkeys. This revealed, unexpectedly, a highly similar topographic lay-out of early dorsal visual cortex in old and new world monkeys, significantly revising the currently prevailing models of visuotopic organisation of this part of the cortex. Moreover, this invites for a re-assessment of the retinotopic organisation of this part of human visual cortex using very high resolution imaging. (PNAS paper, doi: 10.1073/pnas.1805561116, Figure 11). The high resolution retinotopy data is a unique data set. There is not a single other study that acquired whole-brain fMRI at 0.6 mm resolution in the awake rhesus monkeys.

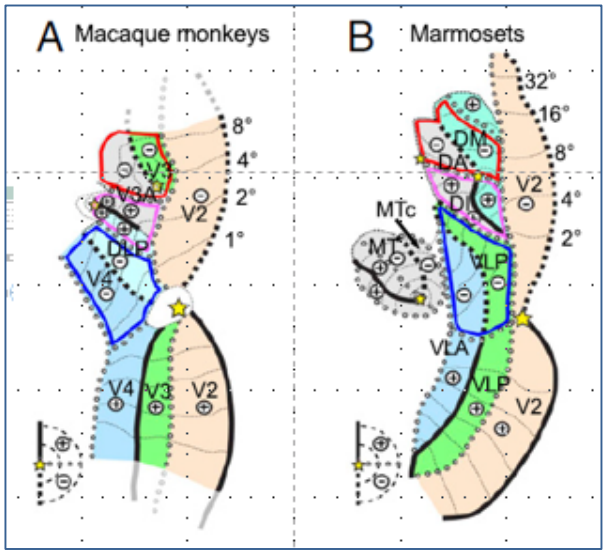


Figure 11: Novel retinotopic model in early visual cortex of NHP.

Finally, we performed a multi-modal (tactile, visual, auditory stimulation) comparative event related fMRI experiment in human and NHP using our recently developed interspecies activity correlation (ISAC) and interspecies beta correlation (ISBC) tools. The data revealed strong evidence

for multi-modal areas in the two species. We use the functional signals derived from one species to analyse those in the other. These cross-species predictors have significantly higher explanatory power compared to the that of the stimulus design. A paper is in preparation.

3.1.15 Output 14 - attentional modulation of sensory processing

Dataset Component: SGA2 T2.5.7 Attentional modulation of sensory processing in monkey and human, ID:C2471

Leader: Wim VANDUFFEL

We identified with fMRI a potential monkey homologue of a region in the human superior parietal lobule (SPL) that is involved in shifting spatial attention (Arsenault *et al*. 2018 and Caspari *et al.*, 2018), see Figure 12. Guided by the fMRI maps in the monkey, we started to record single unit activity with laminar probes while monkeys performed exactly the same attentional paradigm (by which we can dissociate sustained attention and changes in stimulus and motor events from the attentional shifting events). We have now recorded >180 units in one animal from which ~ 50% show shift selectivity. Quite interestingly, we find only shift neurons in the fMRI foci showing shift selectivity. We just started training a second animal on the task and hope to finish the acquisition of the data by SGA2 M18. In addition, we showed fast compensations after reversible inactivation of parietal area LIP during a covert selective attention task (a serial search task and a pop-out search task) by combining fMRI with muscimol injections during task performance. The study is published in Cerebral Cortex (Balan *et al.*, 2018). Finally, Wim VANDUFFEL wrote a commentary in Neuron on a paper promoting data sharing of non-human primate data - which was authored by several HBP members.

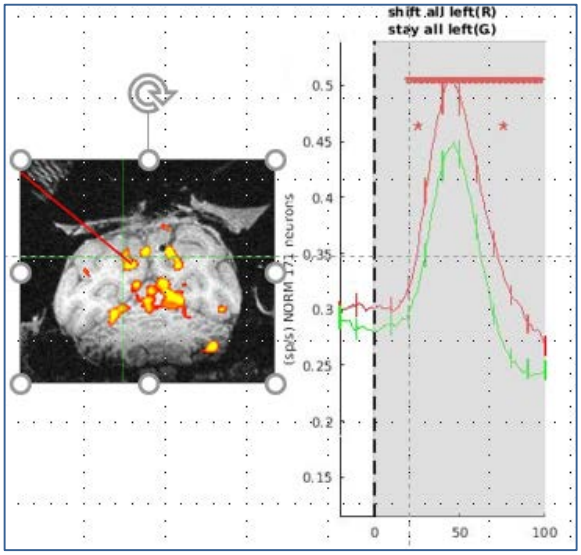


Figure 12: Shift selectivity recorded in shift-selective fMRI patch in V6A of a monkey

Normalised response of all 171 neurons recorded, without making any selection based on their selectivity.

3.1.16 Output 15 - layer- and cell type-specific modulation of excitatory neuronal activity in the neocortex

Dataset Component: Quantitative analysis of neuromodular function in the rodent neocortex, ID:C2345

Leader: Dirk FELDMEYER

We investigated neuromodulation by acetylcholine (ACh) in corticothalamic- and corticocortical-projecting layer 6 (L6) pyramidal cells and found that ACh activates different types of muscarinic (M1 and M4 ACh receptors, respectively) by either depolarising or hyperpolarising and these neurons; likewise, synaptic transmission is either increased or decreased suggesting a differential cholinergic modulation of L6 excitatory neuron activity. In addition, nicotinic ACh receptors are exclusively found in corticothalamic L6 pyramidal cells; their activation results in an enhanced synaptic transmission. This novel cell-specific modulation of synaptic signalling has not been described in previous publications. In a second project, we investigated the effects of the neuromodulator adenosine on the activity of L6 pyramidal neurons in prefrontal and somatosensory cortex. We found that in prefrontal cortex pyramidal cells were much more susceptible to adenosine modulation than in somatosensory cortex. See: Radnikow and Feldmeyer Front Neuroanat 12:1 (2018). doi: 10.3389/fnana.2018.00001

3.1.17 Output 16 – online version of the Economo and Koskinas Atlas

Dataset component: SGA2 T2.6.7 Online version of von Economo and Koskinas atlas, ID:C3026

Leader: Claus HILGETAG

Currently, the classic cytoarchitectonic atlas of von Economo and Koskinas (vEK) is only available as a paper-based 2D atlas in non-standard space. Recent efforts manually mapped the vEK parcellation onto the FreeSurfer Desikan-Killiany atlas, based on textual descriptions and landmarks in 2D drawings. To overcome the limitations of this approach, we explicitly defined a virtual 3D vEK model independent of existing reference geometries, which became possible through 3D scanning of two individual, well-preserved 3D plaster models of the vEK parcellation manufactured in the era of von Economo. Significant efforts have gone into reconstructing the 3D model and preparing the atlas for integration with BigBrain - and by extension, TheVirtualBrain, which will act as the enabling infrastructure for comprehensive large-scale brain network modelling in the HBP.

These data, comprising systematic quantitative macroscopic as well as microscopic anatomical descriptors, such as layer thickness, cell density and cell sizes, are essential for linking fundamental aspects of macroscopic and microscopic cortical organisation and connectivity. The inclusion of the vEK atlas into the mentioned open-source infrastructure offers the prospect of reliably mapping human cytoarchitectonic information into common cortical parcellation schemes, enabling and advancing the enrichment of the human connectome.

This task came in via a Call for Expression of Interest in SGA2 and has started with a delay. Therefore, the online version of the Economo and Koskinas atlas will be delivered in M15 instead of M12.

3.1.18 Output 17 – manifolds of the cortical folding patterns

Task T2.4.1 aims at designing methods to quantify the intersubject variability of the brain architecture and to relate this variability to behaviour and pathologies, taking into account needs emerging from other efforts of the HBP. The first target is the overwhelming variability of the cortical folding pattern, which has never been deciphered and has a negative impact on most of the methodologies used in neuroimaging. In the context of the HBP, we aim at improving our models of the short fibre bundles, which circumvent the cortical folds. The second target is functional connectomes, which variability is difficult to quantify because of noisy and short time-series acquired with resting state fMRI. We need a robust strategy to define these connectomes at the core of modelling activities.

Dataset component: SGA2 T2.4.1 Dictionary of cortical folding patterns, ID:C2362

Leader: Jean-Francois MANGIN

We have inferred ten different 6-dimensional manifolds describing the variability of the morphology of the sensori-motor areas and of the external temporal lobe. This new representation has been

computed from the 1000 subjects of the outstanding Human Connectome Project (HCP) dataset. The heritability of this morphological variability has been inferred from the twin structure of the HCP. The links between morphological variability, functional maps and cognitive tests have been explored. New insight about the hidden links between the cutting edge cortical parcellation of Glaser (Nature, 2016) and the variability of the morphology have been discovered (Figure 13).

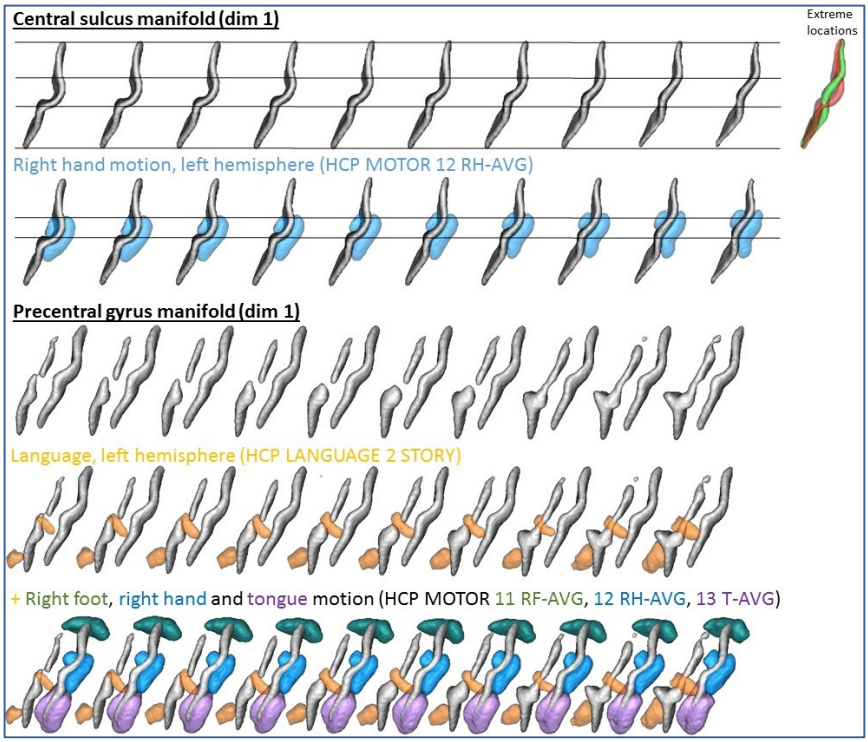


Figure 13: Sulcus variability

Top: variability of the hand area along central sulcus; Bottom: variability of Brodmann 44 language area versus morphology of the motor gyrus morphology.

