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Abstract:	This report describes the implementation and validation of methods developed in SP1 for the mapping of mouse brain. The topics covered include, main achievements, work progress in methods, Milestones, Deliverables, cooperation and limitations during M1-M12, together with a general plan for the next six months. In addition, the values achieved in M6-M12 for the Key Performance Indicators (SKPIs) defined in D1.4.1 are included.
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1. Introduction

1.1 The Human Brain Project (HBP)

The Human Brain Project (HBP) is a major international scientific research project, involving over 100 academic and corporate entities in more than 20 countries. Funded by the European Commission (EC), the ten-year, EUR 1 billion Project was launched in 2013 with the goal "to build a completely new ICT infrastructure for neuroscience, and for brain-related research in medicine and computing, catalysing a global collaborative effort to understand the human brain and its diseases and ultimately to emulate its computational capabilities."

The fields of neuroscience, medicine and information technology each have important roles to play in addressing this challenge, but the knowledge and data that each is generating have been very fragmented. The HBP is driving integration of these different contributions.

During the Ramp-Up Phase, the HBP will collect strategic data, develop theoretical frameworks, and perform technical work necessary for the development of six Information and Communication Technology (ICT) Platforms during the Operational Phase. The ICT Platforms, offering services to neuroscientists, clinical researchers and technology developers, comprise Neuroinformatics (a data repository, including brain atlases and analysing tools); Brain Simulation (building ICT models and multi-scale simulations of brains and brain components); Medical Informatics (bringing together information on brain diseases); Neuromorphic Computing (ICT that mimics the functioning of the brain); and Neurorobotics (allowing testing of brain models and simulations in virtual environments). A High Performance Computing Platform will support these Platforms.

1.2 HBP Subproject 1: Strategic Mouse Brain Data

The aim of SP1 is to acquire strategic datasets describing the molecules, cells and cognitive capabilities of the mouse brain, and to align these with information about the human brain. Assembled data on the cellular and molecular organisation of the brain will set a precedent for the comparison of mouse and human systems, allowing the reconstruction of models and simulations of the brain across all its levels and functions.

Data gathered at the molecular level will reveal the transcriptome and proteome of cell types present in the brain. The main focus will be on single cell transcriptomics and synaptic proteomics – the molecular foundations underlying nervous system function. Datasets on the cellular organisation of the brain will identify the connections between nerve cells and the organisation of brain circuits. Mapping of glial cells and the vascular system will provide a comprehensive architecture for brain simulation. These data will be incorporated into the HBP Mouse Brain Atlas, providing a foundation on which data generated throughout the neuroscience community can be added and allowing the derivation of general principles of structural organisation that are essential for the reconstruction of mouse brain models.

Data collected and generated in SP1 will enable us to decipher the molecular structure of the human brain and map the circuits of nerve cells. Combining the data and generating detailed computer models and simulations of the brain will allow neuroscientists to comprehend the connections that link genes, molecules and cells to human cognition, behaviour and, eventually, disease.

1.3 Purpose of this Document

The report provides a detailed account of the implementation and validation of the methods described in D1.4.1 Methods, Indicators of Progress and Target Values for the Mapping of Mouse Brain in the period M1-M12.

1.4 Structure of this Document

The remainder of this chapter provides an SP-level overview, highlighting the SP's main accomplishments and problems encountered in the period M1-M12. Subsequent chapters look at accomplishments and in issues within individual components of the SP:

- T1.1.2 (UOXF, EBRI): Profiling the transcriptome of different cell types (Transcriptome)
- T1.1.3 (UOXF, EBRI): The cellular and synaptic proteome (Proteome)
- T1.1.4 (IST, UCLM): Neural channelomics and receptomics
- T1.1.5 (SIB, UNIBAS): Potassium channels kinetics
- T1.1.6 (CNR, EBRI, SNS): Trans-synaptic signalling and receptor kinetics
- T1.1.7 (SYLICS, SYNOME). Relationship between genotype and cognitive phenotypes in mice
- T1.2.3 (UPM): Numbers and distributions of excitatory and inhibitory neurons and glia (Synaptome)
- T1.2.4 (WMC, IEM HAS): Morphological analysis of neurons and glia, T1.2.5: Principles of axonal projections (UAM) & T1.2.6 (UPM): Synapse maps of the mouse brain (Connectome)
- T1.2.1 (UZH): Detailed anatomical map of the brain vasculature (Neuro-vascular glia)
- T1.2.2 (LENS): Whole brain cell distribution (Cellome)
- WP1.3 (UEDIN): Data aggregation, analysis and dissemination
- WP1.4 (UPM): Scientific coordination

The Annexes present in tabular form what the Subproject planned to achieve in this period and what it actually achieved, including the Subproject's Scientific Key Performance Indicators (SKPIs).

1.5 Overview of Subproject 1: Achievements

Within this reporting period, SP1 had two SP Deliverables. The first, D1.4.1, was submitted in a timely manner. This report is the second SP1 Deliverable.

The main SP1 achievements in the Period were:

- Single-cell transcriptomics:
  - Methods for identifying all genes expressed in single neurons developed and tested.
  - Large-scale screening of the genetic types of cells in the mouse brain shown to be technically feasible.
  - Draft single cell transcriptomes generated.
- The Cellular and Synaptic proteome:
  - Strategy established for mapping distribution of specific proteins and proteomes across the whole mouse brain.

  - High-resolution, mass spectrometry-based proteomic studies on discrete regions dissected from normal mouse brain can reveal the depth of proteome coverage of 400-1,000 proteins.
- Electron microscopy mapping: Cortex & hippocampus tissue scans:
  - Synapse densities (vital for brain modelling)
- Automated cell counting prototype method:
  - Automated counting of fluorescently stained neurons in whole brain.
- 3D reconstruction methods: neurons, axonal projections & blood vessels:
  - Strategic anatomical data for brain modelling
- Data aggregation:
  - Existing neuronal, glial and synaptic proteomics and gene expression dBases identified.
  - Establishment of informatics analysis and data management pipeline for linking SP1 molecular and cellular data and prepared for integration with the HBP Platforms.

SP1 had seven Milestones planned for the Period M1-M12. All Milestones were completed on time and no significant delays experienced, except for MS6. The achievements in each relevant SP1 Milestone is briefly summarised below.

- MS1 was achieved by T1.1.2 (UOXF) according to schedule in M6, establishing a proof of concept for cell-type transcriptomic analysis as reported in D1.4.1
- MS2 was achieved by T1.1.3 (UEDIN) according to schedule in M12. It ascertained proof of concept and establishing a strategy for mapping distribution of specific proteins and proteomes across the whole mouse brain. Protocols have been optimised to preform in-depth analysis by quantitative mass spectrum on a variety of different brain preparations and across the whole mouse brain.
- MS6 was not achieved in a timely manner in T1.2.3 & T1.2.6 (UPM) - see Section 1.6 for further information.
- MS12 was achieved in a timely manner (M6) by T1.2.1 (UZH). Workflow for data analysis is established and data format for vascular data is defined for synchrotron data. In addition, two-photon microscopy data format has been transformed to the same data format for cross-platform compatibility.
- MS13 was achieved on time in M6 by WP1.3 (UEDIN). MS13 Formats and ontologies for molecular experiments - "Data format and ontology specifications for molecular experiments". A review of data formats and ontologies in use by the groups generating molecular data revealed that on the whole appropriate standards are already in place. This is largely due to their adoption of established community databases. The proteomics and functional genomics work completed to date form the internal tools and pipeline for handling the molecular datasets from SP1.
- MS14 was achieved on time in M12 by WP1.3 (UEDIN). The proteomics and functional genomics work completed to date form the internal tools and pipeline for handling the molecular datasets from SP1.
- M252 was achieved on time in M12 in T1.1.5 by Partners SIB and UNIBAS. Strategy established for modelling procedure of potassium channel kinetics.

1.5.1 Collaboration

The organisation of SP1 is shown in Figure 1.

The following collaborations have resulted in significant progress:

- T1.1.2 UOXD (UK) worked with Karolinksa Insitute (KI, Sweden) to contribute significantly to providing single cell transcriptome data.
- WP1.1 UPM (ES) worked together with WP1.2 UEDIN (UK) to establish a dissection protocol that provides outstanding coverage of whole brain.
- T1.2.4 WMC (VC) collaborated with the Huazhong University of Science and Technology (HUST) in Wuhan (China) to obtain the Golgi mage stack data of a whole rat brain and the fluorescent mage stack data of a whole GFP mouse.
- Several potential new strategies for labelling and 3D analysis of axons with high-resolution LRPN were tested through collaboration between T1.2.5 UAM (ES), T1.2.3 UPM (ES), T1.2.2 LENS (IT), Tissue Vision Inc. (USA) and Caltech (USA).
- There was a discussion about the optimisation of reconstruction methods between the Partners in WP1.2 and about the required data types and formats with Partners in SPs 5 and 6. The importance of precise measurement of dendritic thickness was highlighted. Data from light microscopy must be calibrated at least once using electron microscopic measurements.
- T1.2.1 UZH (CH) and David Kleinfeld, UCAL (U.S.A.) worked together to obtained optic data from two-photon microscopy. The data contains a reconstructed and annotated vascular network from the mouse somatosensory cortex. The Task programmed a parser that is able to read the data and display it.
- T1.2.2 LENS (IT) and T1.1.3 UEDIN (UK) performed some preliminary experiments to assess the feasibility of reconstructing the full distribution of synaptic puncta in the mouse hippocampus using two-photon serial sectioning microscopy.
- T1.2.2 LENS (IT) and T1.2.3 UPM (ES) interacted to compare different analysis methods in cell counting.
- T1.2.2 LENS (IT) from SP1 and UDUS (DE) in SP2 set up a work plan to perform a multimodal investigation of portions of human brain.

There have been several collaborations between SP1 and ICT Platforms and we anticipate having results shortly.

