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Abstract:

The objective of SP1 is to generate neuroscientific concepts, knowledge, experimental datasets and tools, which will be used to build models for the simulation of the brain. These models will be integrated, for example, into neuromorphic systems (SP9) or neurorobotics controllers (SP10) in order to create cost-effective, energy-efficient, high-performance systems. An important role for SP1 is to provide data and knowledge to support activities undertaken by other SPs.

This report provides a detailed account of the Results for SGA1 M01-M12. Within this report we outline the datasets which have been generated in the first 12 months of SGA1 including:

(i) Brain Atlas data package: this data, which will be deposited in the HBP Brain Atlas, includes first drafts of Maps of the vasculature; Whole-brain maps of different cellular types based on gene expression; Microcircuitry analysis, proteins and receptor distributions and fibre architecture; Maps of cellular distributions, long-range axonal projections, and synaptic proteins; reconstructed morphologies of major neuron types; Whole-brain activation maps; Spatial organisation principles in brain activation; Functional maps of cortical activity;

(ii) Brain Modelling data package: This package includes preliminary data on quantitative description of synaptic connections on neurons; numbers, distributions and relative densities of cells in selected regions and where possible across the whole brain; statistical parameters characterising particular cell types and spatial arrangements between neurons, glia and blood vessels; a high-resolution quantitative synaptic map of exemplar brain regions; EM blocks scans and volume analysis of exemplar brain regions with quantification of the neuropil organisation; microcircuit analysis; functional maps of brain activation; morphological and physiological comparative studies of neurons between rodent and human.

Keywords:

Anatomy, electrophysiology, neocortex, cerebellum, hippocampus, basal ganglia, whole brain, ion channels, receptors, IACT-selected antibodies, trans-synaptic signalling, synaptic plasticity, axonal projections, synaptic maps, ultrastructural data, brain vasculature, cell distributions, cell morphologies, whole brain, comparative studies, integration of multilevel data.

Table of Contents

List of Tables

List of Figures

1. SP Leader's Overview

1.1 Key Personnel

Subproject Leader: Javier DEFELIPE (UPM)

Subproject Deputy Leader: Sten GRILLNER (KI), Egidio D'ANGELO (UNIPV)

Subproject Manager: Pilar F. ROMERO (UPM)

1.2 Progress

SP1 has generated neuroscientific knowledge, experimental datasets and tools to build models for the simulation of the brain and to support activities undertaken by other SPs. In general, all Task Leaders have reported a good outcome during this period in spite of some administrative constraints as outlined below. Most of the Task Components have started as scheduled and are progressing well. Furthermore, as a result of the interaction with other researchers within SP1 or in collaboration with other SPs, it has produced additional data that was not originally planned. As indicated below, a number of publications and different dissemination activities have been carried out and several publications are underway. The present report is the first Deliverable planned to be delivered by SP1 in SGA1.

1.3 Deviations

Some delays have occurred in WP1.2 and WP1.4 due to the delay in the SGA1 signature. The delay was produced because the hiring of the planned personnel was postponed until the SGA1 was signed following the practices of the relevant organisations. In addition, once signed the SGA1, the administrative procedure followed to hire personnel took several weeks. Therefore, the effort invested in some Tasks was lower than planned in the first months of the SGA1. These delays have impacted the progress of some activities in T1.2.5, T1.2.7, T1.2.8 and T1.4.2. Four planned Milestones (MSs) have not been achieved in a timely manner or have been partially achieved consequently. These MSs are described in Tables 2 and 4. The effort in these Tasks has been increased as a result of the deviations to avoid further delays.

Moreover, MS1.3.7 from WP1.3 has not been achieved due to hardware issues at the confocal light sheet microscopy (broken rotation stage and delayed implementation of the newly developed autofocus system). It is expected to be achieved in the next few months. In addition, MS 1.5.3 is delayed. The drawing up of the draft data management plan has already started and it is expected to be delivered in M16.

All the delayed activities have now started and are currently running smoothly. It is expected that they will be on schedule in the next few months. No changes to the DoA work plan have been reported. SP1 is on schedule to meet the proposed objectives in SGA1 in M24 as planned.

1.4 Impact of work done to date

The datasets that are being generated in SP1 together with integration of external datasets that complement the core SP1 data are critical to constrain and validate high fidelity models of brain circuits developed in other SPs. In addition, these data are currently being integrated in mouse and human brain atlases. It is also expected that the technological improvements obtained in sample preparation, instrumentation and data analysis by different researchers of SP1 will have considerable impact in the neuroscientific field. In general, the scientific productivity is good. Indeed, some of the results obtained during the first 12 months of SGA1 have been published or has been sent for publication.

1.5 Priorities for the remainder of the phase

SP1 will continue with the proposed research plan in order to generate neuroscientific concepts, knowledge, experimental datasets and tools, which will be used to build models for the simulation of the brain. Furthermore, SP1's objective is to provide data and knowledge to support activities undertaken by other SPs focusing in two major data packages:

(i) Brain Atlas data package (results for SGA1 Period 2): brain maps of the vasculature; Whole-brain maps of different cellular types based on gene expression; Microcircuitry analysis, proteins and receptor distributions and fibre architecture; Maps of cellular distributions, long-range axonal projections, and synaptic proteins; reconstructed morphologies of major neuron types; Whole-brain activation maps; Spatial organisation principles in brain activation; Functional maps of cortical activity.

(ii) Brain Modelling data package (results for SGA1 Period 2): quantitative description of synaptic connections on neurons; numbers, distributions and relative densities of cells in selected regions and where possible across the whole brain; statistical parameters characterising particular cell types and spatial arrangements between neurons, glia and blood vessels; a high-resolution quantitative synaptic maps of exemplar brain regions; EM blocks scans and volume analysis of exemplar brain regions with quantification of the neuropil organisation; microcircuit analysis; functional maps of brain activation; morphological and physiological comparative studies of neurons between rodent and human.

In addition, we will also focus on the Integration of multilevel data and on the application of statistical modelling and machine learning techniques to infer principles of human and mouse brain organisation.

Finally, SP1 is currently working on the detailed plan of data usage and the impact of generated data on models. This strategic data plan will be delivered in M24 and will be implemented in the SGA2 and will continue throughout the Project.

2. WP1.1: Subcellular and Molecular

2.1 Key Personnel

Work Package Leader: Antonino CATTANEO (SNS)

2.2 WP Leader's Overview

- What went particularly well?

Subcellular localisation: the strategy of receptor and ion channel mapping has been set up. Data on the average of density of immunoparticles/µm2 (2D) and immunoparticles/µm3 (3D), size of clusters, composition of clusters, and spatial relation of clusters for different gold particles in the different compartments of pyramidal cells of the hippocampus have been provided, thus mapping the 2D and 3D distribution of neurotransmitter receptors (AMPA, NMDA and GABAB receptors) and ion channels (SK and GIRK channels) in the hippocampus using newly developed immunoelectron microscopy techniques (the SDS-FRL and immunogold FIB/SEM techniques), in four weeks-old male C57BL/6 mice.

Generation of new tools and methods by using IACT-selected antibody domains for next generation brain imaging and mapping: Abeta oligomers - the optimisation of new and comparative protocols of immunodetection in post-mortem human brain in different areas of cortex has been implemented. Anti-NLGN2 - constructs for expression and purification of recombinant proteins developed; Direct labelling of recombinant small antibody domains - optimisation of constructs and protocols with tags for fluorophore conjugation completed.

K Channel Kinetics: the generic kinetic model of K channels with dependency on extra-cellular concentration of K+ is in place and the functional role of key voltage-gated potassium channels based on kinetic modelling is illustrated.

Synaptic plasticity: a model of synaptic plasticity written in Kappa that contains the proteins calmodulin, PSD-95, stargazin, Calcium-Calmodulin dependent kinase II (CaMKII), PP3, I-1 and PP1 has been built. This model is presented with calcium stimuli of varying durations and the synaptic strength in the model can be compared to the wild type traces in Fig. 3 of Carlisle et al (2008), J. Physiol. 586. The next steps are to optimise the parameters to improve the agreement between model and data.

Generation of in vivo functional data on interaction between synaptic proteins and their use for computational modelling of trans-synaptic signalling: the production and validation of intrabodies for synaptic interference have been implemented; the generation of electrophysiological data on the contribution of neuroligins in i) signalling regulation between pre and postsynaptic processing and ii) maintenance of the inhibitory-excitatory balance are currently being carried out; The code of the Graphical User Interface (GUI) to configure the procedure to fit synaptic events is integrated and used in some cases of the Brain Simulation Platform (SP6).

- What didn't go according to plan?

The WP1.1 Tasks have progressed as scheduled.

- Impact of work done

The studies in WP1 are generating and integrating data at the subcellular and molecular levels. During the Ramp-Up Phase, there were rapid technological advances enabling higher resolution analysis of spatial and molecular information including those at the single molecule, single synapse and single cell levels. These datasets include functional data by interference approaches. Moreover, some of these methods were scaled to permit mapping of diversity across many cells, brain regions and whole brain. The generation of these molecular datasets, coordinated and integrated with the anatomical studies that are currently being carried out in WP2, will provide the foundation for key Platform Deliverables and Co-Design Projects.

2.3 Priorities for the remainder of the phase

The work performed in WP1 is generating datasets at the subcellular and molecular levels, based on technological developments during the Ramp-Up Phase. These datasets are being integrated in the models. The priority for the remainder of the phase will be to improve the coordination and integration of the different molecular and cellular datasets obtained in WP1 with the data gathered in the rest of SP1 and with the anatomical studies currently ongoing in SP2. Also, the coordination and integration of the synaptic models being developed (e.g. synaptic plasticity and trans-synaptic signalling) will be a priority.

2.4 Milestones

Table 1: Milestones for WP1.1: Subcellular and Molecular

MS No.	Milestone Name	Leader	Task(s) involved	Expected Month	Achieved Month	Comments
MS1.1.1	Receptor and ion channel mapping strategy	Partner UCLM	T1.1.1	M12	M12	Achieved. We have provided an average of density of immunoparticles/μm^2 (2D) and immunoparticles/μm^3 (3D), size of clusters, composition of clusters, and spatial relation of clusters for different gold particles in the different compartments of pyramidal cells of the hippocampus, thus mapping the 2D and 3D distribution of neurotransmitter receptors (AMPA, NMDA and $GABA_B$ receptors) and ion channels (SK and GIRK channels) in the hippocampus using newly developed immunoelectron microscopy techniques (the SDS-FRL and immunogold FIB/SEM techniques), in four weeks-old male C57BL/6 mice. ImmunoFib/SEM samples from somatosensory cortex, we are investigating the same parameters as in the hippocampus. So far, we have obtained data from 10 neurons of postnatal 27- to 34-day-old male C57BL/6

						mice. Replica samples (SDS-FRL) from the somatosensory cortex have been prepared but not yet analysed. Number of neurons investigated is difficult to count in replica labelling since profiles are only fragments of different neurons, but should be more than 50.
MS1.1.2	Illustration of the functional role of key voltage-gated potassium channels based on kinetic modelling	Partner SIB	T1.1.5	M12	M12	Achieved. In the previous phase of this project we have developed kinetic models describing the activity of different voltage-dependent K channels. These channels present different gating properties and are thus thought to impact differently on the propagation of action potential in neurons. Here our goal was to define a set of computer experiments to illustrate such behaviour. We used the software Neuron and a standard neuron model in which the ionic current associated with Kv channels was in part replaced by the kinetic model of a single type of Kv channels (Ten channels from the Kv1 and Kv2 subfamilies were used as test cases). We have applied different electrophysiological

						protocols that vary in the action potential firing frequency and train length, as well as in the interval between spike trains. This allows us to evaluate how the given Kv channel modulates the action potential amplitude, shape, and firing frequency under different conditions. The same protocol will later be applied to the different Kv channels that were modelled in the Ramp-Up Phase.
MS1.1.3	Computational, dynamic model of LTP and LTD in a wild type Schaffer Collateral synapse	Partner UEDIN	T1.1.6	M12	M12	Achieved. We have built a model of synaptic plasticity written in Kappa that contains the proteins calmodulin, PSD-95, stargazin, Calcium-Calmodulin dependent kinase II (CaMKII), PP3, I-1 and PP1. This model is presented with calcium stimuli of varying durations and the synaptic strength in the model can be compared to the wildtype traces in Fig. 3 of Carlisle et al (2008), J. Physiol. 586. Our next steps are to optimise the parameters to improve the agreement between model and data.

2.5 T1.1.1 Subcellular Localisation: 2D and 3D Mapping of Receptors and Ion Channels

2.5.1 Key Personnel

Task Leader: Rafael LUJÁN (UCLM)

Other Researcher: Ryuichi SHIGEMOTO (IST)

2.5.2 SGA1 DoA Goals

- Investigate the subcellular localisation of different subunits of receptors and ion channels in the hippocampus and neocortex in a compartment-dependent manner.
- Determine the composition of immunoparticle clusters for neurotransmitter receptors and associated ion channels, providing an average of density of immunoparticles/µm2 (2D) and immunoparticles/µm3 (3D), size of clusters, composition of clusters, and spatial relation of clusters for different gold particles.

2.5.3 Component Progress

2.5.3.1 Developing the integrated FIB/SEM and SDS-FRL immunoelectron microscopy technique

Description of Component: We will integrate two newly developed immunoelectron microscopy techniques: 1) an automated dual-beam electron microscope that combines focused ion beam milling and scanning electron microscopy, and 2), we will combine this technology with pre-embedding immunogold reactions (FIB/SEM immunogold) to obtain three-dimensional reconstruction, and with SDS-digested freeze-fracture replica labelling (SDS-FRL) to obtain two-dimensional views of molecular distribution on the surface of neurons.

Progress on Component: Component is progressing as planned. M12 release *Draft report describing the method* is in progress. We are investigating the subcellular localisation of GABAB receptor, SK2 channels, GIRK channels and Cav channels subunits in the hippocampus and neocortex in a compartment-dependent manner. Ryuichi SHIGEMOTO has developed grid-glued replica labelling methods for preparing fully intact replicas covering all layers of CA1 (Harada and Shigemoto, 2016). Samples prepared with this technique can be analysed by SEM or TEM with large (10 nm or more) or small (2-5 nm) gold particles. The method has been applied to other kinds of samples including neocortex, and proved useful to keep good orientation for identification of different neuronal compartments and matching pairs of replicas complementary to each other.

UCLM contributed to replica labelling for GABAB receptor, GIRK and Cav2.1 subunits, and the 3D mapping of GABAB receptor, SK2 channels and GIRK channels. IST contributed to replica labelling for AMPA and NMDA receptors and Cav2.1 subunits for the 2D mapping. In addition, IST contributed to the establishment of fluorescence guided and grid-glued methods for quantitative freeze-fracture replica labelling.

Quality Control:

- No upstream Components.
- Downstream Components: Nanoscale measurements of distributions of individual receptors and ion channels in cortical neurons [essential]; Task responsible: T1.1.1; Status: provided intermediate release.

2.5.3.2 Nanoscale measurements of distributions of individual receptors and ion channels in cortical neurons

Description of Component: This highly innovative approach will provide, for the first time, a mapping and comprehensive characterisation of the cellular and subcellular localisation of

specific ion channel subunits and neurotransmitter receptors along the entire surface of cortical neurons, using single labelling experiments for each receptor and ion channel

Progress on Component: Component is progressing as planned. M12 release (MS1.1.1) *Receptor and ion channel mapping strategy* achieved. All work was done by UCLM & IST. UCLM contributed to nanoscale measurement for GABAB receptor, GIRK and SK2 subunits for the 2D mapping. IST contributed to replica labelling for AMPA and NMDA receptors and Cav2.1 subunits for the 2D mapping.

Quality Control:

- Upstream Component: Developing the integrated FIB/SEM and SDS-FRL immunoelectron microscopy technique; T1.1.1; Status: received; Quality: excellent.
- Downstream Components: SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data [important], SP6-T6.2.4-SGA1-Models of mouse hippocampal neurons [important], SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data [essential], SP1-T1.1.5-SGA1 K channel activity within neuron models [important]; Nothing released.

2.6 T1.1.2 Exploiting the IACT Antibody Platform to Isolate Small Antibody Domains for Next-Generation Brain Imaging & Mapping

2.6.1 Key Personnel

Task Leader: Antonino CATTANEO (SNS)

Other Researcher: Enrico CHERUBINI (EBRI)

2.6.2 SGA1 DoA Goals

Generating new tools and methods by using IACT-selected antibody domains for next generation brain imaging and mapping. The target antigens are: Abeta oligomers, Gephyrin, Neuroligins and Neurexins.

2.6.3 Component Progress

2.6.3.1 Antibodies against targets identified in all genomic and proteomics tasks (data)

Description of Component: We use IACT-SPLINT (Intracellular Antibody Capture Technology - Single Pot Library of Intracellular Antibodies) to obtain, in a validated manner, antibodies against targets identified in genomic and proteomics tasks, without the need to express the proteins (from genes to antibodies), with the additional bonus that also the genes coding for the antibody domain are concurrently isolated, so that the selected antibodies can be used either as proteins (classical use, but with improved properties) or as genes (intrabodies for functional interference).

Progress on Component:

- Anti-Abeta Oligomers small antibody fragments: optimisation of new and comparative protocols of immunodetection (immunofluorescence and confocal microscopy) in post-mortem human brain from cases of Alzheimer's Disease, in different area of cortex (Frontal and parietal). The work was done by EBRI.
- Anti-NLGN2: constructs for expression and purification of recombinant proteins. The work was done by EBRI.
- Direct labelling of recombinant small antibody domains for direct immunohistochemistry: optimisation of constructs and protocols with different tags and fluorophores. The work was done by SNS.

- Evolution of the IACT antibody selection method: Evolution of the IACT antibody selection method for the isolation of antibody domains against post translationally modified proteins and their use for post translational selective silencing in cells (Chirichella et al. (2017) Nature Methods 14, 279–282, doi:10.1038/nmeth.4144 - SNS

Quality control:

The validated antibody domains are being distributed to users of SP1, and we shall explore other interested consumers of these antibodies within other SPs of HBP, in particular those involved in molecular disease investigating tasks.

- No upstream Component.
- Probable release to downstream Components (T1.3.3, T1.3.4, T2.2.2 and T2.2.3, supported by Component linkage; T1.1.3 not supported by Component linkage). At this stage, the release to downstream Component Tasks T6.1.2 and T6.1.3 is being explored.

2.7 T1.1.3 Principles of Glutamate and GABA Receptor Distribution, and Ca2+ and K+ Channel Distribution

2.7.1 Key Personnel

Task Leader: Rafael LUJÁN (UCLM)

Other Researcher: Ryuichi SHIGEMOTO (IST)

2.7.2 SGA1 DoA Goals

- Investigate principles of co-localisation of different subunits of receptors and ion channels in the hippocampus and neocortex.
- Determine the association (co-clustering) of receptors and their effector ion channels in different neuronal compartments using double-labelling approaches with gold particles of different size.
- Elucidate spatial relationships and composition of immunoparticle clusters for neurotransmitter receptors and associated ion channels, and how these parameters differ in different compartment and for different effector channels.
- Provide individual channel distances for the two related receptors and ion channels, channel cluster distances for two kinds of clusters, size of these co-clusters, their composition, and spatial relation of the co-clusters to synapses in different neuronal compartments, done using a MATLAB based software we developed.

2.7.3 Component Progress

2.7.3.1 Association (co-clustering) of receptors and their effector ion channels in different neuronal compartments

Description of Component: We will provide individual channel distances for the two related receptors and ion channels (for example, GABAB and GIRK, Cav2.1 and SK, NMDA and SK), their co-clustering, and channel cluster distances for two kinds of clusters in different neuronal compartments together with size of these co-clusters, their composition, and spatial relation of the co-clusters to synapses obtained from more than 100 neurons, in different subcellular compartments

CDP to which Component contributes (if relevant): NA

Progress on Component: Component is progressing as planned. No releases planned in M1-M12; We have provided the co-clustering of $GABA_B$ receptors and their effector ion channels, GIRK and Cav2.1, in different neuronal compartments using double-labelling approaches with gold particles of different size. UCLM designed and developed a MATLAB based software

for quantitative analyses of clustering and co-clustering in replica materials. We have elucidated spatial relationships and composition of immunoparticle clusters for $GABA_B$ receptors and associated GIRK and Cav2.1, and how these parameters differ in different compartment. We have provided individual channel distances for the $GABA_B$ receptors and GIRK channels, channel cluster distances, size of these co-clusters, their composition, and spatial relation of the co-clusters to synapses in different neuronal compartments. For quantitative analyses for 2D mapping using SDS-FRL technique, we developed a MATLAB based software to provide information on the average of density of immunoparticles/μm^2 (2D) and immunoparticles/μm^3 (3D), size of clusters, composition of clusters, and spatial relation of clusters for different gold particles.

All work was done by UCLM & IST. UCLM contributed to replica labelling for GABAB receptor, GIRK and Cav2.1 subunits. UCLM designed and developed a MATLAB based software for quantitative analyses of clustering and co-clustering in replica materials. IST contributed to the development of MATLAB based software for the analyses of clustering and co-clustering in replica materials.

Quality Control:

- No upstream Components.
- Downstream Components: SP6-T6.2.4-SGA1-Models of mouse hippocampal neurons [added value]; SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data [essential]; SP6-T6.1.3-SGA1-Data-driven modelling of Ca2+ dependent cascades controlling synaptic signalling and homeostasis [important]; SP6-T6.1.2-SGA1-Data-Driven Modelling of G Protein-Coupled Receptor-Dependent Cascades [important]; Status: nothing provided.

2.8 T1.1.4 Generation of *In vivo* Functional Data on Interactions Between Neuroligin and Neuroxin Synaptic Proteins, and their Use for Computational Modelling of Trans-synaptic Signalling

2.8.1 Key Personnel

Task Leader: Enrico CHERUBINI (EBRI)

Other Researcher: Cristina MARCHETTI (EBRI), Silvia MARINELLI (EBRI), Giovanni MELI (EBRI)

Other Researcher: Antonino CATTANEO (SNS, Lead Researcher), Martina GORACCI (SNS), Simonetta LISI (SNS)

Other Researcher: Michele MIGLIORE (CNR, Lead Researcher), Carmen LUPASCU (CNR)

2.8.2 SGA1 DoA Goals

Production of a set of protein interference tools (scFv antibody domains against neuroligins and neurexin) and their biochemical/molecular validation. Generation of electrophysiological data on the contribution of neuroligins and neurexins in i) signalling regulation between pre and postsynaptic processing and ii) maintenance of the inhibitory-excitatory balance. From gathered data, generation of unique computational procedures (currently not available outside the HBP) making them available to the HBP community.

2.8.3 Component Progress

2.8.3.1 Electrophysiological data under neuroligin block

Description of Component: This Component will provide electrophysiological data to study the role played by NLGN-NRXN connections in regulating signalling between post- and pre-synaptic processing in the CA1 region and dentate gyrus of hippocampus.

Progress on Component:

Production and validation of intrabodies:
IACT-selected intrabodies directed against their cytoplasmatic domains of NLGs: a) two anti-NLG2; b) one anti-NLG3; c) one anti-NLG1 intrabodies; d) four anti-NLG3 VH antibody domains directed against the extracellular domain. Use and validation of the lentiviral systems for intrabodies anti-Gephyrin and anti-NLG2 in mouse cultured neurons, organotipic slices and *in vivo*. Functional assays with constitutive lentiviral (dual promoter-EGFP) anti-Gephyrin and anti-NLG2. Ongoing activities towards the generation of new antibodies targeting the extracellular domains of NLGs and Neurexins. Exploiting intrabodies (KDEL tagged) retained in the endoplasmic reticulum and targeting the extracellular domains of NLGs – SNS/EBRI.

Transynaptic signalling in the regulation of synaptic processes:
Work on this Component is still in progress. Hippocampal neurons in culture infected with a lentivirus vector containing intrabodies against gephyrin targeted to the cytoplasmic domain. So far we have characterised the frequency and amplitude of pharmacologically isolated miniature $GABA_A$-mediated inhibitory postsynaptic currents (mIPSCs) in neurons expressing EGFP coupled to intrabodies or EGFP alone (controls). A decrease of mIPSC frequency and amplitude has been revealed in cells containing the intrabodies (respect to controls:EGFP alone) but the number of experiments is still too low to reach a significant level and ongoing experiments will increase the statistic.

Ex vivo recordings of spontaneous action-potential dependent $GABA_A$-mediated inhibitory post synaptic currents (sIPSCs) from dentate gyrus granule cells in hippocampal slices obtained from mice stereotaxically injected 21 days before with lentivirus vector containing intrabodies against neuroligin 2 and EGFP or EGFP alone (controls). In granule cells expressing NL2 intrabodies, (sIPSCs) sIPSCs occurred at lower frequency respect to controls (EGFP alone). Also in this case the number of injected cells is too low to reach a significant level and ongoing experiments will increase the statistic (EBRI).

Quality Control:

- Upstream Components:
  - Antibodies against targets identified in all genomic and proteomics tasks [important], Collaboratory Storage Service [added value]; Tasks responsible: T1.1.2; T5.6.1, T11.3.2; Status: intermediate release; Quality: excellent.
- Downstream Component:
  - STP Model; Task responsible: T1.1.4; Status: intermediate release

2.8.3.2 STP data

Work for this Component is still in progress since it is downstream of activities in this Task, described in paragraph 2.3.1, above. Data will be provided to downstream Tasks 4.3.1 and, possibly to other Tasks.

2.8.3.3 Report on functional *in vivo* interaction data between synaptic proteins of the neuroligin and the neuroxin families, and their use for the computational modeling of trans-synaptic signalling

Work on the functional in vivo interaction data between synaptic proteins of the NLG and NRX families is reported above, in paragraph 2.8.3.1. As for the work on computational modelling using these data, it will start in the next year.

2.8.3.4 Graphical User Interface (GUI) to configure the procedure to fit synaptic events

Description of Component: Graphical User Interface (GUI) to configure the procedure to fit synaptic events (*CNR)*

Progress on Component: planned release achieved. Component has been released by Partner CNR. The code is integrated and used in the following Use Cases of the Brain Simulation Platform:

Synaptic events fitting

Synaptic events fitting (user's model)

Synaptic events fitting (user's data)

Quality Control:

- This Component has nine Upstream Components from different Tasks. We have received high quality Components from all of them. There were no major problems in the implementation process; however, all upstream Components from the Neuroinformatics Platform are still missing, and important weaknesses and limitations of the Collaboratory, especially those related to the use of Jupyter notebooks, reduce user friendliness.
- Its downstream use is planned for a report on functional *in vivo* interaction data between synaptic proteins of the NLGN/NRXN families. This is in preparation and planned for M24.

2.8.3.5 STP model

Description of Component: This Component will produce NEURON mod files reproducing functional interaction between synaptic proteins of the NLGN/NRXN families, ready to be used to implement trans-synaptic signalling of inhibitory CA3-CA1 synapses.

CDP to which Component contributes: CDP No.2, Mouse-Based Cellular Cortical and Subcortical Microcircuit Models, CDP2-UC-001: Single cell modelling (through task T6.2.4)

Progress on Component: The work is proceeding as planned. This Component will generate NEURON mod files reproducing functional interaction between synaptic proteins of the NLGN/NRXN families, ready to be used to implement trans-synaptic signalling of inhibitory CA3-CA1 synapses. Planned release is for M24 (CNR).

Quality Control:

- This Component has eight upstream Components from different Tasks. We have received input from all of them. There are no major problems in the implementation process. The main upstream Components are: Electrophysiological data under neuroligin block (essential), STP data (essential), NEURON (essential), Python (essential)
- The eight downstream Components will receive an early version around M18 and a final release on M24. The main downstream Components are: T1.1.4 Report on functional in vivo interaction data between synaptic proteins of the neuroligin and neuroxin families (important). The connections to T6.1.3, 6.2.4 and 6.4.3 (all "added value")

2.9 T1.1.5 K Channel Kinetics: Modulation of Action Potential Propagation by K Channels

2.9.1 Key Personnel

Task Leader: Simon BERNECHE (SIB)

Other Researcher: Niklaus JOHNER (SIB)

2.9.2 SGA1 DoA Goals

In the Ramp-Up Phase, we designed kinetics models for the different Kv channels (T1.1.5). In SGA1 we aim at understanding how the different channels interact to modulate neural

signalling. The integration of such detailed kinetic models in neuron simulations will provide a view of the landscape of possibilities in terms of signal modulation and help neuroscientists to decide about the properties of Kv channels that need to be retained for accurate modelling of neuron computing.

2.9.3 Component Progress

2.9.3.1 SP1-T1.1.5-SGA1 K channel activity within neuron models

Description of Component: NEURON model that illustrates to what extent neuron signalling can be modulated by the action of K channels using realistic channel distributions and kinetics.

Progress on Component: this Component is progressing as scheduled. M12 release *Neuron models illustrating the activity of K channels* has been achieved. . For reaching Milestone MS1.1.2 "*Illustration of the functional role of key voltage-gated potassium channels based on kinetic modelling"*, an approach was developed based on a neuron model published by A. Destexhe, H. Markram, and colleagues (Minimal Hodgkin–Huxley type models for different classes of cortical and thalamic neurons. Biol. Cybern. 99:427–441 (2008). The K current is replaced in adjustable proportion by the activity of a given Kv channel for which a kinetic model is available. In relation to the Component "*K channel kinetic models including [K+]ext*", the model allows for the control of the extracellular K^+ concentration. All work was done by SIB.

Quality Control:

- Upstream Component name (from PLA)

SP1-T1.1.5-SGA1 K channel kinetic models including [K^+]ext; Task responsible: T1.1.5 (S. Bernèche); Status: received intermediate release; Quality: good.

Nanoscale measurements of distributions of individual receptors and ion channels in cortical neurons (data); Task responsible: T1.1.1 (R. Lujan), Status: nothing received.

2.9.3.2 SP1-T1.1.5-SGA1 K channel kinetic models including [K+]ext

Description of Component: Kinetic models of K channels with dependency on extra-cellulaire concentration of K^+.

Progress on Component: The generic kinetic model is in place. We are awaiting new electrophysiological data for a subset of K channels in presence of different extra-cellular concentration of K+. Using these data, we will determine the corresponding kinetic rates for the different channels. If our colleagues from the Blue Brain Project at EPFL are still unable to provide us with [K+]ext dependent data, we will use data found in the literature for the well characterised *Shaker* channel. Developing such kinetic model for the *Shaker* channel would allow us to illustrate, using single neuron simulations, the key role that extracellular K^+ ions could play in neuron signalling. All work was done by SIB.