3.1.19 Output 18 – multimodal alignment toolbox

We are refining an alignment toolbox aiming first at creating a matrix of nonlinear transformation performing seamless alignment of the different reference spaces embedded in the EBRAINS human atlas. This toolbox will also be embedded in the back end of EBRAINS to allow accurate alignment of some of the data uploaded by the users, including multi-modal datasets. A key feature of the toolbox developed during the past projects is the possibility to force the alignment of some cortical sulci to achieve a controlled alignment of very dissimilar modalities (i.e. *in vivo* and post mortem imaging). It was used to compute the alignment of template spaces (BigBrain, MNI 152, Collins, etc.). Current efforts aim at using the toolbox to control the alignment of large cohorts. While sulcus recognition could be manually corrected for template brains in case of ambiguity, dealing with large cohorts requires improved automatic sulcus recognition. Another ongoing direction of research of the Task lies in the use of multimodal datasets to perform better architectural alignment across subjects.

Software component: SGA2 T2.6.3 Spatial transformation routines for projection of data across templates, ID:C2259

Leader: Jean-Francois MANGIN

CNN-based sulcus recognition: A new sulcus recognition algorithm based on CNN-U-NET has been designed, providing a significant improvement over the past recognition rate (>5%). This new algorithm has been selected from a cross validation performed to compare three original alternative approaches (patch-based approach based on graph representations, patch-based approach based on CNN, U-NET).

Software component: Objective function for matching images to tissue probability maps. ID:C2577

Leader: John ASHBURNER

Multi-modal alignment: The UCL part of the Task is currently underspent because of difficulties recruiting a new post-doc. The UCL PI has done some work on model development for data integration of aligned images. We have a very preliminary prototype tool (https://github.com/WCHN/Label-Training) for projecting information from a small number of labelled brains on to an aligned image of a new subject. The strategy is to use a patch-based dictionary approach to integrate data of different modalities, coming from different individuals, in a way that goes beyond what can be achieved by nonlinear image registration alone.

3.1.20 Output 19 – using manifold structure for functional connectome matrices estimation

Report component: SGA2 - T2.4.1 low-dimensional modelling of inter-subject variability for functional brain data. ID:C2291

Leader: Bertrand THIRION

Using manifold structure for functional connectome matrices estimation: Covariance estimation to infer functional connectome matrices is challenging and calls for penalisation, as with shrinkage approaches. We introduced population shrinkage of covariance estimator (PoSCE): a covariance estimator that integrates prior knowledge of covariance distribution over a large population, leading to a non-isotropic shrinkage. The shrinkage is tailored to the Riemannian geometry of symmetric positive definite matrices. It is coupled with a probabilistic modelling of the individual and population covariance distributions.

3.2 Validation and Impact

3.2.1 Actual Use of Output(s) / Exploitation

Output 1, C2254: The new web-based implementation of JuGEx is currently evaluated in practice by SP2 researchers, who also verify the results compared to the original Matlab implementation. They have reported better usability and flexibility as compared to the original implementation, and already use the tool in their practical work.

Output 2, C2271: None yet.

Output 3, C2315: The human data are currently being anchored to the HBP Human Brain Atlas. This will be fully implemented by March 2020. Tasks T2.3.5 and T2.5.4 included analysis of the rat hippocampus through collaborations with WP2.1, WP2.6, and WP5.2. The data from the rat hippocampus will enrich the data made available to the community via the Waxholm space rat atlas (the main work-load of this project is being carried out in T5.2.1). Rat datasets will allow to perform systematic inter-species comparisons using rat models of cells and circuits, and explore possible strategies for generalisation to the human hippocampus. Furthermore, those Tasks will contribute to refine and extend the cellular level reconstructions of hippocampal neurons and circuitry in rat: datasets will be used in the development of multi-scale scaffold models of plasticity and neuromodulation (T6.2.3, Models of hippocampus and community), by enriching cellular level brain region models with information on receptors densities.

Output 4, C2354: The machine learning algorithm for 3D segmentation and morphochemical classification of neurons is now applied to further tissue samples.

Output 5, C773: The release of a preprint of the paper on an open repository the 5th of February 2019 reveals already at this very early stage an interest of the international scientific community (see Figure 14).

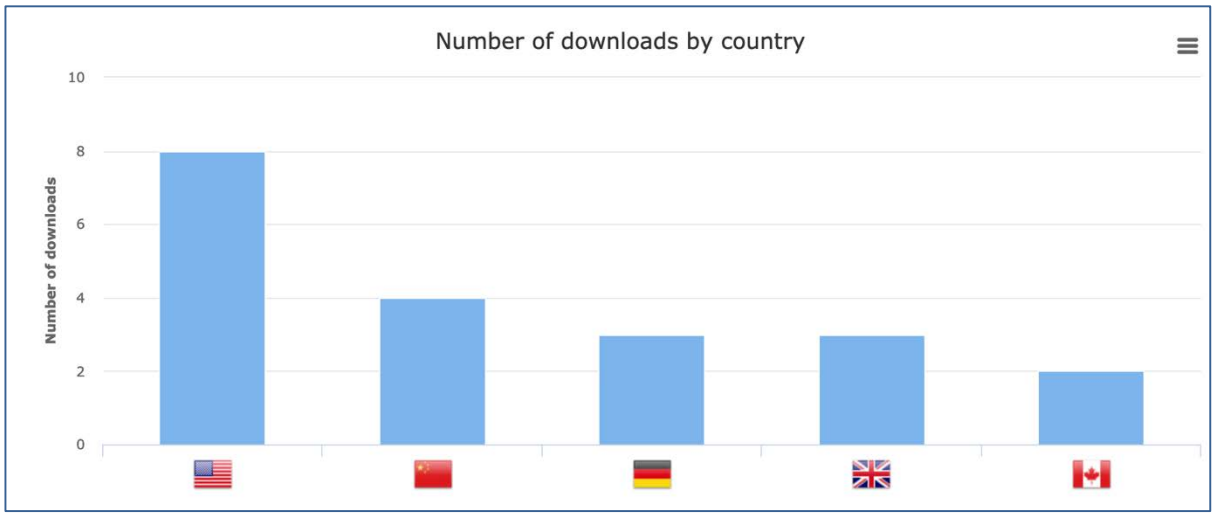


Figure 14: Download numbers of the paper publishing the maps of the hippocampus by country Plachti *et al*. 2019 (status 28.02.19).

Output 6, C2287: The data are complete and the analysis is almost ready. These results contribute to our understanding of complex cognitive processes, specifically those involving visual processing, attention, and motor processes such as saccade planning. These data will provide unique insights into how the neuronal representation of sensory (figure-ground) stimuli are transformed into eye movement plans that will be implemented in the CDP4 visuo-motor integration model. Specifically, these models contribute to the development of saccadic target selection models (CDP4 M24 deliverable, D2.5.1(D12.1)).

Output 7-8, C2352, C2353: In the next year, the combination of the methodologies used in for this work will allow to correlate functional and structural information of specific neurons in large volume of human brain cortex from the same subject.

Output 9, C2293: The implemented method enables characterising large human brain specimens, giving the possibility to expand the histological studies to the third dimension. During the next year, using the built microscope (C2349), we will reconstruct the 3D organisation of excitatory and inhibitory neurons of a whole human hippocampus with micrometre resolution.

Output 10, C2347: Labelling procedure is applied to further samples for data generation.

Output 11, C2349: The implemented methodologies are used by SP2 scientists from Task T2.3.1 to perform three-dimensional reconstructions of specific areas of the human brain.

Output 12, C2469: The data will be of high value to understand the neuronal mechanisms causing (at least some forms of) adult cortical plasticity in primates and perceptual learning.

Output 13, C2470: The laminar dataset will form the basis of a comparison to human and monkey fMRI data that is currently being acquired. The probabilistic retinotopy data (and median polar angle and eccentricity values in 3D and 2D format) will be highly useful for guiding recordings in NHP. They are also used for guiding the high-count electrode implantation experiments of ROELFSEMA (restoring vision in the blind project). The dataset from human V3 has been downloaded 24 times and is being used by other vision scientists to compare responses in humans, monkey and mice. The laminar dataset has formed the basis of a review article which acts as guide for future laminar fMRI studies. The monkey retinotopy data will be uploaded after the probabilistic maps are published. Several groups (USA, Europe and Japan), already requested the data which have been provided.

Output 14, C2471: The group of Sacha VAN ALBADA (Brain Simulation Platform) is already implementing the neuronal data acquired here, in their canonical models of cortex. At the time we provided data, we only had 1 cell selective for shifts of attention (and many other visual and visuo-motor cells recorded from posterior parietal cortex). Meanwhile, we recorded data from > 80 shift-selective cells.

Output 15, C2345: Results are now used for the preparation of publications. The publication of Radnikow has been already cited 3 times.

Output 16, C3026: Validation by anatomy experts ongoing.

Output 17, C2362: The representations of the manifold of the cortex morphology have led to a new brain mapping framework imposing folding pattern compatibility before group analysis. It is a complementary alternative to the new strategies proposed for instance by the HCP consortium aligning subjects using FMRI. It is also exploited to detect abnormal folding patterns signing abnormal developmental events, with a proof of concept on congenital one-handedness (collaboration with Oxford and UCL) and ongoing projects on high prematurity (collaboration with Utrecht hospital) and Autism (collaboration with EU-Aims consortium). The design of a user-friendly toolbox to exploit this methodology is in progress, building upon the sulcus-based alignment toolbox of HBP (T2.6.3).

Output 18, C2259, C2577: The new sulcus recognition method has been applied to the 1,200 subjects of the HCP dataset, in order to study the impact of the improvement on different morphometry benchmarks. It will also be applied on the UKbiobank massive dataset (35,000 subjects), to perform similar comparison with the normative charts about aging-based sulcus opening computed in the context of SP7.

Output 19, C2291: Experiments on two large r-fMRI datasets (HCP n=815, Cam-CAN n=626) show that PoSCE has a better bias-variance trade-off than existing covariance estimates: this estimator relates better functional-connectivity measures to cognition while capturing well intra-subject functional connectivity.

3.2.2 Potential Use of Output(s)

Output 1, C2254: After finalising the tests, we will release the new web version of JuGEx with the HBP atlas services. This release is planned before M18. It will provide the community a very simple and user-friendly, low-threshold access to gene expression data from the Allen atlas combined with HBP atlas parcellations. In particular, not requiring a Matlab license, it will open access to the tool to a broader audience.

Output 2, C2271: The precision of the 3D reconstructions achieved by the novel bisected cell-matching algorithm in brain regions of interest provides a foundation to extract high resolution oblique cuts from the resulting 3D volume a resolution of single cells. Such virtual sections will allow to study the cytoarchitecture of the cortex even in areas of nearly tangential cutting directions, thereby opening new perspectives to create cytoarchitectonic maps of complete human brains. Furthermore, the quality of the now achieved reconstructions is prerequisite for extracting realistic neuronal cell distributions in 3D from histology.

Output 3, C2315: Receptor densities will be used in realistic models of the rodent and human hippocampus. The comparative analysis of densities in the human and rat hippocampi is crucial for the development of novel drugs, since it provided the pharmaceutical companies with information on whether the receptors targeted are evolutionarily conserved.

Output 4, C2354: We will use the software implemented on two-photon images to perform segmentation also on images acquired with light sheet microscopy. The aim is to obtain the information about localisation and shape of the excitatory and inhibitory neurons mapped with LSM of a human hippocampus (Task T2.3.1 and T2.3.2).

Output 5, C773: Our work provided robust models of hippocampus organisation for scientific studies relating neuroimaging makers of hippocampus' structure, function and connectivity to genetic and behavioural markers in heathy and clinical populations.