1.5.2 Significant outcomes

The following outcomes from specific Tasks are particularly significant:

- T1.1.2 UOXF RNA sequencing data has been submitted for publication and will be analysed and pre-processed prior to submission to ICT.
- Since joining the HBP from the Competitive Call, T1.1.7 Partners Synome and Sylics have established strong collaborative links with UEDIN and UT to identify relevant models and are in the process of integrating released BxD and phenotype data into ICT.
- T1.1.6 is currently working with WP6.4 (T6.4.1-3) on integrating data to test for implementation issues.
- T1.2.2 LENS: A fully automated tool for localisation of cell bodies in 3D light sheet whole-brain images has been developed [Frasconi et al., Bioinformatics 2014], in collaboration with computer scientists from the University of Florence (Prof. Frasconi) and from the University Campus Bio-Medico in Rome (Prof. Iannello). The method by counting all Purkinje cells in the cerebellum of an L7-GFP mouse was demonstrated.
- T1.2.3 & T1.2.6 UPM: Techniques for automatic counting of puncta and to generate protein synaptic maps have been developed.
- T1.2.4: Several short programs have been developed in-house to aid the reconstruction and calibration process (3D Reconstruction, Hippocampus).
- WP1.3 UEDIN and SP5 are coordinating with data generation groups to ensure common ontologies and common anatomical location nomenclature for biological tissue samples

used for functional genomic and proteomic analysis, as a prerequisite for later modelling use.

1.6 Overview of Subproject 1: Problems

Two main deviations have been detected during M1-M12. These are as follows:

- MS6 'Scanning and counting methods' was not achieved on schedule by T1.2.3 & T1.2.6 (UPM). By M12, the method of 3D automatic counting for fluorescent puncta was working well in general. However, during M6-M12, we still found that the accuracy of the method was not as good as expected in regions of the brain with very irregular shapes (e. g. Hippocampal region CA3). We are currently working to improve the algorithm used to obtain accurate densities of puncta in any part of the brain. However, this issue should not prevent WP1.2 from accomplishing its Ramp-up phase objectives in a timely manner.
- D1.4.2 'Implementation and validation of methods for mapping of the mouse brain': This Deliverable was submitted late due to resource constraints.

In addition, other minor technical issues have arisen during Year 1 in the activities of some Tasks. Most of them have been successfully overcome. These minor problems have not retarded the SP's work.

1.7 The Next Six Months for Subproject 1

SP1 has seven more Milestones to achieve in the next six months. No delays or changes are foreseen. The scheduled MS are summarised below.

MS3: 1) Transcriptome: several methods have been investigated for sample preparation, efficiency and dataset quality. Work has begun on collecting cell types in cerebellum and hippocampus. Most components of the pipeline have been established. Transcriptomes from the cell types of cerebral cortex and CA1 of hippocampus have been generated by collaborators so we focus on the remaining regions and on human. 2) Proteome – work under way after establishing and validating isolation methods for mouse brain regions.

MS8: Delivery of the synaptic map and ultrastructural data, assigned to T1.2.3 & T1.2.6, is planned for M18.

MS7: Map of mouse vasculature, assigned to T1.2.1, is scheduled to be delivered in M18.

The other Milestones due in this period are MS248, MS253, MS256 and MS259. These are assigned to the Partners who joined us via the Competitive Call.

1.7.1 Other major steps planned in the next six months:

- T1.1.2: Work on human tissue is being optimised. UOXF anticipate collecting cell type-specific data from both mouse and human.
- Calibration of the measurements of dendritic diameters should be finished in 3D Reconstruction (Hippocampus).
- T1.2.3 and T1.2.6: Finalising the validation of the 3D counting method and generation of the first synaptic map of different brain regions.
- T1.2.2 will improve user-friendliness and reliability of the imaging methods, allowing large-scale data production.
- Establishment of a new collaboration between T1.2.1 (UZH), Wuhan University, Sean Hill (SP5) and Felix Schürmann (SP6).

1.7.2 Work that can now start because the necessary preliminaries have been completed:

- As a result of preliminary work now complete, activities assigned to T1.1.3 and T1.1.4 can now begin.

1.8 Integration of new Partners from the Competitive Call

As a result of the Competitive Call, four new Tasks were added to WP1.1. The progress of each is summarised below.

Task 1.1.4 (Neural channelomics and receptomics) Methods have been established for 2D and 3D image capture of neurotransmitter receptor and ion channels for mapping and distribution analysis of major cortical neurons. T1.1.4 anticipate collecting preliminary results for single labelling for AMPA, NMDA and GABAB receptors, as well as for SK, GIRK and P/Q-type channels using the SDS-FRL technique. Preliminary results for density of SK channels using the FIB/SEM technique will also be gathered.

Task 1.1.5 (Potassium channels kinetics) T1.1.5 aims to determine key kinetic states and rates of K potassium channels through activation, ion permeation and inactivation. Since joining the HBP the research teams have been established and M252 was achieved which has allowed for the quality of the generated homology models to be assessed and will improve the pipeline for modelling potassium channels. Differential rates of K channels inactivation have been identified which may be used to classify different K channels.

Task 1.1.6 (Trans-synaptic signalling and receptor kinetics) Protocols, equipment and resources have been established and validated to begin work exploring the relationship between synaptic signalling complex assembly, downstream receptors and channel functionality. This includes recruiting personnel, reformatting intrabodies, construction of baits, identifying suitable mice lines and corroborating libraries. In the next reporting period work will begin in generating recombinant antibodies.

T1.1.7 (Relationship between genotype and cognitive phenotypes in mice) T1.1.7 is well placed to begin studying the cognitive behaviours of BXD recombinant in bred strains and mice with target mutations in genes affecting synaptic function. Since joining the Consortium after the Competitive Call, T1.1.7 has identified target models and has an established pipeline for behavioural analysis. Strong collaborative links between T1.1.7 and UT/UEDIN have resulted in release of novel phenotype date on 4 BxD strains.

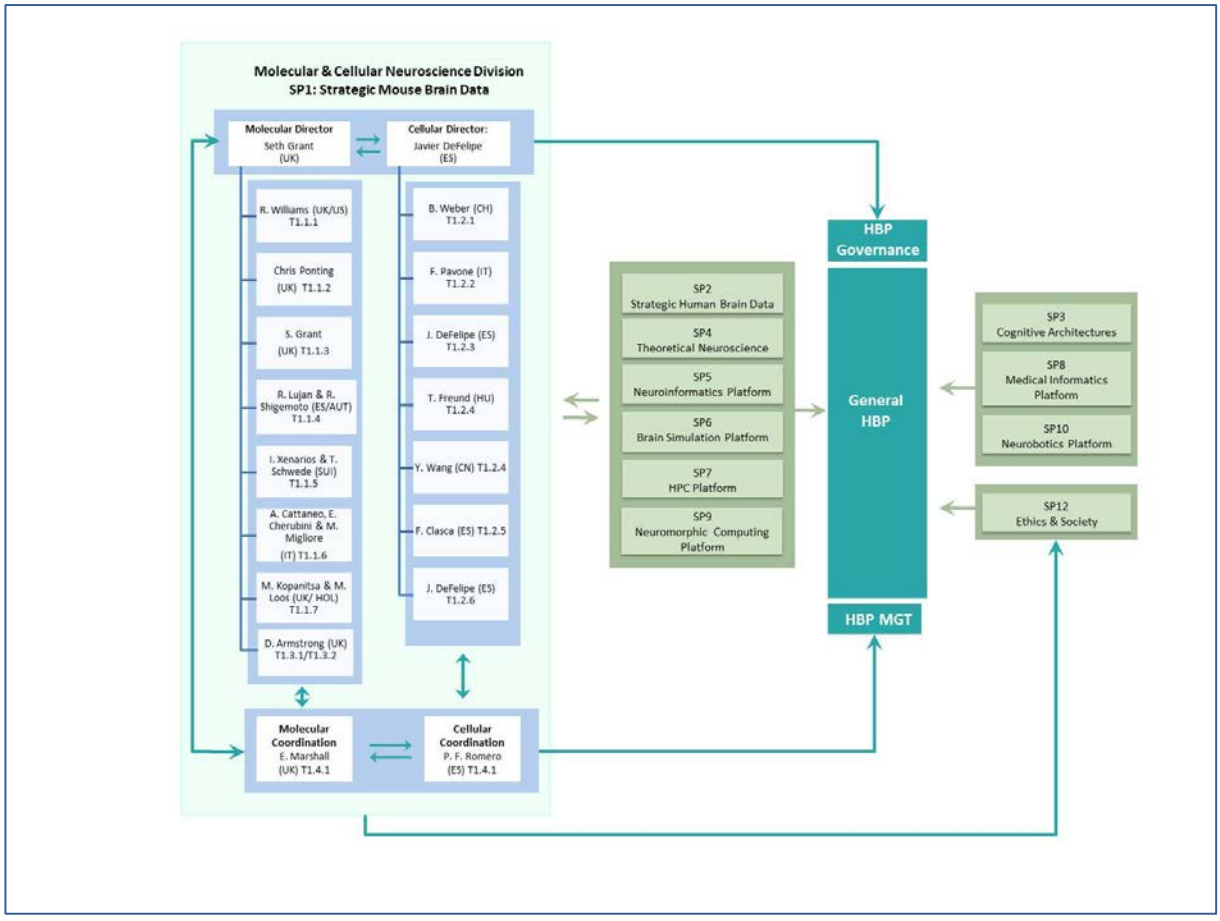


Figure 1: Organisation of SP1 including Partners that joined via the Competitive Call

2. Transcriptome (T1.1.2)

2.1 Transcriptome: Overall Goals

T1.1.2: Quantifying the transcriptomes of different cell types, UOXF (70.68), M01 - M30

T1.1.2 will establish a state-of-art method for obtaining single cell type transcriptomes (SCTs) and use this method to obtain RNA expression data of coding and non-coding RNA for major neurons in the brain with well-studied cellular and synaptic anatomy, physiology and pharmacology. The Task will initially focus on major neuron-types in the neocortex, hippocampus and cerebellum. The age, sex and strain will be standardised. SCTs will be used to establish correlations with the morphological, electrophysiological, pharmacological and synaptic properties of the neurons, allowing the use of SCT to predict these properties in other neurons and to derive the cellular composition of the brain. In the Operational Phase additional SCTs will be obtained for other regions of the mouse brain. The method developed for mouse will be adapted for use in humans, making it possible to predict the properties of any human neuron. Combined with the synaptic proteome data generated in T1.1.3, pairs of transcriptomes will make it possible to predict the synaptic physiology of the synaptic pathway between any pair of neurons belonging to the same or different types.