Quality Control:

- Upstream Component name (from PLA)

SP1-T1.1.5-SGA1 K channel kinetic models including [K^+]ext; Task responsible: T1.1.5 (S. Bernèche); Status: received; Quality: excellent.

- Downstream Component name (from PLA)

SP1-T1.1.5-SGA1 K channel activity within neuron models (model), Task responsible: T1.1.5 (S. BERNÈCHE); Status: provided intermediate release.

SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data (data)(added value); Task responsible: T6.2.2 (J. DEFELIPE); Status: nothing provided.

SP6-T6.2.4-SGA1-Models of mouse hippocampal neurons (model) (added value); Task responsible: T1.2.5 & T1.2.6 (S. KÁLI); Status: nothing provided.

SP6-T6.2.1-SGA1-models of nonlinear human neurons (model)(added value); Task responsible: T6.2.1 (Guy EYAL); Status: nothing provided.

2.10 T1.1.6 Molecular Data-Driven Modelling of Synaptic Plasticity

2.10.1 Key Personnel

Task Leader: Douglas ARMSTRONG (UEDIN)

Other Researcher: David STERRATT (UEDIN)

Other Researcher: Oksana SOROKINA (UEDIN)

2.10.2 SGA1 DoA Goals

The overarching aim of this task is to develop data-driven models of synaptic plasticity and transmission using bioinformatic data now available. The particular goals for this task are:

- Collecting a definitive Protein-protein interaction dataset
- Testing the capabilities of hybrid rule-based/electrophysiological simulation (KappaNEURON)
- Create a model containing one un-modelled but important molecule (PSD-95)
- An additional goal of the task is, in collaboration with others in the HBP (Neil BRUCE, Paolo CARLONI [T6.1.1], Andrew DAVISON [T6.4.3], Michele MIGLIORE [T1.1.4], Daniel KELLER, Jeanette HELLGREN KOTALESKI [T6.1.32]), to write a position paper on synaptic modelling.

2.10.3 Component Progress

2.10.3.1 A mapping of computational models of synapses to proteins

Description of Component: Description: An analysis of what proteins are contained in 15+ existing biophysical models of synaptic plasticity that contain more than one signalling pathway (as defined by Manninen et al 2010, Front Comp. Neur 4). This requires a number of tables:

1) A table of "entities". An "entity" may be: a Protein; a Protein Multimer, which can comprise multiple proteins, (e.g. AMPAR, which contains a combination of GluR1-4); a Protein family (e.g. Calmodulin, which may correspond to one of Calmodulin-1, Calmodulin-2 or Calmodulin-3); an ion; or a second messenger. Each protein entity has a standard, meaningful ID, a full name, and mappings to HGNC Family ID, ENTREZ IDs, and MGI IDs
2) A table of mappings of models to entities. Each row contains the PMID of the paper, the standard name of the entity and the name used for the entity in the paper. There is one row for each entity-paper combination.
3) Additional mappings to resolve mappings of Protein Families and Protein Multimers to genes. There are entities that are implicitly considered protein families by modellers that do not have official protein families in the HGNC or Interpro databases, e.g. SOS. Ideally, mappings we generate will be fed back into the community databases.
4) A table of model characteristics, e.g. region of the brain specified, approximate size of model, simulator used. Some of these data will already exist, e.g. in modeldb or OpenSourceBrain, and these databases would be free to incorporate data we extract.

These tables will be processed to give various views on the data.

Progress on Component: We have added to and refined the tables since the M6 report. UEDIN did all this work.

Quality Control:

- Upstream: SP6-T6.1.3-SGA1-Data-driven modelling of Ca2+ dependent cascades controlling synaptic signalling and homeostasis (T6.1.3). Status: Received nothing, but we are in discussion with the Task Leader
- Downstream: Analytical review of proteins contained in computational models of synaptic plasticity (T1.1.6). Status: intermediate release
- Downstream: SP6-T6.1.3-SGA1-Data-driven modelling of Ca2+ dependent cascades controlling synaptic signalling and homeostasis (T6.1.3). Status: intermediate release (via Analytical review of proteins contained in computational models of synaptic plasticity)
- Downstream: SP6-T6.1.2-SGA1-Data-Driven Modelling of G Protein-Coupled Receptor-Dependent Cascades (T6.1.2). Status: intermediate release (via Analytical review of proteins contained in computational models of synaptic plasticity)

Use Case: The Component contributes to the Use Case Biophysical model of LTP and LTD in wild type and mutant hippocampal CA1 synapse. https://project-lifecycle.herokuapp.com/use_case/89

2.10.3.2 Analytical review of proteins contained in computational models of synaptic plasticity

Description of Component**:** An analytical review paper that has at its core an mapping of proteins contained in biophysical models of synaptic plasticity, in particular models falling into the category of containing more than one intracellular pathway (Manninen et al 2010, Front Comput Neur 4).

Progress on Component: **"**M11 - Manuscript sent to a journal for review: We have not met this release data, but we have written a draft of the manuscript, which we plan to submit in M13. UEDIN did all this work.

Quality Control:

- Upstream: A mapping of computational models of synapses to proteins (T1.1.6). Status: intermediate release.
- Downstream: LTP model (T1.1.4). Status: nothing provided. We will provide the final submitted manuscript.
- Downstream: SP6-T6.1.2-SGA1-Data-Driven Modelling of G Protein-Coupled Receptor-Dependent Cascades (T6.1.2). Status: preliminary draft provided.

Use Case: The Component contributes to the Use Case Biophysical model of LTP and LTD in wild type and mutant hippocampal CA1 synapse. https://project-lifecycle.herokuapp.com/use_case/89

2.10.3.3 KappaNEURON software package

Description of Component: KappaNEURON (https://github.com/davidcsterratt/KappaNEURON) integrates the SpatialKappa simulator with NEURON to allow rule-based simulations of molecular systems embedded in neurons. For example, the package facilitates simulation of dynamic models of the postsynaptic proteome in the context of the spine head. For full details, see Sterratt, D. C., Sorokina, O. and Armstrong, J. D. (2015). "Integration of rule-based models and compartmental models of neurons". Lecture Notes in Bioinformatics 7699: 143-158. Presented to the Third International Workshop on Hybrid Systems Biology Vienna, Austria, July 23-24, 2014 at the International Conference on Computer-Aided Verification 2014.

http://dx.doi.org/10.1007/978-3-319-27656-4_9. Preprint at https://arxiv.org/abs/1411.4980.

Progress on Component: No releases were scheduled, but we have updated KappaNEURON to work with the latest stable version (version 7.4) of NEURON. UEDIN did all this work.

Quality Control:

- Upstream: Plasticity: Proof of concept detailed rule-based synaptic plasticity model (T1.1.6). Status: Finished Component.

Use Case: The Component contributes to the Use Case Biophysical model of LTP and LTD in wild type and mutant hippocampal CA1 synapse. https://project-lifecycle.herokuapp.com/use_case/89

2.10.3.4 Curated list of synaptic protein-protein interactions

Description of Component: A definitive (as of June 2016) curated list of synaptic protein-protein interactions (PPIs), derived from selected PPI databases (currently BIOGRID, INTACT and DIP). Each PPI will be labelled according to whether it is presynaptic and or postsynaptic. We need to be able to store this data somewhere in the Neuroinformatics Platform - currently it is stored locally.

Progress on Component: Tables released at https://collab.humanbrainproject.eu/#/collab/3389/nav However, this work is being rolled into the new Task 1.1.7, which has yet to be entered into the PLA.

Use Case: The Component contributes to the Use Case Biophysical model of LTP and LTD in wild type and mutant hippocampal CA1 synapse. https://project-lifecycle.herokuapp.com/use_case/89

2.10.3.5 LTP data from wild type and PSD-95 mice

Description of Component**:** We will obtain the data from Carlisle HJ, Fink AE, Grant SG and O'Dell TJ (2008, "Opposing effects of PSD-93 and PSD-95 on long-term potentiation and spike timing-dependent plasticity" J. Physiol. 586:5885-900 DOI: 10.1113/jphysiol.2008.163469) put it into a suitable format, and annotate it.

Progress on Component: M9 Data from relevant figures (3 & 4) obtained in excel format from Prof Tom O'Dell. UEDIN did all the work.

Quality Control:

- Downstream Computational, dynamic model of wild-type and PSD-95 KO LTP and LTD in a Schaffer Collateral synapse [essential] (T1.1.6). Status: Provided intermediate Component.
- Computational, dynamic model of LTP and LTD in a wild type Schaffer Collateral synapse [essential] (T1.1.6). Status: Provided intermediate Component.
- Plasticity: Proof of concept detailed rule-based synaptic plasticity model [added value] (T1.1.6). Status: Provided intermediate Component.

Use Case: The Component contributes to the Use Case Biophysical model of LTP and LTD in wild type and mutant hippocampal CA1 synapse. https://project-lifecycle.herokuapp.com/use_case/89

2.10.3.6 Computational, dynamic model of LTP and LTD in a wild type Schaffer Collateral synapse

Progress on Component: Verifying that the model contains PSD-95 and comparing how well the output of the model fits the WT data in Fig 3 of Carlisle, Fink, Grant, and O'Dell, 2008, J. Physiol. 586: 5885

Progress on Component: M12 - MS 1.1.3 Computational, dynamic model of LTP and LTD in a wild type Schaffer Collateral synapse. We have built a model of synaptic plasticity written in Kappa that contains the proteins calmodulin, PSD-95, stargazin, Calcium-Calmodulin dependent kinase II (CaMKII), PP3, I-1 and PP1. This model is presented with calcium stimuli of varying durations and the synaptic strength in the model can be compared to the wild type traces in Fig. 3 of Carlisle et al (2008), J. Physiol. 586. Our next steps are to optimise the parameters to improve the agreement between model and data. UEDIN did all this work.

Quality Control:

- Upstream: Plasticity: STDP with heterosynaptic plasticity and homeostasis for memory formation [added value] (T4.2.1). Status: Nothing received.
- Upstream: Database of paired recordings in hippocampal slices which display *in vivo*-like activity levels and patterns [added value] (T1.2.6). Status: Nothing received.
- Upstream: LTP data from wild type and PSD-95 mice [essential] (T1.1.6). Status: Component received.
- Downstream: Computational, dynamic model of wild-type and PSD-95 KO LTP and LTD in a Schaffer Collateral synapse [added value] (T1.1.6). Status: Provided intermediate Component.
- Downstream: Plasticity: STDP for a multi-compartment model with NMDA spikes (Algo STDPbackprop) [added value] (T4.3.3). Status: Component not yet sent. We would like to do more parameter fitting more before sharing.

2.10.3.7 Position paper on the workflows and strategies for intracellular and synaptic modelling

Description of Component**:** This work seeks to identify issues facing subcellular modellers in the HBP, and to identify points of agreement in order to better align the project. The approach might also be of interest to the wider community. To force us to clarify and publicise our thoughts, we aim to produce an opinion paper by M18 (September 2017).

Progress on Component: No releases within this reporting period. We (UEDIN) have been in discussion with other partners (Dan KELLER, EPFL; Michel MIGLIORE, CNR; Jeanette HÄLLGREN KOTALESKI, KTH; Paulo CARLONI, JUELI; Antoine TRILLER, ENS) about the paper, which is a joint effort.

Use Case: The Component contributes to the Use Case Biophysical model of LTP and LTD in wild type and mutant hippocampal CA1 synapse. https://project-lifecycle.herokuapp.com/use_case/89/

2.10.3.8 Computational, dynamic model of wild-type and PSD-95 KO LTP and LTD in a Schaffer Collateral synapse

Description of Component**:** Comparing the goodness of-fit between the model output with and without PSD-95 and the corresponding data points in Fig 3 of Carlisle, Fink, Grant, and O'Dell, 2008, J. Physiol. 586: 5885

Progress on Component: No releases in this period, and no work started on this.

2.10.3.9 Plasticity: Proof of concept detailed rule-based synaptic plasticity model

Description of Component**:** A model of synaptic plasticity to test the concept of using rule-based modelling to simulate intracellular pathways in which the number of possible complexes can become exponentially large. The model would be of a single synapse in the context of an electrophysiological model of a neuron. The KappaNEURON (or other rule-based simulator) would be used. The model should display LTP and/or LTD and could be based on one from the literature.

Progress on Component: No releases in this period, and no work started on this.

Use Case: The Component contributes to the Use Case Biophysical model of LTP and LTD in wild type and mutant hippocampal CA1 synapse. https://project-lifecycle.herokuapp.com/use_case/89

3. WP1.2: Cell and Microcircuitry

3.1 Key Personnel

Work Package Leader: Javier DEFELIPE (UPM)

3.2 WP Leader's Overview

- What went particularly well?

In general, all experiments planned for the different Tasks in this WP went well. What follows is a brief summary of the progress of research and some main achievements

Neocortex and hippocampus: the geometrical design of the pyramidal neurons (including the dendritic spines) is being explored in the neocortex and hippocampus as scheduled, and the generation of synaptic maps of excitatory and inhibitory inputs are also underway. All tasks are currently in progress and are on schedule to meet the objectives planned in the relevant tasks. 3D reconstructions have been carried out without delay and the synaptic maps are being generated. At present several articles are being prepared regarding these aims. Furthermore, the comparative studies of neuronal morphology and physiology between rodents and human cortex are progressing particularly well. For example, we have started to systematically build a database of all the major excitatory and inhibitory cell types of the hippocampus, using a combination of morphological and electrophysiological classification. Data generated are displayed in relevant publications.

Cerebellum: an optogenetic procedure to stimulate specific neuronal subfields of the cerebellar network and record electrical activity from selected neurons has been implemented. Preliminary results show that the technique is functioning and is generating specific stimulus patterns in the granular layer while recording the corresponding PC responses in whole-cell patch-clamp. In parallel, the first detailed electrical map of cerebellar circuit activity *in vitro* has been provided via a high-density MEA system. In addition, patch-clamp recordings from cerebellar neurons have been performed to construct advanced neuronal models. PC recordings have been combined with optogenetic stimulation. Data generated are displayed in relevant publications.

Basal ganglia: the connectivity, morphology and cellular properties of neurons within striatum and the output stage of the basal ganglia are currently being addressed and the relevant Task progress is as scheduled. The experimental results achieved are being transferred to the relevant modelling Tasks in SP4 and SP6.

- What didn't go according to plan?

Three planned Milestones have not been achieved in a timely manner due to the delay in the SGA1 signature. In particular, MS 1.2.2: Immunostaining of GABAergic subtypes of cortical neuron, MS1.2.5: Reconstruction of hippocampal pyramidal neurons recorded and filled in vivo, and MS 1.2.6: Complete 3D datasets of single-cell somatosensory TC (VP and Po) axons. However, in spite of this delay, all the activities have started and are currently running smoothly. It is expected that they will be on schedule in the next few months and to achieve the relevant MSs planned in M24 as scheduled.

- Impact of work done

The datasets that are being generated in WP1.2 are being used to constrain and validate high-fidelity models of brain circuits. In addition, data are currently being integrated in mouse and human brain atlases. Thus, the impact of WP1.2 in the HBP can be considered highly relevant.

3.3 Priorities for the remainder of the phase

In WP1.2, we would like to continue with the proposed research plan to deal with the problem of imprecise connectomes and incomplete synaptomes in the brain. The priorities of the WP1.2 will be to generate data to contribute to the first steps towards understanding how neuronal circuits contribute to the functional organisation of the brain by defining its detailed structural design and to map its connection matrix. We will continue to use powerful microanatomical techniques at both the optical microscope level in combination with electrophysiological characterisation—for example, intracellular injections of markers, 3D reconstructions, plus physiological data on cellular properties under *in vivo* and *in vitro* conditions—and at the ultrastructural level using automated electron microscopy techniques —for example, Focused Ion Beam milling/Scanning Electron Microscopy; FIB/SEM). Furthermore, we will use and develop new software tools to examine the anatomical design of brain circuits. In this Work Package there are nine Tasks which are dealing with the four major brain circuits that the HBP teams will build in SGA1: neocortex (including the thalamocortical system), hippocampus, basal ganglia and cerebellum.

3.4 Milestones

Table 2: Milestones for WP1.2: Cell and Microcircuitry

MS No.	Milestone Name	Leader	Task(s) involved	Expected Month	Achieved Month	Comments
MS1.2.1	Protocols for the characterisation of unitary transmission and short-term plasticity	IEM HAS	T1.2.6	M06	M06	Achieved. An experimental protocol for the characterisation of unitary transmission and short-term plasticity of hippocampal synapses has been established. Paired whole-cell intracellular recordings are performed in hippocampal slices in a submerged chamber. Action potentials are evoked in one cell in current clamp, while postsynaptic currents are recorded in the other cell in voltage clamp. Trains of presynaptic action potentials at a wide range of frequencies are used to characterise short-term plasticity, and recovery is also measured.
MS1.2.2	Immunostaining of GABAergic subtypes of cortical neuron	UoD	T1.2.7	M10	M16	Not achieved. Progress in this Task was slower than expected and the completion of MS 1.2.2 Immunostaining of GABAergic subtypes of cortical neuron, which is

						planned to be achieved in M10, will be delayed consequently until M16 as reported in the M01-M06 semester report.
MS1.2.3	Reconstruction of pyramidal neurons	UPM	T1.2.1	M12	M12	Achieved. Reconstruction of pyramidal neurons in the neocortex and hippocampus. In the mouse, 300 pyramidal neurons from the somatosensory cortex were reconstructed across layers II-VI and 50 in the CA1 region of the hippocampus. In the rat, 50 cells were reconstructed in the CA1 region. In human tissue, 50 cells in the CA1 region and 200 in the neocortex (temporal, cingulate and frontal) were reconstructed.
MS1.2.4	Synaptic maps (meso-scopic level)	UPM	T1.2.9	M12	M12	Achieved. Immunocytochemical detection and estimation of the density of excitatory (VGluT1) and inhibitory terminals (VGAT) in the mouse neocortex (somatosensory cortex) by confocal microscopy. Five animals have been analysed, 15 confocal stacks per layer. Immunocytochemical detection and estimation of the density of excitatory

						(VGluT1) and inhibitory terminals in the mouse hippocampus (CA1 area) by confocal microscopy. Five animals have been analysed, 15 confocal stacks per layer.
MS1.2.5	Reconstruction of hippocampal pyramidal neurons recorded and filled *in vivo*.	IEM HAS	T1.2.5	M12	TBC	Not achieved. Work on this Component of Task 1.2.5 started late due to the delayed availability of funding. Considerable progress has already been made: several whole-cell intracellular recordings have been made from hippocampal neurons in awake, head-fixed mice, which allows the characterisation of cellular properties and neuronal responses *in vivo*. Cells were filled with biocytin during the recordings, visualised in a confocal microscope, and reconstructed using the Neurolucida software. However, the quality of the morphological data obtained so far is not completely satisfactory: while the dendritic tree can be fully visualised and reconstructed, only a small portion of the axon is visible. This means that the planned release for M12 has not been fully

						achieved. More complete reconstructions of the axonal arbor will likely require changes in, the experimental protocol, and several possible solutions are currently explored. If these changes do not lead to substantial improvements, the completeness of the morphological data may be less than initially expected, which may require adjustments in modelling strategies.
MS1.2.6	Complete 3D datasets of single-cell somatosensory TC (VP and Po) axons	UAM	T1.2.8	M12	M18	Not achieved. We have been unable to hire the two additional researchers in our team for whom we had requested funding until the end of M6. These facts have inevitably delayed the completion of Milestone MS1.2.6 until M18 instead of M12 as we had initially planned.

3.5 T1.2.1: The pyramidal neuron in the cerebral cortex of humans and rodents

3.5.1 Key Personnel

Task Leader: Javier DEFELIPE (UPM)

Other Researcher: Ruth BENAVIDES-PICCIONE (UPM)

3.5.2 SGA1 DoA Goals

Geometrical design of the pyramidal neurons in the neocortex and hippocampus. Unravelling pyramidal cell structure is crucial to understanding cortical structure and information processing in cortical circuits. For this purpose, we are using the systematic light microscopy method of intracellular injection in fixed material. After injections, cells are being fully imaged at high magnification using tile scan mode in a confocal scanning laser system. Dendritic arbors are then being individually reconstructed in 3D using both Imaris 7.6.5 and Neurolucida 360. For further technical details, see Benavides-Piccione et al., 2013 (Cereb Cortex 23:1798-1810). In the mouse, 300 pyramidal neurons from the somatosensory cortex will be reconstructed across layers II-VI and 50 in the CA1 region of the hippocampus. In the rat, 50 cells will be reconstructed in the CA1. In human tissue, 50 cells in the CA1 region and 200 in the neocortex (temporal, cingulate and frontal).

3.5.3 Component Progress

3.5.3.1 3D reconstructions of 300 pyramidal neurons from the mouse somatosensory cortex across layers II-VI

Description of Component: 3D reconstructions of 300 pyramidal neurons from the mouse somatosensory cortex across layers II-VI Related Milestones: MS1.2.3 Reconstruction of pyramidal neurons M12 MS1.2.8 Reconstruction of dendritic arbors and dendritic spines of pyramidal neurons M24 Verification: Data released and data quality validated related task: Task 1.2.1: The pyramidal neuron in the cerebral cortex of humans and rodents.

Progress on Component: the Component is progressing as scheduled. The number of reconstructed cells to date is 30. The quality of data is excellent. M12 planned release has been achieved: Reconstruction of pyramidal neurons. M24 planned release is in progress: Reconstruction of dendritic arbors and dendritic spines of pyramidal neurons. The work was done by UPM.

Quality Control:

- Upstream Components:
  - Immunocytochemical detection of excitatory inhibitory terminals in the mouse neocortex (somatosensory cortex) by confocal microscopy; Task responsible: T1.2.9 (MERCHÁN, Ángel), Status: intermediate release; Quality: excellent.
  - Densities and 3D distributions of synapses using FIB/SEM imaging in the mouse neocortex (somatosensory cortex); Task responsible: T1.2.9 (MERCHÁN, Ángel), Status: intermediate release; Quality: excellent.
  - Tools for the early analysis of morphological data; Task responsible: T1.4.2 (PASTOR, Luis), Status: intermediate release; Quality: excellent.
  - New workflows for embedding exploratory analysis techniques into the early stages of the morphological data extraction and reconstruction processes; Task responsible: T1.4.2 (PASTOR, Luis), Status: intermediate release; Quality: excellent.
- Downstream Components:

- Principal cells morphology comparative models; Task responsible: T1.4.1 (BIELZA, Concha); Status: intermediate release.
- T3.1.4 Dendritic mechanisms of feedback; Task responsible: T3.1.4 (LARKUM, Matthew); Status: provided nothing.
- SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data; Task responsible: T6.2.2 (DEFELIPE, Javier); Status: intermediate release.

3.5.3.2 3D reconstructions of 50 cells in mouse hippocampal CA1 region

Description of Component: 3D reconstructions of 50 cells in mouse hippocampal CA1 region Related Milestones: MS1.2.3 Reconstruction of pyramidal neurons M12 MS1.2.8 Reconstruction of dendritic arbors and dendritic spines of pyramidal neurons M24 Verification: Data released and data quality validated Related task: Task 1.2.1. The pyramidal neuron in the cerebral cortex of humans and rodents

Progress on Component: the Component is progressing as scheduled. The number of reconstructed cells to date is 20. The quality of data is excellent. M12 planned release has been achieved: Reconstruction of pyramidal neurons; M24 planned releases in progress: Reconstruction of dendritic arbors and dendritic spines of pyramidal neurons. The work was done by UPM.

Quality Control:

- Upstream Components:
  - Immunocytochemical detection of excitatory and inhibitory terminals in the mouse hippocampus (CA1) by confocal microscopy; Task responsible: T1.2.9 (MERCHÁN, Ángel), Status: intermediate release; Quality: excellent.
  - Densities and 3D distributions of synapses using FIB/SEM imaging in the mouse hippocampus (CA1); Task responsible: T1.2.9 (MERCHÁN, Ángel), Status: intermediate release; Quality: excellent.
  - Tools for the early analysis of morphological data; Task responsible: T1.4.2 (PASTOR, Luis), Status: intermediate release; Quality: excellent.
  - New workflows for embedding exploratory analysis techniques into the early stages of the morphological data extraction and reconstruction processes; Task responsible: T1.4.2 (PASTOR, Luis), Status: intermediate release; Quality: excellent.
- Downstream Components:
  - SGA2-T6.2.3 - Improved models of hippocampal neurons in the mouse and the rat; Task responsible: T6.2.3 (KÁLI, Szabolcs), Status: provided nothing;
  - Principal cells morphology comparative models; Task responsible: T1.4.1 (BIELZA, Concha); Status: intermediate release.
  - SP6-T6.2.4-SGA1-Models of mouse hippocampal neurons; Task responsible: T6.2.4 (KÁLI, Szabolcs), Status: provided nothing;

3.5.3.3 3D reconstructions of 50 cells n rat hippocampal CA1 region

Description of Component: 3D reconstructions of 50 cells in rat hippocampal CA1 region Related Milestones: MS1.2.3 Reconstruction of pyramidal neurons M12 MS1.2.8 Reconstruction of dendritic arbors and dendritic spines of pyramidal neurons M24 Verification: Data released and data quality validated Related Task: Task 1.2.1: The pyramidal neuron in the cerebral cortex of humans and rodents

Progress on Component: the Component is progressing as scheduled. The number of reconstructed cells to date is 30. The quality of data is excellent M12 planned release has been achieved: Reconstruction of pyramidal neurons; M24 planned releases in progress: Reconstruction of dendritic arbors and dendritic spines of pyramidal neurons. The work was done by UPM.

Quality Control:

- Upstream Components:
  - Tools for the early analysis of morphological data; Task responsible: T1.4.2 (PASTOR, Luis), Status: intermediate release; Quality: excellent.
  - New workflows for embedding exploratory analysis techniques into the early stages of the morphological data extraction and reconstruction processes; Task responsible: T1.4.2 (PASTOR, Luis), Status: intermediate release; Quality: excellent.
- Downstream Components:
  - Synapse segmentation ImageJ plugin and macro; Task responsible: T1.4.1 (LARRAÑAGA, Pedro); Status: intermediate release.
  - SP6-T6.2.4-SGA1-Models of mouse hippocampal neurons; Task responsible: T6.2.4 (KÁLI, Szabolcs), Status: provided nothing;

3.5.3.4 3D reconstructions of 200 cells in human neocortex (temporal, cingulate and frontal)

Description of Component: 3D reconstructions of 200 cells in human neocortex (temporal, cingulate and frontal) Related Milestones: MS1.2.3 Reconstruction of pyramidal neurons M12 MS1.2.8 Reconstruction of dendritic arbors and dendritic spines of pyramidal neurons M24 Verification: Data released and data quality validated. Related Task: Task 1.2.1: The pyramidal neuron in the cerebral cortex of humans and rodents

Progress on Component: the Component is progressing as scheduled. The number of reconstructed cells to date is 185 (83 in the temporal cortex, 58 in the cingular cortex and 44 in the frontal cortex). The quality of data is excellent. M12 planned release has been achieved: Reconstruction of pyramidal neurons; M24 planned releases in progress: Reconstruction of dendritic arbors and dendritic spines of pyramidal neurons. The work was done by UPM.

Quality Control:

- Upstream Components:
  - Densities and 3D distributions of synapses using FIB/SEM imaging in the mouse hippocampus (CA1); Task responsible: T1.2.9 (MERCHÁN, Ángel), Status: intermediate release; Quality: excellent.
  - Tools for the early analysis of morphological data; Task responsible: T1.4.2 (PASTOR, Luis), Status: intermediate release; Quality: excellent.
  - New workflows for embedding exploratory analysis techniques into the early stages of the morphological data extraction and reconstruction processes; Task responsible: T1.4.2 (PASTOR, Luis), Status: intermediate release; Quality: excellent.
- Downstream Components:
  - Principal cells morphology comparative models; Task responsible: T1.4.1 (BIELZA, Concha); Status: intermediate release.

- Synapse segmentation ImageJ plugin and macro; Task responsible: T1.4.1 (LARRAÑAGA, Pedro); Status: intermediate release.
- Simplified neuron models; Task responsible: T4.1.1 (SEGEV, Idan); Status: intermediate release.
- SP6-T6.2.1-SGA1-Models of human dendritic spines; Task responsible: T6.2.1 (EYAL, Guy); Status: intermediate release.

3.5.3.5 3D reconstructions of 50 cells in human hippocampal formation (CA1)

Description of Component: 3D reconstructions of 50 cells in human hippocampal formation (CA1) Related Milestones: MS1.2.3 Reconstruction of pyramidal neurons M12 MS1.2.8 Reconstruction of dendritic arbors and dendritic spines of pyramidal neurons M24 Verification: Data released and data quality validated Related task: Task 1.2.1: The pyramidal neuron in the cerebral cortex of humans and rodents

Progress on Component: the Component is progressing as scheduled. The number of reconstructed cells to date is 40. The quality of data is excellent M12 planned release has been achieved: Reconstruction of pyramidal neurons; M24 planned releases in progress: Reconstruction of dendritic arbors and dendritic spines of pyramidal neurons. The work was done by UPM.

Quality Control:

- Upstream Components:
  - Densities and 3D distributions of synapses using FIB/SEM imaging in the mouse hippocampus (CA1); Task responsible: T1.2.9 (MERCHÁN, Ángel), Status: intermediate release; Quality: excellent.
  - Tools for the early analysis of morphological data; Task responsible: T1.4.2 (PASTOR, Luis), Status: intermediate release; Quality: excellent.
  - New workflows for embedding exploratory analysis techniques into the early stages of the morphological data extraction and reconstruction processes; Task responsible: T1.4.2 (PASTOR, Luis), Status: intermediate release; Quality: excellent.
- Downstream Components:
  - Principal cells morphology comparative models; Task responsible: T1.4.1 (BIELZA, Concha); Status: intermediate release.