We computed the whole-brain functional connectivity pattern of all hippocampus voxels and then examined pattern of differentiation using a parcellation approach. Accordingly, we provided parcellation maps of the human hippocampus. In other words, we used MRI markers of large-scale functional integration to define subregions within the human hippocampus. Thus, the results are provided as parcellation maps.

These maps show substantial differences with the maps based on microstructural features such as cytoarchitecture. Accordingly, both types of maps are strongly complementary and should be used for different purposes. It has been demonstrated in literature (for a review see publication P1550) that maps derived from fMRI data provide a better compression of fMRI data than microstructure-based maps. Accordingly, our maps will be available to the community, as implemented in the HBP atlas and can be used to compress fMRI data. This type of compression is particularly needed for developing and testing machine learning models of phenotype prediction in high-prevalence neurocognitive pathologies, showing alterations of hippocampus' function such as in Alzheimer's Disease, schizophrenia, major depression and anxiety disorders.

Output 6, C2287: Our results have the potential to provide unprecedented insight into the interactions between visual and motor related structures. This knowledge might be used to get more insight in and maybe even cure many diseases related to visual, motor and attentional processes, such as ADHD, Autism and blindness.

Output 7-8, C2352, C2353: The data obtained will be organised for the BigBrain reference space and will serve as input to simulations of brain states in SGA3.

Output 9, C2293: The data obtained from the three-dimensional reconstruction of excitatory and inhibitory neurons of the hippocampus will be organised for the BigBrain reference space and will serve as input to simulations of brain states in SGA3.

Output 10, C2347: Data can be used for brain modelling and simulation.

The complete dataset from T2.3.1 will be released at M24, as planned. Preliminary images obtained on trial samples are already shared in CSCS repository as release of components C2347, C2349 and C2354. The final dataset will be integrated in the Knowledge Graph by M24. The dataset and the maps obtained on hippocampus can be useful for SP6 teams to perform several analyses based on excitatory and inhibitory neuronal distribution.

Output 11, C2349: All the technological implementations obtained here can be used in the future to study different areas of the brain at high resolution. As a matter of fact, the ultra-fast light sheet microscope gives the possibility to study different samples in short time, overcoming the limitation of having just one dataset and, therefore, to study the inter-individual variability.

Output 12, C2469: Results will contribute to a deeper understanding of the neuronal mechanisms causing adult cortical plasticity in primates and perceptual learning.

Output 13, C2470: We expect that the novel retinotopy results will be broadly used in the monkey community. Moreover, they will inspire for re-assessing the retinotopic organisation of early dorsal visual cortex in humans in greater detail (i.e. with very high spatial resolution). The comparative cue fusion results are highly relevant as they reconcile previous discrepancies between human imaging and monkey electrophysiology studies.

Output 14, C2471: Results will be used for cortical brain models (e.g. in SP6).

The acquisition of the complete data set has been completed. The preliminary part has been sent to the laboratory of Sacha VAN ALBADA, who are implementing it in their brain scale models at cellular resolution (Use Case SGA2-SP6-UC002). It is matter of fact that the initial data set contained only very few neurons that were selective for attentional shifts. The newly acquired data (from two subjects) contain a total of about 380 neurons showing shift selectivity -as we predicted based on the fMRI data from both human and monkey. The novel data will be also provided to the VAN ALBADA laboratory.

Output 15, C2345: Data can be used for brain modelling and simulation.

Output 16, C3026: Open source use of vEK atlas for neuroscience/ neuroimaging community for computational modelling, theoretical neuroscience exploitations.

Output 17, C2362: The representations of the manifold of the folding patterns will be used further to improve the HBP short fibre bundle atlas. During SGA1, this atlas has been aligned with the massive iEEG dataset of SP8 to validate each bundle and obtain an estimation of its transmission speed from stimulation performed in the brains of epileptic patients. This feature of connectivity is

of key importance for the simulations planned during SGA3. The sulcus manifolds will also be used as a framework paving the way to new features of the E-brain atlas describing inter-subject variability.

Output 18, C2259, C2577: Achieving robust alignment of any subject into the HBP template spaces will have an impact for a medical use of the HBP portal. The main stream strategies, indeed, do not overcome failure of the spatial normalisation process used as a pre-processing by most of the brain image analysis pipelines.

Output 19, C2291: The workflow of the WP1 of SGA3 aims at validating the global brain simulations performed for instance in the Virtual Brain framework relative to their capacity at relating structural connectomes to functional connectomes. This objective requires robust procedures to estimate individual connectomes, which is the goal of the work performed in this task.

3.2.3 Publications

P1297: Sebastian Bludau, Thomas W. Mühleisen, Simon B. Eickhoff, Michael J. Hawrylycz, Sven Cichon, Katrin Amunts. 2018. Integration of transcriptomic and cytoarchitectonic data implicates a role for MAOA and TAC1 in the limbic-cortical network. Brain Structure and Function.

Significance: JuBrain Gene Expression (JuGEx)

Output: 1

P1667: Plachti, A., Eickhoff, S.B., Hoffstaedter, F., Patil., K.R., Laird, A., Fox, P.T., Amunts, K., & Genon, S. Multimodal parcellations and extensive behavioral profiling tackling the hippocampus gradient. Cerebral Cortex.

Significance: Connectivity based parcellation of the hippocampus.

Output: 5

P1641: Obermayer J, Heistek TS, Kerkhofs A, Goriounova NA, Kroon T, Baayen JC, Idema S, Testa Silva G, Couey JJ, Mansvelder HD. 2018. Lateral inhibition by Martinotti interneurons is facilitated by cholinergic inputs in human and mouse neocortex. Nature Communication.

Significance: Physiological data from human temporal neocortex interneurons were acquired from somatostatinergic, VIP, and parvalbuminergic interneurons.

Output: 7

P1645: Zhu Q, Vanduffel W. 2019. Submillimeter fMRI reveals a new layout of dorsal visual cortex in macaques, remarkably similar to New World monkeys. PNAS 116 (6) 2306-2311

Significance: Novel retinotopic model in early visual cortex of NHP.

Output: 13

P1796: Armendariz M, Ban H, Welchman AE, and Vanduffel W. Areal differences in depth cue integration between monkey and human. 2019. Plos Biol.

Significance: Depth cue fusion in human and monkey.

Output: 13

P980: Radnikow G, Feldmeyer D. 2018. Layer- and Cell Type-Specific Modulation of ExcitatoryNeuronal Activity in the Neocortex. Frontiers Neuroanatomy

Significance: Layer- and cell type-specific modulation of excitatory neuronal activity in the neocortex

Output: 15

P1795: Mehdi Rahim, Bertrand Thirion, and Gaël Varoquaux, Population shrinkage of covariance (PoSCE) for better individual brain functional-connectivity estimation. 2019. Medical Image Analysis

Significance: Population shrinkage of covariance estimator (PoSCE): a covariance estimator that integrates prior knowledge of covariance distribution over a large population, leading to a non-isotropic shrinkage. The shrinkage is tailored to the Riemannian geometry of symmetric positive definite matrices. It is coupled with a probabilistic modelling of the individual and population covariance distributions.

Output: 17

3.2.4 Measures to Increase Impact of Output(s): Dissemination

Results were presented as posters or oral presentation at several scientific conferences, e.g. OHBM 2018. In addition, results were communicated via articles in the HBP newsletter, at the HBP website and via press releases of the institutions.

The main dissemination activities for this KR were:

HBP School 'The HBP Human Brain Atlas: Neuroscientific basis, tools and applications', 3.-7. September 2018 in Düsseldorf, Maastricht and Jülich.

Significance: Presentation of background, methods and results of SP2. School was attended by 36 HBP internal and external PhD and young postdocs.

Output: 1-18

Mazzamuto, G., Costantini, I., Neri, M., Roffilli, M., Silvestri, L., Pavone, F. S. "Automatic Segmentation of Neurons in 3D Samples of Human Brain Cortex". 21st International Conference, EvoApplications 2018, Parma, Italy, 4-6 April 2018

Significance: Presentation of the results Automatic Segmentation of Neurons in 3D to the scientific community.

Output: 4, C2354

The dissemination (press releases) of the new conceptual ideas derived from Genon et al. and Plachti *et al.* 2019 has been promoted by outreach publications and communications in media:

https://medium.com/brain-byte-blog/what-does-this-part-of-brain-do-8098cace3b7d

https://www.spektrum.de/magazin/hirnkartierung-welche-funktionen-haben-hirnareale/1612188

http://www.fz-juelich.de/SharedDocs/Pressemitteilungen/UK/EN/2018/2018-03-16-hbp-brain-function.html

https://blogs.fz-juelich.de/zweikommazwei/en/

http://blogs.discovermagazine.com/neuroskeptic/2018/03/09/what-does-part-of-brain-do/#.WrIKF5NuaL8.

Significance: Communication of results of the two publications to scientific community and public.

Output: 5, C773

The laminar dataset and the human V3 dataset have been presented by P.R. ROELFSEMA and M.W. SELF at international meetings of the Society for Neuroscience (3-7 November 2018, San Diego, USA), Vision Sciences Society (8 May 2018 in Florida, USA), and HBP Summit (October 2018 in Maastricht).

Significance: Presentation of output 13 to scientific community.

Output: 13, C2470

Doctoral theses: PhD thesis of Danqing Yang, M.Sc. entitled 'Characterization of synaptic connections and cholinergic modulation of layer 6A microcircuitry in rat barrel cortex' (12 November 2018, Aachen, Germany), http://publications.rwth-aachen.de/record/749817/files/749817.pdf, PhD thesis by Chao DING, M.Sc. entitled 'Characterisation of neuronal microcircuitry and adenosine

modulation on layer 6 of rat medial prefrontal cortex' (13 November 2018, Düsseldorf), https://docserv.uni-duesseldorf.de/servlets/DocumentServlet?id=47807

Significance: Doctoral thesis on the layer- and cell type-specific modulation of excitatory neuronal activity in the neocortex are publicly available. Education of the next generation of researchers.

Output 15, C2345

Abstract for FENS Forum of Neuroscience on 7-11 July 2018 in Berlin by Danqing Yang et al. 'Differential cholinergic modulation of L6A microcircuitry in rat barrel cortex'

Significance: Presentation of the differential cholinergic modulation of L6A microcircuitry in rat barrel cortex.

Output: 15, C2345:

Borne L., Mangin JF., Rivière D. A Patch-Based Segmentation Approach with High Level Representation of the Data for Cortical Sulci Recognition: 4th International Workshop, Patch-MI 2018, Held in Conjunction with MICCAI 2018, Granada, Spain, 20 September 2018, Proceedings.

Significance: Presentation of one of the three algorithmic models designed to improve the toolbox.

Output: 18, C2259:

Brudfors M, Balbastre Y & Ashburner J. "Nonlinear Markov Random Fields Learned via Backpropagation". Paper accepted for 26th international conference on Information Processing in Medical Imaging (IPMI). Preprint available from http://arxiv.org/abs/1902.10747 (27 February 2019).

Significance: This paper bridges the gap between traditional generative models for image segmentation, and convolutional neural network approaches. Aspects of this work provide a framework for the patch-based approach for integrating data across different modalities, whereby neighbouring patches are linked via a conditional random field.