T1.1.1 will identify and, where necessary, produce mouse lines suitable for cell-type specific studies. T1.1.2 will isolate fluorescent cells from these animals and determine their single cell-type transcriptomes. The Task will involve collaboration with all major international initiatives on labelling single cell-types in transgenic mice.

2.2 Transcriptome: Main Achievements

During period M6-M12 significant progress has been achieved in the validation and implementation of methods to be used in quantifying the transcriptomes of different cell types.

2.2.1 Completion of Milestones

UOXD has established proof of concept for cell-type transcriptomics and achieved Milestone 1 (MS1). In doing so, several methods have been investigated for sample preparation, efficiency and dataset quality.

A mouse Purkinje cell transcriptome was acquired, mapped and quantified with bulk RNA-Seq on Laser Capture Microdissected (LCM) neurons. To maximise the information content of the sequence obtained, the transcriptomes of individual cells for production purposes will be targeted. Furthermore, we are now using cell-sorting methods that greatly increase our throughput in comparison to LCM methods.

Pilot transcriptome datasets have been generated from individual induced pluripotent stem cell (iPSC)-derived neurons with two or three copies of chromosome 21, but which are otherwise isogenic. Although elevated expression from chromosome 21 in the trisomy can be seen among these cells, the variability in these data suggests that increased numbers of cells will be required to obtain robust information at the single gene level. Analyses of small pilot datasets will continue to guide experimental design before the sequencing is scaled up for production.

Individual cultured primary mouse striatal neurons had their transcriptomes sequenced (via Rob Williams). Although these data sets show some expected medium spiny neuron

markers, some notable markers are conspicuously absent. We speculate that this absence may be attributable to the culturing. Thus any methods we scale up will directly use dissociated tissue. The antibody-based methods developed for mouse can be adapted for use in humans, which should facilitate predicting some physiological properties of individual human neurons.

2.2.2 WP progress

UOXF is targeting cell types in cerebellum and the remaining areas of hippocampus, as well as rare cell types in cortex and hippocampus not covered by the transgenics.

UOXF have also set up a procedure in Oxford for obtaining fresh human tissue for single cell transcriptome sequencing, from both post-mortem tissue and unaffected resected cortical tissue of patients with temporal lobe epilepsy. Antibody-based methods will be used and/or shotgun methods to obtain human counterparts of the neuronal transcriptomes we are gathering in mice, with an initial focus on the cerebellum.

2.2.3 Collaboration

Two recent collaborations are contributing well to the progress of the remaining associated MS. Sten Linnarsson's group at the Karolinksa Institut (KI, SE) has sequenced single cell transcriptomes from ~1,500 neocortical cells and ~1,500 cells from CA1 (hippocampus), collectively representing approximately 45 cell types. These cells were randomly sequenced from a pool of both neuronal and glial cells, and as such the types need to be computationally identified before being contributed to HBP. The sequencing is now complete for these cells and the paper has been submitted. These data will undergo additional analyses prior to being uploaded to the ICT.

In collaboration with Jesper Ryge from the Brain Mind Institute (EPFL, CH) Sten's lab is also sequencing transgenically targeted cells that were underrepresented in the shotgun approach, specifically targeting Emx1+, Sst+, Pvalb+, DAT+ and VIP+ cells.

2.2.4 Internal monitoring and quality control activities

T1.1.1 Mouse models for transcriptomes and proteome analysis

- SP1_SKPI-01 No of suitable mouse lines for cell-type studies – already over 90 lines available
- SP1_SKPI-14 Identification of mouse lines:
  - Generation of methods. Planned: 2014/02/28 - 2014/03/31 – in progress
  - Methods development. Planned: 2014/02/28 - 2014/03/31 – in progress
  - Identification of models. Planned: 2014/03/31 - 2016/03/31 – in progress

T1.1.2 Quantifying the transcriptomes of different cell types

- SP1_SKPI-02 No. of neocortical neuron types with first draft SCTs – in progress
- SP1_SKPI-03 No. of hippocampal neuron types with first draft SCTs – in progress
- SP1_SKPI-04 No. of cerebellum neuron types with first draft SCTs – in progress
- SP1_SKPI-15 Establishment of method for single cell transcriptomics (SCTs) from well characterised neuron-types:
  - Method generation. Planned: 2014/02/28 - 2014/03/31 - done
  - Method optimisation. Planned: 2014/03/31 - 2014/09/30 – in progress
  - Transcriptome data generation. Planned: 2015/02/28 - 2016/03/31

WP structure: No changes have been done to WP objective, structure or personnel.

2.3 Transcriptome: Main Problems

Although several challenges have been encountered, UOXF did not suffer any delays or miss any Milestones for T1.1.2. One challenge has been successfully implementing SCRB-seq using antibody-sorted cells. Although three upstream techniques were tried for this (including modifications made in consultation with the original developers of both the upstream chemistry and the library preparation protocols), none has produced high quality libraries. UOXF are therefore pressing forward with the more established single cell transcriptome sequencing methods.

2.4 Transcriptome: The Next Six Months

By April 2015, UOXF anticipates that it will have achieved the transcriptomic components of M3 (cerebral cortex), partially achieved the transcriptomic components of M4 (hippocampus), and to have made substantial progress towards the transcriptomic parts of M5 (cerebellum) now that all the necessary SCT components are in place in Oxford (cell sorting, C1, single cell sequencing and basic computational analysis pipeline). We hope also to get a pilot SCT dataset from human tissue, pending tissue availability. We will keep trying to get the single cell library preparation to work downstream of antibody selection of cells. We anticipate that this will involve an inactivation of proteinase K on the C1 machine and are working with a Fluidigm scientist to make this modification to the C1 script.

Although we are now capable of producing STRT libraries in Oxford, we do not yet have a suitable sequencing centre that uses the custom primers to sequence these. We are working with the research core at the Sanger Institute to build this capability in the coming months, so that our targeting of cortical and hippocampal cell types can be equivalent to the data generated at KI. In the meantime, we have implemented Smart-seq2 and will collect SCTs of cerebellar neurons once the cell sorting difficulties have been resolved.

3. Proteome (T1.1.3)

3.1 Proteome: Overall Goals

T 1.1.3: The cellular and synaptic proteome, UEDIN (P60), M01 - M30

T1.1.3 will use state-of-art techniques in proteomics to identify the complete set of brain proteins expressed in cells and synapses. It will focus on protein complexes that play an important role in electrophysiology, neuromodulation, synapse identification and synapse function. In the Operational Phase, the Task will identify differential expression of proteins in the different cell-types identified in Task 1.1.2 and compare the mouse proteomic data with human data in order to establish generalising principles for specifying the molecular properties of human neurons, synapses and glia.

3.2 Proteome: Main Achievements

T1.1.3 achieved significant progress during the period M6-M12

3.2.1 Completion of Milestones

UEDIN has achieved MS2: Strategy established for mapping distribution of specific proteins and proteomes across the whole mouse brain. UEDIN has developed protocols for performing high-resolution mass spectrometry-based proteomic studies on discrete regions dissected from normal mouse brain. Performing quantitative mass spectrometry on these samples revealed the depth of proteome coverage allowed by each sample and preparation type: neuro-synaptosomes preparations at a depth of 400 proteins; whole brain tissue extracts at a depth of 600 proteins; crude PSD preparations at a depth of 900-1,000 proteins and pure PSD preparations at a depth of 820 proteins (SP1_SKPI-16).

Pure PSD preparations can be isolated from as little as 200 mg of brain tissue. From this, the identity and relative abundance of 820 proteins can be identified. Furthermore, it is possible to quantify 550 proteins in the PSD (including all MAGUKs and NMDARs) with a minimum of 4 unique peptides. This capability allows for the differential, label-free quantitation of each of these 550 proteins in the PSD in numerous samples acquired throughout the brain. This pilot study will provide valuable insight into the stoichiometry of proteins in the PSD, synapses and whole brain tissue.

3.2.2 WP progress

Having now developed a strategy to examine the synapse proteome across various regions of the mouse brain, work will progress to obtain a first draft of the proteome across the brain (MS3). In collaboration with Javier de Felipe's laboratory, the methodology for the dissection of various mouse brain regions has been developed for 7 integral regions of the mouse brain. Additionally, methods for the dissection of entire mouse brains by micropunch dissection have been established. This second method allows for high-resolution mass spectrometry-based proteomic profiles to be obtained for each 1mm3 volume throughout the entire mouse brain (SP1_SKPI-05).

3.2.3 Collaboration

Working closely with UPM has led to the successful development of mouse brain dissection protocols and two strategies for sampling various regions of the entire mouse brain as outlined above; a sampling strategy that will allow for a draft of the mouse brain proteome to be achieved at the necessary depth.

3.2.4 Internal monitoring and quality control activities

Timeline for development of Methods:

1) Optimisation of synaptosome and PSD complex isolation: deployment
2) Analysis of human brain tissue: development
3) Western blotting, imaging and quantitation: Deployment
4) Mass spectrometer analysis of PSD protein samples: development

- SP1_SKPI-05 No. of brain region synapse proteomes - synapse proteomes of 10 brain regions generated
- SP1_SKPI-16 Development of proteomic techniques:
  - Generation of methods - Development of proteomic techniques allowing the identification of sets of brain/synaptic proteomes. Planned: 2014/02/28 - 2014/03/31 - completed
  - Methods have been tested, optimised, and validated. Planned: 2014/03/31 - 2014/09/30 - completed
  - Synaptic proteasome data generation is now underway. Planned: 2014/08/31 - 2016/03/31

3.2.5 WP structure:

No changes have been done to WP objective, structure or personnel.