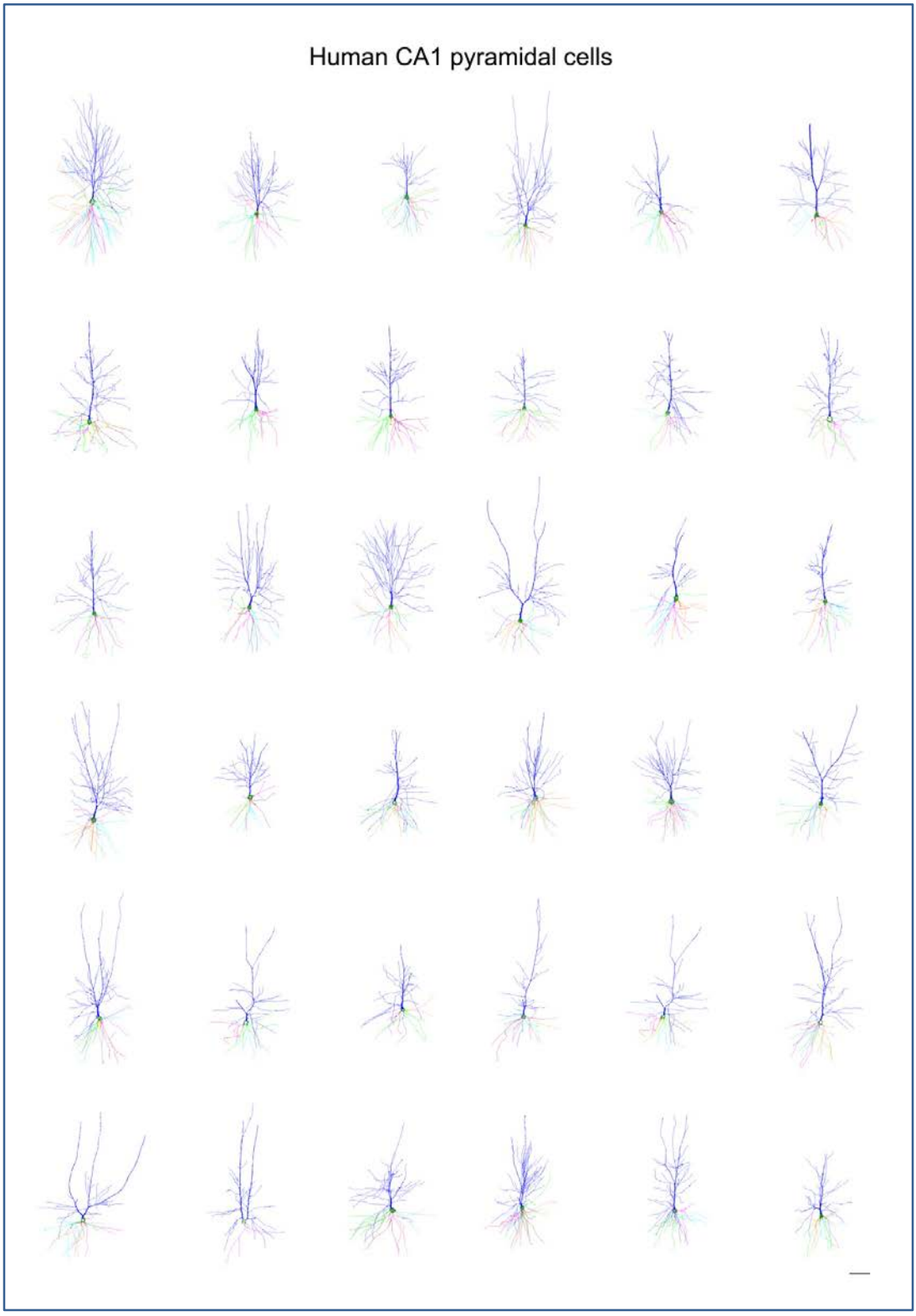

Figure 1: Schematic drawings of apical and basal arbors of CA1 pyramidal neurons reconstructed in 3D from human hippocampal formation. Scale bar = 100 µm

3.6 T1.2.2 - Comparative Studies of Neuronal Morphology and Physiology in Adult Mouse, Rat and Human Cortex

3.6.1 Key Personnel

Task Leader: Huib MANSVELDER (VU)

3.6.2 SGA1 DoA Goals

During the Ramp-Up Phase of HBP (Task 1.2.4), we developed and fine-tuned procedures for precise morphological reconstruction and electrophysiological characterisation of hippocampal neurons in 600-micrometer-thick slices from 8-week-old Black6 mice. In this phase, we aim to further apply these methods to start systematically building a database of all the major excitatory and inhibitory cell types of the hippocampus, using a combination of morphological and electrophysiological classification, and also utilising transgenic animals expressing cell-type-specific fluorescent markers. In addition, we will carry out whole-cell recording and labelling of hippocampal neurons *in vivo*. Reconstructing these neurons will likely lead to more complete morphologies than labelling in the slice (plus physiological data on cellular properties under *in vivo* conditions), although it is expected to have a considerably lower yield.

3.6.3 Component Progress

3.6.3.1 Morphological and physiological data from same neurons in adult mouse (data):

Description of Component: morphological (dendrites AND axons) and physiological data (e-codes) from the same cortical pyramidal neurons in different layers in temporal and frontal cortex of the adult rodent will be recorded.

Progress on Component: the Component progress is as scheduled. Pilot set of data has been released in M12. The work was done by VU.

Quality Control:

Data generated has been sent to T4.1.1 (SP4) and T6.2.1 (SP6). These data are in the publication: Unique membrane properties and enhanced signal processing in human neocortical neurons. Guy Eyal, Matthijs B Verhoog, Guilherme Testa-Silva, Yair Deitcher, Johannes C Lodder, Ruth Benavides-Piccione, Juan Morales, Javier DeFelipe, Christiaan PJ de Kock, Huibert D Mansvelder, Idan Segev. eLife. 2016; 5: e16553. Published online 2016 Oct 6. doi: 10.7554/eLife.16553.

3.6.3.2 Morphological and physiological data from same neurons in adult human (data)

Description of Component: Description: Morphological (dendrites AND axons) and physiological data (e-codes) from the same cortical pyramidal neurons in different layers in temporal and frontal cortex of the adult human will be recorded.

Progress on Component: the Component progress is as scheduled. Pilot set of data has been released in M12. The work was done by VU.

Quality Control: see the Component above.

3.6.3.3 Template of morphing rules to translate pyramidal neuron function from rodent brain to microcircuits in the human brain (model)

Description of Component: The morphological and physiological data will be used for feature extraction using existing models of these neuron types (Eyal et al., 2014). Using the modelling and simulation efforts of SP4 and SP6 (Segev and Giugliano) we will derive from these data and their models a template of morphing rules to translate pyramidal neuron function from rodent brain to microcircuits in the human brain.

Progress on Component: the Component progress is as scheduled. This Component is on schedule to meet the release in M24. The work was done by VU.

Quality Control

This Component will ultimately result from the work of SP4, mainly T4.1.1, using the data of T1.2.2.

3.7 T1.2.3 - Basal Ganglia Circuitry

3.7.1 Key Personnel

Task Leader: Sten GRILLNER (KI)

3.7.2 SGA1 DoA Goals

The connectivity, morphology and cellular properties of neurons within striatum and the output stage of the basal ganglia will be addressed, as well as the projection patterns of the different types of neurons from cortex as well as thalamus. The experimental results achieved in this Task will be of critical importance for the modelling Tasks T6.2.5 (Models of basal ganglia) and T4.4.3 (Models of motor control). The techniques that will be used to capture the data include single- and multi-electrode whole cell patch clamp, morphological reconstructions with neuronal labelling and tracing using fluorescence bright field and confocal microscopy, immunolabelling, two-photon microscopy and live-cell imaging.

3.7.3 Component Progress

3.7.3.1 Cellular properties of neurons within striatum

Description of Component: The cellular properties of neurons within striatum and the output stage of the basal ganglia will be addressed, as well as the projection patterns of the different types of neurons from cortex as well as thalamus. The techniques that will be used to capture the data include single- and multielectrode whole cell patch clamp.

Progress on Component: the Component is progressing as scheduled. M12 planned release *Pilot recordings from five striatal cell types* has been achieved. The work was done by KI.

Quality Control:

- No upstream Components.
- Downstream Components:
  - SP6-T6.2.5-SGA1-Models of basal ganglia nuclei [essential]; Task responsible: T6.5.2 (KOZLOV, Alexander); Status: intermediate release.
  - SP 4 T4.4.3 Motor control

3.7.3.2 Connectivity and morphology of neurons within striatum

Description of Component: The connectivity and morphology properties of neurons within striatum and the output stage of the basal ganglia will be addressed, as well as the projection patterns of the different types of neurons from cortex as well as thalamus. The techniques that will be used to capture the data include morphological reconstructions with neuronal labelling and tracing using fluorescence bright field and confocal microscopy, immunolabelling, two-photon microscopy, live-cell imaging.

Progress on Component: the Component is progressing as scheduled. M12 planned release *First set of reconstructions* has been achieved. The work was done by KI.

Quality Control:

- No upstream Components.
- Downstream Components:
  - SP6-T6.2.5-SGA1-Models of basal ganglia nuclei [essential]; Task responsible: T6.5.2 (KOZLOV, Alexander); Status: intermediate release.

– SP 4 T4.4.3 Motor control

3.8 T1.2.4 - Morphological Reconstruction and Physiological Characterisation of Cerebellar Neurons

3.8.1 Key Personnel

Task Leader: Egidio D'ANGELO (UNIPV)

3.8.2 SGA1 DoA Goals

In this Task, essential data for cerebellar microcircuit construction and validation will be provided. In particular, T1.2.4 is currently working on:

- Construction: patch-clamp recordings from cerebellar neurons to construct advanced Purkinje cell models and stellate cell models.
- Validation: combined optogenetic, two-photon imaging and electrophysiological recordings to determine microcircuit dynamics and plasticity in response to the specific patterns used for simulations.

3.8.3 Component Progress

3.8.3.1 Combined optogenetic, two-photon imaging and electrophysiological recordings from cerebellar neurons (data)

Description of Component: Combined optogenetic, two-photon imaging and electrophysiological recordings from cerebellar neurons will be made in order to determine microcircuit dynamics and plasticity in response to the specific patterns used for simulations in modelling tasks. About 50 experimental recordings will be performed.

CDP to which Component contributes: CDP 2-5.

Progress on Component: In this phase we have first implemented an optogenetic procedure to stimulate specific neuronal subfields of the cerebellar network and record electrical activity from selected neurons. Preliminary results show that the technique is functioning and now being used to generate specific stimulus patterns in the granular layer while recording the corresponding PC responses in whole-cell patch-clamp. In parallel, a high-density MEA system has been installed and is providing the first detailed electrical maps of cerebellar circuit activity *in vitro*. VSD imaging techniques have been combined with biomolecular assessments to investigate the spatio-temporal patterns of gene expression during synaptic plasticity. The data that will be produced will be used primarily for microcircuit model validation. The task is on time. All the work was done by UNIPV.

Quality Control:

Downstream Components: T1.2.4; Task responsible: Egidio D'Angelo; Status: intermediate release

- Simulation of brain lesion and cortical bistability on complexity
- TMS/EEG non-invasive perturbation recordings
- Slow waves and complexity relationships explored by perturbations: definition of models
- Cerebellum scaffold models

3.8.3.2 Patch-clamp recordings from cerebellar neurons (data)

Description of Component: Patch-clamp recordings from cerebellar neurons will be made to assist with constructing advanced Purkinje cell models and stellate cell models. The

experiments will be designed to achieve optimal alignment with HBP cerebellar modelling, thus allowing efficient model construction. About 50 neurons will be recorded and analysed.

Progress on Component: The reconstruction of neuronal models requires specific data sets from neurons. In this task we are performing patch-clamp recordings from cerebellar neurons to construct advanced neuronal models. PC recordings have been combined with optogenetic stimulation. Some granule cell recordings have been used for model reconstruction (one paper already published by Masoli et al., 2017) and others are being used for further modelling. We have already transferred 30 granule cell recordings to SP5 for data-basing. Golgi cell whole-cell patch-clamp recordings have been carried out with fluorescent dyes and analysed together with UPM-CSIC for morphological reconstruction. Stellate cell recordings are ongoing and will be used for model optimisation. The task is on time. All the work was done by UNIPV.

Quality Control:

Downstream Components: T1.2.4; Task responsible: Egidio D'Angelo; Status: intermediate release

- Cerebellum electrophysiological pipeline
- Cerebellum scaffold models

3.9 T1.2.5 Morphological reconstruction and physiological characterisation of hippocampal neurons

3.9.1 Key Personnel

Task Leader: Tamás F. FREUND (IEM HAS)

3.9.2 SGA1 DoA Goals

During the Ramp-Up Phase of HBP (Task 1.2.4), we developed and fine-tuned procedures for precise morphological reconstruction and electrophysiological characterisation of hippocampal neurons in 600-micrometre-thick slices from 8-week-old Black6 mice. In this phase, we aim to further apply these methods to start building systematically a database of all the major excitatory and inhibitory cell types of the hippocampus, using a combination of morphological and electrophysiological classification, and also utilising transgenic animals expressing cell-type-specific fluorescent markers. In addition, we will carry out whole-cell recording and labelling of hippocampal neurons *in vivo*. Reconstructing these neurons will likely lead to more complete morphologies than labelling in the slice (plus physiological data on cellular properties under *in vivo* conditions), although it is expected to have a considerably lower yield.

3.9.3 Component Progress

3.9.3.1 Database of all the major excitatory and inhibitory cell types of the mouse hippocampus, using a combination of morphological and electrophysiological classification

Description of Component: During the Ramp-Up Phase of HBP (Task 1.2.4), we developed and fine-tuned procedures for precise morphological reconstruction and electrophysiological characterisation of hippocampal neurons in 600-micrometre-thick slices from 8-week-old Black6 mice. InSGA1, we aim to further apply these methods to start building systematically a database of all the major excitatory and inhibitory cell types of the hippocampus, using a combination of morphological and electrophysiological classification, and also utilising transgenic animals expressing cell-type-specific fluorescent markers.

CDP to which Component contributes (if relevant):

Progress on Component: Recording, filling, and reconstruction of neurons in mouse hippocampal slices has continued. The number of reconstructed neurons has increased to approximately 120. Experiments now target specific populations of neurons based on the frequency of different cell types in the existing database, and on feedback from the hippocampal modelling task in SP6 (T6.2.4). All the work was done by IEM HAS.

Quality Control:

Upstream Components:

- Morphological reconstructions of mouse hippocampal neurons (RUP): an extensive database of morphological reconstructions and physiological data has been received.
- SP6-T6.2.4-SGA1-Models of mouse hippocampal neurons: Feedback on the data required for modelling has been received.
- New workflows for embedding exploratory analysis techniques into the early stages of the morphological data extraction and reconstruction processes (T1.4.2 – SGA1): No input has been received so far.
- Tools for the early analysis of morphological data (T1.4.2 – SGA1): No input has been received so far.

Downstream Components:

- SP6-T6.2.4-SGA1-Models of mouse hippocampal neurons (T6.2.4 – SGA1): New data are regularly made available for the modelling task.

3.9.3.2 Whole-cell recording and labelling of hippocampal neurons *in vivo*

Description of Component (from PLA): We will reconstruct and record from hippocampal neurons labelled *in vivo*. This will likely lead to more complete morphologies than labelling in the slice (plus physiological data on cellular properties under *in vivo* conditions), although it is expected to have a considerably lower yield.

CDP to which Component contributes (if relevant):

Progress on Component: Work on this Component started late due to the delayed availability of funding. However, considerable progress has already been made: several whole-cell intracellular recordings have been made from hippocampal neurons in awake, head-fixed mice, which allows the characterisation of cellular properties and neuronal responses *in vivo*. Cells were filled with biocytin during the recordings, visualised in a confocal microscope, and reconstructed using the Neurolucida software. The quality of the morphological data obtained so far is not completely satisfactory: while the dendritic tree can be fully visualised and reconstructed, only a small portion of the axon is visible. This means that the planned release for M12 MS1.2.5 Reconstruction of hippocampal pyramidal neurons recorded and filled *in vivo* has not been fully achieved. More complete reconstructions of the axonal arbor will likely require changes is the experimental protocol. All the work was done by IEM HAS.

Quality Control:

Downstream Components:

- SP6-T6.2.4-SGA1-Models of mouse hippocampal neurons (T6.2.4 – SGA1): New data are regularly made available for the modelling task.

3.10 Task 1.2.6 Physiological characterisation of hippocampal synapses

3.10.1 Key Personnel

Task Leader: Tamás F. FREUND (IEM HAS)

3.10.2 SGA1 DoA Goals

We will perform paired recordings in hippocampal slices which display *in vivo*-like activity levels and patterns. Using this approach, we will describe the basic properties of synaptic transmission and characterise short-term synaptic plasticity between identified cells, employing morphological and electrophysiological classification as well as transgenic animals expressing cell-type-specific fluorescent markers to target specific connections.

3.10.3 Component Progress

3.10.3.1 Protocol for paired recordings in hippocampal slices which display *in vivo*-like activity levels and patterns

Description of Component (from PLA): Simulating the function of brain networks also requires a detailed characterisation of neuronal interactions at the physiological level, using conditions and activity patterns which are characteristic of the intact brain. The relevant synaptic parameters (including the properties of activity-dependent short-term plasticity) have been determined only for a small and almost arbitrary subset of connections, and mostly under very artificial (silent) conditions. By contrast, we will perform paired recordings in hippocampal slices which display *in vivo*-like activity levels and patterns.

CDP to which Component contributes (if relevant):

Progress on Component: The protocol for characterising synaptic transmission (including short-term plasticity) between hippocampal neurons has been established. It involves making paired somatic whole-cell recordings from excitatory and inhibitory neurons in the same subfield of the hippocampus in either thin (silent) or thick (active) hippocampal slices. Pre-synaptic neurons are induced to fire action potentials in trains of various frequencies, and in patterns characteristic of different physiological and pathological conditions, while synaptic currents are recorded in the postsynaptic cell in the voltage-clamp configuration. Specific types of neuron (e.g., parvalbumin-containing and cholecystokinin-containing interneurons) can be targeted using mouse lines expressing the appropriate cell-type-specific fluorescent markers. The effects of background synaptic activity can be assessed by comparing results from thin and thick slices. This completes the M6 (final) release of this Component MS1.2.1 Protocol of paired recordings. The work was done by IEM HAS.

Quality Control:

Downstream Components:

- Database of paired recordings in hippocampal slices which display *in vivo*-like activity levels and patterns (T1.2.6 – SGA1): The experimental protocol has been defined and tested, and is now used in the downstream Component.

3.10.3.2 Database of paired recordings in hippocampal slices which display in vivo-like activity levels and patterns

Description of Component (from PLA): Using our protocol for performing paired recordings in hippocampal slices which display *in vivo*-like activity levels and patterns, we will describe the basic properties of synaptic transmission and characterise short-term synaptic plasticity between identified cells, employing morphological and electrophysiological classification as well as transgenic animals expressing cell-type-specific fluorescent markers to target specific connections.

Progress on Component: Paired recordings have been performed and synaptic connections have been characterised in the CA3 area of the hippocampus, using the protocol established in the first Component of this task. The connections studied so far include connections between parvalbumin-containing or cholecystokinin-containing interneurons and pyramidal cells, as well as connections between interneurons of the same type. The work was done by IEM HAS.

Quality Control:

Upstream Components:

- Protocol for paired recordings in hippocampal slices which display *in vivo*-like activity levels and patterns (T1.2.6 – SGA1): The experimental protocol has been defined and tested, and is now used in this Component.
- SP6-T6.2.4-SGA1-Circuit model of the mouse hippocampus: Feedback on the data required for modelling has been received.

Downstream Components:

- SP6-T6.2.4-SGA1-Circuit model of the mouse hippocampus (T6.2.4 – SGA1): New data are regularly made available for the modelling task.
- STP model (T1.1.4 – SGA1) (added value): The exact nature of the data required by this Component needs to be clarified.
- Computational, dynamic model of LTP and LTD in a wild type Schaffer Collateral synapse (T1.1.6 – SGA1) (added value): No data have been recorded for this connection.

3.11 T1.2.7 Polyneuronal Innervations of Dendrites: EM Data on Synaptic Coverage of GABAergic Neuron Subtypes

3.11.1 Key Personnel

Task Leader: Zoltan KISVARDAY (UoD)

Other Researcher: Petra TALAPKA (UoD)

3.11.2 SGA1 DoA Goals

The overarching aim is to establish a quantitative electron microscopic (qEM) database of the complete synaptic coverage of subtypes of GABAergic neurons.

3.11.3 Component Progress

3.11.3.1 Morphological categorisation and clustering of synaptic coverage of GABAergic neuron subtypes

Description of Component (from PLA): The polyneuronal innervation of the dendrites will undergo a thorough examination for unravelling compartmentalisation and clustering features. A wide range of quantitative criteria will be applied to this, allowing morphological categorisation and clustering signatures of synapses to be detected. The following main parameters will be determined: bouton size and shape, vesicle and mitochondria content, surface extent of the active zone, location of boutons along the dendrite segments.

Progress on Component: This Component is delayed due to the delay in the SGA1 signature and the effective work in T1.2.7 was starting in January 2017 consequently. In spite of this delay, M24 release *Synaptic coverage of GABAergic subtypes of cortical neuron* is expected to be achieved as planned. The work was done by UoD.

Use Case:

Quality Control:

- Upstream Component:
  - Quantitative electron microscopic (qEM) database of Synaptic Coverage of GABAergic Neuron Subtypes [essential]; Task responsible: T1.2.7 (KISVARDAY, Zoltan); Status: delayed;
- Downstream Component:

- SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data; Task responsible: T6.2.2 (Javier DEFELIPE); Status: nothing released.

3.11.3.2 Quantitative electron microscopic (qEM) database of Synaptic Coverage of GABAergic Neuron Subtypes

Description of Component: Neocortical GABAergic neurons will be labelled *in vivo* and characterised post hoc for chemical content using a battery of immunohistochemical markers for somatostatin, parvalbumin, calretinin, calbindin, vasoactive intestinal polypeptide, cholecystokinin, and neuropeptide Y, choline-acetyltransferase and NO-synthase in adult mice. Our aim is to establish a quantitative electron microscopic (qEM) database of the complete synaptic coverage of subtypes of GABAergic neurons. To generate a qEM database, a representative number of subtypes of GABAergic neurons will be selected to map out synaptic coverage along the soma-dendritic surface and estimate the contribution of various input types. This immunostaining will be carried out in brain tissue of C57BL/6J adult mice (8-week-old male) and for GABAergic cells of Slc32altm2(cre) Lowl and Ai14 mice strains. Tissue samples will include the primary visual cortex (V1) and the primary somatosensory cortex (SI). Labelled cells of all six cortical layers will be selected for a detailed EM analysis. At least two labelled cells per cortical layer will be subjected to qEM.

Progress on Component: Part of the activities planned in this Component is delayed due to the delay in the SGA1 signature and the effective work in T1.2.7 was starting in January 2017 consequently. In spite of this delay, the immunohistochemical characterisation of the GABAergic interneurons has started and the identification of three sub-types could be achieved. In particular, vasoactive intestinal polypeptide, somatostatin and choline acetyltransferase producing cells were successfully identified in the above mentioned mice strains; and calbindin, calretinin, parvalbumin in brain tissue of C57BL/6J miceM10 release *Immunostaining of GABAergic subtypes of cortical neuron* was not achieved but it has already started (see in table 2, MS 1.2.2 for further information). Data is expected to be released from M12. The work was done by UoD.

Quality Control:

- No upstream Components.
- Downstream Components:
  - SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data; Task responsible: T6.2.2 (Javier DEFELIPE); Status: nothing released.
  - Morphological categorisation and clustering of synaptic coverage of GABAergic neuron subtypes [essential]**;** Task responsible: T1.2.7 (Zoltan KISVARDAY); Status: nothing provided.

3.12 T1.2.8 Labelling, 3D reconstructing and quantifying selected populations of long-range projection neurons in the mouse forebrain

3.12.1 Key Personnel

Task Leader: Francisco CLASCA MD, PhD (UAM)

Other Researcher: Lucia PRENSA DVM, PhD (UAM)

Other Researcher Maria GARCIA-AMADO, PhD (UAM)

Other Researcher Cesar PORRERO, PhD (UAM)

Other Researcher: Marian EVANGELIO (Graduate Student), (UAM)

Other Researcher: Javier RODRIGUEZ-MORENO (Graduate Student), UAM

3.12.2 SGA1 DoA Goals

To implement a workflow for a) selective, high signal-to-noise ratio labelling, b) 2D and 3D reconstruction (Neurolucida DAT. with landmarks for precision atlas indexation) c) measurement (axon length and varicosity/bouton number); and d) indexation with ancillary metadata (Serial high-resolution tissue image stacks) of representative populations of long range projection neurons linking the thalamus with the somatic and visual areas of the mouse cerebral cortex.

3.12.3 Component Progress

3.12.3.1 3D digital reconstruction of thalamocortical Projection neurons

Description of Component: We will produce 3D digital reconstruction and quantification of a significant number (30-40) of individual cells from four populations of thalamocortical LRPNs (40 in total), each from a different thalamic nuclei, (two specific/sensory and two multispecific/higher order nuclei), with particular focus on their terminal synaptic site in different cortical layers. We chose thalamocortical cells because of their “hub” position in forebrain networks and because the homogeneity in cellular composition of thalamic nuclei makes it technically easier to obtain a significant number of well-labelled cells of the same axonal type.

CDP to which Component contributes (if relevant): CDP1. Development of Whole Mouse Brain Model and Mouse Brain Atlas.

Progress on Component: At UAM, we have microinjected / electroporated *in vivo*, perfusion-fixed and processed the brains of 91 adult C57BL6 male mice (>10.000 brain tissue sections). Of these, we have obtained nine cells complete and valid for analysis (five visual, four somatosensory): At present have already Neurolucdida-reconstructed four of these cells. Examples of two such cells, one higher-order visual and a somatosensory thalamocortical cell are shown in Figure 2.

A substantial effort has been invested on improving the efficiency and reliability of the 3D microscopic analysis across large series of sections for the measurement of key structural parameters such as axonal length and varicosity number referenced to identified neuropil spaces cortical layers, etc.). We keep in close contact with the Neurolucida software development team (Microbrightfield Inc.) in Willinston VT, USA in order to optimise their software tools for the analysis of the axonal compartment.

We have released two complete 3D Neurolucida reconstructions (.DAT files) to two SP5 groups (SKU, R. Bakker, P. Tiesinga and UIO, T. Brauns, J. Bjaalie) that are developing the tools for registering in 3D atlas space reconstructed morphologies. In addition to frequent exchanges of digital materials, have had three long topic meetings with these groups on the subject, two physical (10/2016, 2/2017) and a videoconference (3/2017). From these interactions we have identified a number of critical steps such as the inclusion of stereotaxic 3D spatial markers at standard in specific points at the time of microscope reconstruction. We have also identified a number of critical capabilities that should be implemented in the atlasing software tools of SP5. M12 release *Complete 3D datasets of single-cell somatosensory TC axons* has been partially achieved (see MS 1.2.6 in table 2 for further information). The work was done by UAM.

Use Case:

Quality Control:

- Upstream Components:

  - *Thalamocortical cells full 3D reconstructions* and *Workflow for labelling, reconstruction, quantification and indexing of Long-range projection neurons*; Task responsible: 1.2.8 (CLASCÁ); Status: intermediate release; Quality: excellent

- Downstream Component:
  - SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data; Task responsible: T6.2.2 (DEFELIPE); Status: intermediate release.

3.12.3.2 Workflow for labelling, reconstruction, quantification and indexing of Long-range projection neurons

Description of Component: Our laboratory's main contribution will be to set up and implement a workflow for: a) labelling (Adenovirus plasmid and Sindbis Pal-eGFP RNA electroporation), b) 2D and 3D reconstructing (Neurolucida DAT with landmarks for precision atlas indexation), c) quantifying (axon length and varicosity/bouton number) and d) indexing (along with ancillary data such as serial high-resolution tissue section images) the long-range projection neurons (LRPNs) that will populate in the HBP Mouse Brain Atlas, as well as HBP-based simulations of brain networks. The morphologies will be indexed in the HBP digital Mouse Brain Atlas.

Progress on Component: the Component progress is as scheduled. M9 release *Draft of protocol* has been achieved. The work was done by UAM.

Quality Control:

- No upstream Components
- Downstream Component:
  - 3D reconstruction of thalamocortical projection neurons; Task responsible: T1.2.8 (CLASCÁ); Status: intermediate release.
  - Dendritic mechanisms of feedback T3.1.4 (LARKUM, Matthew); Status: intermediate release.