Output: 18

4. Key Result KR2.2 High-resolution reconstruction of nerve fibre architecture applying 3 different imaging techniques in the same brain sample

4.1 Outputs

4.1.1 Overview of Outputs

Output 1: long and U-fibre bundle atlas ID: C339

Output 2: HPC-based simulation of white matter tissue ID: 2366

Output 3: Gobal tractography tool ready and usable for high resolution dMRI ID: C2367

4.1.2 Output 1 – long and U-fibre bundle atlas

Dataset component: SGA1/SGA2 - T2.1.3 Map of human fibre bundles and their microstructure, ID:C339

Leader: Cyril POUPON

Long and U-fibre bundle atlas computed for the whole HCP dataset.

800 subjects of the HCP dataset have been submitted to a multiple-shell diffusion MRI protocol, thus allowing to investigate their connectivity. We are currently designing two novel long and U-fibre bundle atlases from the dMRI HCP dataset scanned at 1.2mm isotropic resolution.

To this aim, connectograms were computed for the 800 subjects using a constrained spherical deconvolution model of ODFs (spherical harmonics order 8, Laplace-Beltrami regularisation factor of 0.006) and a streamline regularised fibre tracking algorithm.

Two fibre clustering pipelines are still currently running individually on each connectogram, targeting the construction of individual long (100-240mm) and short (25-100mm) U-fibre fascicles, as shown in Figure 15. 490 subjects out of the 800 subjects have been processed. A novel HCP-based bundle atlas will be delivered during the second trimester of 2019.

The atlasing pipeline was designed to be usable on large datasets, and should allow to design a novel bundle atlas including all the subjects of the HCP dataset, which goes beyond the current state of the art where at most 100 subjects are used to design atlases.

Piece by piece UHF diffusion MRI of a whole hemisphere

During the first year, the team acquired a post-mortem mesoscopic resolution anatomical and diffusion MRI dataset over an entire left hemisphere (called Chenonceau, see Figure 16) provided by Christophe DESTRIEUX (CHU Tours, INSERM iBrain) using a preclinical UHF MRI system at 11.7T and equipped with strong gradients (780mT/m & 9600T/m/s). Resolutions were 100µm and 150µm for the anatomical MRI scans, and 200µm for the multiple shell diffusion MRI scan.

Acquisitions were performed during a long campaign lasting 12 months during which 28 fields of view corresponding to 7 blocks and 4 FOVs of 4.2cmx4.2cmx5.6cm per block were scanned during 107 hours (total scan duration: 2996 hours) and should finish by the end of April 2019 (see Figure 17). Acquisition of the right hemisphere will follow.

To the best of our knowledge, there does not exist such a rich dataset available in the world yet, thus providing the first mesoscopic ultra-high field diffusion MRI dataset to the community.

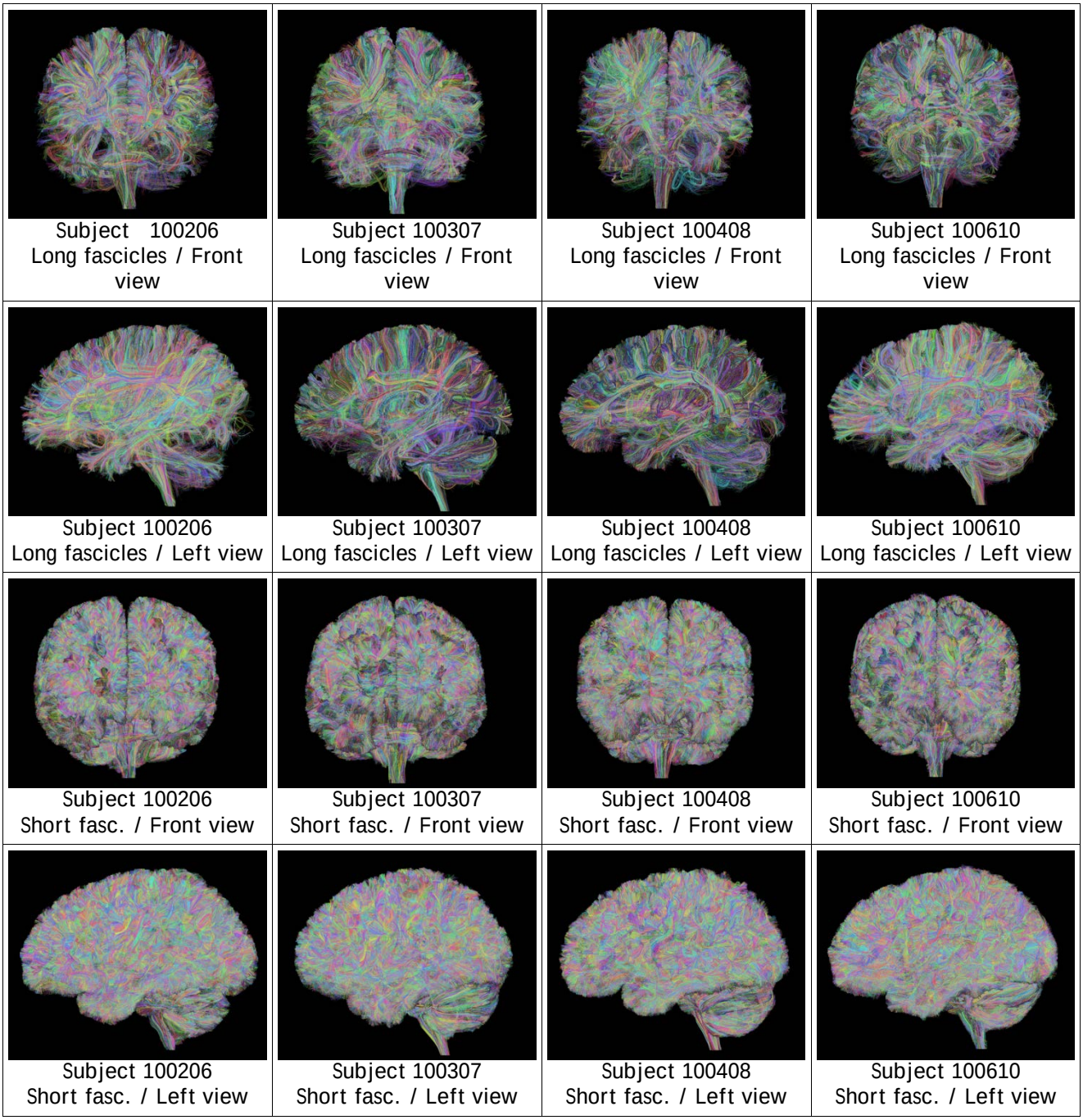


Figure 15: Long fibre bundles in 4 subjects.

Computation of long and short white matter fascicles of 4 subjects of the HCP database using an optimised fibre clustering algorithm.

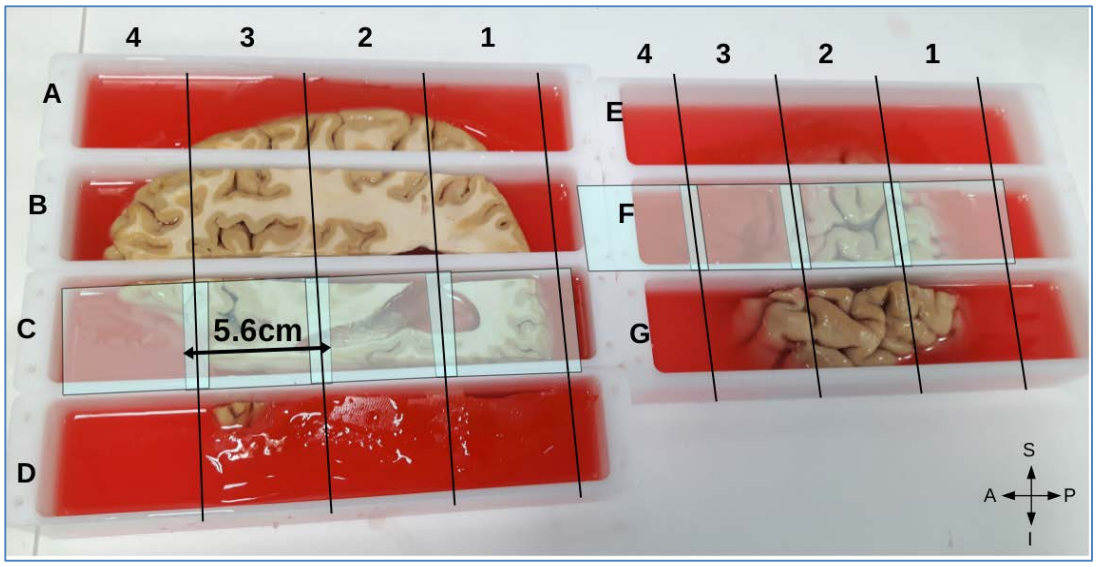


Figure 16: Tissue preparation for the 11.7T MRI.

Preparation of the Chenonceau left hemisphere into 7 blocks compatible with the preclinical UHF 11.7T MRI system of Neurospin.

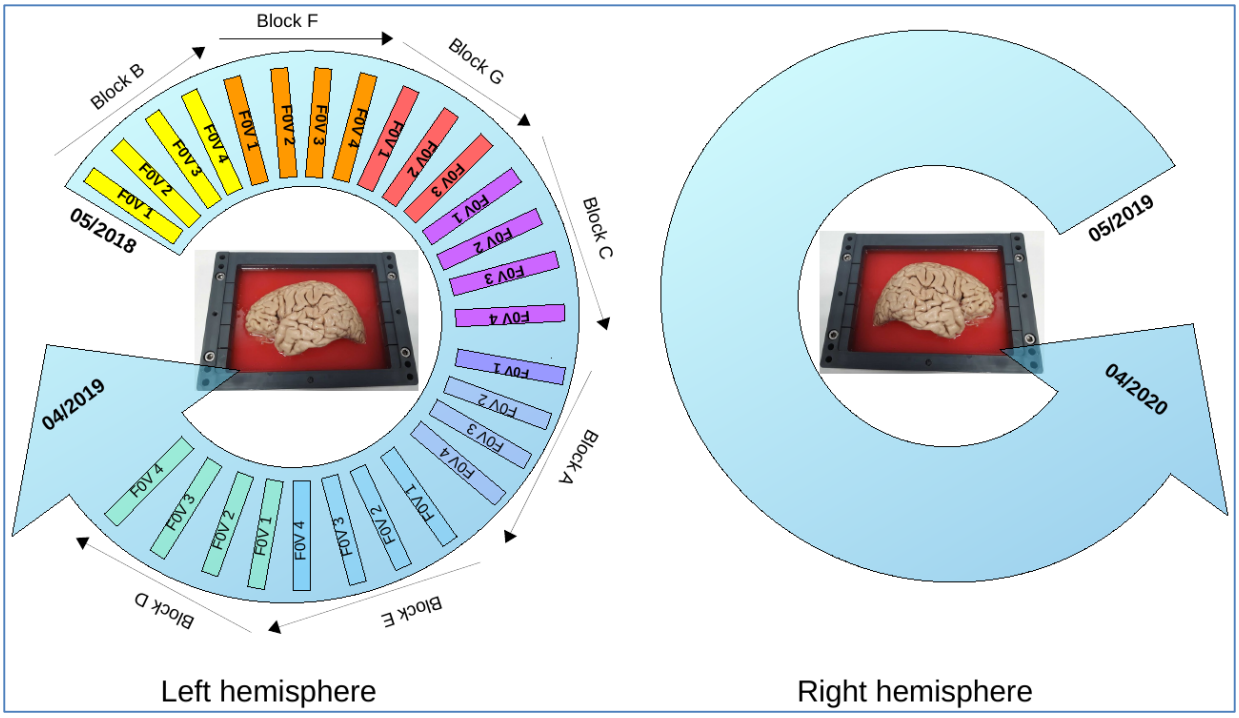


Figure 17: Acquisition roadmap

Roadmap of the acquisition of mesoscopic scale UHF MRI dataset on the Chenonceau brain

The current status of the work consists of aggregating the 28 field of views to create a whole left hemisphere dataset, involving several post-processing steps among which are imaging noise removal, diffeomorphic registration, intensity bias correction, resampling in q-space, image regularisation to finally obtain a high quality mesoscopic (200µm) diffusion MRI dataset (see Figure 18) able to provide mesoscale structural connectivity information to the community. The reconstructed dataset is planned to be delivered during the 3rd trimester of 2019.