3.3 Proteome: Main Problems

UEDIN have encountered no problems in work towards T1.1.3 and anticipate no delays in achieving future MS.

3.4 Proteome: The Next Six Months

Due to the success in optimising dissection techniques, protein isolation and establishing quantitative mass spectrometry in M1-M12, work is now under way to collect pilot proteomic data for all 7 integral regions of the mouse brain, as well as every 1mm^3 volume of the entire mouse brain. UEDIN anticipate delivering the first draft synapse proteome in M18 as scheduled. Also, as a direct result of acquiring pilot data for various sample preparation and PSD extraction methods, mass spectrometry-based label-free quantitation and relative abundance of PSD proteins in the mouse brain can begin.

UEDIN anticipate no structural changes or delays in achieving T1.1.3 goals.

4. Neural Channelomics and Receptomics (T1.1.4)

4.1 Neural channelomics and receptomics: Overall Goals

Task 1.1.4: Neural channelomics and receptomics, UCLM (P40), IST (P32), M07 – M30

T1.1.4 will use strategically relevant mice and state-of-the-art techniques in electron microscopy localisation to obtain two- and three-dimensional maps of neurotransmitter receptor and ion channel distributions in different subcellular compartments of major cortical neurons. The Task will identify the precise cellular localisation of major neurotransmitter receptor and ion channels and map the distribution of these channel subunits in different parts of the dendritic tree of cortical cells. The methods developed for mice will be adapted for use on human tissue. The Task will also develop small gold and palladium particles and generate differently shaped nanoparticles for immunolabelling with high sensitivity and resolution. Labelling of receptor and ion channel subunits using these particles conjugated with secondary (or primary) antibodies will also allow multiple labelling. This technical innovation will be valuable for detecting subunit composition of receptors and ion channels. The data generated by this Task will be uploaded to the neuroinformatics Platform. This Task is led by the Universidad de Castilla – La Mancha, a Partner added via the Competitive Call.

4.2 Neural channelomics and receptomics: Main Achievements

UCLM and IST joined the Consortium via the Competitive Call. During period M9-M12, significant progress was achieved in establishing the strategy for 2D and 3D ion channel mapping.

4.2.1 WP achievements

For the two-dimensional maps of neurotransmitter receptor and ion channel distribution freeze-fracture replica labelling has been established using carbon replica, which gave nearly twice as much sensitivity. For the three-dimensional maps of neurotransmitter receptor and ion channel distribution the technical requirements for FIB/SEM are in place to obtain uniform labelling through the tissue allowing for collection of image stacks.

4.2.2 Other WP progress

Generation of methods for two- and three-dimensional ion channel mapping. Optimisation of coating for replica and use of 2nm gold particles have enabled the higher sensitivity for Cav2.1 subunit of P/Q-type voltage-dependent calcium channels, and also higher resolution of double labelling combined with conventional 5nm gold particles for SDS-FRL. Optimisation of immunogold and direction of tissue cutting for FIB/SEM.

4.2.3 Collaboration

Dr Rafael Lujan visited Dr Ryuichi Shigemoto at IST Austria in August to plan collaboration on SDS-FRL of potassium channel subunits and $GABA_B$ receptors, and to establish the common criteria that will be employed for the systematic quantitative analysis of hippocampal pyramidal cells.

4.2.4 Internal monitoring and quality control activities

Appropriate controls using the corresponding knockout animals to make sure on the use of specific antibodies and to set the level of background in our immune-histochemical reactions.

- SP1_SKPI-31 Maps generation - Neural channelomics and receptomics:
  - Generation of methods. Planned: 2014/04/30 - 2015/09/30 – started and tested
  - Generation of maps of receptor and ion channel distribution. Planned: 2014/04/30 - 2016/03/31 – in progress
  - Data integration. Planned: 2016/01/31 - 2016/03/31 – not started
- SP1_SKPI-32 No. of maps for receptors and ion channels will start in month 13.

4.2.5 Lessons learned

Biochemical optimisation is indispensable for obtaining maximum labelling efficiency.

4.2.6 WP structure

No changes have been made to the WP objective, structure or personnel.

4.3 Neural channelomics and receptomics: Main Problems

UCLM and IST have encountered no problems in their work on T1.1.4 and anticipate no delays in achieving future MS.

4.4 Neural channelomics and receptomics: The Next Six Months

It is expected that MS248 (Strategy established for 2D and 3D ion channel mapping), will be achieved by M18. This will deliver quantitation of ion channel densities in surfaces and volumes in different compartments of cortical neurons.

Within the period M13-M18, the WP will accomplish several additional things:

- Collecting preliminary results for single labelling for AMPA, NMDA and $GABA_B$ receptors, as well as for SK, GIRK and P/Q-type channels using the SDS-FRL technique.
- Collecting preliminary results for density of SK channels using the FIB/SEM technique.

Due to progress in the first three months, SDS-FRL under conventional conditions can start now for a few subunits for AMPA and NMDA receptors, for subunits of SK and GIRK channels, and for the GABAB1 subunit of the $GABA_B$ receptor. FIB/SEM under conventional conditions can start now for GIRK and SK channels and for $GABA_B$ receptors.

UCLM and ISTA anticipate that no changes are necessary to the WP's original Ramp-Up Phase plan.

5. Potassium Channels Kinetics (T1.1.5)

5.1 Potassium channels kinetics: Overall Goals

Task 1.1.5: Potassium channels kinetics, SIB (P40), UNIBAS (P12), M07 – M30

Work in T1.1.5 will determine the key states visited by potassium channels through activation, ion permeation, and inactivation. The Task will consider the impact of external factors such as transmembrane voltage and concentration of permeant and non-permeant ions. The Task will also curate selected publications describing the function of potassium channels and the associated kinetic rates. The Task will construct tri-dimensional homology models of relevant neural mouse and human potassium channels based on the available experimental ion channel structures. The Task will integrate functional data and homology models with the HBP ion channel database by coordinating with WP1.3. This Task will define a set of rules to classify qualitatively the rates of the channels for which data are not available. Whenever possible, rates for the following transitions will be given: activation, deactivation, conductance, inactivation, and recovery from inactivation. The data generated by this Task will be uploaded to the neuroinformatics Platform. This Task is led by the Universitaet Basel, a Partner added via the Competitive Call.

5.2 Potassium channels kinetics: Main Achievements

SIB and UNIBAS joined the Consortium via the Competitive Call. During the period M7-M12, significant progress has been achieved in establishing the strategy for 2D and 3D ion channel mapping.

5.2.1 WP achievements

During the period covered by this report, Milestone MS252 was achieved. Homology models were generated for all the human potassium channels using an automated procedure based on the SwissModel pipeline. Specifically, models were built for the 40 channels in the Kv family, the 16 channels of the Kir family, the 15 in the K2P family and the 8 channels of the KCa family. Improvements to the pipeline are being made to specifically address important challenges in the modelling of potassium channels and the assessment of the quality of potassium channel models. Notably modelling of oligomeric assemblies has been incorporated in the pipeline and a statistical potential used for model quality prediction has been re-parameterised and tested specifically for transmembrane proteins.

5.2.2 Other WP progress

Investigation on the mechanisms of activation and inactivation in eukaryotic and prokaryotic potassium channels are in progress. Molecular dynamics simulations and free energy calculations were performed showing that the selectivity filter of potassium channels is not only involved in the selectivity and inactivation processes, but also in the mechanism of activation. Through molecular mechanics simulations and electrophysiology measurements we have highlighted that the inactivation mechanism at the selectivity filter of potassium channels is not unique. At least two different mechanisms with rates that differ by many orders of magnitude exist and are displayed by different channels. The first one, which is extremely fast, involves the ion depletion of the selectivity filter and seems to highly depend on a glutamate residue in the vicinity of the selectivity filter. The second mechanism remains elusive, though we have shown that it does not involved the release of more than one ion, most likely leaving two ions in the selectivity filter. Such

distinct inactivation mechanisms with different rates will constitute one among other criteria to classify the different potassium channels.

5.2.3 Internal monitoring and quality control activities

SP1_SKPI-34 Potassium channel kinetics - has been almost reached in terms of number of homology models and we are now proceeding with their quality control.

5.2.4 WP personnel

The team was rapidly put in place following the announcement of the results of the Competitive Call. Florian Heer was recruited by the Swiss Institute of Bioinformatics in April 2014 for the study of the molecular gating mechanisms. Mr. Heer pursue PhD studies at the Biozentrum of the University of Basel, Switzerland. Dr. Niklaus Johner was recruited by the University of Basel for the homology modelling of the different potassium channels. For the last three years, Dr. Johner was a post-doc at the Weill Cornell Medical School. He started in his new position in Basel in July 2014.

5.3 Potassium channels kinetics: Main Problems

The development of the project is in line with the planned work and no problems have been encountered.

5.4 Potassium channels kinetics: The Next Six Months

MS253 will be achieved in the next six months. A draft of the kinetic models of the individual regulatory processes has been sketched. In the coming months work will proceed with the integration of the different gating mechanisms, notably the activation, inactivation, and deactivation, into a unique kinetic model.

Completion of Milestone MS252 and the introduction of a new statistical potential for membrane proteins (see section 5.2) will allow the quality of the generated homology models to be assessed and will improve the pipeline for modelling potassium channels. Many of the potassium channels comprise several domains: a transmembrane domain and intracellular domains (e.g. the T1 domain in the Kv family or gating-ring in the KCa family). As for certain channels, no template covering the full length protein are available whereas there are templates for the individual domains; combining information from multiple template will be an important task to further improve some of the models.

Before proceeding with the biocuration of published data and knowledge surrounding the different potassium channels of interest, it is essential to establish a clear set of annotations priorities specific to ion channels and the needs of the HBP, going beyond the requirements for a more general usage of the data, or a mere text-mined data integration approach (which only skim the surface of the published data). It should contain the list of the ion channel properties to be reported and the set of requirements. T1.1.5 will use the Swiss-Prot knowledge representation for each of them as they will directly be used by a wide variety of scientists, providing the outreach mechanism for HBP. Such set of priorities should provide a clear and realistic view of the landscape of potential dataset and a ranking of channels of interest. The voltage-gated potassium channel Kv2.1 has been selected as a test case to define the canvas. Kv2.1 is a well-studied channel for which different kinds of functional and structural data are available.