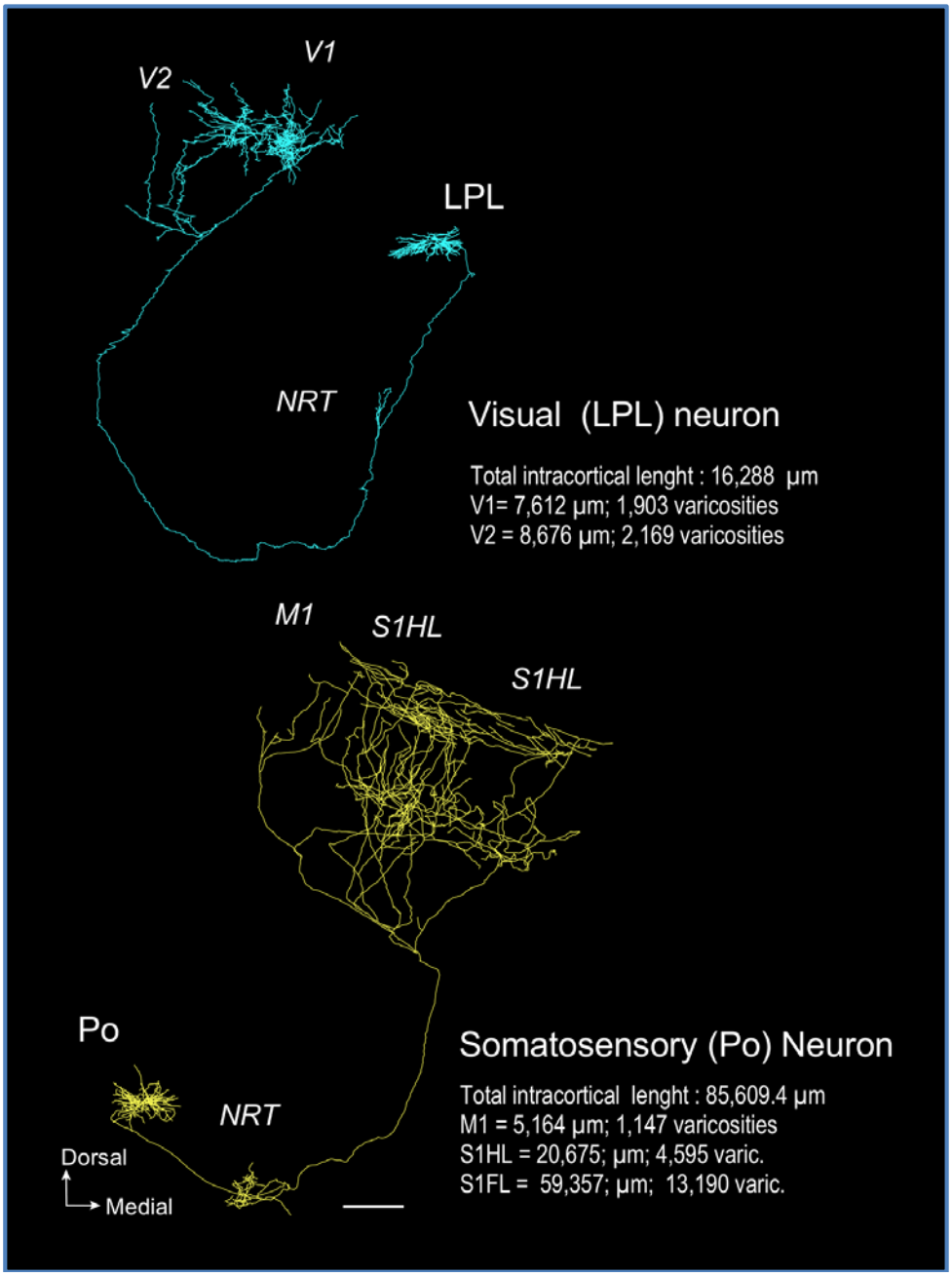

Figure 2: Examples of two cells, one higher-order visual and a somatosensory thalamocortical cell

3.13 T1.2.9 High-resolution synaptic maps in the neocortex and hippocampus using confocal microscopy and FIB/SEM

3.13.1 Key Personnel

Task Leader: Angel Merchan PEREZ (UPM)

Other Researcher: Alberto Muñoz CESPEDES (UPM)

Other Researcher: Lidia Alonso NANCLARES (UPM)

3.13.2 SGA1 DoA Goals

We focus on the quantification and measurement of synapses in the neocortex (hindlimb region of the primary somatosensory cortex in the mouse and temporal cortex in human) and hippocampus (CA1 of both the mouse and human). The number of synapses per unit volume will be calculated. The three-dimensional distributions of synapses will be analysed with spatial statistical tools. The target of asymmetric and symmetric synapses (dendritic spines or shafts) will be determined. Synaptic junction sizes will be measured. Data obtained with FIB-SEM will be correlated with data obtained with confocal microscopy regarding the distributions of excitatory and inhibitory terminals identified by immunocytochemistry; in particular, axonal boutons in apposition to neuronal somata, dendrites and axon initial segments. In this way, data obtained with confocal microscopy in extended regions of the brain will be validated with electron microscopy. The data will be used to reconstruct synaptic connections in brain models.

3.13.3 Component Progress

3.13.3.1 Immunocytochemical detection of excitatory and inhibitory terminals in the mouse neocortex (somatosensory cortex) by confocal microscopy

Description of Component: Immunocytochemical detection of excitatory an inhibitory terminals in the mouse neocortex (somatosensory cortex) by confocal microscopy. Five animals, 15 confocal stacks per layer. Related Milestone: MS1.2.4 Synaptic maps (mesoscopic level) M12 Verification: Data released and data quality validated Related Milestone: MS1.2.7 High-resolution synaptic maps in neocortex and hippocampus M16 Verification: Segmented images released; correct light microscopy reconstructions validated with correlative FIB/SEM electron microscopy 3D reconstructions, also with real images labelled by experts; software released and tested Related task: Task 1.2.9: High-resolution synaptic maps in the neocortex and hippocampus using confocal microscopy and FIB/SEM.

Progress on Component: Component progress is as scheduled. M12 release *Synaptic maps, mouse neocortex (mesoscopic level)* has been achieved (see table 2 MS1.2.4 for further information). The work was done by UPM.

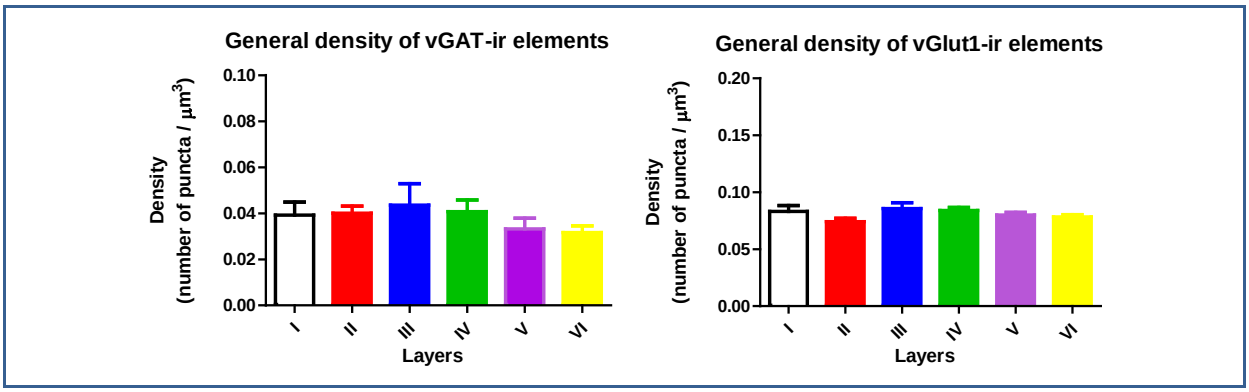

Figure 3: Histograms showing the mean density values of punctate elements (putative axon terminals) immunoreactive for vGAT (GABAergic, left) or vGlut-1 (glutamatergic) in the different layers of the mouse hindlimb primary somatosensory cortex.

Quality Control:

- No upstream Components.
- Downstream Component:
  - 3D reconstructions of 300 pyramidal neurons from the mouse somatosensory cortex across layers II-VI [important]; Task responsible: T1.2.1 (DEFELIPE); Status: intermediate release.

- T3.1.4 Dendritic mechanisms of feedback [added value]; Task responsible: T3.1.4 (LARKUM, M.); Status: nothing provided yet.
- SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data [essential]; Task responsible: T1.2.1 (DEFELIPE); Status: intermediate release.

3.13.3.2 Immunocytochemical detection of excitatory and inhibitory terminals in the mouse hippocampus (CA1) by confocal microscopy

Description of Component: Immunocytochemical detection of excitatory an inhibitory terminals in the mouse hippocampus (CA1) by confocal microscopy. Five animals, 15 confocal stacks per layer. Related Milestone: MS1.2.4 Synaptic maps (mesoscopic level) M12 Verification: Data released and data quality validated Related Milestone: MS1.2.7 High-resolution synaptic maps in neocortex and hippocampus M16 Verification: Segmented images released; correct light microscopy reconstructions validated with correlative FIB/SEM electron microscopy 3D reconstructions, also with real images labelled by experts; software released and tested Related task: Task 1.2.9: High-resolution synaptic maps in the neocortex and hippocampus using confocal microscopy and FIB/SEM.

Progress on Component: Progress on Component: Component progress is as scheduled. M12 release *Synaptic maps, mouse neocortex (mesoscopic level)* has been achieved (see table 2 MS1.2.4 for further information). The work was done by UPM.

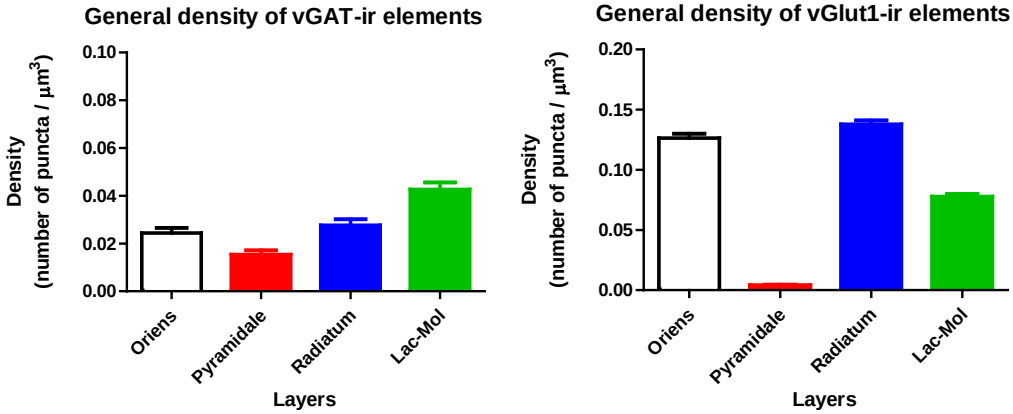

Figure 4: Histograms showing the mean density values of punctate elements (putative axon terminals) immunoreactive for vGAT (GABAergic, left) or vGlut-1 (glutamatergic) in the different layers of the hippocampal CA1 region of the mouse.

Quality Control:

- No upstream Components.
- Downstream Components:
  - SP6-T6.2.4-SGA1-Circuit model of the mouse hippocampus [important]; Task responsible: T6.2.4 (ROMANI, A); Status: intermediate release.
  - 3D reconstructions of 50 cells in mouse hippocampal CA1 region [important]; Task responsible: T1.2.1 (DEFELIPE); Status: intermediate release.

3.13.3.3 Densities and 3D distributions of synapses using FIB/SEM imaging in the mouse neocortex (somatosensory cortex)

Description of Component: Densities and 3D distributions of synapses using FIB/SEM imaging in the mouse neocortex (somatosensory cortex). Three animals, one FIB/SEM stack per layer Related Milestone: MS1.2.7 High-resolution synaptic maps in neocortex and hippocampus M16 Verification: Segmented images released; correct light microscopy reconstructions

validated with correlative FIB/SEM electron microscopy 3D reconstructions, also with real images labelled by experts; software released and tested Related Milestone: MS1.2.9 Synaptic maps (nanoscopic level) M24 Verification: Data released and data quality validated Related Task: Task 1.2.9: High-resolution synaptic maps in the neocortex and hippocampus using confocal microscopy and FIB/SEM.

Progress on Component: Component progress is as scheduled. To date, 18 FIB/SEM stacks have been obtained from the somatosensory cortex of three mice. They are being segmented and analysed with Espina software. M16 release *High-resolution synaptic maps in the mouse neocortex* is in progress. The work was done by UPM.

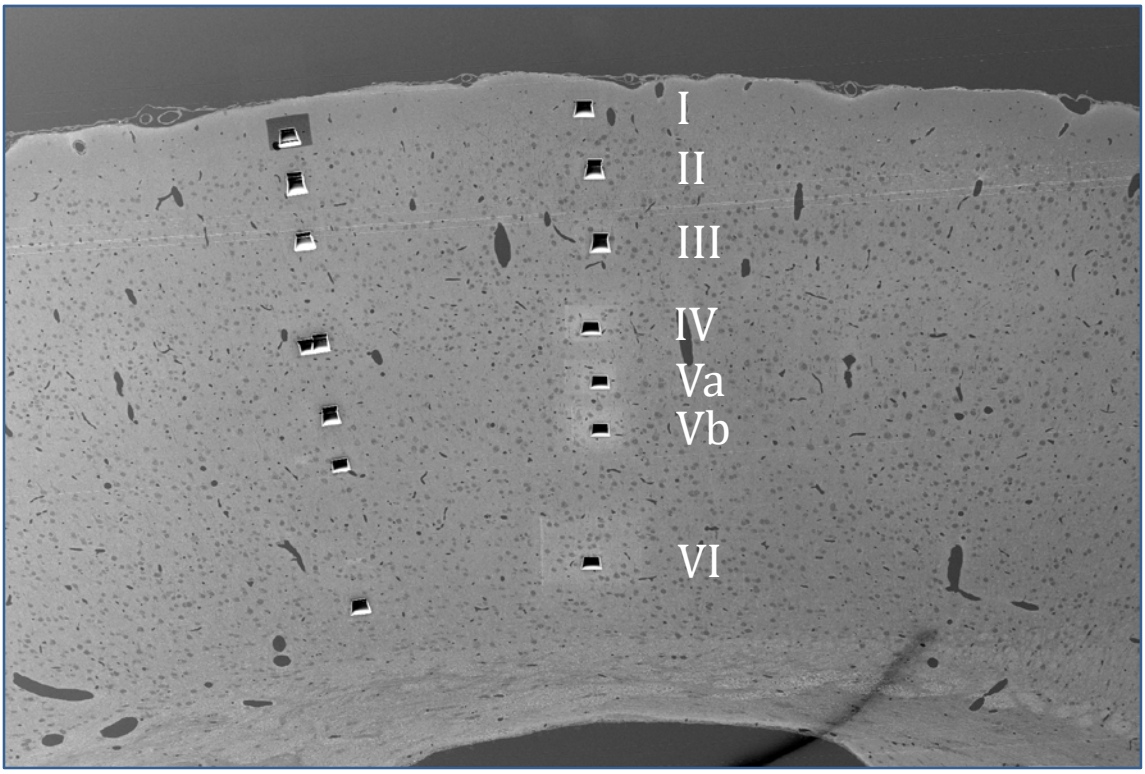

Figure 5: Panoramic view of the block face of a specimen of the mouse somatosensory cortex. The small trenches shown have been milled and imaged using focused ion beam milling and scanning electron microscopy (FIB/SEM). Samples have been taken from the six cortical layers.

Quality Control:

- Upstream Components:
  - Tools for the early analysis of morphological data [important]; Task responsible: T1.4.2 (PASTOR, L.); Status: intermediate release; Quality: excellent.
  - New workflows for embedding exploratory analysis techniques into the early stages of the morphological data extraction and reconstruction processes [important]; Task responsible: T1.4.2 (PASTOR, L.); Status: intermediate release; Quality: excellent.
- Downstream Components:
  - Synapses spatial distribution comparative models [essential]; Task responsible: T1.4.1 (BIELZA, Concha); Status: intermediate release.
  - 3D reconstructions of 300 pyramidal neurons from the mouse somatosensory cortex across layers II-VI [important]; Task responsible: T1.2.1 (DEFELIPE, Javier); Status: intermediate release.

- T3.1.4 Dendritic mechanisms of feedback; Task responsible: T3.1.4 (LARKUM, Matthew); Status: provided nothing.
- SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data; Task responsible: T6.2.2 (DEFELIPE, Javier); Status: intermediate release.

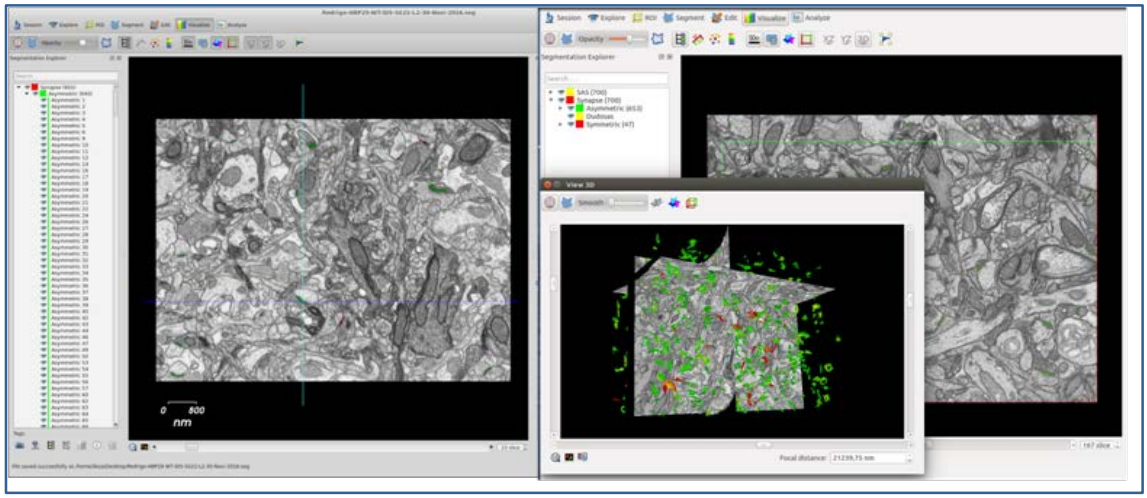

Figure 6: Screenshots of Espina software. This software is being used for the segmentation and analysis of FIB/SEM stacks.

3.13.3.4 Densities and 3D distributions of synapses using FIB/SEM imaging in the mouse hippocampus (CA1)

Description of Component: Densities and 3D distributions of synapses using FIB/SEM imaging in the mouse hippocampus (CA1). Three animals, one FIB/SEM stack per layer Related Milestone: MS1.2.7 High-resolution synaptic maps in neocortex and hippocampus M16 Verification: Segmented images released; correct light microscopy reconstructions validated with correlative FIB/SEM electron microscopy 3D reconstructions, also with real images labeled by experts; software released and tested Related Milestone: MS1.2.9 Synaptic maps (nanoscopic level) M24 Verification: Data released and data quality validated. Related Task: Task 1.2.9: High-resolution synaptic maps in the neocortex and hippocampus using confocal microscopy and FIB/SEM.

Progress on Component: Component progress is as scheduled. To date, eight FIB/SEM stacks have been obtained from the hippocampus of three animals. They are being segmented and analysed with Espina software. M16 release *High-resolution synaptic maps in the mouse neocortex* is in progress. The work was done by UPM.

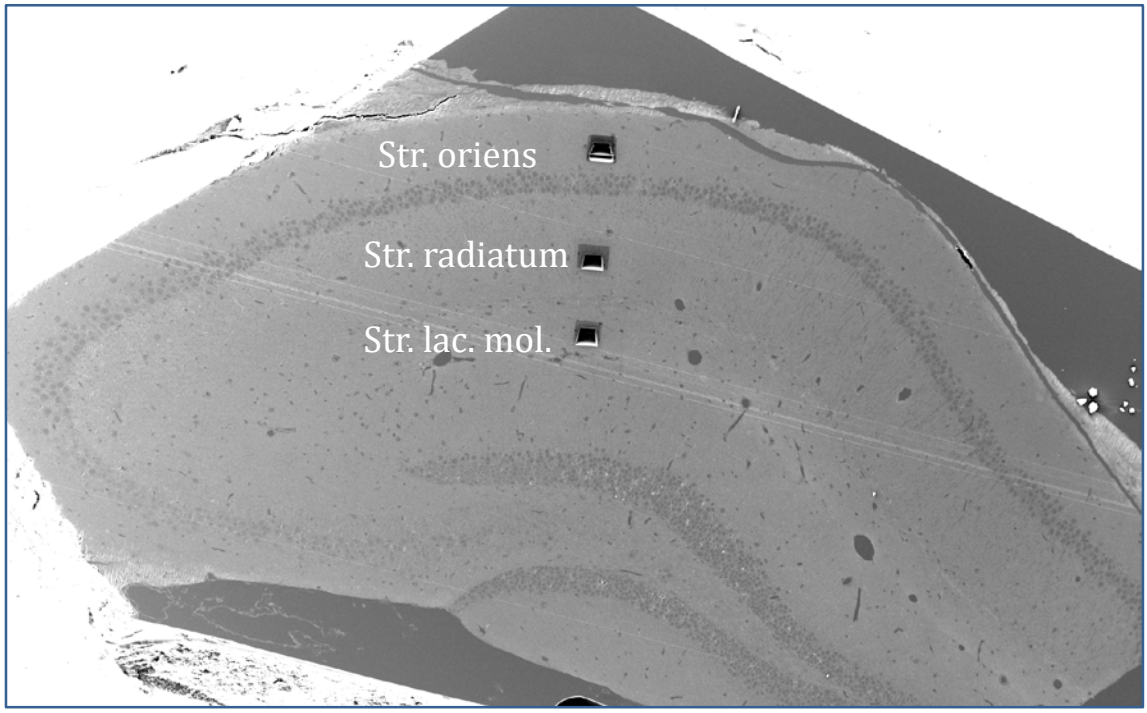

Figure 7: Panoramic view of the block face of a specimen of the mouse hippocampus. FIB/SEM samples have been obtained from strata oriens, radiatum and lacunosum-moleculare, as indicated.

Quality Control:

- Upstream Components:
  - Tools for the early analysis of morphological data [important]; Task responsible: T1.4.2 (PASTOR, L.); Status: intermediate release; Quality: excellent.
  - New workflows for embedding exploratory analysis techniques into the early stages of the morphological data extraction and reconstruction processes [important]; Task responsible: T1.4.2 (PASTOR, L.); Status: intermediate release; Quality: excellent.
- Downstream Component:
  - Synapses spatial distribution comparative models [essential]; Task responsible: T1.4.1 (BIELZA, Concha); Status: intermediate release.
  - SP6-T6.2.4-SGA1-Circuit model of the mouse hippocampus [important]; Task responsible: T6.2.4 (ROMANI, A); Status: intermediate release.
  - 3D reconstructions of 50 cells in mouse hippocampal CA1 region [important]; Task responsible: T1.2.1 (DEFELIPE); Status: intermediate release.

3.13.3.5 Densities and 3D distributions of synapses using FIB/SEM imaging in the human neocortex (Temporal cortex, T2)

Description of Component: Densities and 3D distributions of synapses using FIB/SEM imaging in the human neocortex (Temporal cortex, T2). Three human subjects, nine FIB/SEM stacks Related Milestone: MS1.2.9 Synaptic maps (nanoscopic level) M24 Verification: Data released and data quality validated Related Task: Task 1.2.9: High-resolution synaptic maps in the neocortex and hippocampus using confocal microscopy and FIB/SEM.

Progress on Component: Progress on Component: Component progress is as scheduled. M24 release Synaptic maps, human neocortex (nanoscopic level) is in progress. The work was done by UPM.

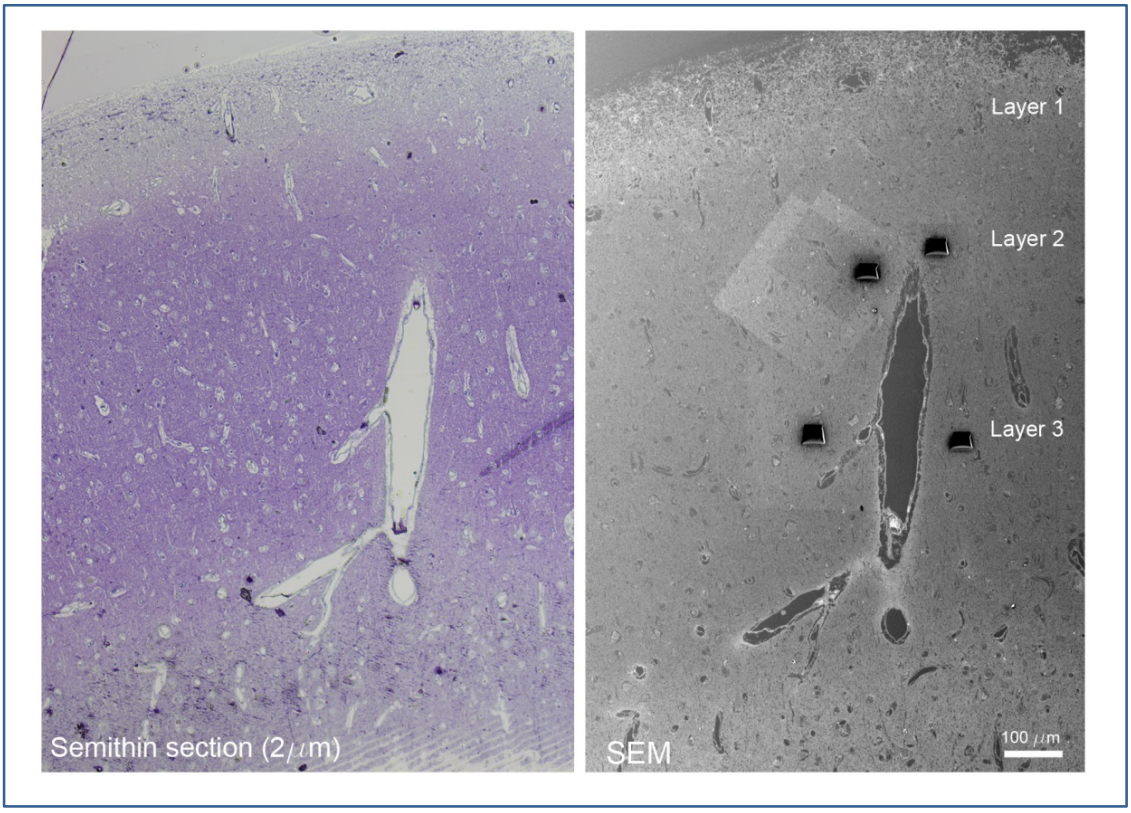

Figure 8: Panoramic pictures of temporal human cortex (T2), from a semithin section and the adjacent EM sample. FIB/SEM samples have been were obtained in layers 2 and 3

Quality Control:

- Upstream Components:
  - Tools for the early analysis of morphological data [important]; Task responsible: T1.4.2 (PASTOR, L.); Status: intermediate release; Quality: excellent.
  - New workflows for embedding exploratory analysis techniques into the early stages of the morphological data extraction and reconstruction processes [important]; Task responsible: T1.4.2 (PASTOR, L.); Status: intermediate release; Quality: excellent.
- Downstream Component:
  - Synapses spatial distribution comparative models [essential]; Task responsible: T1.4.1 (BIELZA, Concha); Status: intermediate release.
  - Synapse segmentation ImageJ plugin and macro [essential]; Task responsible: T1.4.1 (LARRAÑAGA, Pedro); Status: intermediate release.
  - 3D reconstructions of 200 cells in human neocortex (temporal, cingulate and frontal) [important]; Task responsible: T1.2.1 (DEFELIPE, Javier); Status: intermediate release.

3.13.3.6 Densities and 3D distributions of synapses using FIB/SEM imaging in the human hippocampus (CA1)

Description of Component: Densities and 3D distributions of synapses using FIB/SEM imaging in the human hippocampus (CA1). Three human subjects, one FIB/SEM stack per layer Related Milestone: MS1.2.9 Synaptic maps (nanoscopic level) M24 Verification: Data released

and data quality validated Related task: Task 1.2.9: High-resolution synaptic maps in the neocortex and hippocampus using confocal microscopy and FIB/SEM.

Progress on Component: Progress on Component: Component progress is as scheduled. M24 release Synaptic maps, human neocortex (nanoscopic level) is in progress. The work was done by UPM.

Quality Control:

- Upstream Component name (from PLA) + Task responsible + status (i.e. you have received nothing / intermediate release / finished Component) + quality of upstream Component
  - Tools for the early analysis of morphological data [important]; Task responsible: T1.4.2 (PASTOR, L.); Status: intermediate release; Quality: excellent.
  - New workflows for embedding exploratory analysis techniques into the early stages of the morphological data extraction and reconstruction processes [important]; Task responsible: T1.4.2 (PASTOR, L.); Status: intermediate release; Quality: excellent.
- Downstream Component name (from PLA) + Task responsible + status (i.e. you have provided nothing / intermediate release / finished Component)
  - Synapses spatial distribution comparative models [essential]; Task responsible: T1.4.1 (BIELZA, Concha); Status: intermediate release.
  - 3D reconstructions of 50 cells in human hippocampal formation (CA1) [important]; Task responsible: T1.2.1 (DEFELIPE, Javier); Status: intermediate release.

4. WP1.3: Whole Brain

4.1 Key Personnel

Work Package Leader: Francesco S. PAVONE (LENS)

4.2 WP Leader's Overview

- What went particularly well?

Concerning technological development, we improved and streamlined sample preparation and data acquisition protocols, and started implementation of optimised tools for data analysis on high-end GPUs. Together with UIO group (SP5) we performed spatial anchoring to atlas on some pilot scans and stacks. We also set up a first prototype of serial sectioning multi-spot confocal two-photon microscope.

We obtained full datasets of wide-field calcium registrations on mice performing the motor (pulling) task while recording the applied forces. The collaboration with SP4 and SP6 on cortical and whole-brain simulations are set up now and preliminary results are obtained on the in silico stroke brain. The simulation of the rehabilitation platform from SP10 also led to encouraging preliminary results. Both collaborations are within the frame of CDP1.

Regarding whole-brain mapping, we obtained all the relevant ethical authorisation, and set up the animal colonies. Animals of the right age will be available in the next weeks.

A novel computational model to estimate intravascular and tissue partial pressure of oxygen has been developed based on the flow of discrete red blood cells moving in reconstructed vascular networks.

- What did not go according to plan?

We had a slight delay in the production of whole brain datasets, because we could only place the order for the first animals to start the colony in October 2016, after the SGA1 was signed. However, we expect to be on time with the deliverable of datasets.

We had some hardware issues with sample rotation in the light sheet microscope, which prevented acquisition of multiview vasculature data with nearly isotropic resolution.

- Impact of work done

The technological improvements obtained in sample preparation, instrumentation and data analysis will have considerable impact in the neuroscientific field. We already presented some results at international conferences (SfN, BIOS Photonics West) with very positive feedback, and we will submit journal articles in the next months.

4.3 Priorities for the remainder of the phase

Regarding whole-brain mapping, the largest part of technological development has been carried out, we should now concentrate on data acquisition, and on streamlining data integration and sharing within the consortium. On this respect, it is crucial to terminate software porting on high-end GPUs as soon as possible (3-4 months) in order to have enough time to process all the acquired data.

Also, proper dissemination within HBP of the technological advancements obtained (both in instrumentation and software) will maximise the impact of Task 1.3.3.

Concerning T1.3.2, we will focus on obtaining and analysing the last portion of data on the treated mice on the robotic rehabilitation platform. We also characterised the cortical optogenetic stimulation of the motor cortex; in the remainder of the phase, we will interrogate inter-hemispheric connectivity in rehab mice. We plan to submit a paper on this in the next few months, before the end of SGA1.

Concerning vasculature imaging, rotation stage is now fully operative. We need to focus on multi-view image acquisition in the next months to deliver Milestone MS1.3.7.

4.4 Milestones

Table 3: Milestones for WP1.3: Whole Brain

MS No.	Milestone Name	Leader	Task(s) involved	Expected Month	Achieved Month	Comments
MS1.3.3	Production of maps of cortical activation by wide field microscopy in GCaMP6 mice	LENS	1.3.2	09	09	We stored in the CINECA platform repository (URL>https://www.repo.cineca.it/davrods/DRES_HBPprjt/Isilvest/HBP_data/Calcium_traces) files recording cortical activation of GCaMP6 mice in the awake state, while not performing any specific task. These files are in multi-tiff format. For each mouse (4), we have daily (5 days tot) imaging sessions lasting 40 sec, 25 Hz framerate acquisition. Each file size is around 1GB, but is later down-sampled by a factor of 4 for easier processing.
MS1.3.7	Optimisation of acquisition protocol for confocal lightsheet microscopy	UZH	1.3.5	12	TBC	Not achieved. Project is delayed due to hardware issues at the confocal light sheet microscopy (broken rotation stage and delayed implementation of the newly developed autofocus system).