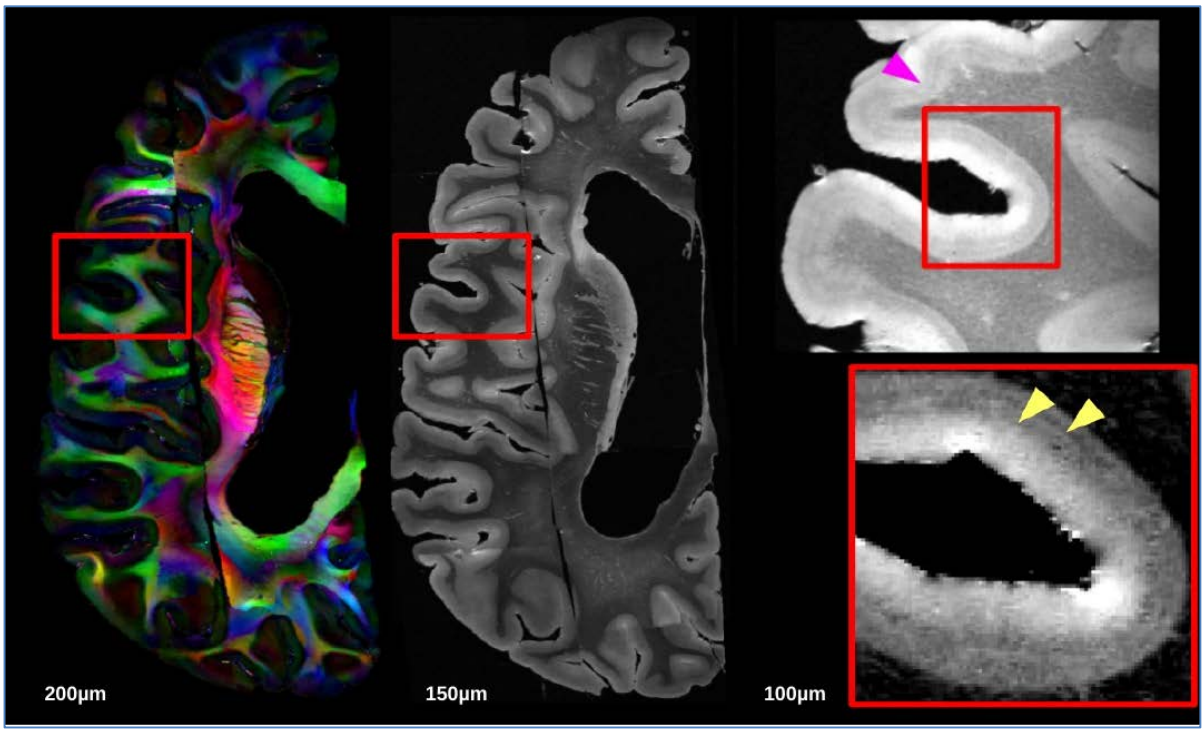


Figure 18: Reconstruction of the mesoscale diffusion UHF 11.7T MRI dataset.

Preliminary whole left hemisphere reconstruction of the mesoscale diffusion UHF 11.7T MRI dataset demonstrating the high level of orientational and anatomical details available in the data.

Figure 19 shows a comparison of a corpus callosum scanned on a conventional clinical MRI system versus our Chenonceau dataset. One can clearly see the impact of the enhancement of the spatial resolution in terms of anatomical details.

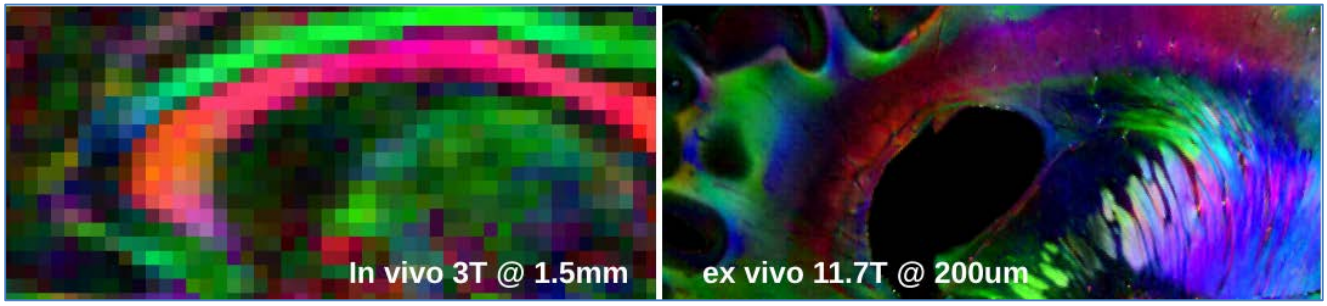


Figure 19: Comparison of *in vivo* 3T and *ex vivo* 11.7T.

Comparison of the level of anatomical details observed at the level of the corpus callosum between a standard acquisition performed on a clinical 3T MRI system at 1.5mm and a high-resolution acquisition performed on a preclinical 11.7T MRI system at 200µm.

4.1.3 Output 2 – HPC-based simulation of white matter tissue

Software component: SGA2 T2.6.5 Simulator of polarised light and water diffusion in biological tissues, ID: C2366

Leaders: Markus AXER, Cyril POUPON

In order to go beyond current state-of-the-art analytical models for the local orientation distribution functions (ODFs), the team has developed a novel simulator to generate ultra-realistic white matter virtual tissues. The algorithm allows to create virtual cellular organisations including not only populations of axons, but also populations of glial cells (oligodendrocytes and astrocytes) with a reduced set of parameters to control the characteristics of the cell populations (size distribution of cells, density of cell for every cell population, tortuosity of axons, characteristics of the myelin

sheath, branching level of astrocytes, ...). A key feature of the developed algorithm is its ability to prevent the existence of any overlap between neighbouring cells (see Figure 20). Descriptions of the developed algorithms and tools as well as their applications have already been published or will be published soon.

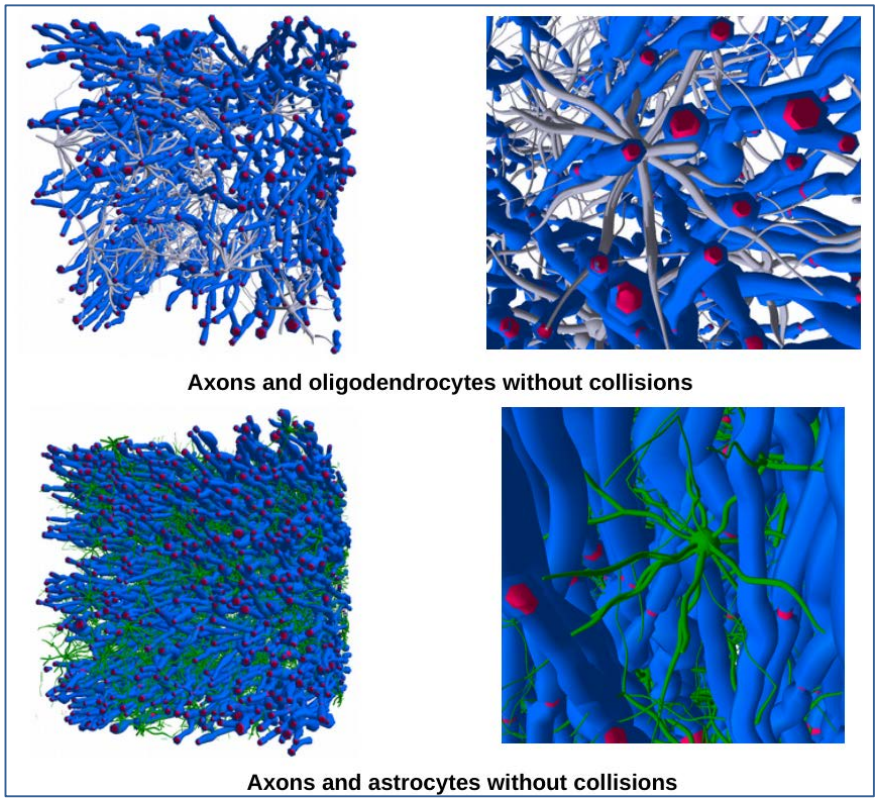


Figure 20: Simulated white matter tissue.

Example of virtual white matter tissues resulting from the novel ultra-realistic tissue simulator developed in the frame of the project.

4.1.4 Output 3 – global tractography tool ready and usable for high resolution dMRI

Software component: SGA2 T2.6.6 HPC compatible global tractography, ID: C2367

Leaders: Cyril POUPON, Markus AXER

We developed a novel global tractography framework which allows inferring the structural connectivity of the human brain from high-resolution diffusion MRI datasets, like the mesoscopic Chenonceau diffusion MRI dataset, acquired at a resolution of 200µm.

We developed software to scale up the spin-glass-based original algorithm designed by the team (Teillac *et al.* 2017) for high resolution datasets, using a two-fold strategy:

- parallelisation of the algorithm at two different levels, i.e. by first solving the problem at the scale of sub-fields of view (corresponding to the individual "pieces" of the Chenonceau UHF dMRI hemisphere), and second solving the problem at the interfaces between sub-fields of view to reconnect fibres stemming from different pieces. The inference of global solutions can therefore be performed either sequentially on a single large-memory HPC node, or in parallel if several HPC nodes are available. Solving the connectivity at the interfaces is then performed on a single node, taking as inputs all the global solutions obtained for the individual sub-fields of view
- optimisation of the computation time by allowing the modification of several individual spin-glasses at the same time; in practice, the number of simultaneously selected spin-glasses must

match the number of CPU cores of the HPC node (typically up to 80 cores) and their positions must be as far as possible from each other.

A key feature of this global fibre tracking approach relies on its ability to take prior anatomical and microstructural knowledge into account (when available, for instance using advanced diffusion models like NODDI) to better monitor the trajectories of fibres entering the cortical ribbon and thus adequately deal with a putative sharp turn.

International benchmarks have shown that global tractography is the most efficient method to disentangle fibre crossing. It is based on an algorithmic strategy organising a competition between bundles to estimate the configuration, providing the best explanation of the signal generated by fibres. The refined approach aims at 1) dealing with sharp turns of the fibres occurring at the outstanding resolutions targeted by the HBP, 2) scaling up to the huge datasets generated with postmortem MRI, PLI and TPFM, which requires HPC-adapted software.