6. Trans-Synaptic Signalling and Receptor Kinetics (T1.1.6)

6.1 Trans-synaptic signalling and receptor kinetics: Overall Goals

Task 1.1.6: Trans-synaptic signalling and receptor kinetics, SNS (P81), EBRI (P74), CNR (P30), M7-30

T1.1.6 will exploit the IACT-SPLINT approach to generate recombinant antibodies in scFvs format against the NLGs/NRXs system and their interactors. The SPLINT-selected scFvs will be expressed as intracellular antibodies (intrabodies) in mammalian cells and *in vivo*. The Task will perform neuronal-specialised targeting of intrabodies, and use derivative formats of monoclonal antibodies for different applications (*in vitro* and *in vivo*). The Task will biochemically, anatomically and electrophysiologically validate newly generated intrabodies. The protein silencing will allow us to assess the relationship between protein assembly, downstream receptors and channel functionality. The Task will implement a general method to generate automatically computational models of trans-synaptic protein network influence on receptor kinetics. These models will represent the effects of proteins silencing under different conditions. The method and the models will follow the format and requirements of the HBP data and Brain Simulation Platform. The data generated by this Task will be uploaded to the Neuroinformatics Platform. This Task is led by the European Brain Research Institute, Rita Levi-Montalcini Fondazione, a Partner added via the Competitive Call.

6.2 Trans-synaptic signalling and receptor kinetics: Main Achievements

SNS, EBRI and CNR joined the Consortium following success in the Competitive Call. During period M7-M12 progress has been achieved in establishing the strategy for trans-synaptic signalling and receptor kinetics.

6.2.1 WP achievements

The existing available anti gephyrin scFv intrabodies have been reformatted for Tet inducible all-in-one Lentiviral vector, to ensure good inducible expression of the intrabodies in primary neurons and organotypic cultures. Protocols for the organotypic cultures have been transferred to EBRI from SISSA and equipment has been purchased for culture preparations. Neuroligin-3 deficient mice breeding at EBRI has started. Implementation of an initial computational model of Gephyrin action, integrated into the Tsodyks-Markram scheme. The construction of the neuroligin antigen baits, for IACT selections, has started by existing personnel.

Work has started to validate and implement SPLINT libraries of scFv fragments. In particular, besides the available SPLINT library of mouse origin, a SPLNT library of human variable regions has been generated. Validation of the latter is underway. A new procedure for the experimental determination of antibody diversity and of the library complexity has been developed, using next-generation sequencing facility available at the BioSNS Laboratory of Scuola Normale Superiore.

6.2.2 Internal monitoring and quality control activities

SP1_SKPI-33 Trans-synaptic signalling and receptor kinetics:

- Isolation and biochemical validation of antibodies against gephyrin and neuroligin proteins. Planned: 2014/04/30 - 2015/03/31 – in progress

- Electrophysiological functional validation of intrabodies against gephyrin and neuroligin protein. Planned: 2015/04/30 - 2015/09/30 – not started yet
- Computational method development for synaptic model. Planned: 2015/10/31 - 2016/03/31 – not started yet

6.2.3 WP personnel

A Post-doc has been recruited to start electrophysiological experiments. The selection procedure for the recruitment of a dedicated post doc to work on the IACT selection is under way. A Post-doc has been recruited, for the implementation of the IACT selections against neuroligin baits. A postdoc has been recruited, to implement of the modelling part.

6.3 Trans-synaptic signalling and receptor kinetics: Main Problems

The set up at EBRI of protocols for the organotypic cultures has had some delay, due to the technician involved entering maternity leave. In the process of getting the organotypic cultures in full speed, planned recordings will be performed from acute hippocampal slices of control and anti-gephyrin scFv infected mice.

No problems have been encountered for the modelling part. The program is being carried out as planned.

6.4 Trans-synaptic signalling and receptor kinetics: The Next Six Months

It is expected that M256 will be achieved by M18. As planned, IACT selections for the isolation of scFv fragments against different neuroligin domains will be performed, from mouse and human SPLINT libraries.

Electrophysiological recordings will be performed from both acute and organotypic hippocampal slices from WT and NL3KO mice. Acute slices will be also obtained from mice infected with anti gephyrin scFv to functionally test how knocking down gephyrin affects local glutamatergic and GABAergic networks.

In collaboration with WP 6.4 (T6.4.1-3) the model for gephyrin will be integrated into the Optimiser and used with a CA1 neuron to test for implementation issues.

7. Relationship between Genotype and Cognitive Phenotypes in Mice (T1.1.7)

7.1 Relationship between genotype and cognitive phenotypes in mice: Overall Goals

T1.1.7: Relationship between genotype and cognitive phenotypes in mice, SYNOME (P48), SYLICS (P24), M07-M30

This Task will explore the relationship between genotype and phenotype by using BXD recombinant inbred strains and mice with targeted mutations in genes affecting synaptic functions and/or associated with brain disorders. The Task will study cognitive behaviours of these mice using conventional and touchscreen-based operant testing chambers (BXD strains and synaptic mutants), as well as automated PhenoTyper homecages (BXD strains). In addition, the Task will conduct proteomic profiling of brain tissue samples from BXD strains to identify the impact of random genetic variation on synaptic protein abundance. The Task will integrate the data with publically available strategic datasets on molecular, cellular, electrophysiological and anatomical data existing for these mouse strains. The data generated by this Task will be uploaded to the Neuroinformatics Platform. This Task is led by Synome and Sylics, Partners added via the Competitive Call.

7.2 Relationship between genotype and cognitive phenotypes in mice: Main Achievements

T1.1.7 joined the Consortium after being winning Competitive Call Topic 2. The successful project is a partnership between 2 SMEs: Synome Ltd (SYNOME) and Synaptologics BV (SYLICS). Below is a summary of the main achievements.

7.2.1 WP achievement and progress

In collaboration with Prof. Robert W. Williams (UT/ UEDIN) and Prof. Seth Grant (UEDIN), SYNOME and SYLICS have achieved significant progress towards the MS of T1.1.7.

MS259 (18M): Behavioural assessment of relevant strains: the set of recombinant inbred BxD mouse strains have been established that will be studied in the touchscreen object-location Paired-Associates Learning (PAL) task. The selected strains have been ordered from the Jackson Laboratory and the first set of these mice was scheduled to be received by SYNOME during M11. The use of a dedicated housing room and an experimental room with 24 touchscreen chambers in the Babraham animal facility for HBP have been approved following a UK Home Office inspection in August 2014, and new touchscreen chambers have been delivered and set up (SP1_SKPI-27).

Partner SYLICS approved the set of recombinant inbred BxD mouse strains to be analysed in its automated home-cage phenotyping system and operant task of impulsivity and attention. Availability of these strains at the Jackson Laboratory has been confirmed and the shipment of mice from USA to the Sylics facility in Amsterdam is being organised. Partner SYLICS has two dedicated rooms in the animal facility and respective equipment to start behavioural phenotyping as soon as mice arrive (SP1_SKPI-29).

MS 260 (30M): Proteomic profiling: For these experiments, Partner SYLICS will use the brain tissue of BxD mice following completion of the experiments described in MS259 (SP1_SKPI-30).

Data of 1 and 2 will be integrated with publically available strategic datasets by international collaboration in the GeneNetwork platform. Recent additions of valuable strategic BXD datasets to GeneNetwork (including cognitive function, metabolomics, proteomics, neurocognitive ageing and gene expression) as well improvements in GeneNetwork interface and mapping algorithms will facilitate this integration.

MS 261(30M): Establishment and touchscreen-based analysis of gene-targeted mutant mouse cohorts: SYNOME has identified available mouse lines with gene-targeted mutations in genes encoding synaptic/brain disease-relevant proteins for touchscreen assessments. Two of these lines (SynGAP and Shank2) have been rederived into the Experimental Area of the Babraham animal facility in M10, in preparation for breeding cohorts for our experiments (SP1_SKPI-28).

7.2.2 Internal monitoring and quality control activities:

- SP1_SKPI-27 Touchscreen analysis of recombinant inbred BxD lines, testing of 24 mouse lines – in preparation
- SP1_SKPI-28 Touchscreen analysis of gene-targeted mutant mice – in preparation
- SP1_SKPI-30 Proteomic analysis of BxD prefrontal cortex specimen, total around 600 proteins – in preparation
- SP1_SKPI-29 Behavioural analysis of BxD mice at Synaptologics:
  - First data set. Planned: 2014/04/30 - 2015/03/31
  - Final data set. Planned: 2015/04/30 - 2016/03/31

7.2.3 WP personnel and structure

Two Research Technicians have been recruited; initiated rent of two dedicated rooms in the animal facility; ordered 24 touchscreen boxes to start the experiments as soon as mice arrive. The touchscreen boxes have already arrived at Synome and we are waiting for the last batch of BXD mice and some minor accessories for touchscreens to start the experiments.

7.2.4 Collaboration

Prof. Williams (UT/UEDIN) added to the GeneNetwork.Org database a data set of novel 26 traits based on performance of BxD mice in touchscreen visual discrimination/reversal and extinction tasks in a recently published study by Graybeal et al. (PMID 24586288).

7.3 Relationship between genotype and cognitive phenotypes in mice: Main Problems

Milestones 259, 260 and 261 are generally well on track, although due to a slight delay in the delivery to Partner SYNOME of selected BxD lines, their phenotyping in touchscreen chambers may finish 1-2 months later than expected, making it problematic to obtain the full planned set of data for the last group of gene-targeted mutants (M261) before March 2016. In that eventuality, Partner SYNOME would complete touchscreen testing of the last cohort of these mice as an “in-kind” contribution to the Project, and report it when all analyses are complete.