4.5 T1.3.1 (SGA1) Micro- to mesoscopic multilevel and multimodal maps of the mouse brain

4.5.1 Key Personnel

Task Leader: Francesco Saverio PAVONE (LENS)

Other Researcher: Leonardo SACCONI (CNR)

4.5.2 SGA1 DoA Goals

The activity aims to provide a useful reference atlas of the whole mouse brain with the spatial distribution of different cell types (parvalbumin interneurons, somatostatin interneurons, VIP interneurons, pyramidal cells) across the entire brain. The data acquisition is obtained by an improved light sheet microscopy. Moreover, spatial distribution of smaller features (synapses characterised by different PSDs, receptors, proteins) over large areas (hippocampus, basal ganglia, motor cortex, cerebellum) will complement whole-brain measurements with finer details.

4.5.3 Component Progress

4.5.3.1 Whole-brain images of selected neuronal types

Description of Component (from PLA): Image stack of different cell types (parvalbumin interneurons, somatostatin interneurons, VIP interneurons and pyramidal cells) across the entire mouse brain

CDP to which Component contributes (if relevant): CDP1, Use Case CDP1-P1: Reference set-up of the experiment

Progress on Component: we collected pilot images to validate the methodology. Transgenic mouse strains needed for the task have been acquired and are currently in breeding. We expect to have animals of the right age (PND 56) in the next couple of months. Overall, we expect to be on time for release at M18 [MS1.3.6 (SGA1-M24)]. The work was done by CNR-INO and LENS.

Quality Control:

- Upstream Component:
  - Optimisation of Clarity for whole brain imaging [important]- T1.3.3 (Task Leader: Sacconi, Owner: Costantini) – M10: finished Component with good result
  - Improved light-sheet microscopy for whole brain imaging [essential] – T1.3.3 (Task Leader: Sacconi, Owner: Silvestri) – M14: Component almost finished with good preliminary results.
- Downstream Component:
  - T3.3.3 Multi-area recordings from visual and somatosensory cortices, perirhinal and entorhinal cortex and hippocampal CA1 [added value] – nothing provided yet, waiting for the planned release at M18 T3.3.3 Optogenetic manipulation of episodic encoding and retrieval in hippocampal, parahippocampal and mesencephalic dopamine cells [important] – nothing provided yet, waiting for the planned release at M18
  - SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data [added value] – nothing provided yet, waiting for the planned release at M18

- Analysis of network-level mechanisms constraining the *in vivo* implementation of learning rules and implementing integration, encoding and recall of multisensory memories [important] – nothing provided yet, waiting for the planned release at M18

4.5.3.2 Whole-brain maps of selected neuronal types

Description of Component (from PLA): Spatial distribution of different cell types (parvalbumin interneurons, somatostatin interneurons, VIP interneurons and pyramidal cells) across the entire brain

CDP to which Component contributes (if relevant): CDP1, Use Case CDP1-P1: Reference set-up of the experiment, CDP1-P2: A virtual anatomy lab app (should be added CDP1-P3: A virtual imaging lab app)

Progress on Component: we have implemented the analysis pipeline and tested on pilot datasets. Once data from Component 3.3.1 will be available, we will extract from them neuron positions. Overall, we expect to be on time for releases at M18 [MS1.3.9 (SGA1-M18)]. The work was done by CNR-INO and LENS.

Quality Control:

- Upstream Component:
  - Whole brain images of selected neuronal types [essential]: T1.3.1 – waiting for data, should be obtained in the next 2-3 months.
- Downstream Component:
  - T3.3.3 Decoded spike patterns of neural ensembles in cortex and hippocampus during multimodal scene representation [added value] – nothing provided yet, waiting for the planned release at M18T3.3.3 Multi-area recordings from visual and somatosensory cortices, perirhinal and entorhinal cortex and hippocampal CA1 [added value] – nothing provided yet, waiting for the planned release at M18
  - SP6-T6.2.4-SGA1-Circuit model of the mouse hippocampus [important] – nothing provided yet, waiting for the planned release at M18
  - SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data [added value] – nothing provided yet, waiting for the planned release at M18
  - Point-neuron model of the whole mouse brain [important] – nothing provided yet, waiting for the planned release at M18
  - Analysis of network-level mechanisms constraining the *in vivo* implementation of learning rules and implementing integration, encoding and recall of multisensory memories [important] – nothing provided yet, waiting for the planned release at M18

4.6 T1.3.2 (SGA1) Cortical plasticity associated with motor recovery in mouse models of stroke triggered by a robotics task

4.6.1 Key Personnel

Task Leader: Francesco Saverio PAVONE (LENS)

4.6.2 SGA1 DoA Goals

The main goal of this Task is to monitor longitudinally cortical plasticity following focal cerebral ischemia. We will characterise the modifications of motor representation and functional connectivity in the ipsi and contralateral hemisphere. In addition, we will

investigate how robotic rehabilitation of the affected arm aids motor recovery by boosting cortical remodelling.

4.6.3 Component Progress

4.6.3.1 Fluorescence imaging of cortical activity after stroke

Description of Component (from PLA): 2D lapse recording of calcium-activity in the cortex of fluorescent mice of three experimental groups: control, stroke, rehabilitated. Five mice per group, 15 datasets total.

CDP to which Component contributes (if relevant): CDP1, Use Case CDP1-P1: Reference set-up of the experiment

Progress on Component: We acquired 15 datasets (1/day, 5 days, three mice) of calcium activity in awake mice while performing the motor task (i.e. pulling of forelimb). The work was done by LENS.

Releases:

M9 - 2D Time lapse cortical recording maps of learning – it is the Milestone MS1.3.3

already done: URL

https://www.repo.cineca.it/davrods/DRES_HBPprjt/lsilvest/HBP_data/Calcium_traces

M18 - 2D Time lapse cortical recording maps after stroke-> Milestone MS1.3.5 Production of maps of cortical activity by wide field microscopy in GCaMP6 mice after stroke due M18

M24 - 2D Time lapse cortical recording maps during rehabilitation

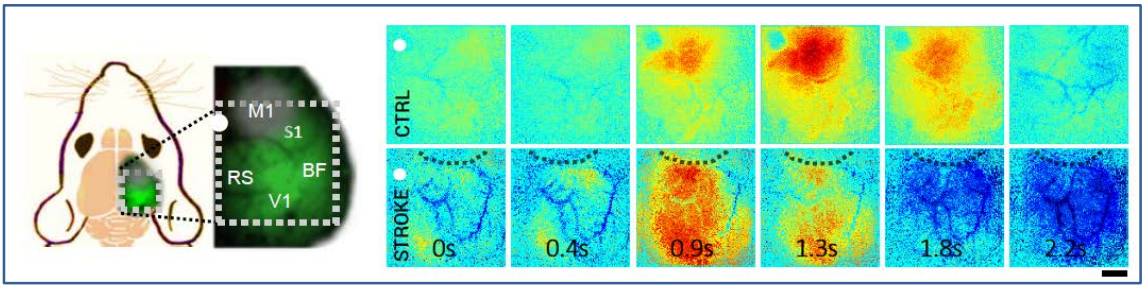

Figure 9: Example from fluorescence imaging of cortical activity after stroke.

Quality Control:

- Upstream Component:
  - Wide-field mesoscope [important] received finished Component
  - Single-photon system for optogenetic actuation [important] received finished Component
  - Optimisation of rehabilitation platform [essential] received finished Component
- Downstream Component:
  - Structural and functional connectivity at different scales [important] – provided intermediate release
  - Allen Mouse Atlas (AMA) based brain network [important] – received finished Component
  - Effective connectivity changes inferred from optogenetic brain interrogation and calcium imaging [essential] – nothing provided, waiting for release planned at M18

- Validation of the virtual rehabilitation scenario [important] - provided intermediate release
- Analysis of meso-scale fluorescence functional data [important] - provided intermediate release

4.6.3.2 Optimisation of rehabilitation platform

Description of Component (from PLA): Technique of sample and environment set-up for rehabilitation platform

CDP to which Component contributes (if relevant): CDP1, Use Case CDP1-P1: Reference set-up of the experiment

Progress on Component: We set up the platform for robotic-rehabilitation of the mouse. Release M12 - draft technique for internal use. The work was done by LENS.

Quality Control:

- Upstream Component: none
- Downstream Component:
  - Specifications of the motor rehabilitation experiment [essential] provided intermediate release
  - Fluorescence cortical recording of mouse activity after stroke [essential] provided intermediate release

4.6.3.3 Analysis of meso-scale fluorescence functional data

Description of Component (from PLA): software development for 2D time lapse cortical recording maps

CDP to which Component contributes (if relevant): CDP1, Use Case CDP1-P1: Reference set-up of the experiment

Progress on Component: we are setting up the software; we got in contact with SP5 for uploading it on the NIP and sharing it with HBP internal users. The work was done by LENS. The developed software could be useful for the entire neuroscientific community within and outside HBP working on functional imaging. The analysis software is consistent with but is not based on the Allen Brain Atlas as for now.

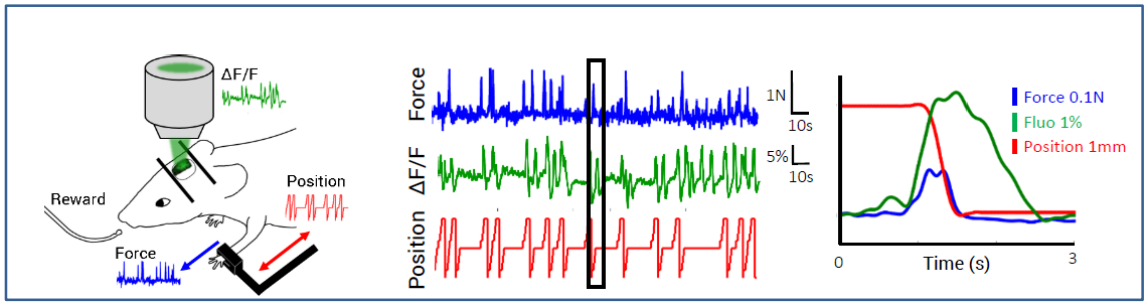

Figure 10: Example from analysis of meso-scale fluorescence functional data.

Quality Control:

- Upstream Component:
  - Allen Mouse Atlas (AMA) based brain network [important] – received intermediate release
  - Fluorescence cortical recording of mouse activity after stroke [important] – received intermediate release

- Downstream Component:
  - Report on the virtual motor rehabilitation experiment on mice [essential] - provided intermediate release
  - Validation of the virtual rehabilitation scenario [essential] - provided intermediate release

4.7 T1.3.3 (SGA1) Methodical development in optical imaging, data analysis, integration and atlasing

4.7.1 Key Personnel

Task Leader: Leonardo SACCONI (CNR)

Other Researcher: Francesco Saverio PAVONE (LENS)

4.7.2 SGA1 DoA Goals

This Task is devoted to develop the adequate technologies for the imaging acquisition performed by LENS in SP1 and SP2. In detail the activity consists of: the set-up of four apparatus (Fast serial section 2-photon microscope, Improved light-sheet microscopy for whole brain imaging, Single-photon system for optogenetic actuation, Wide-field mesoscope), the optimisation of Clarity technique and the development of a tool for cell counting and shape recognition.

4.7.3 Component Progress

4.7.3.1 SP1 - Software for cell counting and shape recognition

Description of Component (from PLA): Suite of fast and scalable algorithms for fully automated cell identification, cell shaping and fibre segmentation.

CDP to which Component contributes (if relevant): none

Progress on Component: a preliminary work for defining a stitching protocol for 3D high resolution microscopy imagery has been laid out. This is needed for having a good quality of the image on which cell counting algorithms will be developed. The work was done by LENS.

Quality Control:

- Upstream Component: none
- Downstream Component:
  - T3.3.3 Optogenetic manipulation of episodic encoding and retrieval in hippocampal, parahippocampal and mesencephalic dopamine cells [important]
  - Modelling of network-level mechanisms from T3.6.3a [important]
  - Analysis of network-level mechanisms constraining the *in vivo* implementation of learning rules and implementing integration, encoding and recall of multisensory memories [important]

4.7.3.2 Fast serial section 2-photon microscope

Description of Component (from PLA): set-up of Fast serial section 2-photon microscope

CDP to which Component contributes (if relevant): none

Progress on Component: a preliminary prototype has been developed exploiting the advantage of diffractive optical elements to produce a multi-focal excitation. A preliminary investigation underlined that a system has a signal-to-noise ratio comparable to a single

beam implementation, but with the possibility of tenfold increase in imaging speed. The work was done by LENS and CNR-INO.

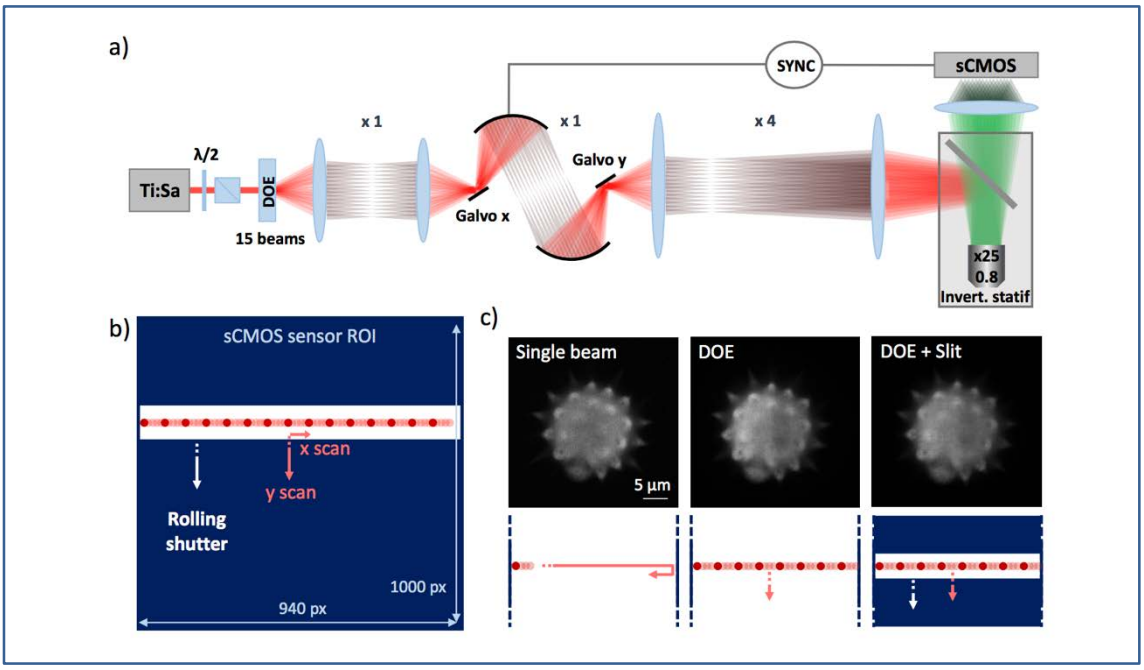

Figure 11: a) Schematic of the microscope.

The two galvanometer mirrors scanned the spot line parallel and perpendicularly to its axis, in order to uniformly excite a 90 µm x 96 µm field of view (FOV) on the object plane. The FOV resulted from the full DOE diffraction angle (here 5.27°) and total magnification. b) Scheme of the rolling shutter as a synchronised confocal slit on the sCMOS camera sensor. The white band represents the rolling shutter. c) Fluorescent images of pollen grain for three differing microscope configuration. From left to right: a single beam is scanned, the 15 beams are scanned, the 15 beans are scanned with synchronised 10 px slit.

Quality Control:

- Upstream Component: none
- Downstream Component:
  - SGA2_T2.3.2 Integration of ultra-fast large area fluorescence microscope with Switch clearing techniques [essential] – activity not yet started
  - SP2 - Mapping of cellular structures onto molecular architecture [essential] - An internal report has been produced and passed to this Component.
  - SP2 - Multilevel maps of quantitative cell distributions and morphologies [essential] - An internal report has been produced and passed to this Component.
  - SP2 - Maps of different human neuronal circuits [important]

4.7.3.3 Optimisation of Clarity for whole brain imaging

Description of Component (from PLA): Optimisation of Clarity technique for whole brain imaging

CDP to which Component contributes (if relevant): CDP1, Use Case CDP1-P1: Reference set-up of the experiment

Progress on Component: the clarity protocol has been optimised using various incubation time and different percentage of hydrogel solutions. A passive clearing chamber has been built and tested. Thy1-GFPM mouse brains were finally imaged with LSFM to validate the results. The work was done by LENS and CNR-INO.

Quality Control:

- Upstream Component:
  - Antibodies against targets identified in all genomic and proteomics tasks [added value] – not received
- Downstream Component: the resulted protocol has been passed to the following Components:
  - SP2 - Maps of different human neuronal circuits [essential]
  - Images of neuronal activation of whole mouse brain [important]
  - Whole-brain images of selected neuronal types [important]
  - SP2 - Mapping of cellular structures onto molecular architecture [essential]

4.7.3.4 Improved light-sheet microscopy for whole brain imaging

Description of Component (from PLA): Set-up of Improved light-sheet microscopy for whole brain imaging

CDP to which Component contributes (if relevant): none

Progress on Component: The existing light-sheet microscope has been improved to obtain a better resolution and image accuracy by introducing Bessel beam illumination and a real time auto-focus system. (RAPID, manuscript in preparation), see figure 12 below. The work was done by LENS and CNR-INO.

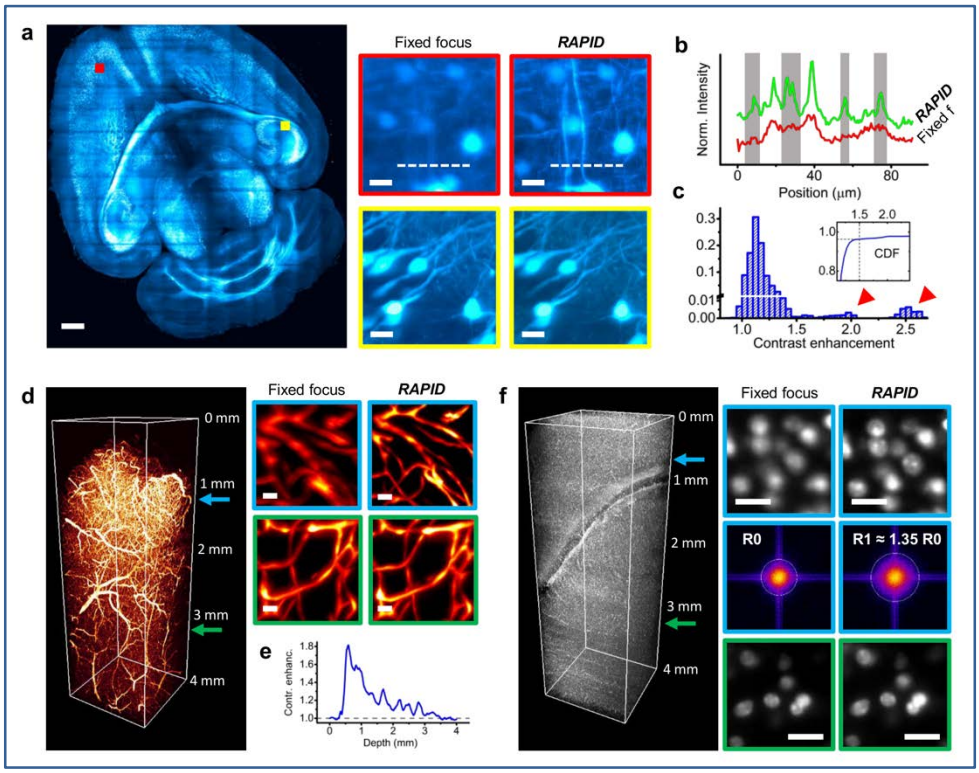

Figure 12: RAPID autofocusing in high-resolution light-sheet microscopy.

A virtual slab (500 µm thick) from the brain of a thy1-GFP-M transgenic mouse (a). RAPID defocus correction across different tiles (insets). Intensity profiles along the dashed with lines (b). Gray regions highlights fine sample details lost without autofocus. Histogram of contrast enhancement (c) for all the images forming the slab in (a). Red arrowheads highlight positive outliers, whereas the inset shows the cumulative density function (CDF). 3D rendering of an image stack from a vasculature-stained mouse brain showing insets at different depths (d). RAPID contrast enhancement as a function of depth for this stack is shown in (e). 3D rendering of an image stack from a mouse brain with nuclear staining. The constant shape of the nuclei allows evaluation of resolution enhancement with RAPID looking at the radius of the Fourier transforms (insets, middle line). Scale bars: 1 mm (a), 20 µm (insets).

Quality Control:

- Upstream Component: none
- Downstream Component:
  - 3D image of the entire vascular system of the mouse brain [important]
  - Whole-brain images of selected neuronal types [essential] – the results obtained hitherto have been shared internally provided internal intermediate release
  - Images of neuronal activation of whole mouse brain [essential] - the results obtained hitherto have been shared internally provided internal intermediate release
  - SP2 - Multilevel maps of quantitative cell distributions and morphologies [essential] provided internal intermediate release

4.7.3.5 Single-photon system for optogenetic actuation

Description of Component (from PLA): set-up description of single-photon system for optogenetic actuation

CDP to which Component contributes (if relevant): CDP1, Use Case CDP1-P1: Reference set-up of the experiment

Progress on Component: A wide-field mesoscope (see below) has been implemented with an ultra-fast scanning system allowing the stimulation of the cortical area with a blue laser to optogenetic investigation. The system has been used to dissect the inter-hemisphere connectivity and plasticity during robotic rehabilitation. The work was done by LENS and CNR-INO. The optogenetic system is integrated into the wide field mesoscope (see Component below).

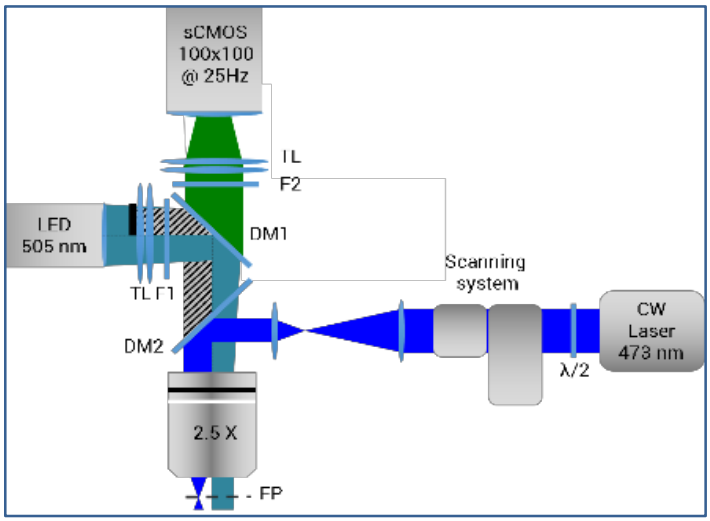

Figure 13: Example from a single-photon system for optogenetic actuation.

Quality Control:

- Upstream Component: none
- Downstream Component:
  - Fluorescence cortical recording of mouse activity after stroke [important] – A report of the set-up has been shared internally

4.7.3.6 Wide-field mesoscope

Description of Component (from PLA): set-up of a wide-field "mesoscope" for high-speed calcium imaging on the whole mouse cortex

CDP to which Component contributes (if relevant): CDP1, Use Case CDP1-P1: Reference set-up of the experiment

Progress on Component: A wide field has been developed (see picture above) allowing the large area fast detection during s robotic rehabilitation task. The work was done by LENS and CNR-INO.

Quality Control:

- Upstream Component: none
- Downstream Component:
  - Fluorescence cortical recording of mouse activity after stroke [important] - An internal report of the set-up has been shared internally

4.8 T1.3.4 (SGA1) Whole-brain cell-resolution activity maps

4.8.1 Key Personnel

Task Leader: Francesco Saverio PAVONE (LENS)

4.8.2 SGA1 DoA Goals

The goal of this activity is to provide whole-brain functional data on the entire brain volume and with cellular quantification, in order to integrate them in the atlas available on the NIP and allow the validation of whole mouse brain models with high accuracy. The integration of these data in the HBP atlas will be a general benchmark for functional brain models.

4.8.3 Component Progress

4.8.3.1 Images of neuronal activation of whole mouse brain

Description of Component (from PLA): Whole-brain images of neuronal activation in mouse brain acquired with light-sheet microscopy. Animal models will be used to detect immediate early genes (IEGs) expression.

CDP to which Component contributes (if relevant): CDP1, Use Case CDP1-P1: Reference set-up of the experiment and CDP1-P3: A virtual imaging lab app

Progress on Component: we have optimised animal procedure and performed whole-brain scans in pilot mice. Mice for larger cohort studies are currently breeding; we expect to collect images within the next 2-3 months. Overall, we expect to be on time for Release 18 - 3D Images of neuronal activation of whole mouse brain. The work was done by LENS.

Quality Control:

- Upstream Component:
  - Optimisation of Clarity for whole brain imaging [important] Finished with good results.
  - Antibodies against targets identified in all genomic and proteomics tasks [added value] Not yet finished, we are in contact with Component owner to obtain test antibodies.
  - Improved light-sheet microscopy for whole brain imaging [essential] Almost finished with good intermediate results.
- Downstream Component:

- T3.1.4 Dendritic mechanisms of feedback [important] nothing provided yet, waiting for the planned release
- SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data [added value] nothing provided yet, waiting for the planned release
- Maps of neuronal activation of whole mouse brain [essential] nothing provided yet, waiting for the planned release
- Whole-brain maps of selected neuronal types [essential] nothing provided yet, waiting for the planned release

4.8.3.2 Maps of neuronal activation of whole mouse brain

Description of Component (from PLA): Whole-brain maps of neuronal activation with cellular resolution

CDP to which Component contributes (if relevant): CDP1, Use Case CDP1-P1: Reference set-up of the experiment and CDP1-P3: A virtual imaging lab app

Progress on Component: we have implemented the analysis pipeline and tested on pilot datasets. Once data from the other Component of this task will be available, we will extract from them positions of activated neurons. Overall, we expect to be 1-2 months late for the first release, and on time for the second one, MS1.3.4 due M14. The work was done by LENS.

Quality Control:

- Upstream Component:
  - SP2 - Computational architecture of the functional organisation in visual and auditory processing streams in human and macaque monkey [added value] result not received
  - Images of neuronal activation of whole mouse brain [essential]. Not yet finished. We expect to have images in the next 2-3 months.
- Downstream Component:
  - T3.1.4 Dendritic mechanisms of feedback [important] – nothing provided yet, waiting for the planned release
  - SP6-T6.2.2-SGA1-Initial model validation tests implemented to compare rat model with mouse data [added value] – nothing provided yet, waiting for the planned release
  - Point-neuron model of the whole mouse brain [important] – nothing provided yet, waiting for the planned release

4.9 T1.3.5 - Vasculature Maps and Computation of Oxygen Delivery Key Personnel

4.9.1 Key Personnel

Task Leader: Task Leader: Bruno WEBER (UZH)

Other Researcher: Matthias SCHNEIDER (UZH)

4.9.2 SGA1 DoA Goals

This Task will use multimodal and multiscale approaches to achieve high-resolution maps of the murine cerebral vasculature. We have set up an informatics and data management platform in the last two years, which includes the implementation of novel approaches for efficient filtering and segmentation that are run on supercomputers. For SGA1, the main

objective will be to achieve a three dimensional reconstruction of the entire vascular system of the mouse brain for the first time. This will be achieved by using light-sheet microscopy and/or synchrotron-radiation based X-ray microscopy. Very big data sets (~ 15 TB per mouse brain) will be generated and translated into a three-dimensional graph. The resulting graph will be used as a spatial scaffold for the cellular elements and will be the basis for simulating the brain's energy and oxygen delivery. The vascular network is a key element for Task T4.2.2.

4.9.3 Component Progress

4.9.3.1 3D image of the entire vascular system of the mouse brain

Description of Component: For SGA1, the main objective is to achieve a three dimensional image of the entire vascular system of the mouse brain for the first time. This will be achieved by using light-sheet microscopy and/or synchrotron-radiation based X- ray microscopy. Very big datasets (~ 15 TB per mouse brain) will be generated.

CDP to which Component contributes (if relevant): CDP1-P1: Reference set-up of the experiment.

Progress on Component: A novel computational model to estimate intravascular and tissue partial pressure of oxygen is developed based on the flow of discrete red blood cells moving in reconstructed vascular networks. This will be extended for the use in larger networks. Such data are not available to date and both the anatomical data and the numerical modelling will contribute to a fundamental understanding of the neuroenergetic constraints of the brain's information processing. M15 release *Acquisition of first image datasets* is in progress. MS1.3.7 Optimisation of acquisition protocol for confocal light sheet microscopy was not achieved due to technical hardware problems. Work was done by UZH.

Quality Control:

- Upstream Component:
  - Improved light-sheet microscopy for whole brain imaging [important]; Task responsible: T1.3.3 (SILVESTRI, Ludovico); Status: intermediate release; Quality: excellent.
- Downstream Components:
  - Internal report on acquisition of vascular images and post-processing protocol [essential]; Task responsible: T1.3.5 (WEBER, Bruno); Status: intermediate release.
  - 3D reconstruction of the entire vascular system of the mouse brain [essential]; Task responsible: T1.3.5 (WEBER, Bruno); Status: intermediate release.

4.9.3.2 3D reconstruction of the entire vascular system of the mouse brain

Description of Component: The 3D image will be translated into a three-dimensional graph. The resulting graph will be used as a spatial scaffold for the cellular elements and will be the basis for simulating the brain's energy and oxygen delivery. The vascular network is a key element for T4.2.2.

CDP to which Component contributes (if relevant): CDP1-P1: Reference set-up of the experiment.

Progress on Component: We have not been able to proceed to this stage as planned due to methodological issues. Reason: Full mouse brain reconstructions of the vascular system has not been achieved due to methodological issues, Synchrotron radiation-based X-ray tomography could not be performed in large samples due to an unexpectedly high absorption and as a consequence image reconstruction failure. Phase contrast X-ray tomography was

tested as an alternative but did not yield sufficient contrast for vessel segmentation. We now switch to optical methods but the data are not yet available.

Quality Control:

- Upstream Component:
  - 3D image of the entire vascular system of the mouse brain [essential]; Task responsible: T1.3.5 (WEBER, Bruno); Status: intermediate release; Quality: excellent.
- Downstream Components:
  - Internal report on acquisition of vascular images and post-processing protocol [essential]; Task responsible: T1.3.5 (WEBER, Bruno); Status: intermediate release.
  - Model of intravascular and tissue partial pressure of oxygen [essential]; Task responsible: T1.3.5 (WEBER, Bruno); Status: intermediate release.