Figure 21 depicts results of global tractography obtained on the Chenonceau high-mesoscopic-resolution diffusion MRI dataset at the level of a gyrus, showing precise fibres trajectories.

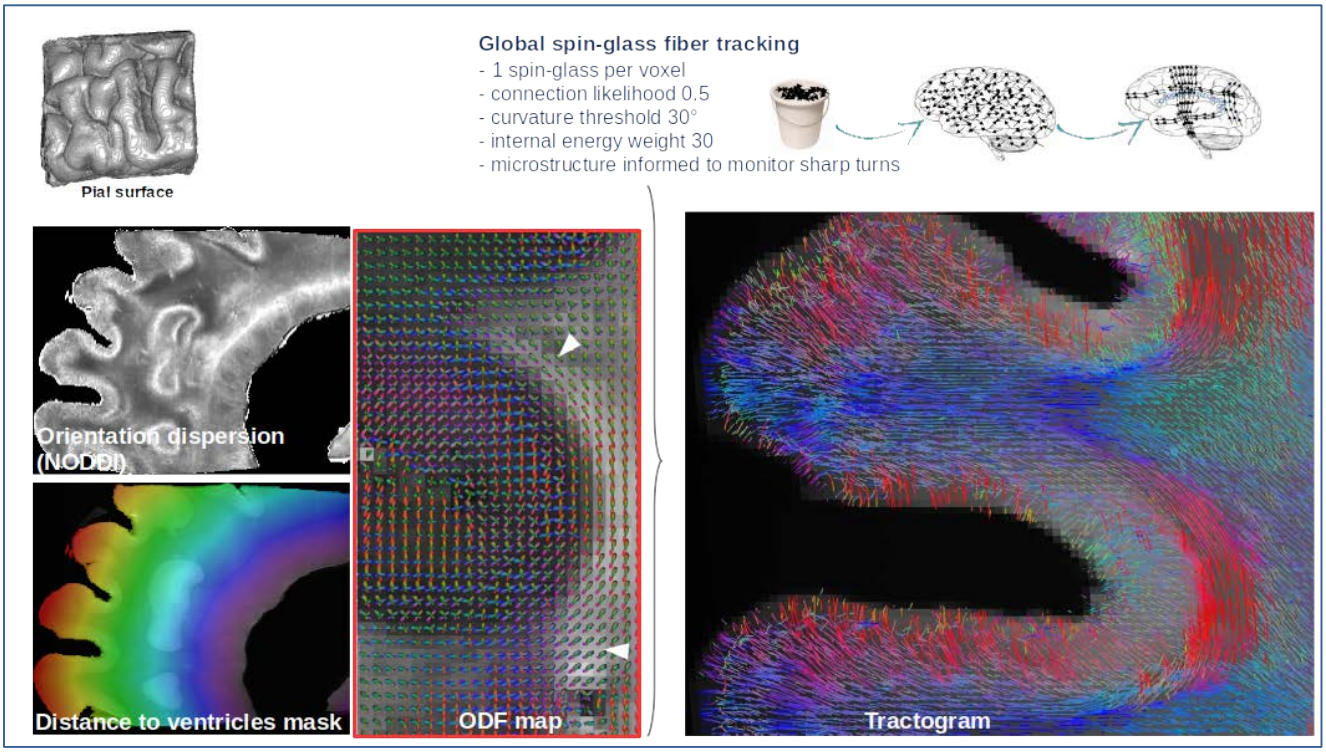


Figure 21: Fine connectivity of the cortical ribbon and subcortical areas.

Inference of the fine connectivity in the cortical ribbon and sub-cortical areas obtained using our anatomy- and microstructure- informed global fibre tracking algorithm; fibres typically show rectilinear trajectories when located deep inside the gyrus and higher curvature trajectories depicting some sharp turns when located closer to the side-surface of sulci.

4.2 Validation and Impact

4.2.1 Actual Use of Output(s) / Exploitation

Output 1, C339: Long and U-fibre bundle atlas computed for the whole HCP dataset - Since the atlas are not finished yet, the Neurospin team is the only user: (i) construction of the short U-fiber HCP-based bundle atlas under the supervision of J.-F. MANGIN (involved people: Nicole LABRA-AVILA, PhD student), (ii) construction of the long HCP-based bundle atlas under the supervision of C. POUPON (involved people: Ivy USZYNSKI, post-doc). When the atlas is ready, it will be delivered to the HBP Brain Atlas and will allow to establish for any parcellation available in the HBP Brain Atlas to look at

the connectivity profile of any parcel. A web-service will also be made available to allow any external user to segment the long and short white matter bundles of any new subject through the HBP portal from an adequate clinical diffusion MRI dataset.

Piece by piece UHF diffusion MRI of a whole hemisphere - Until the end of the acquisition campaign (scheduled in April 2019), the piece by piece UHF 11.7T diffusion MRI dataset covering an entire left hemisphere is exploited by the Neurospin team in order to reconstruct a dataset aggregating all the pieces and perform the required data curation on the individual pieces. The Jülich team will be the first HBP user of the curated dataset in order to jointly define one piece to be scanned using 3D PLI in order to compare the structural connectivity of the piece through the two microscopic and mesoscopic scales. After publication, the UHF diffusion MRI dataset will be delivered to be integrated to the HBP Atlas (scheduled 3rd trimester 2019) and open to external users for exploitation under conditions fixed by the HPB partners.

Output 2, C2366: HBP users: Neurospin (Diffusion MRI), Jülich (3D PLI); External user(s): ATHENA Project-Team, Inria Sophia Antipolis – Méditerranée, France.

Output 3, C2367: Some work is still required to finalise the software code dedicated to the connection/optimisation of fibres at the interface of sub-fields (end of developments scheduled in May 2019). The Neurospin team will deliver the tool to the Jülich team to be tested on 3D PLI dataset. At the end of the acquisition campaign of the Chenonceau dMRI left hemipshere (schedules in April 2019), and after the curation and aggregation of the mesoscopic Chenonceau dataset, the Global Tractography tool will be used to infer the structural connectivity of the whole hemisphere at high resolution. The obtained connectogram will be delivered to the HBP Brain Atlas. In parallel, a web-service is actually under development to provide access to the tool for the HBP and external communities. A web-service is actually under development to give access to the tool to any HBP or external user an interface to compute a connectogram from low to high resolution *in vivo* or *ex vivo* diffusion MRI or 3D PLI datasets.

4.2.2 Potential Use of Output(s)

Output 1, C339: Long and U-fibre bundle atlas computed from the whole HCP dataset - the potential use of the novel long and short U-fibre bundle atlas computed from the HCP dataset is twofold: (i) feeding the HBP Atlas with information about the structural connectivity that will allow to explore the connectivity of any parcel, may it stem from functional imaging (eEEG or fMRI), cytoarchitectonics or myeloarchitectonics, (ii) allowing any HBP or external user to segment the long and short connectivity of any subject from a diffusion MRI dataset acquired *in vivo*; this possibility is strongly related to the current development of a dedicated web-service to offer the service. For instance, in the frame of the HBP project, it would allow to segment the white matter bundles of the UK BioBank dataset, to potentially look at the heritability of genes.

Piece by piece UHF diffusion MRI of a whole hemisphere - Because the mesoscopic UHF diffusion MRI dataset is unique in the field of diffusion MRI, generally limited to the millimetre resolution, it establishes a bridge between the microscopic scale of optical methods and the macroscopic scale of *in vivo* imaging methods, thus opening a large range of applications from the enhancement of diffusion conventional and microstructural MRI models and fibre tracking methods to the more fundamental inference of the fine brain connectivity in humans. Examples of such applications are: (i) a cross-modality study of the human brain connectivity from microscopic to mesoscopic scales, in particular to compare results from UHF diffusion MRI, 3D PLI and two-photon or light-sheet microscopy, (ii) the improvement of new global tractography approaches benefiting from prior knowledge learned from the Chenonceau dataset (example of sharp turns of fibres entering the cortical mantel), (iii) the construction of a mesoscopic diffusion MRI-based atlas of the human brain connectivity and of brain structures at 11.7T and the validation of bundle atlases inferred from large cohorts acquired *in vivo* (example of HCP or UK-BioBank datasets), (iii) the construction of an atlas of the human brain white matter microstructure using the novel deep learning approach developed in the frame of Task T2.6.5, (iv) the investigation of the cortex lamination using cytoarchitectural features and myeloarchitectural features provided within the Chenonceau dataset, (v) the inference

of a novel parcellation of the cortex by exploiting its laminar structure and the associated cyto- and myelo- architectural features using clustering methods.

Output 2, C2366: The numerical tissue phantoms enable simulations of different imaging technologies (e.g. dMRI, 3D-PLI or bright-field microscopy) based on the same tissue composition. This opens up new possibilities (i) to study the impact of underlying structures on modality-specific measurements, (ii) to underpin cross-modality comparisons, (iii) to develop novel deep learning approaches to decode the local cellular organisation of white matter tissue, (iv) to replace the analytical ODF information used to monitor the creation of connections in the current global tractography algorithm by richer information, including not only the angular profile of axons populating a voxel, but also some further microstructural features, like their orientation, dispersion, their density or the density of glial cells.

Output 3, C2367: As mentioned earlier, because a web-service is under construction to open access to the tool, the potential use of the Global Tractography tool is quite large. Contrary to existing non-global fibre tracking methods, global tractography is able to better manage complex fibre configurations by 1) taking advantage of anatomical and even microstructural priors, 2) solving the problem at a global scale and not local, thus providing more coherent trajectories than with conventional methods. Of course, dealing with such global approach is more computationally demanding, and the HPC compatible implementation plus the parallelisation strategy provides a framework that does not suffer from computational limitations. The tool, actually available to process high resolution dMRI datasets, will then be scaled up to process 3D PLI datasets (corresponding to MS2.6.15, scheduled at M24). In the frame of the HBP, there is also an opportunity to process large cohorts like the HCP or UK-BioBank cohorts with the Global Tractography tool. The obtained connectograms should include much less false positives than non-global methods, which might allow to decrease the variability of connectivity matrices mainly explained by limitations of tractography methods today. The Global connectograms might also be used to establish new bundle atlases, targeting the fine connectivity beyond long and short U-fibre bundles.

4.2.3 Publications

The main publications of this Key Result are:

P956: K Ginsburger, F Poupon, J Beaujoin, D Estournet, F Matuschke, JF Mangin, M Axer, C Poupon, 2018. Improving the Realism of White Matter Numerical Phantoms: A Step toward a Better Understanding of the Influence of Structural Disorders in Diffusion MRI. Frontiers in Physics 6.

Significance: Development of a novel algorithm to produce a wide variety of biomimicking numerical phantoms representing more realistic white matter tissue configurations from a reduced set of control parameters.

Output: 2

P1705: Menzel M, Axer M, Amunts K, De Raedt H, Michielsen K. Diattenuation Imaging reveals different brain tissue properties. 2019. Scientific Reports.

Significance: Demonstration that the diattenuation signal depends not only on the nerve fibre orientations but also on other brain tissue properties like tissue homogeneity, fibre size, and myelin sheath thickness. This allows to use the diattenuation signal to distinguish between brain regions with different tissue properties and establishes Diattenuation Imaging as a valuable imaging technique.