7.4 Relationship between genotype and cognitive phenotypes in mice: The Next Six Months

Partners SYNOME and SYLICS will complete all experimental work towards M259 (Behavioural assessment of relevant strains), comprising the assessment of recombinant inbred BXD mouse lines in the touchscreen object-location Paired-Associates Learning task (SYNOME) and in automated home cages (SYLICS). All the necessary equipment has been purchased and first BXD lines will arrive at Partners' labs in September 2014 (M12). The first sets of behavioural data for submission into the GeneNetwork database should be available by January 2015 (Month 16). As part of this work, our HBP Partner, Robert W. Williams (UT), has entered relevant behavioural data for the BXD strains, generated by C. Graybeal and colleagues (PMID 24586288), into the GeneNetwork system (open source data). We will exploit this and other extant BXD data sets as part of this Project.

In preparation for the work with gene-targeted strains (M261), Partner SYNOME has already rederived mice with loss-of-function mutations in *Syngap1* and *Shank2* genes into its animal facility, and rederivation of mice with mutations in *Dlgap1* and *Dlgap2* genes will occur in September 2014. This will enable SYNOME to breed a sufficient number of mice with gene-targeted mutations for touchscreen testing to commence straight after completion of our BxD study in Month 18.

Our HBP Partners are now updating GeneNetwork webservices with genotypes and touchscreen data four new BXD strains (BXD122, BXD124, BXD138 and BXD149) as well as another inbred strain C57BL/6NJ. Also, data on neurocognitive aging and hippocampal gene expression during aging is being generated at UT and entered into GeneNetwork database for genome-to-cognitive phenotypes comparison. Below is an example of data that continue to be added to the GeneNetwork database:

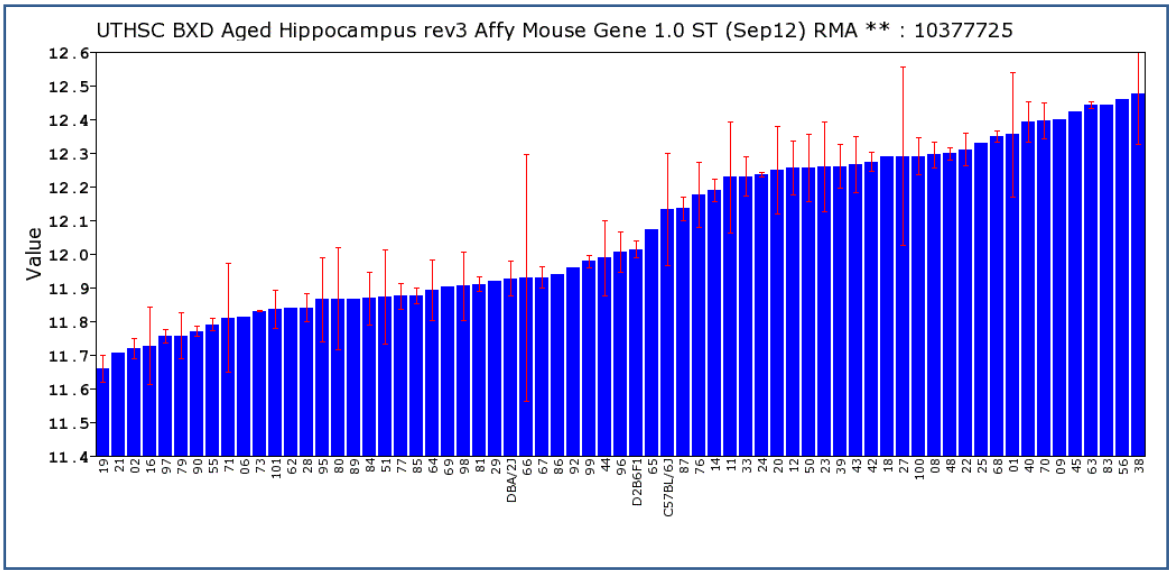


Figure 2: Expression of Psd95 mRNA (DLG4) in the hippocampus of aged BXD strains (350 to 900 days of age).

The distribution is bimodal with approximately a 0.5 unit 1.5-fold difference between parental strains (DBA/2J and C57BL/6J) (log2 scale) and the many derivative BXD strains, (All data openly available from GeneNetwork.org).

8. Synaptome (T1.2.3)

8.1 Synaptome: Overall Goals

Numbers and distributions of excitatory and inhibitory neurons and glia (T1.2.3)

We are developing a new 3D segmentation algorithm that currently allows neurons (stained with anti-NeuN antibody) to be automatically segmented in 3D from image stacks obtained by confocal microscopy, providing accurate data concerning their spatial distribution and size. This method is now being adapted for its use in the 3D segmentation of all cells in sections stained for DAPI (which labels the nuclei of both neurons and glia). This work is mostly being performed in collaboration with SP5 (WP5.2: Tools for Structural Data Analysis (Peña, MIDAS-UPM and Fua, CVL-EPFL). The 3D segmentation algorithm generated will be used, along with spatial statistical techniques in collaboration with SP5 (WP5.4: Predictive Neuroinformatics, T5.4.2: Neuronal Structural Design and Predictions, Larrañaga, CIG-UPM), to characterise the density and principle patterns of spatial distribution of (i) different subpopulations of GABAergic inhibitory neurons and (ii) subpopulations of excitatory neurons. The cerebral cortex and other major brain regions will be characterised. For this purpose we have started to use both wild-type mouse brain tissue stained immunocytochemically for markers for selective neuronal populations, and brain tissue from transgenic mouse lines that express GFP in specific neuronal populations. These colonies have already been established at the animal facility of the Cajal Institute (CSIC, Spain).

8.2 Synaptome: Main Achievements

8.2.1 Completion of Milestones

MS6 (Scanning and counting methods), was not achieved in a timely manner (see Section 8.3 for further information). In spite of the delay with MS6, the development of automated methods is being implemented according to the scientific KPIs defined in the report D1.4.1. These methods are currently being tested and validated (SP1_SKPI-19).

8.2.2 Other WP progress

In addition, the method for automatic segmentation of cells to improve the accuracy for counting neuronal and non-neuronal cells has been further developed.

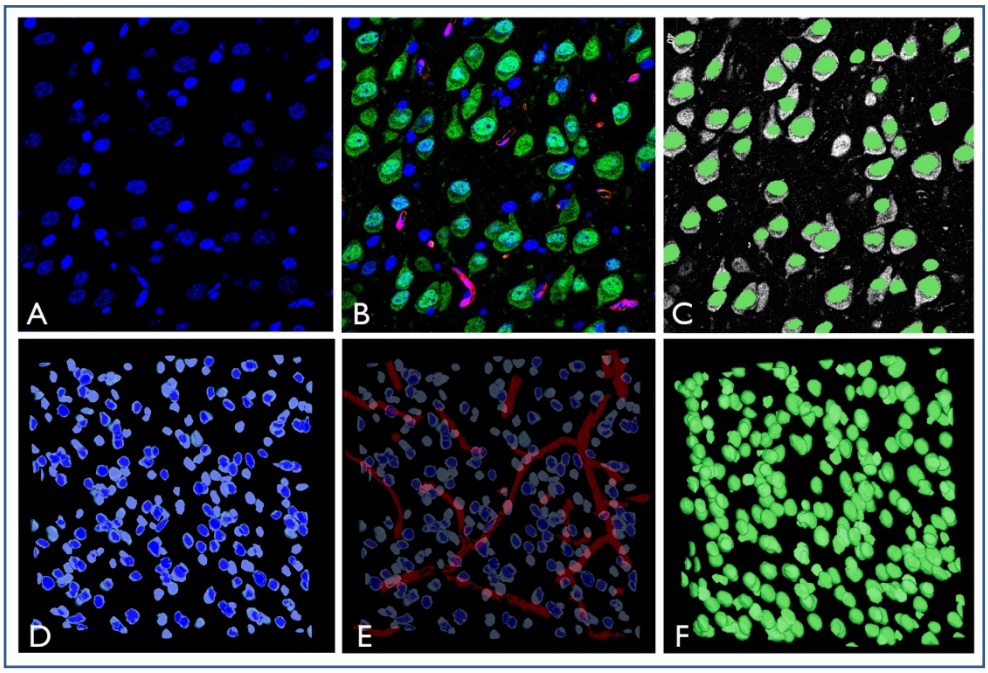


Figure 3: Improved method for automated 3D segmentation.

A-C: Images obtained by confocal laser microscope from the neocortex. A: DAPI stained nuclei of all cell types (blue) from a single slice. B: z-projection of confocal channels from the same single slice to visualise DAPI nuclei (blue), NeuN positive neurons (green), and sulforhodamine stained blood vessels (red). C: Segmented neuronal nuclei (green) superimposed to a single section of NeuN staining. D-F: 3D reconstructions of cells and blood vessels performed with EspINA software. D: DAPI stained nuclei (all cell types, blue). E: sulforhodamine stained blood vessels (red) merged with DAPI stained nuclei (blue). F: Only nuclei from neuronal cells (green) are visualised and segmented.

8.2.3 Results from collaboration between Partners in different countries or with other WPs

In general, all Tasks were successfully performed in collaboration with several laboratories from several countries and within the same or other WPs. In particular, a challenging research topic has been established between DeFelipe's (WP1.2) and Grant's (WP1.1) laboratories, 'synaptic mapping of the whole brain': Quantitative analysis of synaptic density and 3D distribution based on GPF-labelled proteins (GPF-transgenic mice) and electron microscope validation using FIB/SEM technology.