5. WP1.4 Integration of Micro-anatomical Data

5.1.1 Key Personnel

Work Package Leader: Javier DEFELIPE (UPM), Douglas ARMSTRONG (UEDIN)

5.1.2 WP Leader's Overview

- What went particularly well?

Some relevant toolsets and plugins needed for the toolsets have been released during M1-M12, such as **obj_detection_toolset** v0.1.2. Moreover, a multi-platform desktop application to visualise, edit, create and analyse (e.g. estimate counts and density of points in user selected ROIs) spatial data anchored to maps and an alpha version of the library NeuroSTR have been released. In addition, network spatial analysis has been used to model the locations of spines along dendritic arborisations.

- What didn't go according to plan?

Some delays have occurred in WP1.4 affecting mainly the progress of some activities in T1.4.2. In particular, MS1.4.2 *Prototype of early exploratory analysis techniques for morphological data* has been partially achieved due to the delay in hiring personnel. It is expected to finalise this MS in M15. In spite of some activities are delayed, WP1.4 is on schedule to meet the proposed objectives in M24.

- Impact of work done

In WP1.4, proteomic, structural and electrophysiological datasets produced in SP1 are currently being integrated. WP1.4 is also assisting with the integration of external datasets that complement the core SP1 data. Statistical modelling, including network analysis of molecular networks, is expected to reveal relationships between the linked datasets and will inform the modelling work of SP4 and SP6. Statistical and machine learning techniques are applied to infer principles of human and mouse neuron morphology and neuroanatomical organisation.

In addition, strategic multi-level datasets are being integrated, such as the characterisation of neocortical GABAergic neurons by immunohistochemical markers to establish a quantitative electron microscopic database on synaptic coverage of subtypes of GABAergic neurons.

5.1.3 Priorities for the remainder of the phase

We will continue to integrate the genetic, transcriptomic, proteomic, structural and electrophysiological datasets produced in SP1. WP1.4 will also assist with the integration of external datasets that complement the core SP1 data. In particular, statistical modelling and machine learning techniques are being applied to infer principles of human and mouse neuron morphology and neuroanatomical organisation. Furthermore, multi-level datasets will be further developed, including: (i) broad behavioural screening of mouse models carrying genetic variation or treatment with reference compounds; (ii) integration of single cell genomic datasets as they become available; (iii) characterisation of neocortical GABAergic neurons by immunohistochemical markers in order to establish a quantitative electron microscopic database on synaptic coverage of subtypes of GABAergic neurons.

5.2 Milestones

Table 4: Milestones for WP1.4: Integration of Microanatomical Data

MS No.	Milestone Name	Leader	Task(s) involved	Expected Month	Achieved Month	Comments
MS1.4.1	Predictive models of the position of pyramidal dendritic spines and synapses along dendritic arbors	Partner UPM	T1.4.1	M8	M8	Achieved. MS achieved on 20 November 20 2016. Dendritic spines cannot lie anywhere but they are constrained to lie on the dendritic network, so the application of network spatial analysis, i.e., the application of statistical and computational methods for analysing events occurring on and along networks, can provide much more complete models than traditional spatial statistic techniques. We have used network spatial analysis to model the position of spines, which correspond to most of the excitatory synapses in the brain, along dendritic arbors of pyramidal neurons, in both basal and apical dendrites
MS1.4.2	Prototype of early exploratory analysis techniques for morphological data	Partner URJC	T1.4.2	M12	Expected in M15	Task 1.4.2 has been severely affected by the impossibility of hiring people at URJC until July 2016, since URJC did not allowed us to start the hiring procedures before the HBP

						Project was signed. MS1.4.2 could not be finished on time (M12) due to these reasons. It has been estimated that Milestone MS1.4.2 will be achieved in M15.

5.3 T1.4.1 - Analysis of micro-anatomical data

5.3.1 Key Personnel

Task Leader: Concha BIELZA (UPM)

5.3.2 SGA1 DoA Goals

We have three goals related to the study of the anatomical design principles of cortical circuits (neocortex and hippocampus):

1) Pyramidal dendritic architecture requires predictive models of the position of dendritic spines and synapses along dendritic arbors. The models could be inspired in the recent approach of spatial models along networks allowing to place synapsis locations over the dendritic shaft.

2) High-resolution synaptic maps in neocortex and hippocampus, based on correlative light and electron microscopy will require spatial sampling techniques to avoid the fully reconstruction of all brain regions.

3) Characterising the main types of GABAergic interneurons. Semi-supervised clustering techniques together with multi-expert labelled cells will lead this goal allowing for new types discovery, whose names will be agreed upon by the scientific community.

5.3.3 Component Progress

5.3.3.1 Synapse segmentation ImageJ plugin and macro

The plugin filters a stack of images from confocal microscopy to separate background from foreground. Foreground pixels are further connected to create 3D objects, the synapses.

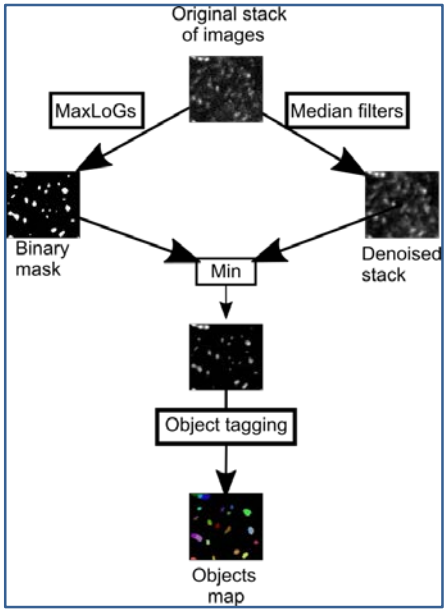

Figure 14: Example from synapse segementation ImageJ plugin and macro.

Use Case: High-resolution synaptic maps in the mouse neocortex and hippocampus https://project-lifecycle.herokuapp.com/use_case/64

Progress: We have released obj_detection_toolset v0.1.2, an ImageJ/FIJI macro toolset. The code is hosted at github (https://github.com/ComputationalIntelligenceGroup/obj_detection_toolset) where documentation is also available. The toolset is registered in the software catalogue (obj detection toolset) of the HBP collaboratory. Moreover, we have also released two plugins needed by the toolset (ObjCounter to connect and count segmented objects and MaxLoGs a maximum of Laplacians of Gaussian filter). The link to the code can be found in the obj

detection toolset documentation. Release planned in M8 achieved. All the work was done by UPM.

Quality Control:

- Upstream Component: Densities and 3D distributions of synapses using FIB/SEM imaging in the human neocortex; Task responsible: T1.2.9, Status: received finished Component; Quality: good quality
- Downstream Component: Interactive synaptic map, T1.4.1, we have provided a finished Component

5.3.3.2 Interactive synaptic map

Web-based end-user interface to interactively analyse regions of the brain previously segmented to detect synapses. Error model is used to validate segmentation.

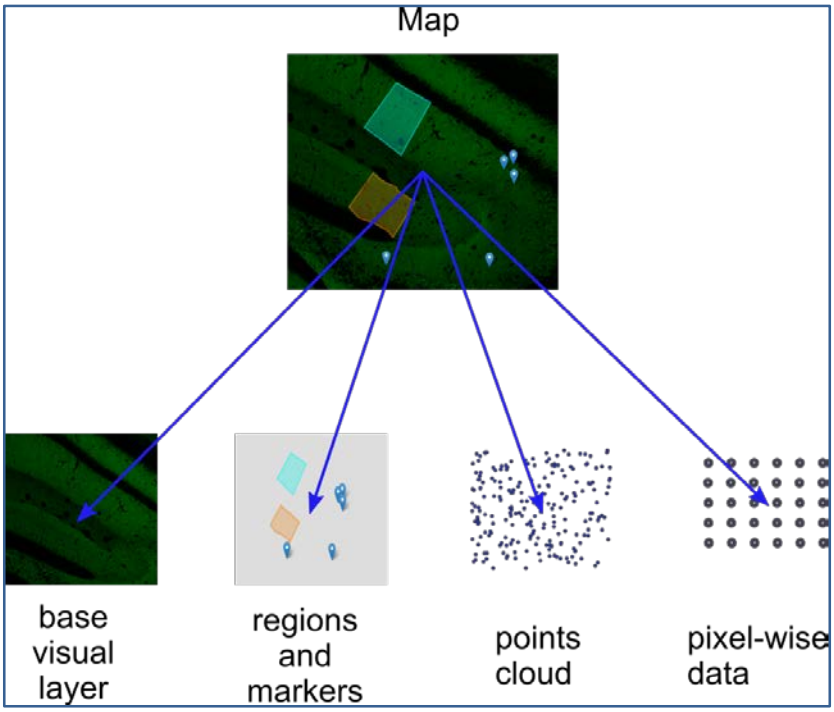

Figure 15: Example from interactive synaptic map.

Use Case: High-resolution synaptic maps in the mouse neocortex and hippocampus https://project-lifecycle.herokuapp.com/use_case/64

Progress: We released Atlas (this is provisional name) v0.1.3 (https://github.com/ComputationalIntelligenceGroup/Atlas/releases/tag/v0.1.3) a multi-platform desktop application to visualise, edit, create and analyse (e.g. estimate counts and density of points in user selected ROIs) spatial data anchored to maps. This application is registered in the software catalogue (Atlas) of the HBP collaboratory. No releases planned in M1-M12. All the work was done by UPM.

Quality Control:

- Upstream Component: Synapse segmentation ImageJ plugin and macro; Task responsible: T1.4.1; Status: received finished Component; Quality: good quality

5.3.4 Synapse spatial location on the dendritic shaft model

Spatial probabilistic model that describes and predicts the distribution of synapses along the dendritic shaft.

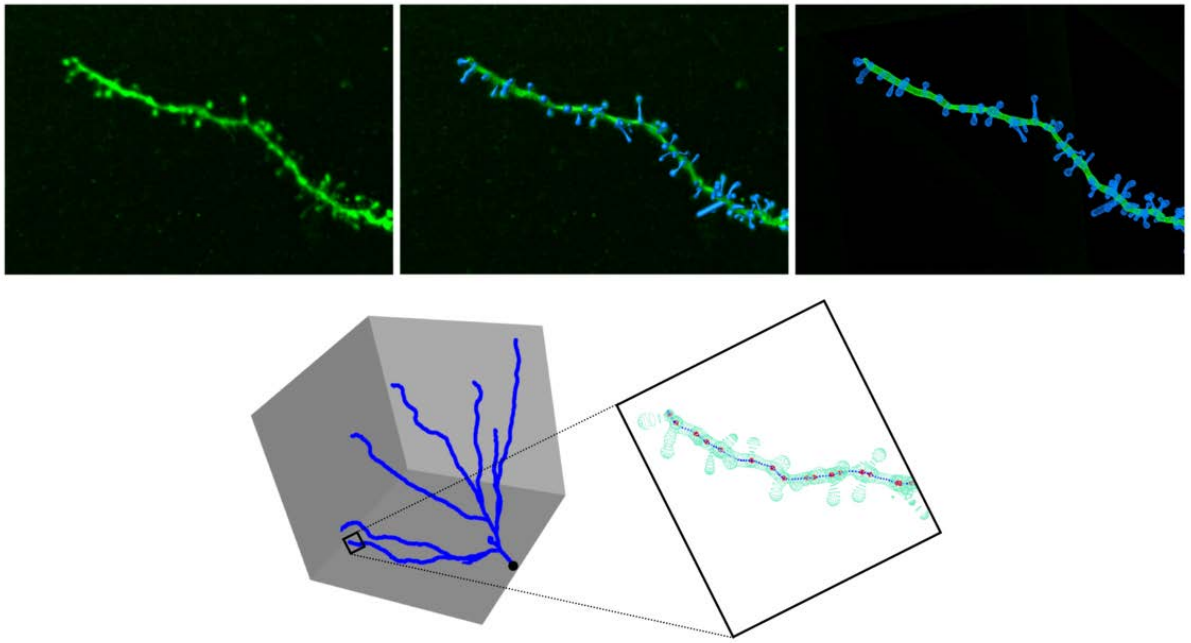

Figure 16: Modelling spine distribution along the dendritic networks.

Use Case: Spatial models along networks allowing to place synapses locations over the dendritic shaft https://project-lifecycle.herokuapp.com/use_case/63

Progress: We use network spatial analysis to model the locations of spines along dendritic arborisations. In a first version (released in M8), each dendritic tree was analysed separately. Later, we have found that spine intensity depends on the distance to the cell body. In addition, we have used a replicated point pattern-based analysis to detect differences between groups, for example between apical and basal dendrites or between different neurons. We have submitted an article with this work to PLOS ONE . The progress is as expected. Release planned in M8 achieved, MS1.4.1 Predictive models of the position of pyramidal dendritic spines and synapses along dendritic arbors. All the work was done by UPM.

Quality Control:

- Upstream Component: 3DSynapsesSA; Task responsible: T5.4.2 (RUP); Status: received finished Component; Quality: good quality.

5.3.4.1 NeuroSTR: C++ Neuroanatomy library

The Neurostr library provides analysis and editing functionalities for three-dimensional traced neurons. It imports traced neurons written in SWC and 'Neurolucida' DAT and ASC format and validates them. It includes a large set of predefined measures, selectors, etc. but new measures and functionality can be added easily.

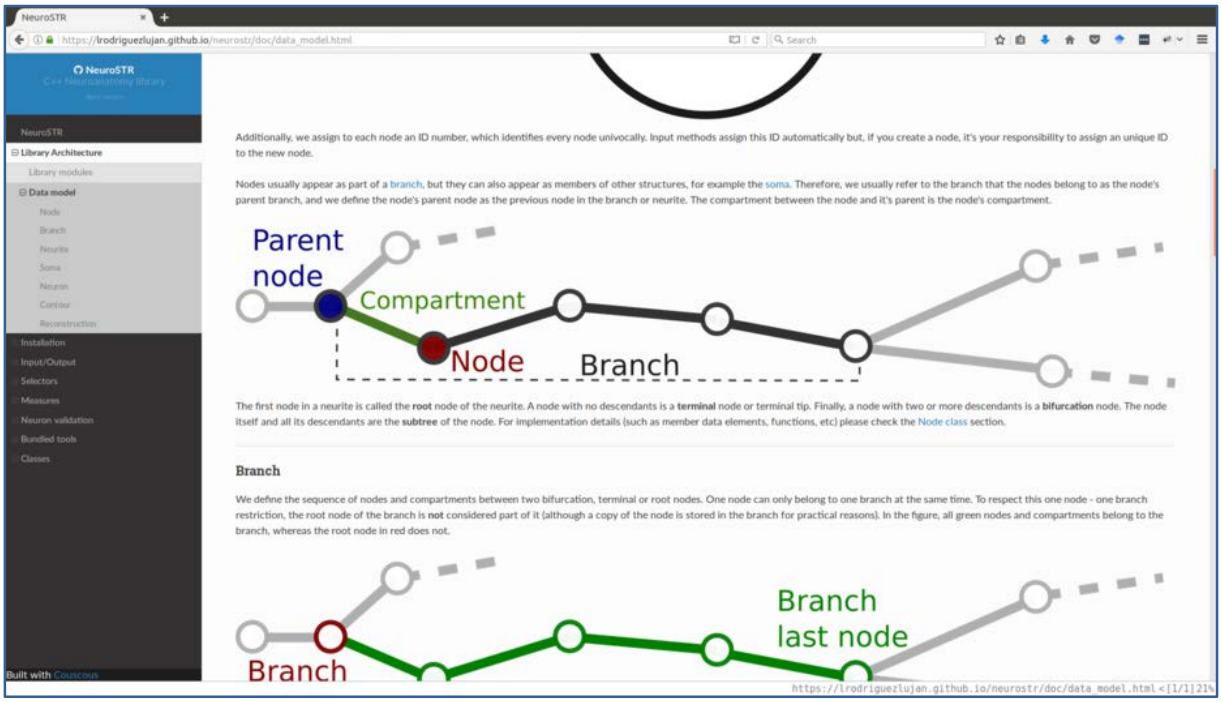

Figure 17: A screenshot of the library's online documentation.

Progress: We have released an alpha version of the library in M8, including a number of pre-packaged executables (e.g., morphology validator tool) and comprehensive online documentation . The library can compute all morphometrics provided by L-Measure in addition to many others. It is extensively documented. It is available the Github (https://github.com/ComputationalIntelligenceGroup/neurostr) and registered in the software catalogue (NeuroSTR) of the HBP Collaboratory. We are actively testing and maintaining the library. Release planned in M8 achieved. All the work was done by UPM.

Quality Control:

- Upstream Component: 3DPyrStructure; Task responsible: T5.4.2 (RUP); Status: received finished Component; Quality: good quality.
- Downstream Component: GABAergic interneuron classifier; Task responsible: T1.4.1; Status: intermediate release

5.3.4.2 GABAergic interneuron classifier

R package that implements a model that automatically classifies interneurons into agreed-upon interneuron types based on their morphology features.

Use Case: GABAergic interneurons characterisation https://project-lifecycle.herokuapp.com/use_case/65

Progress: We have had no planned releases in M7-M12. We have been working on the classification of a set of rat interneurons provided by the Blue Brain Project, using NeuroSTR++ to compute morphometrics and handling class imbalance with over- and under-sampling. The progress on this Component is as expected. No releases planned in M1-M12. All the work was done by UPM.

Quality Control:

- Upstream Component: NeuroSTR: C++ Neuroanatomy library, Task responsible: T1.4.1 (RUP); Status: received intermediate release; Quality: good quality.

5.3.4.3 Microscopy error model (model)

Model-based sampling plan to estimate the error due to the lower resolution of the confocal microscopy compared against the "ground truth" given by the EM.

Use Case: High-resolution synaptic maps in the mouse neocortex and hippocampus https://project-lifecycle.herokuapp.com/use_case/64

Progress:

Quality Control:

Downstream Component: Interactive synaptic map [important].

5.4 T1.4.2 - Visual analysis tools for microanatomical data

5.4.1 Key Personnel

Task Leader: Luis PASTOR (URJC)

Other researchers: Sofía BAYONA, Marcos GARCÍA (URJC)

5.4.2 SGA1 DoA Goals

The main goal of Task T1.4.2 is to enhance user productivity through the development of alternative workflows that integrate new data analysis tools, facilitating the acquisition and exploitation of morphological data from cells and microcircuits.

For this purpose, work in this task addresses two different issues:

1) The development of exploratory tools for morphological data that will facilitate the integration of analysis stages within the data acquisition process, yielding early feedback about both the whole data acquisition process and the data being gathered.
2) The establishment of new workflows that embed these analysis tools within the acquisition procedures and incorporate stages of data analysis and assessment. This will facilitate analysing the collected data early during the acquisition process, guiding subsequent acquisition actions.

5.4.3 Component Progress

5.4.3.1 Tools for the early analysis of morphological data

Description of Component: Tools for the 1) early analysis of morphological data, in order to provide feedback to steer the data extraction process and to correct possible errors or even redesign experiments if necessary, 2) analysis of complex systems, exploiting the ability of the human visual system to extract information from visual scenarios

Progress on Component: Progress on Component: Component delayed due to the hiring staff process. Release planned in M12 MS1.4.2 *Prototype of early exploratory analysis techniques for morphological data* has been partially achieved. This delay has been estimated at 3 months, and it should not affect significantly downstream work, such as in T6.2.2 (some preliminary, non-integrated tools have been available before M12). All work was done by URJC.

Quality Control:

- No upstream Components.
- Downstream Components: there are 13 downstream Components linked to this task; Task responsible: T1.2.1, T1.2.5, T1.2.9, T1.4.2, T6.2.2 & T7.3.9; Status: intermediate release to some tasks. Nothing received from others (except for the dataset used for tool development and testing)

5.4.3.2 New workflows for embedding exploratory analysis techniques into the early stages of the morphological data extraction and reconstruction processes

Description of Component: New workflows will be designed to embed exploratory analysis techniques into early stages of the morphological data extraction and reconstruction processes within Tasks T1.2.1 and T1.2.2. Thus the tools developed in T1.4.2 will be applied to the analysis of the pyramidal cell structure in T1.2.1 and to the production of high-resolution synaptic maps to identify principles of organisation in the reconstruction of synaptic connections in T1.2.9. Additionally, new stages to refine or even define new features, or to include new analysis procedures, will be added to these acquisition and early analysis workflows whenever advisable, in order to potentiate the analysis process. The selection of these new features will be performed taking into consideration the specific particularities of the acquisition process and the data to be produced.

Progress on Component: some activities of this Component are delayed due to the hiring staff process. No releases planned in M12. and it is expected that the delays caused by the hiring process will not affect the planned Component release. All work was done by URJC.

Use Case: 3D reconstruction and visualisation of microanatomical parameters of pyramidal cells: PyramidalExplorer 1.2

Quality Control:

- Tools for the early analysis of morphological data; Component partially delayed. Task responsible: T1.4.2; status: intermediate release;
- Downstream Components: there are 11 downstream Components; Task responsible: T1.2.1, T1.2.5, T1.2.9 & T6.2.2; Status: intermediate release

6. WP1.5 Management and Scientific Coordination

6.1 Key Personnel

Work Package Leader: Javier DEFELIPE (UPM)

SP1 Manager: Pilar F. ROMERO (UPM)

Task Leader: Douglas ARMSTRONG (UEDIN)

Task Leader: David STERRATT (UEDIN)

6.2 WP Leader's Overview

Work is progressing as scheduled in WP1.5 and is being efficiently organised and appropriately documented in a timely manner.

We have continued a good network of communication between our Lead Contractors and have evidence of collaborative work between the Tasks within SP1. As displayed in this report at Task Component level, SP1 activities interact with many activities planned in other SPs, and we are keen to foster further links between the SP1 and other SPs within the HBP.

Within the SP1, an internal flow of communication has been set up to ensure that work is being performed as scheduled, to share information and results when achieved, and for planning new strategies for the HBP next phases. These regular meetings are being mainly carried out by electronic means (email, Skype, call-conferences), take place weekly (or even more frequently) and are being held at any level within the SP (SP1 Leader & Co-leaders; SP1 Managers; SP1 Collaborators; between SP1 collaborators). Additionally, SP1 meets with other SPs based on any needs that arise. Some of these meetings have not been reported onto EMDESK at task and WP level, since inputting these data was not considered an efficient use of resources.

All the SP1 members had a significant input to responding to requests for reporting in M1-M12 and the SGA2 proposal preparation. MS1.5.1 MS1.5.2 have been achieved in a timely manner, as well as the M12 Deliverable D1.5.1.

In WP1.5, MS 1.5.3 'Data Management Plan (first draft)' is delayed. The drawing up of the draft of data management plan has already started and it is expected to be delivered in M16. In general, all delayed activities in SP1 have already started and are currently running smoothly. It is expected that they will be on schedule in the next few months. No changes in the DoA work plan have been reported. SP1 is on schedule to meet the proposed objectives in SGA1 in M24 as planned.

6.3 Priorities for the remainder of the phase

For the remainder of the phase WP1.5 will be focus on the continuation of the planned activities according to DoA in terms of scientific management, data coordination and ethics. SP1 is on schedule for meeting WP1.5 Milestones and Deliverables from M12 to M24.

6.4 Milestones

Table 5: Milestones for WP1.5: Management and Scientific Coordination

MS No.	Milestone Name	Leader	Task(s) involved	Expected Month	Achieved Month	Comments
MS1.5.1	First draft of molecular, structural and functional datasets for the Brain Atlas	Partner UPM	All SP1 tasks	M12	M12	Achieved
MS1.5.2	First draft of molecular, structural and functional datasets for brain modelling	Partner UPM	All SP1 tasks	M12	M12	Achieved
MS1.5.3	SP1 Data Management Plan Report (First Draft)	Partner UPM	All SP1 tasks	M12	M16 (expected)	MS1.5.3 has not been achieved. It is expected to be delivered in M16.

6.5 T1.5.1 Management and Scientific Coordination

6.5.1 Key Personnel

Task Leader: Javier DEFELIPE (UPM)

SP1 Manager: Pilar F. ROMERO (UPM)

6.5.2 SGA1 DoA Goals

This Task includes the following activities:

- Supporting SP1 Subproject Leaders in the coordination of SP1 Partners and SP1's relationships with other SPs.
- Fixing the agenda for meetings and leading scientific and technical discussions
- Organisation of SP1 events and other events related with the Project.
- Planning, assembling, and editing all reports to be produced by the Subproject at Task, WP and SP level.
- Reviewing the quality of SP1 Work Package contributions to SP1 Deliverables, applying the methodology described and the specific indicators defined, and proposing corrective action where this is required.
- Defining KPIs and drawing up SP1's semester and periodic reports.
- Integrating new Partners that join the Project via open calls and activities carried out via the relevant Partnering Projects.
- T1.5.1 coordinates the planning of SP1 scientific activities for the SGA2.
- Community management (Coordinate documentation and dissemination of SPs non-technical results; organise SP outreach and communication activities; liaise with the central communications team to produce dissemination material relevant for all HBP activities).

6.5.3 Component Progress

6.5.3.1 D1.5.1 SP1 Mouse Brain Organisation - Results for SGA1 Period 1

Brain Atlas data package (Period 1):

This data, which will be deposited in the HBP Brain Atlas, will include: Maps of the vasculature; Whole-brain maps of different cellular types based on gene expression; Microcircuitry analysis, proteins and receptor distributions and fibre architecture; Maps of cellular distributions, long-range axonal projections, and synaptic proteins; reconstructed morphologies of major neuron types; Whole-brain activation maps; Spatial organisation principles in brain activation; Functional maps of cortical activity.

Brain Modelling data package (Period 1):

This package will include strategic data on quantitative description of synaptic connections on neurons; numbers, distributions and relative densities of cells in selected regions and where possible across the whole brain; statistical parameters characterising particular cell types and spatial arrangements between neurons, glia and blood vessels; a high-resolution quantitative synaptic map of exemplar brain regions; EM blocks scans and volume analysis of exemplar brain regions with quantification of the neuropil organisation; microcircuit analysis; functional maps of brain activation; morphological and physiological comparative studies of neurons between rodent and human.

Progress on Component: M12 releases (MS1.5.1 & MS1.5.2) - First draft of molecular, structural and functional datasets for brain modelling, First draft of molecular, structural and functional datasets for the Brain Atlas and D 1.5.1 SP1 Mouse Brain Organisation - Results

for SGA1 Period 1 - have been achieved. Work has been planned, assembled and edited by UPM and all the SP1 partners have contributed.

Quality Control:

- Upstream Component: SP1 Roadmap for SGA2; Essential; DEFELIPE, Javier; Finished Component; Excellent.
- Downstream Component: M1-M12 Periodic Report; Essential; WILLIS, Guy; Status: the reporting procedure was updated. The new light report for M1-M12 is currently underway. SP1 contributions to this report were sent out to the PCO in M12.

6.5.3.2 D1.5.1 SP1 Mouse Brain Organisation - Results for SGA1 Period 2

Description: WPs involved: WP1.1, WP1.2, WP1.3, WP1.4 & WP1.5

Brain Atlas data package:

This data, which will be deposited in the HBP Brain Atlas, will include: Maps of the vasculature; Whole-brain maps of different cellular types based on gene expression; Microcircuitry analysis, proteins and receptor distributions and fibre architecture; Maps of cellular distributions, long-range axonal projections, and synaptic proteins; reconstructed morphologies of major neuron types; Whole-brain activation maps; Spatial organisation principles in brain activation; Functional maps of cortical activity.

Brain Modelling data package:

This package will include strategic data on quantitative description of synaptic connections on neurons; numbers, distributions and relative densities of cells in selected regions and where possible across the whole brain; statistical parameters characterising particular cell types and spatial arrangements between neurons, glia and blood vessels; a high-resolution quantitative synaptic map of exemplar brain regions; EM blocks scans and volume analysis of exemplar brain regions with quantification of the neuropil organisation; microcircuit analysis; functional maps of brain activation; morphological and physiological comparative studies of neurons between rodent and human.

Progress on Component: M12 release - First draft of molecular, structural and functional datasets for the Brain Atlas and modelling, MS1.5.1 & MS1.5.2, has been achieved. Work has been planned, assembled and edited by UPM and all the SP1 partners have contributed.

Quality Control:

- No upstream Components.
- Downstream Component: M13-M24 Periodic Report; Essential; WILLIS, Guy; Status: Not started yet.

6.5.3.3 D1.5.3 Detailed plan of data usage and the impact of generated data on models

Description: WPs involved: WP1.1, WP1.2, WP1.3, WP1.4 and WP1.5.

From M1 to M24, a detailed plan for data usage via cross-SP working meetings will be drawn up. We will provide strategic structural and functional data to model and simulate the four major brain circuits and to set up their use in modelling at sub-cellular, cellular and circuit levels. Due to the possible constraints to produce new data in terms of time and resources, the data generated will be sent to the platforms as it is produced, i.e., not only the initial (and also intermediate), but also incomplete datasets to test all the pipelines. The identification of the gaps between what data is available and what data is needed will be implemented following a scale of priorities for SP2, SP3, SP4, SP5 and SP6. The modelling work and the coordination with the relevant SPs will ensure that data generated is strategic and is used in HBP models. This strategic data plan will be delivered in M24 and will be implemented in the SGA2 and will continue throughout the project.

Progress on Component: M12 release (MS1.5.3) - SP1 Management Plan Report (First draft) is delayed. The drawing up of this draft has already been started but the draft has not been finalised in a timely manner. In spite of this delay, it is expected to achieve this MS in M16 and to deliver the strategic plan in M24 as scheduled.

Quality Control:

- Upstream Components: Data standards document; Essential; STERRATT, David; Nothing received: This Component is delayed as it is depending for an HBP-wide data standards document to be drafted, which will then be augmented to cover any special cases in SP1.
- No downstream Components.

6.5.3.4 SGA2 Roadmap for SGA2

Description: Coordination of planning of SP1 scientific activities for SGA2

Progress on Component: M13 release (MS1.5.4) - SP1 roadmap for SGA2 is due to be released in M13. In M12 this roadmap was almost finished and in good shape to achieve this MS1.5.4 in a timely manner. Work has been planned, assembled and edited by UPM and all the SP1 partners have contributed.

Quality Control:

- No upstream Components.
- Downstream Components:
  - SAG2 proposal; Essential; FAUTEUX, Christian; Status:
  - D1.5.1 SP1 Mouse Brain Organisation - Results for SGA1 Period 1; Essential; DEFELIPE, Javier; Status: Finished Component.