Output: 2

4.2.4 Measures to Increase Impact of Output(s): Dissemination

HBP School 'The HBP Human Brain Atlas: Neuroscientific basis, tools and applications', 3.-7. September 2018 in Düsseldorf, Maastricht and Jülich.

Significance: Presentation of background, methods and results of SP2. School was attended by 36 HBP internal and external PhD and young postdocs.

Output: 1-3

PhD thesis: Justin Beaujoin 'Post mortem inference of the human brain microstructure using ultra-high field magnetic resonance imaging with strong gradients.' 2018, https://tel.archives-ouvertes.fr/tel-01977910

Significance: Doctoral thesis on high-field MRI is publicly available. Education of the next generation of researchers.

Output: 1

Poster presentation of Schmitz D et al. 'Oblique views through unstained brain sections: a new module to 3D-Polarized Light Imaging.' OHBM, 19.-21. June 2018 in Singapore.

Significance: Presentation of 3D-PLI to scientific community.

Output: 2

5. Conclusion and Outlook

This Deliverable only includes a minor part of the results which will be delivered by SP2 during the entire SGA2 period. The majority of results will only be available by the end of SGA2 because of time-consuming data acquisition, mappings and tool development etc. In addition, data curation in the Neuroinformatics Platforms requires more time as expected and a close collaboration between the researchers in SP2 and data curators in SP5. A delay in the process of the calls for expression of interest for SGA2 lead to a 2-3-month delay of the start and progress of the selected tasks in SP2 (see output 16). Besides these delays, SP2 is well on track to achieve its objectives by the end of SGA2. SP2 is working on providing whole-brain structural, functional and connectivity maps; a model of the multilevel organisation of visuo-motor integration tasks in the human brain for theory, modelling and atlasing; a multi-modal, ultra-high resolution model of the human hippocampus, including cells, fibres, receptors, gene expressions for theory, modelling, simulation, and atlasing; a quantification of inter-subject variability of the human brain and its relation to genotype and phenotype; an analysis of inter-species differences and commonalities in mouse, rat, monkey and human brains in visuo-motor areas and the medial temporal lobe; and tools developed and applied in Co-Design Projects to bring the knowledge and the results to the scientific community.

In summary, SP2 published during SGA2 M1-M12 60 papers in peer reviewed journals. We organised a one-week School on the HBP Human Brain Atlas: Neuroscientific basis, tools and applications in Düsseldorf, Maastricht and Jülich which was well attended by HBP internal but also external PhD and young postdocs in this field.

For the next year, highest priority will be given to the development of the Human Brain Atlas, making the data accessible for the scientific community and promote its usage. This atlas is unique. In addition, the results derived by 3D-PLI, dMRI and TPMF provide ground-breaking insights into the micro-to-macro organisation of the complex human brain fibre architecture with an until now unknown high resolution and details using HPC and simulations.

Annex A: Component Details

Table 1: Overview of components related to Key Results KR2.1

ID	Component Name	Type	Contact	Info
C2254	SGA2 T2.6.1 Query and analysis tool for Allen human gene expressions grouped by atlas regions	software	Timo DICKSCHEID	Software links: • http://www.fz-juelich.de/inm/inm-1/DE/Forschung/_docs/JuGex/JuGex_node.html • https://object.cscs.ch/v1/AUTH_227176556f3c4bb38df9feea4b91200c/JuGEx/jugex.zip
C2271	SGA2 T2.6.2 Layer-specific 3D cell densities in human visual cortex	dataset	Timo DICKSCHEID	Manuscript on the reconstruction based on vessels is under revision. Manuscript on the reconstruction with bisected cells is in preparation. Planned release by M24.
C2315	SGA2 T2.3.5 Quantification of multiple receptor distributions for hippocampal regions and layers	dataset	Nicola PALOMERO-GALLAGHER	Data and metadata are released and implemented in the Knowledge Graph: • https://object.cscs.ch/v1/AUTH_227176556f3c4bb38df9feea4b91200c/Receptors/data_derived/Hippocampus-CA-cell/CA-cell_metadata_20190410.zip • https://object.cscs.ch/v1/AUTH_227176556f3c4bb38df9feea4b91200c/Receptors/data_derived/Hippocampus-CA-mol/CA-mol_metadata_20190410.zip • https://object.cscs.ch/v1/AUTH_227176556f3c4bb38df9feea4b91200c/Receptors/data_derived/Hippocampus-CA1/CA1_metadata_20190410.zip • https://object.cscs.ch/v1/AUTH_227176556f3c4bb38df9feea4b91200c/Receptors/data_derived/Hippocampus-CA2/CA2_metadata_20190410.zip • https://object.cscs.ch/v1/AUTH_227176556f3c4bb38df9feea4b91200c/Receptors/data_derived/Hippocampus-CA3/CA3_metadata_20190410.zip • https://object.cscs.ch/v1/AUTH_227176556f3c4bb38df9feea4b91200c/Receptors/data_derived/Hippocampus-DG/DG_metadata_20190410.zip
C2354	SGA2 T2.6.4 Morphological classification of neurons with immunohistological staining	software	Giacomo MAZZAMUTO, Timo DICKSCHEID	Release of the software is planned for M24. Pipeline description is uploaded in the HBP Collab: • https://collab.humanbrainproject.eu/#/collab/63460/nav/431840
C773	SP2 - Selected multi-modal regional maps with cognitive features	dataset	Simon EICKHOFF, Sarah GENON	Papers: • http://anima.fz-juelich.de/studies/Plachti_Hippocampus_2019 • https://doi.org/10.1093/cercor/bhy336 Data and metadata are released and implemented in the Knowledge Graph: • https://kg.humanbrainproject.org/proxy/export?container=https://object.cscs.ch/v1/AUTH_227176556f3c4bb38df9feea4b91200c/hbp-data-000773

C2287	SGA2 - T2.2.2 Neuronal mechanism transforming a visual stimulus into an eye movement plan	dataset	Pieter ROELFSEMA	Data are complete and about to be released, the analysis is almost ready. Data will be uploaded in the CSCS (HBP) repository and implemented in the Knowledge Graph.
C2352	SGA2 T2.5.2 Physiological data recording of interneurons from human and mouse temporal neocortex	dataset	Huib MANSVELDER	Papers: • https://doi.org/10.5061/dryad.83dv5j7 • https://doi.org/10.1038/s41467-018-06628-w Metadata are released via Knowledge Graph, data are curated but under embargo: • https://object.cscs.ch/v1/AUTH_227176556f3c4bb38df9feea4b91200c/hbp-data-000043/EPhys_Morpho_Human_PYR.zip
C2353	SGA2 T2.5.2 Integration of physiological data recording from human temporal neocortex and 3D morphological reconstruction of the same interneurons	dataset	Leonardo SACCONI, Irene COSTANTINI	Release of morphological characterisation of interneurons with two-photon fluorescence microscopy is planned for M24. Data related to C2353 are still preliminary and therefore, not yet implemented in the Knowledge Graph.
C2293	SGA2 - T2.3.1 Layer-specific excitatory and inhibitory neuronal maps of hippocampus	dataset	Francesco PAVONE, Irene COSTANTINI	Release of 3D organisation of excitatory and inhibitory neurons of a whole human hippocampus with micrometre resolution is planned for M24. Data are still preliminary. The release is planned for M24.
C2347	SGA2 T2.3.2 Multiple staining for characterisation of different neuronal types	report	Irene COSTANTINI	Labelling procedure implemented. Labelling procedure uploaded in the HBP Collab: • https://collab.humanbrainproject.eu/#/collab/64427/nav/438168?state=uuid%3D98b21da5-4567-42dc-a801-37074a7e5052
C2349	SGA2 T2.3.2 Integration of ultra-fast large area fluorescence microscope with Switch clearing techniques	report	Leonardo SACCONI, Irene COSTANTINI	Ultra-fast large area light sheet microscope in place. Data related to this component are under embargo and will be available at M24. Report uploaded in the HBP Collab: • https://collab.humanbrainproject.eu/#/collab/64428/nav/438173?state=uuid%3D5fd7e440-2cd5-4f8a-9b93-d9cc63399749
C2469	SGA2 T2.5.5 Feedback interactions in monkey and human	report	Wim VANDUFFEL	Paper resubmitted. Release by M24.

C2470	SGA2 T2.5.6 Comparative mapping of visuo-motor cortex in monkey and human	report	Wim VANDUFFEL	Papers: • osf.io/qzj2v • https://doi.org/10.1073/pnas.1805561116 Data related to this component are under embargo and will be available at M24. https://collab.humanbrainproject.eu/#/collab/64541/nav/438916 Click on C2470 (the effects of context and attention on human early visual cortex) to see all datasets related to this component.
C2471	SGA2 T2.5.7 Attentional modulation of sensory processing in monkey and human	dataset	Wim VANDUFFEL	Papers: • https://doi.org/10.1523/JNEUROSCI.1111-17.2017 • https://doi.org/10.1093/cercor/bhx114 • https://doi.org/10.1093/cercor/bhy128 Data have still to be uploaded in the CSCS (HBP) repository and implemented in the Knowledge Graph.
C2345	Quantitative analysis of neuromodular function in the rodent neocortex	dataset	Dirk FELDMEYER	Paper: • https://doi.org/10.3389/fnana.2018.00001 Datasets are now uploaded in the HBP Collab: • https://collab.humanbrainproject.eu/#/collab/64541/nav/438916 Click on "Acetylcholine_Dopamine modulation of deep layer 6A of S1 cortex"; "Acetylcholine modulation in S1 cortex layer 6"; "Adenosine modulation in PFC layer 6" to see each dataset.
C3026	SGA2 T2.6.7 Online version of von Economo and Koskinas atlas	dataset	Claus HILGETAG	Online version will be released at the end of February 2020.
C2362	SGA2 T2.4.1 Dictionary of cortical folding patterns	dataset	Jean-Francois MANGIN	Data are uploaded in the CSCS (HBP) repository and under curation • https://object.cscs.ch/v1/AUTH_227176556f3c4bb38df9feea4b91200c/hbp-d000014_CorticalFoldingPatternDict_dev
C2291	SGA2 - T2.4.1 low-dimensional modelling of inter-subject variability for functional brain data	report	Bertrand THIRION	• https://github.com/mrahim/posce/
C2259	SGA2 T2.6.3 Spatial transformation routines for projection of data across templates	software	Jean-Francois MANGIN	Software is now available in the HBP Collab: • https://collab.humanbrainproject.eu/#/collab/19/nav/2108?state=software,hbp-spatial-backend
C2577	Objective function for matching	software	John ASHBURNER	Software available at: • https://github.com/WCHN/Label-Training

	images to tissue probability maps			Software is now also available in the HBP Collab: • https://collab.humanbrainproject.eu/#/collab/19/nav/2108?state=software,C2579:%20Patch-based%20Bayesian%20CCA%20method A very preliminary prototype tool for projecting information from a small number of labelled brains on to an aligned image of a new subject.