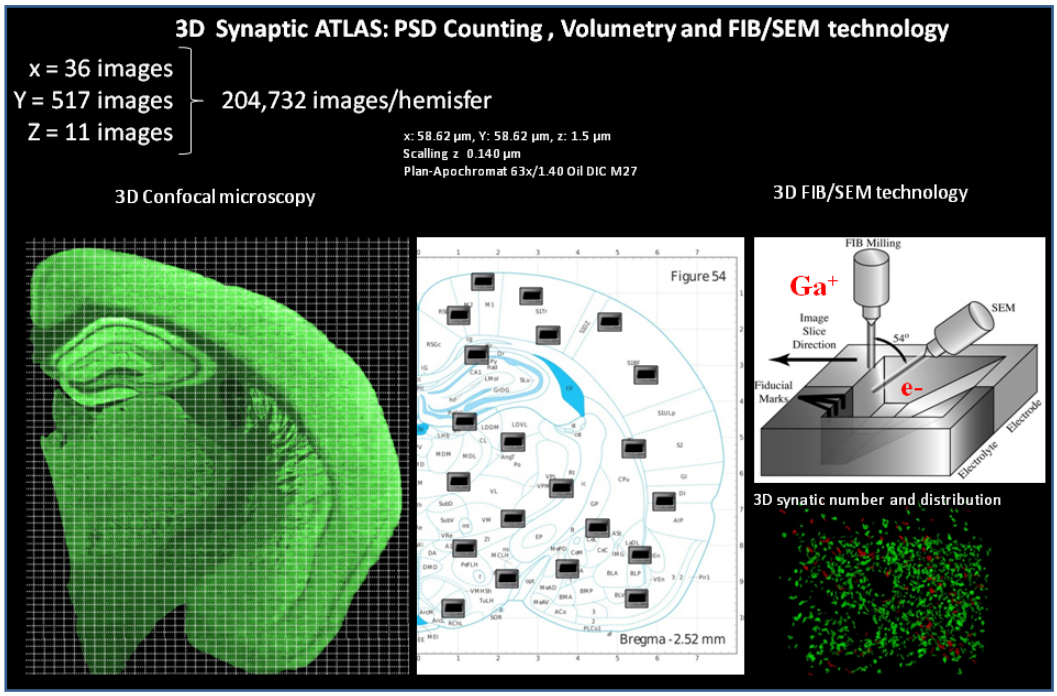


Figure 4: Synaptic mapping of the whole brain

Left: Consecutive high-resolution confocal microscope stacks of images showing an hemisphere of a GFP-transgenic mouse brain. Middle: Brain atlas image to illustrate possible regions of interest where 3D PSD counting can be performed. Right: FIB/SEM technology to validate and estimate the number and volume of PSDs

8.2.4 Results from combination of data and ICT

We have developed techniques for automatic counting of puncta and to generate protein synaptic maps

8.2.5 Internal monitoring and quality control activities

From the beginning of the Project, a regular internal communication flow has been established to ensure the information exchange among the WP participants as well as with the Molecular Section of the SP1. This communication flow has been used as the main resource to ensure that work has been performed as scheduled and to detect and correct deviations, where appropriate. Specifically, we have been in direct contact on a weekly basis with the laboratories involved in the WP by electronic means (email, Skype calls, etc.), In addition, the Milestones scheduled in the Project work plan, together with the scientific KPIs defined in the report D1.4.1 have been used as an internal tool for monitoring the work progress in the Tasks involved in the WP.

SP1_SKPI-19 Establishment of automated methods to generate maps of inhibitory and excitatory neurons and glia:

- Generation of methods. Planned: 2014/02/28 - 2014/03/31 – done
- Set up of the methods for 3D counting. Planned: 2014/02/28 - 2014/03/31 – done, fine-tuning going on.
- Maps generation. Planned: 2015/02/28 - 2016/03/31

8.2.6 Lessons learned

We found that sharing with SP5 rough quantitative data instead of elaborated final data is very useful to avoid mistakes and accelerates the multiple steps of analysis required to generate the final data.

8.3 Synaptome: Main Problems

MS6, Scanning and counting methods, was not achieved in a timely manner. We have developed a punctuate (PSDs) volume segmentation and quantification algorithm designed to be executed in a supercomputer in order to obtain 3D quantitative data in of all brain regions. This algorithm has been validated in several regions of the hippocampus. However, the algorithm does not work well in certain brain regions due to significant variations on the shape of the PSD. Regarding cell counting, we have been able to successfully segment neuron's nuclei, and therefore we are able to count automatically the total number of neurons. We are now working in the next step that consists in automatic counting other types of cells (glia). We hope to improve these algorithms and solve these problems in the next weeks. Nevertheless, this issue is not representing a constraint to accomplish the general WP objectives.

8.4 Synaptome: The Next Six Months

We are on schedule for meeting the planned Milestone MS8 for this period: *Synaptic map and ultrastructural data*, which is expected to be delivered in M18.

8.4.1 Work that can now start because necessary preliminaries have been completed

Finalising the validation of 3D counting method and generation of the first synaptic map of different brain regions (M18)

9. Connectome (T1.2.4, T1.2.5 & T1.2.6)

9.1 Connectome: Overall Goals

9.1.1 Morphological Analysis of Neurons and Glia (in T1.2.4)

9.1.1.1 3D reconstruction with Neurolucida (Hippocampus)

In order to obtain morphological reconstructions from pyramidal cells and different types of inhibitory neurons in the mouse hippocampus, we carry out whole-cell patch-clamp recordings in the CA3 area of 600 micrometre thick in vitro hippocampal slices of 8-week-old Black6 mice. Following electrophysiological characterisation, neurons are filled with biocytin. To facilitate sampling of specific cell types we use, where available, transgenic animals expressing fluorescent markers in subsets of inhibitory neurons. After preserving the slices by aldehyde fixation the intracellularly filled neurons are visualised using immunofluorescent staining against biocytin. We optimised this procedure to be able to visualise the processes of the neurons throughout the whole depth of the sections without the need of re-sectioning, thus improving the accuracy of the reconstructions as well as the throughput. This protocol allows us to scan the whole section using a confocal microscope and reconstruct the dendritic and axonal arbour of the neuron in 3D using the Neurolucida program. The structure of the dendritic tree is recorded with special emphasis on the thickness of the processes, since this is the most sensitive parameter when modelling signal propagation in model neurons. The results of the reconstructions will be forwarded to the collaborators responsible for data management in the required file formats. Recording and filling neurons in 8-week-old animals requires the fine-tuning of slice preparation procedures to increase the rate of successful visualisation. When the workflow is fully optimised, we expect to be able to reconstruct approximately 10 neurons per month (SP1_SKPI-09).

9.1.1.2 3D reconstruction with Neurolucida (Neocortex)

This involves reconstruction of different types of neurons and their synaptic connections in the mouse brain. After recording, mouse brain slices are histochemically stained, and different types of neurons and their synaptic connections are reconstructed using Neurolucida system. Additionally, collaboration is in progress with Wuhan Institute of Chinese Academy of Sciences in China. The institute has established a new technique called MOST, by which different types of images from whole mouse brains can be scanned and integrated. The collected images include cell density, blood vessel network, and Golgi stained neurons, and single neurons specifically labelled by transgenic and virus-mediated expression of fluorescence. Hopefully, this collaboration will benefit HBP in multiple aspects. For instance, using their image data, the cell density and blood vessel networks can be reconstructed and mapped at the scale of the whole mouse brain. Using image data of fluorescently labelled neurons, the neuron reconstruction can be done at a level of the whole structure, which will be much faster compared with previous techniques based on in vivo labelling and subsequent serial sectioning. Using image data of Golgi stained neurons of a whole mouse brain, the reconstruction of different neuron types can be systematically carried out and compared among different brain regions (SP1_SKPI-10).

9.1.1.3 In utero electroporation technique

An *in utero* electroporation (IUE) method is being developed to improve the analysis of neuronal morphology and to correlate neuronal morphology with specific molecules in the mouse cerebral cortex. IUE is a method that allows rapid gene delivery in a spatially- and temporally-controlled manner in the developing central nervous system. The progenitor cells in ventricular zone carrying the DNA will undergo neurogenesis, migration, and final

differentiation to become mature neurons positioned in distinct cortical layers according to their birth date. In addition, by controlling the direction of electroporation, a specific cortical area can be targeted. IUE is being used to study the development of neuronal morphology and the correlation of neuronal morphology and specific molecules in the mouse cerebral cortex. Using null mutant mice and by overexpression with this technique, we are studying the function of a layer specific transcription factor in regulating the physiological properties of cortical pyramidal cells (PCs). It was found that the target gene promotes basal dendritic growth and regulates intrinsic electrophysiological and synaptic properties of layer 5 PCs in mouse cortex.

9.1.1.4 Internal monitoring and quality control activities:

- SP1_SKPI-09 No. of mouse cell morphologies reconstructed (hippocampus) - target reached by Month 12.
- SP1_SKPI-10 No. of mouse cell morphologies reconstructed (neocortex) – target exceeded by Month 12.
- SP1_SKPI-20 Reconstruction and classification of mouse neurons and glia:
  - Method generation. Planned: 2014/02/28 - 2014/03/31 – in progress
  - Optimisation of methods. Planned: 2014/02/28 - 2014/09/30 – in progress
  - Generation of neocortical and hippocampal cell morphologies. Planned: 2014/02/28 - 2016/03/31 – in progress

9.1.2 Principles of Axonal Projections (in T1.2.5)

Research efforts have been mainly focused on setting up and refining the methods and instrumentation for conducting this Task in seven specific areas.

Three pilot experiments have been conducted to adjust the optimal brain tissue processing protocols to allow quantification at the optical/electron microscope levels (both standard and FIB/SEM) of anterogradely labelled thalamocortical axon boutons and synapses.

Two independent lines of instrumentation have been tested for achieving efficient visualisation and generation of 3D digital analysis on the complete morphologies of individual GFP-labelled axons on whole mice brains. First, the adequacy of light-sheet fluorescence microscopy (LSFM) on chemically cleared whole cerebral hemispheres is being explored in collaboration with LENS. Several mice brains injected in our lab are being examined at LENS. Second, joint experiments have been conducted and established a collaboration with TissueVision Inc. (Cambridge MA, USA) for designing and implementing technical modifications to the first commercial serial two-photon tomography scanner (STP; Tissue Cyte1000) to make it able of working in supervised mode for directed high-resolution Z scanning. STP has been applied for 3D imaging of GFP-labelled individual projection thalamocortical mice axons.