6.6 T1.5.2 Data Coordination

6.6.1 Key Personnel

SP Science Coordinator: David STERRATT (UEDIN)

Task Leader: Douglas ARMSTRONG (UEDIN)

6.6.2 SGA1 DoA Goals

This Task includes the following activities:

- Documentation and dissemination of SPs data standards
- Ensure implementation of data Components follows FPA described Project Lifecycle
  - Coordinate data validation implementations (producers AND consumers)
  - SP internal assessment and communication of TRLs for data produced/curated
- Implementation of the Data Accessibility Criteria according to HBP and SP strategy
- Align SP scientific activities with:
  - on-going scientific developments
  - emerging HBP external trends
  - Co-Design Projects
- Attend conferences/events on behalf of HBP, participation in HBP booths
- Supporting SP1 planning for SGA2 by helping with entering dependencies in the PLA, validating that both connected parties agree with the link.

6.6.3 Component Progress

6.6.3.1 SP1 Science coordination

Description: Science coordination for SP1, including encouraging and assisting partners to put information into the Project Lifecycle App, checking this information, liaising with science coordinators from other SPs via the weekly science coordinators video conference and by email.

Progress on Component: There are no planned releases in this period. Work has involved regular (mostly weekly) video conferences with other science coordinators to discuss aspects of SGA1 implementation and how science coordinators can help with SGA2 planning. We have worked closely with the SP1 manager and leadership to help partners enter information into the PLA and establish links with other HBP partners. We have validated that the owners of both upstream and downstream tasks and their respective science coordinators agree to dependencies being created.

Quality Control: No upstream nor downstream Components have been set up for this Component.

6.6.3.2 SP1 Data Standards Document

Description: A document describing the SP's data standards

Progress on Component: M10 - Data standards document drafted. This is delayed as we are depending for an HBP-wide data standards document to be drafted, which we will then augment to cover any special cases in SP1.

Quality Control:

- No upstream Components
- Downstream Component: D1.5.3: Detailed plan of data usage and the impact of generated data on models; Essential; DEFELIPE, Javier; this Component has not started yet. It is planned to be delivered in M24.

6.7 T1.5.3: Ethical Issues

6.7.1 Key Personnel

Task Leader: Douglas ARMSTRONG (UEDIN)

6.7.2 SGA1 DoA Goals

This Task will be led by an Ethics Rapporteur (ER). The ER will be responsible of all individual projects proposals within the SP1 that may be associated with ethical issues to the attention of the Ethics Advisory Council of the HBP. The ER will be the contact person between SP1, the Ethics Advisory Council and SP12. In particular, the ER will:

- report to Ethics Advisory Council (and potentially SP12) if he becomes aware of any emerging overarching ethical issues
- brief Ethics Advisory Council on a regular basis on technical progress in the SP1 (in short, concise update papers)
- bring any individual project proposals that may be associated with ethical issues to the attention of the Ethics Advisory Council

6.7.3 Component Progress

6.7.3.1 SP1 ethics reporting

Description: Ethics reporting from SP1, which we will link to the Periodic Reports.

Progress on Component: Release M12: 12 - Ethics contribution to report for period. Contributed to the Rapporteur One Pagers and took part in reviews with the EAB. Identified

examples of best practice in 3Rs and highlighted these to SP1 task leaders. Briefed SP1 task leaders on feedback from EU Ethic review ahead of SGA02. Currently providing input to Data Policy document.

Quality Control:

- Upstream Components:
  - Ethics Training Workshops and Videos: Essential; RAINEY, Stephen; Status: intermediate release – workshops have been scheduled for summer 2017 at KTH.
  - Data Policy Manual; Essential: STAHL, Bern; Status; intermediate release – draft released for review at 2017 Ethic workshop, March in Bristol.
  - HBP SGA2 Ethical Issues and Approvals Form: Essential; HARRIS, Emma; Status: Not available
- Downstream Component name (from PLA) + Task responsible + status (i.e. you have provided nothing / intermediate release / finished Component):
  - Ethics Advisory Board. Essential; PATEL, Shamim; Status: finished Component. Presented SP1 issues to EAB in March 2017.
  - HBP SGA2 Ethical Issues and Approvals Form. Essential; HARRIS, Emma; Status: Nothing provided.
  - Rapporteur One Pagers. Essential; GRIMES, Kevin; Status: 2016/2017 version completed in collaboration with EAB status finished Component.

7. CDP1 Development of Whole Mouse Brain Model and Mouse Brain Atlas

7.1 Key Personnel

CDP Science Leader: Francesco Saverio PAVONE (LENS)

CDP Implementation Leader: Marc-Oliver GEWALTIG (EPFL)

7.2 CDP Leader's Overview

- What went particularly well?

The coordination of efforts between SP4 and SP10 for simulation of brain activity and of the in silico experiment from our SP1 data is leading to encouraging preliminary results. Spatial anchoring of whole-brain data in SP5 is paving the way for building mouse brain atlas. SP4 set up a preliminary version of the simulation of meso-scale bilateral cortical activity in healthy and stroke conditions. In addition, SP10 team provided a first version of the simulation of the rehabilitation platform with neuronal activity (NEST)-driven motors and mouse body model. We provided SP6 with meso-scale resting state calcium imaging data for constraining the point-neuron simulation of the whole brain.

- Impact of work done

The simulation of a complete experiment, with integration of brain activity and mouse body into a specific behaviour within an in silico lab environment on the NRP will lead to a new approach to experimental science. In this context, closed-loop simulation will provide a new tool for refining experiments and vice-versa.

The building of a quantitative mouse whole-brain atlas and of a related whole-brain model will provide a new great tool for neuroscientists, allowing exploring and simulating the brain as a whole – although with limited granularity in this moment. Within HBP, the presence of a simplified whole brain model together with realistic local models built in CDP2 will allow meeting in between to reach one of the overall goals of the flagship, i.e. a realistic simulation of the whole brain.

7.3 Use Case Progress

The Use Cases CDP1-01 and CDP1-02 are not operational Use Cases but represent the overarching goals of the CDP1. Therefore, they do not have direct links to involved Tasks in the PLA.

7.3.1 CDP1-01 - Spatio-temporal coordinated activity during motor learning

Use Case Description (from PLA): We want to investigate to which extent the fundamental relationship between motor cortex activity and movement is shaped by learning. To this aim we will study the remodelling of activation maps both *in vivo* and *ex vivo*. In detail, we will perform longitudinal mapping of cortical activity using calcium indicators coupled with a multi-level imaging system based on both a wide-field macroscope and a cellular-resolution two-photon microscope. In parallel, we will analyse whole-brain cell-resolution activation maps by detecting early gene expression *ex vivo*. The mouse will learn a motor task driven by milk reward in a robotic platform. The Platform is integrated in a wide-field one-photon fluorescence macroscope. This configuration allows obtaining functional maps (via calcium indicators) in the awake mouse during execution of the motor task (i.e. pulling a handle) in the whole cortex. Cortical connectivity will be dissected by integration of optogenetic tools with genetically encoded indicator of activity.

Use Case Leader: SILVESTRI, Ludovico (LENS)

Contributing Tasks & Components:

- Collaboratory Storage Service (service)> T11.3.2 (SGA1) Collaboratory and Research Infrastructure Integration Web Services + T13.3.1 (RUP) IT services
- Collaboratory Storage UI (service)> T11.3.2 (SGA1) Collaboratory and Research Infrastructure Integration Web Services + T13.3.1 (RUP) IT services
- Collaboratory Web UI (service)> T11.3.2 (SGA1) Collaboratory and Research Infrastructure Integration Web Services + T13.3.1 (RUP) IT services

Progress summary:

- The activity is performed trough the development of Products 1 to 6.

7.3.2 CDP1-02 - Robotic platform for the study of rehabilitation-induced cortical remapping after stroke

Use Case Description (from PLA): Brain remapping after stroke is supposed to support the recovery of limb functionality. We will examine in a mouse model of stroke the features of neuronal plasticity relevant for functional motor recovery. We will assess impairments and motor recovery in a quantitative way by training mice on a robotic platform that mimics a human robotic device for upper limb stroke rehabilitation. The platform is integrated in a wide-field one-photon fluorescence mesoscope. This configuration allows obtaining functional maps (via calcium indicators) in the awake mouse during execution of the motor task (i.e. pulling a handle) in the whole cortex. Cortical connectivity will be dissected by integration of optogenetic tools with genetically encoded indicator of activity. Differences in early gene expression will be evaluated *ex vivo* to assess differences in whole-brain activation profiles induced by robotic rehabilitation.

Use Case Leader: ALLEGRA, Letizia (LENS)

Contributing Tasks & Components:

- Collaboratory Storage Service (service)> T11.3.2 (SGA1) Collaboratory and Research Infrastructure Integration Web Services + T13.3.1 (RUP) IT services
- Collaboratory Storage UI (service)> T11.3.2 (SGA1) Collaboratory and Research Infrastructure Integration Web Services + T13.3.1 (RUP) IT services
- Collaboratory Web UI (service)> T11.3.2 (SGA1) Collaboratory and Research Infrastructure Integration Web Services + T13.3.1 (RUP) IT services

Progress summary:

- The activity is performed trough the development of Products 1 to 6
- We are now integrating on the NIP the datasets we produced on mouse cortical activation while performing a motor task. Our data are now shared with SP6, SP4 and SP10 collaborators, which are using them to build their simulation as for the following products.

7.3.3 CDP1-P1: Reference set-up of the experiment

Use Case Description (from PLA): The reference set-up is a physical realisation of the experiment. It is used to acquire the functional data in custom mouse models (e.g. specific pathology or behaviour), to provide reference instances of the stimulation and recording equipment that needs to be modelled by CDP1 Product 4 and to provide validation data for the *in silico* models developed as part of CDP1 Products 2-4. Data include functional cortical maps (via calcium or voltage indicators), whole-brain activity maps via immediate early genes expression, optogenetics stimulation, single neuron electrophysiology. All data will be seamlessly integrated in the atlas via CDP1 Product 5.

Use Case Leader: SILVESTRI, Ludovico (LENS)

Contributing Tasks & Components:

- 3D reconstruction of the entire vascular system of the mouse brain (data), 3D image of the entire vascular system of the mouse brain> T1.3.5 (SGA1) Vasculature maps and computation of oxygen delivery
- Analysis of meso-scale fluorescence functional data (software)> T1.3.2 (SGA1) Cortical plasticity associated with motor recovery in mouse models of stroke triggered by a robotics task
- Optimisation of rehabilitation platform)> T1.3.2 (SGA1) Cortical plasticity associated with motor recovery in mouse models of stroke triggered by a robotics task
- CINECA Archive Repository Service (service)> T7.5.2 (SGA1) Data Services
- CSCS Archive Repository Service (service) > T7.5.2 (SGA1) Data Services
- Effective connectivity changes inferred from optogenetic brain interrogation and calcium imaging (report)> T4.4.1 (SGA1) Models of spontaneous brain activity
- HPC Platform Guidebook website repository (report)> T7.6.1 (RUP) HPC platform documentation and user training
- Maps of neuronal activation of whole mouse brain (data)> T1.3.4 (SGA1)
- Whole-brain cell- resolution activity maps>T1.3.3 (SGA1) Methodical development in optical imaging, data analysis, integration and atlasing
- Optimisation of Clarity for whole brain imaging (report)> T1.3.3 (SGA1) Methodical development in optical imaging, data analysis, integration and atlasing
- Single-photon system for optogenetic actuation (report)> T1.3.3 (SGA1) Methodical development in optical imaging, data analysis, integration and atlasing
- SP1 Data Standards Document (report)> T1.5.2 (SGA1) Data Coordination
- Whole-brain images of selected neuronal types (data)> T1.3.1 (SGA1) Micro- to mesoscopic multilevel and multimodal maps of the mouse brain
- Whole-brain maps of selected neuronal types (data)> T1.3.1 (SGA1) Micro- to mesoscopic multilevel and multimodal maps of the mouse brain
- Wide-field mesoscope (report)> T1.3.3 (SGA1) Methodical development in optical imaging, data analysis, integration and atlasing

Progress summary:

- Set-up of the integrated rehabilitation platform with the wide-field imaging setup; first simultaneous recordings of forces applied by the forelimb and cortical meso-scale calcium activity. Set-up of the stroke model and integration of the optogenetic stimulation system into the wide-field imaging apparatus.
- The dataset “2D Time lapse cortical recording maps of learning” already obtained. Acquisition of simultaneous registrations of applied forces and cortical activity are in progress on stroke observation (after 15 days from the stroke).
- The imaging data acquired so far have been pre-processed and aligned to the Allen Brain Atlas by SP5.
- The same data has been shared with SP4 and SP6 for validation of the whole-brain simulation.
- Thanks to continuous interaction with SP10, we could reproduce the technical specification of the rehabilitation platform on the simulation that is available on the NeuroRobotic Platform.
- 180 TB archival repository has been set up on CINECA

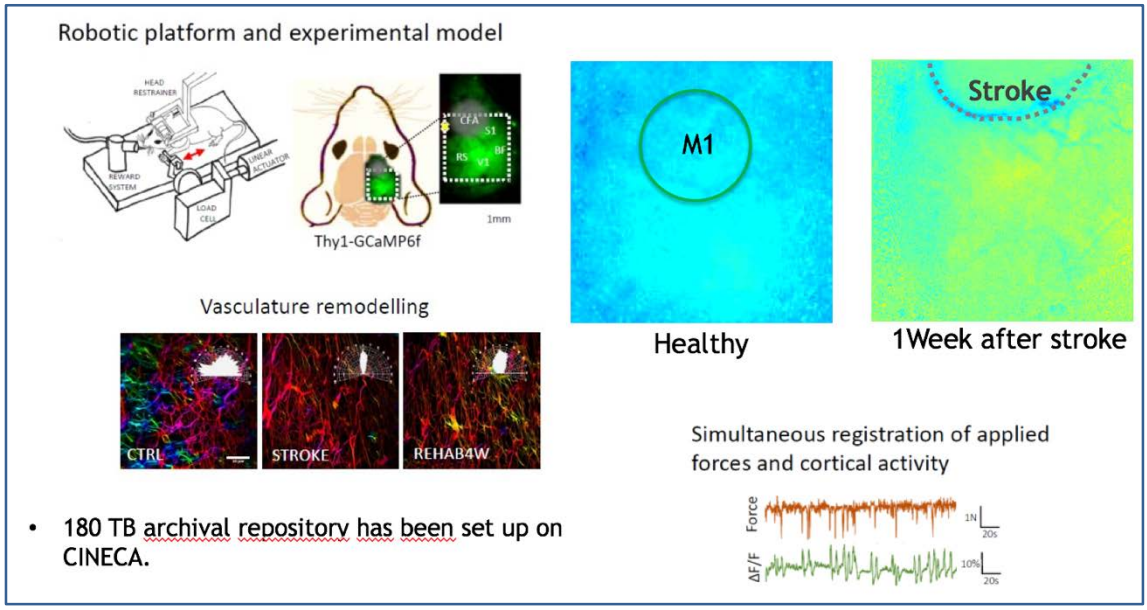

Figure 18: Example from the robotic platform and experimental model.

7.3.4 CDP1-P2: A virtual anatomy lab app

Use Case Description (from PLA): Users can obtain quantitative morphological and molecular data from user-selected brain regions, described either as standard atlas partitions (e.g. M1 cortex) or in geometrical terms (e.g. arbitrary cut slice). Data will include cell type positions and densities, gene expression, fibre densities and orientations, etc. Users can also obtain long-range connectivity data (ingoing and outgoing) relative to user-selected brain regions. Furthermore, the app will provide appropriate cutting geometry needed to preserve user-selected long-range projections intact after slicing, which would be useful in both real and virtual experiments. Data extraction from the atlas will rely on CDP1 Product 5. Custom data (e.g. related to pathology) can be integrated from CDP1 Product 1.

Use Case Leader: BJAALIE, Jan (UIO)

Contributing Tasks & Components:

- Allen Mouse Atlas (AMA) based brain network (model)> T4.5.2 (SGA1) Mouse brain function from structure
- Data store sources for interactive visualisation for selected data types (software)> T7.2.2 (SGA1) Coupling data analytics and visualisation to simulation
- HPC systems at Cineca (hardware)> - T7.5.1 (SGA1) HPC and Cloud Services
- Whole-brain maps of selected neuronal types (data)> T1.3.1 (SGA1) Micro- to mesoscopic multilevel and multimodal maps of the mouse brain
- Meta-Data used to Enriched SGA1 data and models> SGA1-T5.1.1 - Data and Parameter Workbench Support and Curation Team
- Meta-Data used to Enriched RUP data and models> SGA1-T5.1.1 - Data and Parameter Workbench Support and Curation Team
- Waxholm Space rat brain reference atlas enriched with additional receptor data> SGA1-T5.2.1 - Maintenance of Rodent Atlases
- Expanded and improved Waxholm Space rat brain reference atlas (with additional and corrected structure delineations) based on registered multimodal data> SGA1-T5.2.1 - Maintenance of Rodent Atlases

- Allen mouse brain reference atlas with white matter structures parcellated> SGA1-T5.2.1 - Maintenance of Rodent Atlases
- Customised versions of Allen mouse brain atlas tailored for different analyses> SGA1-T5.2.1 - Maintenance of Rodent Atlases
- Procedure for non-linear warping of 3D image data to reference atlas (report)> SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Procedure for non-linear warping of 2D image data to reference atlas (report)> SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Procedure for anchoring of 3D image data to reference atlas (report) > SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Optimised procedure for anchoring of 2D image data to reference atlas (report)> SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Curation of semantic spatial metadata delivered in T5.4.1. > SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Validation and approval of spatial metadata before final entry in KnowledgeGraph > SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Tutorials, training and supervision in assignment of spatial metadata > SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Metadata and links to data shared via the mouse connectome project > SGA1-T5.2.3 - Harvesting and Curation of Strategic Metadata from Existing Third Party Data Repositories
- Metadata and links to Allen Institute mouse brain data repositories > SGA1-T5.2.3 - Harvesting and Curation of Strategic Metadata from Existing Third Party Data Repositories
- Region-wise key characteristics of connectivity > SGA1-T5.2.4 - Strategic Mining of Data Anchored to Rodent Atlases
- Region-wise key characteristics of subcellular elements > SGA1-T5.2.4 - Strategic Mining of Data Anchored to Rodent Atlases
- Region-wise key characteristics of neuronal morphologies > SGA1-T5.2.4 - Strategic Mining of Data Anchored to Rodent Atlases
- SGA1/SGA2 - T5.2.5 Connectomic composition predictor (rodent) > SGA1-T5.2.5 - Prediction of Cellular, Synaptic and Connectomic Composition, Distributions and Properties of the Rodent Brain
- Parameter Workbench (API, WebApp, MetaData DB)> SGA1-T5.4.1 - Tools for Metadata Curation
- Data Workbench (API, WebApp, MetaData DB)> SGA1-T5.4.1 - Tools for Metadata Curation
- MeshView v2.0: updated functionality, viewing of annotations from LocaliZoom > SGA1-T5.4.2 - Integrating 2D Atlas Viewers and Manual Spatial Registration Tools
- Transformation inverter > SGA1-T5.4.2 - Integrating 2D Atlas Viewers and Manual Spatial Registration Tools
- Non-linear warping of whole brain 3D volume to reference atlas > SGA1-T5.4.2 - Integrating 2D Atlas Viewers and Manual Spatial Registration Tools
- Non-linear warping of 2D images anchored to a reference atlas > SGA1-T5.4.2 - Integrating 2D Atlas Viewers and Manual Spatial Registration Tools

- LocaliZoom: viewer for series of 2D images with reference atlas superimposed > SGA1-T5.4.2 - Integrating 2D Atlas Viewers and Manual Spatial Registration Tools
- QuickNII v 2.0: updated functionality and new procedures for propagation of anchoring information through large series of images > SGA1-T5.4.2 - Integrating 2D Atlas Viewers and Manual Spatial Registration Tools
- HBP Knowledge Graph Service > SGA1-T5.5.1 - Development and Maintenance of KnowledgeGraph
- HBP Knowledge Graph Indexer > SGA1-T5.5.2 - Search Application
- Collaboratory integrated Search UI > SGA1-T5.5.2 - Search Application
- KG Search Indexer > SGA1-T5.5.2 - Search Application
- Web Atlas viewer > SGA1-T5.5.4 - Data and Image Services
- Spatial Search API > SGA1-T5.5.7 - Spatial Search Application
- Connection of ilastik to other HBP services > SGA1-T5.6.2 - Workflow for Populating Brain Atlases with Features, Automatically Extracted by ilastik

Progress summary:

Representative experimental data provided by the teams of Pavone and Jirsa have been mapped in mouse brain reference atlas space, and spatial metadata have been prepared for registration in the KnowledgeGraph. The pilot data handled includes 1) three-dimensional lightsheet imaging data showing propidium-iodide labelled neurons (Pavone team), that were anchored to the Allen mouse brain atlas (v3) using the QuickNII tool, and 2) cortical activity maps (Jirsa team) that were mapped to the Allen mouse brain atlas (v3) by translation of stereotaxic coordinates to atlas coordinates, using the QuickNII and MeshView tools (see picture below). Analytic steps have been piloted by extracting spatial coordinates for specific data points (labelled neurons or activity signals) using the new LocaliZoom tool. Next steps will be to a) organise experimental metadata in the SP5 data workbench, b) optimise spatial anchoring procedures, and c) evaluate performance of computational image analysis with the Ilastic toolkit to enumerate labelled objects in atlas-defined regions of interest.

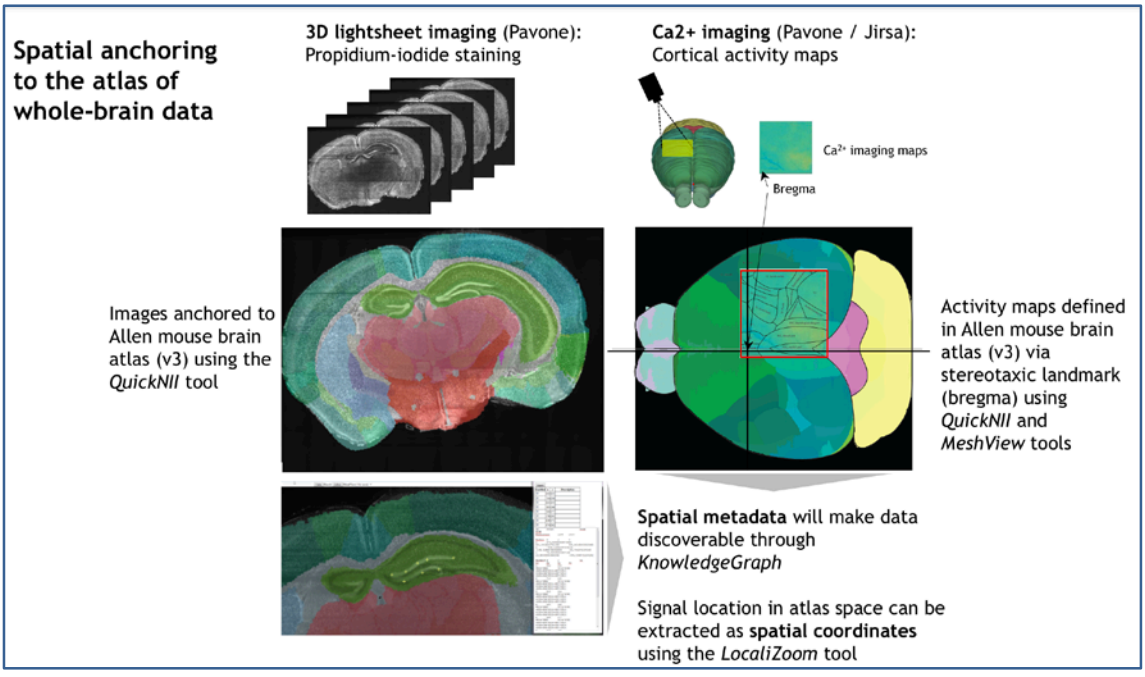

Figure 19: Spatial anchoring to the atlas of the whole-brain data.

7.3.5 CDP1-P3: A virtual imaging lab app

Use Case Description (from PLA): Users can obtain virtual imaging data from selected brain regions, described either as standard atlas partitions (the entire cortex, M1 area, etc.) or in geometrical terms (e.g. arbitrary cut slice). The user can specify the portion of the brain to simulate (e.g. whole brain or a single slice – missing at least part of long range projections), the model to be used (e.g. high-dimensional, spiking point neurons, population level), physiological/pathological conditions, the type of imaging (calcium, VSD, early gene expression, fMRI, PET, electrophysiology), the details of imaging system (resolution, acquisition speed, field of view, spatial orientation effects). Data extraction from the atlas will rely on CDP1 Product 5. Custom data (e.g. related to pathology) can be integrated via CDP1 Product 1. This app can run simultaneously with CDP1 Products 2, 4.

Use Case Leader: JIRSA, Viktor (AMU)

Contributing Tasks & Components:

- Collective behaviour of mean-field and neural population models: A comparative study (report)> T4.4.1 (SGA1) Models of spontaneous brain activity+ T4.4.5 (SGA1) Development of a large-scale, mean field model on sensorimotor integration
- HPC systems at Cineca (hardware)> T7.5.4 (RUP) The HBP supercomputer for massive data analytics+ T7.5.1 (SGA1) HPC and Cloud Services
- HPC systems at JSC (hardware)> T7.5.1 (RUP) The HBP Supercomputer for brain modelling and simulation+ T7.5.1 (SGA1) HPC and Cloud Services
- Mean-field models of interacting populations of rate and spiking neurons (model)> T4.1.3 (SGA1) Mean-field and population models
- Model of biologically-realistic network states (model)> T4.3.3 (RUP) Models of biologically realistic network states; wakefulness & sleep
- Model of calcium imaging signals (model)> T4.1.4 (SGA1) Models of brain signals
- NEST code with abstracted neuron model representations (software)> T7.1.3 (SGA1) Code generation for neuron and synapse models for software and neuromorphic backends
- NEST in situ framework (software)> T7.3.1 (SGA1) In situ visualisation for neuronal network simulations
- NEST Requirements Management (service)> T7.5.5 (SGA1) Simulator NEST as a Service
- NEST Support for Modellers (service)> T7.5.5 (SGA1) Simulator NEST as a Service
- NEST Support for Providers (service)> T7.5.5 (SGA1) Simulator NEST as a Service
- NEST - The Neural Simulation Tool (software)> T7.4.1 (SGA1) Co-design of applications to enable dynamic resource management + T7.1.4 (SGA1) Massively parallel methods for network construction from rules and data + T7.1.3 (SGA1) Code generation for neuron and synapse models for software and neuromorphic backends + T7.1.1 (SGA1) Integration methods for continuous-time population models
- NEST with enabled malleability (software)> T7.4.1 (SGA1) Co-design of applications to enable dynamic resource management
- Point-neuron model of the whole mouse brain (model)> T6.2.6 (SGA1) Models of whole mouse brain
- population activity equations: Finite-N mean-field model for interacting populations (with adaptation) (model)> T4.1.3 (SGA1) Mean-field and population models

- Population density techniques for the simulation of populations and neural circuits (model)
- SP6-T6.2.7-SGA1-Simplified brain models (model)> T6.2.7 (SGA1) Simplification
- SP6-T6.3.5-SGA1-Prototype NEST simulation kernel with directed spike exchange (software) > T6.3.5 (SGA1) Tools for network simulation
- SP6-T6.3.6-SGA1-Tools for configuring stimulation and recording in NEST simulations (software)> T6.3.6 (SGA1) Whole brain level *in silico* instrumentation, services and apps (point neuron models)
- VisNEST (software)> T7.3.4 (RUP) Integrative visualisation and analysis tools for the HBP cockpits

Progress summary:

We have implemented the open source tracer dataset of the Allen Institute (Oh et al., 2014) and its atlas into The Virtual Brain (TVB) (Sanz-Leon et al. 2015), thus allowing detailed Structural Connectivity (SC) to be obtained (Melozzi et al 2017 submitted). This is then used to build large-scale brain network models for the Functional Connectivity (FC) from different modalities, fMRI or calcium imaging.

The resolution of the long-range structural connectivity can be as small as 0.1 mm leading to maximum number of 540 nodes, with 88 belonging to the isocortex. In addition to this, we have also applied homogeneous local connectivity for the isocortex, thus increasing its spatial resolution to several thousand nodes.

Modelling and stimulation can be performed in the same way as for the human connectome. Hence, users can obtain virtual imaging data from selected brain regions, described either as standard atlas partitions (the entire cortex, M1 area, etc.) or in geometrical terms (e.g. arbitrary cut slice). The user can specify the portion of the brain to simulate (e.g. whole brain or a single slice – missing at least part of long range projections), the model to be used (e.g. high-dimensional, spiking point neurons, population level), physiological/pathological conditions, the type of imaging (calcium, VSD, early gene expression, fMRI, PET, electrophysiology), the details of imaging system (resolution, acquisition speed, field of view, spatial orientation effects).

For the network nodes we have investigated the dynamics using the reduced Wong Wang model in the bistable regime, as was shown (Hansen et al. 2015) to capture the FC dynamics in humans, but we have also applied different oscillatory models, i.e. Hopf and Kuramoto oscillators. This has allowed the Allen's data to be verified against resting state functional connectivity.

Different lesioning or resection (stroke) strategies and their influence to propagation of excitability are systematically analysed. We have demonstrated proof of concept for the resection of the structural connectivity, and the impact that it has on the observed functional connectivity.

In addition, resting state have been stimulated and propagation patterns (excitability) due to connectivity have been analysed for in line with the experimental data.