Table 2: Overview of components related to Key Results KR2.2

ID	Component Name	Type	Contact	Info
C339	SGA1/SGA2 - T2.1.3 Map of human fibre bundles and their microstructure	dataset	Cyril POUPON	Long and U-fibre bundle atlas will be released by M24. Data and metadata are released via Knowledge Graph: • https://kg.humanbrainproject.org/proxy/export?container=https://object.cscs.ch/v1/AUTH_227176556f3c4bb38df9feea4b91200c/hbp-d000339_DeepWhiteMatterBundles_DWM_pub • https://kg.humanbrainproject.org/proxy/export?container=https://object.cscs.ch/v1/AUTH_227176556f3c4bb38df9feea4b91200c/hbp-d000339_SuperficialWhiteMatterBundles_pub
C2366	SGA2 T2.6.5 Simulator of polarised light and water diffusion in biological tissues	software	Markus AXER, Cyril POUPON	Software will be released at M24. Software is now uploaded in the HBP Collab: • https://collab.humanbrainproject.eu/#/collab/19/nav/2108?state=software,Fiber%20Architecture%20Constructor • https://github.com/3d-pli/FAConstructor
C2367	SGA2 T2.6.6 HPC compatible global tractography	software	Cyril POUPON, Markus AXER	Software code will be released by M24.

Appendix: Fibre bundles and connectivity

SP2 produces unique datasets, protocols and tools for brain preparation, scanning, and big data analytics to reconstruct the brain's nerve fibre architecture from the system's level (i.e. pathways) down to the cellular level (i.e. individual axons) in a given brain sample. Below, we present the list of the multimodal connectivity datasets and describe their exploitation in other SPs:

1) ***In vivo* diffusion MRI (CEA)**

This dataset includes a map of human fibre bundles and their microstructure: the first release (V1) of the long and short bundle atlas, inferred from the HBP ARCHI dataset, has been delivered during SGA1 and has been integrated in the HBP Human Brain Atlas. This bundle atlas is now at the core of a **strong collaboration between SP2 and SP8** (WP8.8, originated from CEoI "Federated analysis of human intracerebral stimulation and recording data"), which aims to estimate **axonal conduction velocities** along each bundle, a key feature for the simulations planned in SGA3/WP1. For this purpose, the HBP bundle atlas has been aligned with the F-TRACT dataset of SP8 (T8.8.1, Olivier DAVID), consisting of electrical stimulations in 200 epileptic patients. A first proof of concept has been achieved for about 120 bundles and this has been presented at the Human Brain Mapping conference in Rome in June 2019 (E937).

In parallel, the **HBP bundle atlas has been aligned with the Human Connectome Project** (HCP) dataset to estimate, for each bundle, the variability of different features in the general population: bundle size and shape, diffusion properties (FA, MD, etc.). During SGA3, we will try to **predict the velocities from the microstructural properties of the bundles** in order to fine-tune the simulation to individual subject specificities.

Hence, at the end of SGA2, we will release the V1 bundle atlas for each bundle, implemented with axonal conduction velocity and diffusion properties (FA, MD, etc). This bundle atlas will be redefined during the first year of SGA3.

At the end of SGA2, we will also release a new version (V2) of the HBP bundle atlas developed from the HCP dataset and including about 300 bundles. A milestone of this work has been presented at the Organization for Human Brain Mapping conference 2019:

Labra Avila N, Lebenberg J, Rivière D, Auzias G, Fischer C, Poupon F, Guevara P, Poupon C, Mangin JF. Inference of an Extended Short Fiber Bundle Atlas Using Sulcus-Based Constraints for a Diffeomorphic Inter-subject Alignment (E1259).

2) Parcellation of the cortical surface (CEA)

At the end of SGA1, SP2 had delivered a proof of concept of **connectivity-based multiscale nested parcellations of the cortical surface.** This concept of nested parcellation is at the core of the connectome descriptions planned by SGA3/WP1. In SGA2, the structural connectivity matrices have been computed for the HBP Archi dataset as well as for the HCP dataset and they have been **exploited in SP4** for modelling of brain dynamics inferred from resting state fMRI data. This collaboration has recently led to a **first publication**: Gilson M, Kouvaris NE, Deco G, Mangin JF, Poupon C, Lefranc S, Rivière D, Zamora-López G. Network analysis of whole-brain fMRI dynamics: A new framework based on dynamic communicability (P2039).

Further interactions with SP5 and SP8 (CDP8, Petra RITTER) aim to integrate this nested parcellation scheme in the virtual brain (TVB), taking advantage of the identical first level parcellation (Freesurfer's Desikan parcellation). A first proof of concept of this integration will be performed through **a dedicated container chaining TVB and the Constellation pipeline, applied on UKbiobank massive dataset on ICEI-Fenix**.

During SGA3, an alternative connectivity-based parcellation scheme based on the HBP architectonics atlas will be added into EBRAINS.

3) Post mortem diffusion MRI (CEA)

During SGA2, an *ex vivo* human brain, cut into fourteen 4.2cmx4.2cmx5.6cm blocks (7 blocks per hemisphere) was scanned in an ultra-high field preclinical 11.7T MRI system, producing a unique mesoscopic resolution anatomical (100µm & 150µm), relaxometric (200µm) and diffusion (200µm, 3 shells at b=1,500, 4,500, 8,000s/mm2) MRI dataset. This unique dataset will outperform the best diffusion MRI dataset available today, limited to 400µm at a b-value of 3,000s/mm2. The acquisition of the left hemisphere is now achieved (3,300 hours of scan in total), the one of the right hemisphere is ongoing and scheduled to be finished in July 2020. Curation, reconstruction of the entire left hemisphere and registration to the HBP Human Brain Atlas frame will be finished by the end of SGA2. Preliminary results were presented at the 2019 ISMRM conference (Montreal, Canada):

Beaujoin J, Popov A, Yebga Hot R, Poupon F, Mangin C, Destrieux C, Poupon C. CHENONCEAU: towards a novel mesoscopic (100/200um) post mortem human MRI atlas at 11.7T (E1125).

The post mortem diffusion MRI dataset will be analysed using the following processes:

- manual segmentation of the deep and brainstem structures performed by our team and anatomists (C. Destrieux and coll.)
- segmentation of the cortical laminar structure using cytoarchitectural / myeloarchitectural features, inferred from diffusion and quantitative MRI, using the approach presented during the 2018 ISMRM conference (Paris, France) (Beaujoin J, Destrieux C, Poupon F, Zemmoura I, Mangin JF, Poupon C. Post-mortem mapping of cortical layers using combined multicompartmental relaxometry and diffusometry at ultra-high field (7T and 11.7T) (E1254).

  In SGA3, this laminar structure will allow establishing novel parcellations of the cortical surface based on the local thickness of layers, as well as on cyto-/myelo-architectural features, stemming from quantitative and diffusion MRI
- inference of the structural connectivity using global spin-glass tractography, using prior anatomical and microstructural knowledge in order to discard false positives (ICEI-FENIX).

  This processing, combined with fibre-clustering techniques, will allow to reconstruct long and short white matter bundles in subcortical areas, with a high degree of precision. It will also allow the computation of high-resolution structural connectome matrices with respect to available cortex parcellations;
- computation of quantitative profiles of axon density and axon dispersion on the set of segmented long and short white matter bundles. These profiles will be correlated with velocities stemming from Direct Electrical Stimulation (F-Tract consortium).

  All results will be integrated to the HBP Human Brain Atlas and a journal publication about this unique mesoscopic ex vivo diffusion MRI dataset has been submitted:

  Beaujoin J, Popov A, Yebga Hot R, Poupon F, Mangin C, Destrieux C, Poupon C. CHENONCEAU: the HBP mesoscopic (100/200um) post mortem human MRI atlas at 11.7T

4) Post mortem 3D PLI (Juelich)

During SGA2, the same human hippocampus imaged with dMRI was cryo-sectioned at 60 µm thickness and imaged with 3D-PLI at 1,3 µm pixel size. Each section measurement has undergone an HPC-based workflow to determine the local orientations of fibres and small fibre tracts (FOMs) to complement the high-resolution dMRI results (Beaujoin J, Palomero-Gallagher N, Boumezbeur F, Axer M, Bernard J, Poupon F, Schmitz D, Mangin JF, Poupon C. Post-mortem inference of the human hippocampal connectivity and microstructure using ultra-high field diffusion MRI at 11.7 T (P1748)). In addition to the 3D-PLI specific modalities (FOMs, transmittance, and retardation), blockface images were acquired during the sectioning process to make 3D FOM reconstruction and cross-modality alignment possible.

The reconstructed volume of blockface images (aligned with the corresponding dMRI volume data sets) is currently being curated and anchored to the BigBrain by SP5 colleagues. 3D-PLI data will be integrated step-by-step. By the end of SGA2, the joint dataset will be enriched with all reconstructed 3D-PLI modalities.

The data will become an integral part of the **HBP Human Brain Atlas**. Tractography, as developed in the context of T2.6.6, will be applied to the reconstructed volume, and this is an important next step to interface with the **theory/simulation groups in HBP** focusing at the hippocampus (SP6).

5) Combined application of TPFM, 3D-PLI, and dMRI to the same sample (LENS, Juelich)

In order to obtain a combinative approach between different technologies, new protocols were implemented to make **consecutive scanning of the same tissue with MRI, 3D-PLI and two-photon fluorescence microscope (TPFM) possible. The TPFM-specific protocol called MAGIC "Myelin Autofluorescence imaging by Glycerol Induced Contrast enhancement"** enables to visualise the spatial courses of auto-fluorescent myelinated axons within unstained brain sections, previously prepared for 3D-PLI. The optical sectioning of TPFM can achieve a resolution of 0.5 x 0.5 x 1 µm, providing integrative connectivity information.

During M1-M18, the protocol was successfully applied to sections from mouse, rat, monkey and human brains, previously imaged with 3D-PLI. In M19-M24 we will perform measurements on sample previously imaged with MRI and then with 3D-PLI; the TPFM acquisition will focus on the hippocampus area. To provide complementary connectivity information, datasets obtained from the different methodologies will be aligned and (wherever possible) visualised with tractography. This complementary approach permits to obtain information of dedicated regions of interest, such as the white/grey matter transition zones of u-fibre systems, which are very difficult to analyse about directionality using only the 3D-PLI approach. **All the data will be curated and integrated into the Knowledge Graph in strict collaboration with SP5.**

Hence, at the end of SGA2, we will release acquisitions and derived modalities of the hippocampus sample, using MRI, 3D-PLI and TPFM. The datasets will be aligned amongst each other.

In SGA3 this correlative approach will be used to analyse even larger human brain volumes of interest and will be extended by a fourth approach, i.e. immunohistochemistry staining. Combining the different methodologies (MRI, 3D-PLI, TPFM and immunohistochemistry), applied to the same sample helps to overcome the huge microstructural variability challenge that arises from the analysis of different samples by means of different techniques.

The correlation with the high-resolution imaging obtained with TPFM will permit to reach the goal of an exhaustive connectome descriptions planned by SGA3/WP1.

Publication is in preparation; data were presented at the following conference:

Costantini I "A label-free fluorescence approach for high resolution imaging of myelined fiber" INO annual symposium, 2019, Florence, Italy (E1255)

Costantini I, Menzel M., Silvestri L., Schubert N., Axer M., Amunts K., Pavone F.S. "Correlative Polarized Light Imaging and Two-Photon Fluorescence Microscopy for 3D myelinated fibers reconstruction" ECBO 2017 (P816)