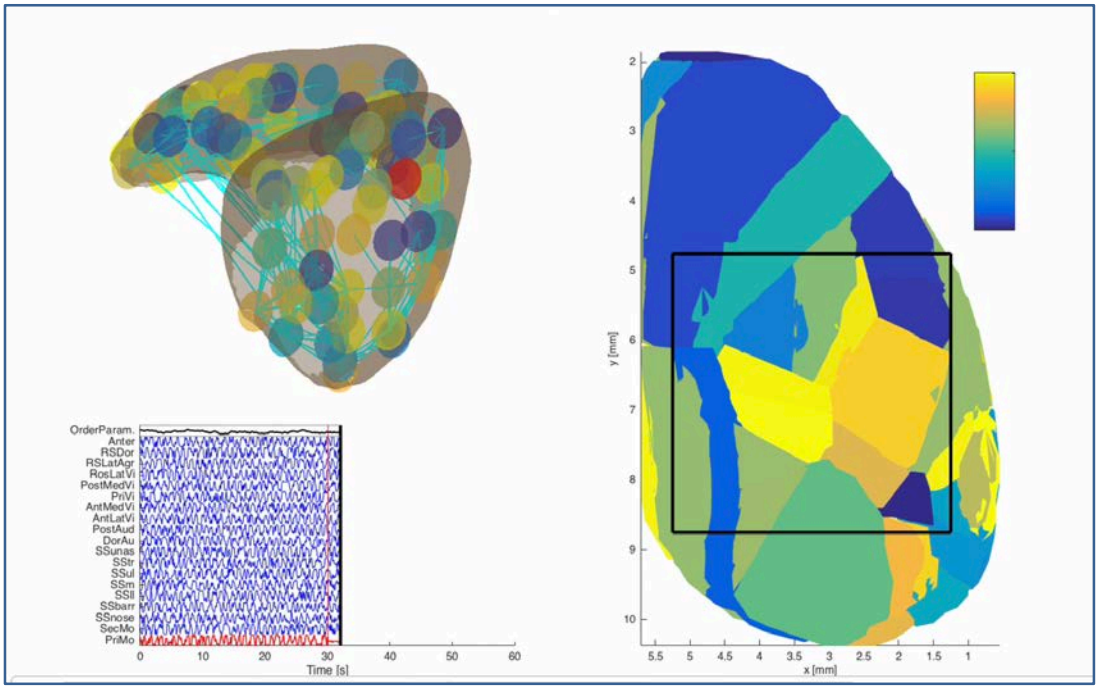

Figure 20: Image from virtual imaging lab app.

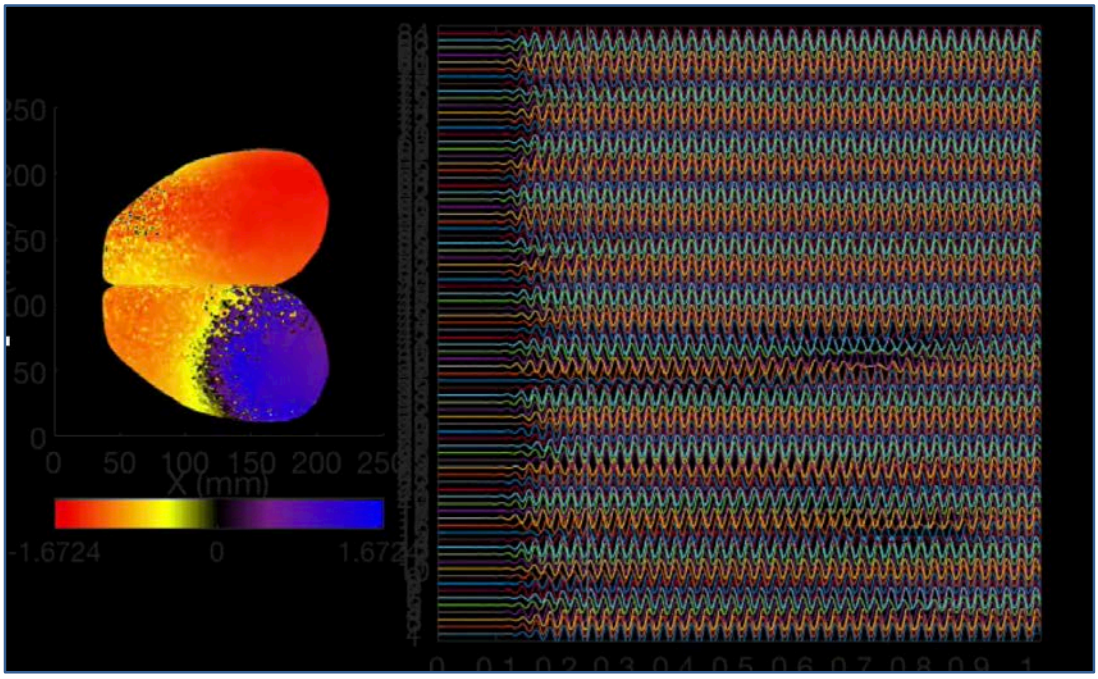

Figure 21: Image from virtual imaging lab app

7.3.6 CDP1-P4: A virtual behaviour lab app

Use Case Description (from PLA): Users can simulate selected behavioural experiments. They can specify the experiment Components such as robotic platform for motor learning and rehabilitation, reward systems, the brain model to use, physiological/pathological conditions. Data extraction from the atlas will rely on CDP1 Product 5. Custom data (e.g. related to pathology) can be integrated from CDP1 Product 1. This app can run simultaneously with CDP1 Product 3.

Use Case Leader: GEWALTIG, Marc-Oliver (EPFL)

Contributing Tasks & Components:

- Allen Mouse Atlas (AMA) based brain network (model)> T4.5.2 (SGA1) Mouse brain function from structure
- Mean-field models of interacting populations of rate and spiking neurons (model)> T4.1.3 (SGA1) Mean-field and population models
- Musculoskeletal models of rodents for the Neurorobotics Platform (model)> T10.3.2 (SGA1) Muscolo-skeletal models of rodents
- NEST in situ framework (software)> T7.3.1 (SGA1) In situ visualisation for neuronal network simulations
- NEST Support for Modellers (service)> T7.5.5 (SGA1) Simulator NEST as a Service
- NEST Support for Providers (service)> T7.5.5 (SGA1) Simulator NEST as a Service
- NEST - The Neural Simulation Tool (software)> T7.4.1 (SGA1) Co-design of applications to enable dynamic resource management + T7.1.4 (SGA1) Massively parallel methods for network construction from rules and data + T7.1.3 (SGA1) Code generation for neuron and synapse models for software and neuromorphic backends + T7.1.1 (SGA1) Integration methods for continuous-time population models
- population activity equations: Finite-N mean-field model for interacting populations (with adaptation) (model)> T4.1.3 (SGA1) Mean-field and population models
- Population density techniques for the simulation of populations and neural circuits (model)> T4.1.3 (SGA1) Mean-field and population models
- Rodent Body Model for the Neurorobotics Platform (model)> T10.3.1 (SGA1) Rodent body model
- Rule- and data-based connectivity generation in NEST (software)> T7.1.4 (SGA1) Massively parallel methods for network construction from rules and data
- Sensory models (model)> T10.1.2 (SGA1) Sensory-motor integration
- Simulation of a motor rehabilitation scenario (model) > T10.1.6 (SGA1) Simulation of motor rehabilitation experiment in rodents
- VisNEST (software)> T7.3.4 (RUP) Integrative visualisation and analysis tools for the HBP cockpits

Progress summary:

In this first period the integration of the M-platform in the NRP for stroke rehabilitation experiment has been performed (see picture of CDP1-P6 below). The CAD model of the main Components of the M-Platform (i.e. linear actuator, linear slide, handle), previously designed (see CDP1-P1) was converted in a suitable format for the Gazebo simulator. Properties of the model such as link weights, joint limits and frictions have been adjusted according to the real characteristics of the slide. The actuator was connected to a PID controller whose parameters have been tuned to reproduce the behaviour of the real motor.

A biologically model of proprioceptive sensory information, a necessary feature for the design of brain-inspired neurorobotic controllers that include complete action-perception loops, has been integrated and tested in the Neurorobotics Platform (see picture below). The translation is achieved implementing a computational model of neural activity of type Ia and type II sensory fibres connected to muscle spindles. The model also includes activity of both static and dynamic gamma-motoneurons, that provide fusimotor activation capable of regulating the sensitivity of the proprioceptive feedback, through the contraction of specific intrafusal fibres. The proprioceptive model is implemented on NEST, in order to provide an easy integration inside the NRP. The proposed Component can be coupled to both biomechanical models, like musculo-skeletal systems, and common robotic platforms (via

suitable conversions from encoder values to simulated muscle length). Figure 1 shows the implementation on the simulated mouse in the NRP.

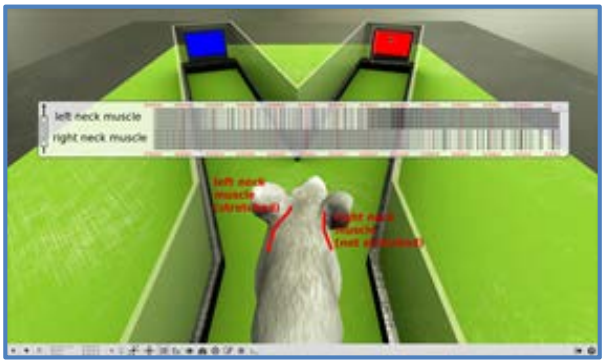

Figure 22: The model of proprioceptive sensory feedback implemented in the mouse experiment simulated in the Neurorobotics Platform.

7.3.7 CDP1-P5: A data explorer and importer app

Use Case Description (from PLA): The user can import, explore and export molecular (genomics, transcriptomics, proteomics, etc.), anatomical (morphologies, cell spatial distribution, projections, etc.), and functional data (CDP1 Product 1 and external data sources for fMRI) from and to the atlas. The app will assure standardisation of metadata and alignment with reference space where applicable. The atlas will be fed initially with 3rd parties data (e.g. Allen Institute) and strategic missing data from SP and it will feed selected data to the modelling pipeline of SP6.

Use Case Leader: MULLER, Jeff (EPFL)

Contributing Tasks & Components:

- Active data repositories (service)> T7.5.7 (SGA1) User Support Services + T7.5.2 (SGA1) Data Services
- Archive data repositories (service)> T7.5.2 (SGA1) Data Services
- CINECA Active Repository Service (service)> T7.5.2 (SGA1) Data Services
- CINECA Archive Repository Service (service)> T7.5.2 (SGA1) Data Services
- Definition of APIs for Job schedulers (report)> T7.4.2 (SGA1) Holistic dynamic resource management
- HPC systems at Cineca (hardware)> T7.5.1 (SGA1) HPC and Cloud Services
- SGA1 - Atlas viewer web frontend for 3D interaction (service)>
- SOFTWARE > HDB > Federated Query (software)> T8.1.7 (SGA1) Management of query templates and workflows + T8.1.6 (SGA1) User Defined Functions (UDFs) and query templates + T8.1.5 (SGA1) Distributed complex workflow engine
- Meta-Data used to Enriched SGA1 data and models> SGA1-T5.1.1 - Data and Parameter Workbench Support and Curation Team
- Meta-Data used to Enriched RUP data and models> SGA1-T5.1.1 - Data and Parameter Workbench Support and Curation Team
- Waxholm Space rat brain reference atlas enriched with additional receptor data> SGA1-T5.2.1 - Maintenance of Rodent Atlases

- Expanded and improved Waxholm Space rat brain reference atlas (with additional and corrected structure delineations) based on registered multimodal data> SGA1-T5.2.1 - Maintenance of Rodent Atlases
- Allen mouse brain reference atlas with white matter structures parcellated> SGA1-T5.2.1 - Maintenance of Rodent Atlases
- Customised versions of Allen mouse brain atlas tailored for different analyses> SGA1-T5.2.1 - Maintenance of Rodent Atlases
- Procedure for non-linear warping of 3D image data to reference atlas (report)> SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Procedure for non-linear warping of 2D image data to reference atlas (report)> SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Procedure for anchoring of 3D image data to reference atlas (report) > SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Optimised procedure for anchoring of 2D image data to reference atlas (report)> SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Curation of semantic spatial metadata delivered in T5.4.1. > SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Validation and approval of spatial metadata before final entry in KnowledgeGraph > SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Tutorials, training and supervision in assignment of spatial metadata > SGA1-T5.2.2 - Curation of Rodent Atlas Data
- Metadata and links to data shared via the mouse connectome project > SGA1-T5.2.3 - Harvesting and Curation of Strategic Metadata from Existing Third Party Data Repositories
- Metadata and links to Allen Institute mouse brain data repositories > SGA1-T5.2.3 - Harvesting and Curation of Strategic Metadata from Existing Third Party Data Repositories
- Parameter Workbench (API, WebApp, MetaData DB)> SGA1-T5.4.1 - Tools for Metadata Curation
- Data Workbench (API, WebApp, MetaData DB)> SGA1-T5.4.1 - Tools for Metadata Curation
- MeshView v2.0: updated functionality, viewing of annotations from LocaliZoom > SGA1-T5.4.2 - Integrating 2D Atlas Viewers and Manual Spatial Registration Tools
- Transformation inverter > SGA1-T5.4.2 - Integrating 2D Atlas Viewers and Manual Spatial Registration Tools
- Non-linear warping of whole brain 3D volume to reference atlas > SGA1-T5.4.2 - Integrating 2D Atlas Viewers and Manual Spatial Registration Tools
- Non-linear warping of 2D images anchored to a reference atlas > SGA1-T5.4.2 - Integrating 2D Atlas Viewers and Manual Spatial Registration Tools
- LocaliZoom: viewer for series of 2D images with reference atlas superimposed > SGA1-T5.4.2 - Integrating 2D Atlas Viewers and Manual Spatial Registration Tools
- QuickNII v 2.0: updated functionality and new procedures for propagation of anchoring information through large series of images > SGA1-T5.4.2 - Integrating 2D Atlas Viewers and Manual Spatial Registration Tools

- HBP Knowledge Graph Service > SGA1-T5.5.1 - Development and Maintenance of KnowledgeGraph
- HBP Knowledge Graph Indexer > SGA1-T5.5.2 - Search Application
- Collaboratory integrated Search UI > SGA1-T5.5.2 - Search Application
- KG Search Indexer > SGA1-T5.5.2 - Search Application
- Web Atlas viewer > SGA1-T5.5.4 - Data and Image Services
- Spatial Search API > SGA1-T5.5.7 - Spatial Search Application
- Connection of ilastik to other HBP services > SGA1-T5.6.2 - Workflow for Populating Brain Atlases with Features, Automatically Extracted by ilastik

Progress summary: the integration in the KnowledgeGraph will be performed when a significant amount of different datasets will be available.

7.3.8 CDP1-P6: In silico *setup of the motor rehabilitation experiment*

Use Case Description (from PLA): This product is the *in silico* analog to CDP1-P1 and is implemented in SP10 WP 10.1 and WP 10.3.

Use Case Leader: GEWALTIG, Marc-Oliver (EPFL)

Contributing Tasks & Components:

- NRP Brain-Body Integrator (BIBI) (software)> T10.5.2 (SGA1) World Simulation and Closed-loop engine
- NRP – Documentation (report)> T10.6.3 (SGA1) Documentation, user support and user training> T10.5.1 (SGA1) Simulation of physics (mechanics, kinematics, sensor models etc.)
- NRP - Physics simulation (software)> T10.5.1 (SGA1) Simulation of physics (mechanics, kinematics, sensor models etc.)
- NRP software packages (software)> T10.5.9 (SGA1) Software integration, packaging and release
- NRP Web cockpit (ExDFrontend) (software)> T10.5.2 (SGA1) World Simulation and Closed-loop engine
- NRP - Environment Designer (software)> T10.5.4 (SGA1) Environment and experiment designer
- NRP - Virtual Coach (software)> T10.5.7 (SGA1) Virtual coach

Progress summary: An experiment has been designed in order integrate and test the M-Platform in the Neurorobotics Platform. The experiment includes a 100-neuron brain model, divided in two populations of 90 and 10 neurons respectively. In this closed loop experiment, the first neuron population spikes randomly, and the spike rate of the population is converted to a force value picked out of a predefined range, compatible with the range of forces possibly performable by the mouse through its forelimb.

The computed force values are continuously applied to the handle and can move the slide until the starting position. Once there, the second neural population, wired to suppress the first population spike rate when active, is triggered, so there is no more force acting on the slide. The motor pushes the slide until the maximum extension position and it then comes back to its starting position, letting the loop start again (Figure 23 below).

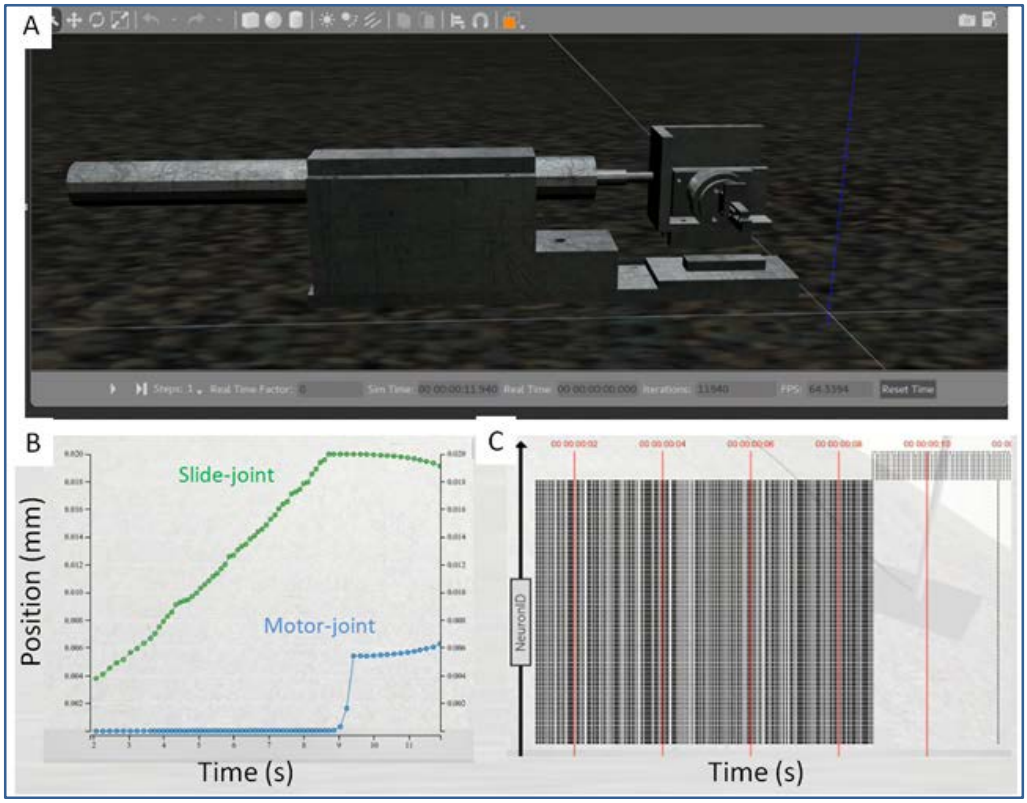

Figure 23: (A) M-Platform on the Gazebo Simulator. (B) The position of slide-join is changing because of the application of a force converted from the spiking activity of neurons population (in C).

8. Publications

- Aguado C, García-Madrona S, Gil-Minguez M, Luján R. (2016) Ontogenic Changes and Differential Localization of T-type Ca(2+) Channel Subunits Cav3.1 and Cav3.2 in Mouse Hippocampus and Cerebellum. *Frontiers in Neuroanatomy;* 10:83. doi: 10.3389/fnana.2016.00083. T1.1.1 (Components 1 & 2) & T1.1.3 (Component 1)
- Aguado C, Orlandi C, Fajardo-Serrano A, Gil-Minguez M, Martemyanov KA, Luján R. (2016). Cellular and Subcellular Localization of the RGS7/Gβ5/R7BP Complex in the Cerebellar Cortex. *Frontiers in Neuroanatomy;* 10:114. T1.1.1 (Components 1 & 2) & T1.1.3 (Component 1)
- María E. Rubio, Ko Matsui, Yugo Fukazawa, Naomi Kamasawa, Harumi Harada, Makoto Itakura, Elek Molnár, Manabu Abe, Kenji Sakimura and Ryuichi Shigemoto. (2017). The number and distribution of AMPA receptor channels containing fast kinetic GluA3 and GluA4 subunits at auditory nerve synapses depend on the target cells. *Brain Structure and Function*, in press. T1.1.1 (Components 1 & 2) & T1.1.3 (Component 1)
- Takafumi Miki, Walter A. Kaufmann, Gerardo Malagon, Laura Gomez, Katsuhiko Tabuchi, Masahiko Watanabe, Ryuichi Shigemoto and Alain Marty. (2017). Correspondence between presynaptic Ca2+ channel clusters and functionally defined vesicular docking sites in single central synapses. *PNAS* (submitted). T1.1.1 (Components 1 & 2) & T1.1.3 (Component 1)
- Alejandro Antón-Fernández, Guillermo Aparicio-Torres, Silvia Tapia, Javier DeFelipe, Alberto Muñoz. (2017). Morphometric alterations of Golgi apparatus in Alzheimer´s disease are related to tau hyperphosphorylation. *Neurobiology of Disease;*(97):11-23. T1.2.9 (Components 1-6)
- Carolina Gonzalez-Riano, Silvia Tapia-Gonzalez, Antonia García, Alberto Muñoz, Javier DeFelipe, Coral Barbas (2017). Metabolomics and neuroanatomical evaluation of post

mortem changes in the hippocampus. *Brain Structure and Function*. DOI: 10.1007/s00429-017-1375-5. T1.2.9 (Components 1-6)

- Cazemier JL, Clascá F, Tiesinga PH. (2016) Connectomic Analysis of Brain Networks: Novel Techniques and Future Directions. Frontiers in Neuroanatomy;10:110. eCollection 2016 Nov 9 Open Access https://doi.org/10.3389/fnana.2016.00110. T1.2.8 (Components 1 & 2)
- Rodriguez-Lopez C., Clasca F, Prensa L. (2017) The Mesoaccumbens Pathway: A Retrograde Labeling and Single-Cell Axon Tracing Analysis in the Mouse. (2017) *Frontiers in Neuroanatomy*;11:25 eCollection Mar 27 https://doi.org/10.3389/fnana.2017.00025. T1.2.8 (Components 1 & 2).
- Anton-Sanchez L, Bielza C, Benavides-Piccione R, DeFelipe J, Larrañaga P. (2016). Dendritic and Axonal Wiring Optimization of Cortical GABAergic Interneurons. *Neuroinformatics*;14 (4):453-64. doi: 10.1007/s12021-016-9309-6.
- Bosch C, Masachs N, Exposito-Alonso D, Martínez A, Teixeira CM, Fernaud I, Pujadas L, Ulloa F, Comella JX, DeFelipe J, Merchán-Pérez A, Soriano E. (2016). Reelin Regulates the Maturation of Dendritic Spines, Synaptogenesis and Glial Ensheathment of Newborn Granule Cells. *Cerebral Cortex*; [Epub ahead of print] T1.2.9 (Components 1-6).
- Fernandez-Gonzalez P, Benavides-Piccione R, Leguey I, Bielza C, Larrañaga P, DeFelipe J. (2016). Dendritic-branching angles of pyramidal neurons of the human cerebral cortex. *Brain Structure and Function*; [Epub ahead of print] T1.2.1 (Components 1-5).
- Eyal G, Verhoog MB, Testa-Silva G, Deitcher Y, Lodder JC, Benavides-Piccione R, Morales J, DeFelipe J, de Kock CP, Mansvelder HD, Segev I. (2016). Unique membrane properties and enhanced signal processing in human neocortical neurons. *Elife*;5. pii: e16553. doi: 10.7554/eLife.16553. [Epub ahead of print] T1.2.2 (Component 1), T1.2.1 (Components 1-5).
- Urrecha M, Romero I1, DeFelipe J, Merchán-Pérez A. Influence of cerebral blood vessel movements on the position of perivascular synapses. *PLoS One*;12(2):e0172368. doi: 10.1371/journal.pone.0172368. T1.2.9 (Components 1-6).
- Kohus Z, Káli S, Rovira-Esteban L, Schlingloff D, Papp O, Freund TF, Hájos N, Gulyás AI. (2016). Properties and dynamics of inhibitory synaptic communication within the CA3 microcircuits of pyramidal cells and interneurons expressing parvalbumin or cholecystokinin. *The Journal of Physiology*;594:3745-3774. T1.2.6

9. Dissemination

- Collaborative Development of Data-Driven Models of Neural Systems meeting, Janelia Farm, USA, 18 September 2016, Michele MIGLIORE, T1.1.4, Components 1 and 2.
- Brain Simulation Platform booth and talk at the HBP Open Day, Florence, Italy, 12 October 2016, Michele MIGLIORE, T1.1.4, Components 1 and 2
- Satellite Symposium, Society for Neuroscience Meeting 2016: Using the Neuroscience Gateway Portal for Parallel Simulations, San Diego, USA, Carmen LUPASCU, Michele MIGLIORE, T1.1.4, Components 1 and 2
- Annual Biophysical Society Meeting, New Orleans, 10-15 February 2017. T1.1.5, Simon BERNECHE.
- Data science in Neuroscience and Industry. An Academic Vision. In “Women in Data Science”, 2017 Madrid. Invited talk. Tasks T1.4.1 and T5.3.4. Components ‘Synapse spatial location on the dendritic shaft model’, ‘GABAergic interneuron classifier’, ‘Spine morphology clustering’, and ‘Single cell arborization model’. T1.4.1 BIELZA, C. Components 1-5.
- Conference: Nuevas tecnologías para el estudio microscópico del cerebro. Hospital Universitario La Princesa. Madrid, April 2016. SP1, DEFELIPE.
- Conference: Nuevas tecnologías del estudio del Cerebro. Universidad Europea de Madrid. Madrid, April 2016. T1.2.1 (Components 1-5) & T1.2.9 Components 1-6), DEFELIPE.

- Conference: Nuevas tecnologías para el estudio de la enfermedad de Alzheimer. Facultad de Medicina de la Universidad Autónoma de Madrid. Madrid. April 2016. T1.2.1 (Components 1-5) & T1.2.9 Components 1-6), DEFELIPE.
- Radio interview: Coloquio abierto sobre cerebro, nuevas tecnologías etc. RNE. Madrid, April 2016. T1.2.1 (Components 1-5) & T1.2.9 Components 1-6), DEFELIPE.
- Nuevas tecnologías para el estudio del cerebro: "Human Brain Project". Hospital de la Santa Creu i Sant Pau. Barcelona, October 2016. SP1, DEFELIPE.
- Conference: Nuevas estrategias pero el estudio de enfermedades del cerebro. EM Forum. Madrid. June 2016. T1.2.1 (Components 1-5) & T1.2.9 Components 1-6), DEFELIPE.
- Radio interview: RNE. Madrid. September 2016. SP1, DEFELIPE.
- TV Interview: Programa ConCiencia, Telemadrid. Madrid. October 2016. SP1, DEFELIPE.
- Conference: Ibercaja Obra Social. Ciclo: Ciencia por descubrir. ¿Podremos construir un modelo virtual del cerebro? La apuesta del Human Brain Project. October 2016, Zaragoza. SP1, DEFELIPE,
- Conference: Avanzando en el conocimiento del cerebro. Proyectos Cajal Blue Brain y Human Brain. XIV Reunión Anual de la Asociación Madrileña de Neurología. 21 October 21, 2016, Madrid, SP1, DEFELIPE.
- Conference: Nuevas tecnologías para el análisis del cerebro: aplicaciones en la enfermedad de Alzheimer. XII Ciclos seminarios de Biomedicina. Madrid. November 2016. T1.2.1 (Components 1-5) & T1.2.9 Components 1-6), DEFELIPE.
- Radio Interview: Cadena SER. Madrid. Diciembre 2016. SP1, DEFELIPE.
- Conference: Nuevas tecnologías para el estudio microanatómico del cerebro de pacientes con EM. V SYMPOSIUM SOBRE NEUROINMUNOLOGÍA: Esclrerosis múltiple. Madrid. December 2016. SP1. DEFELIPE.
- Presentation Flag-Era HBP Madrid. Universidad Autónoma de Madrid. Madrid. January 2017. SP1. DEFELIPE.
- Conference: Los pilares del HBP desde la óptica científica. 1º Foro HBP y su impacto en la Insdustria Española. Madrid. February 2017. SP1, DEFELIPE.
- Conference: Cognición y Circuitos Corticales. Reunión de Expertos: tratamiento clínico de la depresión. Madrid. March 2017. T1.2.1 (Components 1-5) & T1.2.9 Components 1-6), DEFELIPE.
- Conference: The Blue Brain/ Human Brain Project. WADD. Madrid. March 2017. SP1, DEFELIPE.
- Conference: La Conectividad cerebral: el Cajal visionario. XIV Reunión de la Sociedad Andaluza de Epilepsia. Málaga. March 2017. SP1, DEFELIPE.

10. Education

SP1 has been deeply involved in the educational activities carried out by the HBP Education Team. In particular, the young researchers have participated with oral presentation and posters in the events organised by this team such as the FENS Satellite event 2016 (July 2016, Copenhagen, Denmark), the 3rd HBP School: Future Neuroscience. The Multiscale Brain: From Genes to Behaviour (December 2016, Obergurgl, Austria), and the first HBP Student Conference: Transdisciplinary research linking neuroscience, brain medicine and computer science (February 2017, Vienna, Austria).

In addition, SP1 students have presented their relevant results in international meetings such as the 9th Forum of Neuroscience (Federation of European Neurosciences, FENS. Copenhagen, Denmark, July 2017); 12th Göttingen Meeting of the German Neuroscience Society (NWG, Göttingen, Germany, March 2017). Moreover, some students are currently planning their attendance in other high-level conferences such as the Twenty-sixth Annual Computational Neuroscience Meeting (CNS2017, Antwerp, Belgium, 15-20 July 2017).

Finally, a PhD Thesis has been finalised in WP1.2 (T1.2.8): Porrero C. (6 September 2016) Arquitecturas axónicas y organización de las neuronas de proyección multiespecífica del

tálamo. Estudio en el núcleo posterior del ratón. Graduate Program in Neuroscience, Autonoma University of Madrid. Advisor: F. Clasca.

11. Ethics

The participation of SP1 in Ethics and Society activities has been implemented via Task T1.5.3. In particular, following the Component *SP1 ethics reporting*: Ethics reporting from SP1, which we will link to the periodic reports. Release M12: Ethics contribution to report for period. Activities carried out are as follows:

- Contributed to the Rapporteur One Pagers and took part in reviews with the EAB
- Identified examples of best practice in 3Rs and highlighted these to SP1 task leaders
- Briefed SP1 task leaders on feedback from EU Ethic review ahead of SGA02
- Currently providing input to Data Policy document.

12. Innovation

No innovation-related actions in M01-M12 have been reported in SP1.

13. Open Research Data

Zsolt Kohus, Attila I Gulyás. (2016) Temporal properties of mouse hippocampal CA3 area PV and CCK inhibitory neuron transmission measured by physiologically relevant action potential sequences. CRCNS.org.

DOI: 10.6080/K0MK69T5

Accessible, reusable.

Linked to the publication Kohus et al. (2016), DOI: 10.1113/JP272231.