Importantly, our STP trial experiments have revealed that, even under the best epifluorescence optics and working distance conditions, the fluorescent signal coming from the thin distal segments of a single Sindbis-transfected axon are too weak for detection and imaging. Our interpretation is that weak fluorescent staining results from the relatively quick cytopathic effects of the Sindbis promoter, which kills the infected neuron in about 72 hours, thus precluding sufficient labelling saturation of distal axonal branches. In fact, we and the only other lab in the world that uses this technique (Dr. Takeshi Keneko in Kyoto University, Japan) had always relied on tissue sectioning and immunolabelling against GFP, not on direct fluorescent imaging, for visualising distal axonal processes.

Since techniques for the efficient acquisition of 3D fluorescent imaging (Laser Sheet microscopy or STP) are rapidly improving and may become available for the SP1, the development of innovative strategies for obtaining intense fluorescence in the distal axons of individually transfected neurons have been decided. Specifically we have begun testing adenoassociated viral vectors. We are currently testing AAV1.hSyn.eGFP.WPRE.bGH pseudoviruses and vector plasmids that we have obtained from the University of Pennsylvania Vector Core.

To increase the spatial precision and efficacy of our single-neuron labelling by transfection with Sindbis viral vectors, a new electro-osmotic procedure has been developed. We are currently writing the corresponding methodological report for publication in a science methods journal. In parallel, experiments to explore the feasibility of combining single-cell extracellular recodings with Sindbis RNA electroporation have been started. If successful, this technique could be crucial to correlate cell morphologies and functional profiles. Besides, a systematic population-level quantitative analysis has been started of thalamocortical afferents to primary and higher order visual areas in C57BL6 mice.

The use of stereological measurements has been tested on the axonal length and bouton number on the various terminal arborisations of a single projection axon in different brains regions. The optical fractionator and virtual planes methods are applied using an Olympus CAST-GRID Advanced Stereology System. This procedure may turn out to be an efficient compromise for generating precise data on a large number of local arborisation morphologies of complex multi-branched projection axons.

9.1.2.1 Internal monitoring and quality control activities:

- SP1_SKPI-11 No. of axonal projections traced – target reached by Month 12
- SP1_SKPI-12 No. of terminal axon branches and monosynaptic targets identified – target not reached by Month 12
- SP1_SKPI-21 Tracing of axonal projections:
  - Generation of methods. Planned: 2014/02/28 - 2014/03/31 - done
  - Optimisation of methods. Planned: 2014/02/28 - 2014/03/31 - done
  - Tracing of single cell axonal projections. Planned: 2014/02/28 - 2016/03/31 – in progress
- SP1_SKPI-22 Identification and distributions of terminal axon branches and monosynaptic targets:
  - Generation of methods. Planned: 2014/02/28 - 2014/03/31 - done
  - Optimisation of methods. Planned: 2014/02/28 - 2014/03/31 - done
  - Determination of the distribution of terminal axons branches. Planned: 2014/02/28 - 2016/03/31 – in progress

9.1.3 Synapse Maps of the Mouse Brain (in T1.2.6)

A new methodology is under development for automated 3D counting of fluorescent puncta in stacks of confocal microscope images. This work has been performed in collaboration with Seth Grant's laboratory-UEDIN (WP1.1 - Mouse Brain Transcriptomics and Proteomics). Specifically, we have used genetically modified PSD95 EGFP mice to develop a novel 3D quantification methodology to analyse the number, distribution and size of the PSD95 protein, which is one of the main components of the postsynaptic densities. We are currently working on an automated counting process using 3D connected components. This technology will also be applied to the characterisation of the density and principles of spatial distribution of excitatory and inhibitory axon terminals in different brain regions using transgenic mouse lines with GFP labelling proteins that label glutamatergic and

GABAergic terminals. Breeding animals have recently been acquired, and colonies for these transgenic animals have already been established at the animal facility of the Cajal Institute.

3D FIB-SEM Technology: Improvement of perfusion-fixation of brain samples to be used for confocal and FIB/SEM imaging. Intravascular perfusion with paraformaldehyde is performed so that it is adequate for conventional light and confocal microscopy. Sections of the same tissue to be used for electron microscopy (FIB/SEM) have been improved by postfixation with osmium tertroxide and potassium ferricyanide in a microwave oven. This technique renders a better contrast and a higher signal-to-noise ratio. Secondary methodologies that are currently being used provide data to be integrated with the transcriptome, proteome, synaptome and connectome data. These methodologies are summarised below.

9.1.3.1 Internal monitoring and quality control activities:

- SP1_SKPI-08 No. of synaptic maps of individual identified neurons – in progress
- SP1_SKPI-13 No. of synaptic maps of mouse brain - in time
- SP1_SKPI-23 Generation of synaptic maps of brain regions:
  - Generation of methods. Planned: 2014/02/28 - 2014/03/31 - done
  - 3D methods development. Planned: 2014/02/28 - 2015/03/31 – in progress
  - Synaptic maps and ultrastructural data. Planned: 2015/07/31 - 2016/03/31 – not started yet

9.2 Connectome: Main Achievements

9.2.1 Completion of Milestones

MS6, *Scanning and counting methods*, was not achieved in a timely manner. See section 8.3 for further information.

9.2.2 Other WP progress:

Synapse Maps of the Mouse Brain: In spite of the delay with MS6, the development of automated methods is being implemented according to the scientific KPIs defined in the report D1.4.1. These methods are currently being tested and validated.

3D Reconstruction (Hippocampus): We reconstruct in 3D in vitro filled neurons of different types from hippocampal slices of 8-week-old Black6 mice using the Neurolucida program. We have optimised full penetration visualisation of neuronal labelling and confocal imaging in the 600 micrometre slice. As a final step in fine-tuning the reconstruction workflow, we are now calibrating the measurement of dendritic diameters from fluorescent images using electron microscopy. As of September 1, 2014, two cells are fully reconstructed, including soma, dendrites, axon terminal arbour and proximal axon branches. For eight other cells, the reconstruction of the soma and dendrites has been completed. In the case of an additional 15 cells, the visualisation and confocal scanning have been performed, and they will get into the reconstruction phase soon.

3D Reconstruction (Neocortex): WP progress is on the schedule for meeting the scientific KPIs planned in this activity according to D1.4.1. The generation and optimisation of methods to reconstruct and classify mouse neurons and glia have been completed. These methods have also been tested and validated by M12 as planned SP1_SKPI-20.The generation of neocortical cell morphologies was started in M1. In particular, the numbers of mouse cell morphologies in neocortex reconstructed have been 8 & 20-40 for M6 & M12, respectively, as scheduled (see Table 2 & 3 & 4 in D1.4.1) SP1_SKPI-09 and -10. In addition, more cortical neurons that are not in the scheduled plan but requested by BBP

team have also been reconstructed including 30 single PCs and interneurons from rat SSC, and 19 neurons in different cortical layers of the SSC from the image stack of a Golgi stained whole rat brain.

Furthermore, we are working on the Golgi image stacks, focusing on the reconstruction of PCs in the SSC first. The PC types reconstructed from Golgi image stacks will be compared with those obtained from slice recording in Henry Markram's laboratory.

Axonal projections: T1.2.5 set up and tested innovative methods to improve reliability of cell labelling to explore axonal projections.

9.2.3 Results from collaboration between Partners in different countries or with other WPs:

All Tasks included in the WP are being successfully performed in collaboration with several laboratories from several countries and within SP1 or other WPs. Most relevant results from these collaborations are as follows:

There has been an ongoing discussion about the optimisation of reconstruction methods between the Partners in WP 1.2, and about the required data types and formats with Partners in SPs 5 and 6. We drew attention to the importance of precise measurement of dendritic thickness. Data from light microscopy must be calibrated at least once using electron microscopic measurements.

A collaboration has been started with the Huazhong University of Science and Technology (HUST) in Wuhan China. The Golgi mage stack data of a whole rat brain and the fluorescent mage stack data of a whole GFP mouse have been given by the HUST.

- Golgi image stacks allow reconstruct dendrites of neurons in all areas of the brain. A systematic study on PC classification for different regions could be possible with this data. But axons are labelled very sparsely. Therefore, it won't work for the interneuron classification because of the limitation of axon labelling.
- In the GFP fluorescent image stacks, clear axonal clusters were found to emerge from axonal fibres in the white matter (possibly from subcortical regions such as thalamus). This may allow tracing the axonal clusters formed from subcortical regions and find out source regions. However, the fine axonal collaterals cannot be visualised clearly.
- We've setup the new version of offline reconstruction system, NL 360, which allows tracing neuron in 3D. So it makes reconstruction much more accurate than it is done in 2D. It also has a function to detect putative synapses automatically.

Through the collaborations with Profs Clasca (SP1, UAM), DeFelipe (SP1, UPM) and Pavone (SP1, LENS), Tissue Vision Inc. (Cambridge, MA, USA), and Dr. Carlos Lois (Caltech, USA) we have tested --and found sub-optimal-- several potential new strategies for labelling and 3D analysing with high-resolution LRPN axons. Additional techniques are currently in our testing pipeline. In any case, we have made big strides towards our goal of devising an efficient, high-throughput protocol for the analysis of these cortical brain circuits with cellular resolution.

9.2.4 Results from combination of data and ICT:

With regards to methods of automated 3D counting, we have developed techniques for automatic counting of puncta and to generate protein synaptic maps. Several short programs have been developed in-house to aid the reconstruction and calibration process (3D Reconstruction, Hippocampus).

9.2.5 Internal monitoring and quality control activities:

From the beginning of the Project, a regular internal communication flow has been established to ensure the information exchange among the WP participants as well as with the Molecular Section of the SP1. This communication flow has been used as the main resource to ensure that work has been performed as scheduled and to detect and correct deviations, where appropriate. Specifically, we have been in direct contact on a weekly basis with the laboratories involved in the WP by electronic means (email, Skype calls, etc.). In addition, the control points scheduled in the Project work plan, such as Milestones, as well as the scientific KPIs defined in the report D1.4.1 have been used as an internal tool for monitoring the work progress in the Tasks involved in the WP.

Moreover, personnel involved in reconstruction crosscheck each other at crucial steps of the reconstruction, i.e. axonal arbour characteristics, classification of dendrite types and thickness assignments (3D Reconstruction, Hippocampus).

9.2.6 Lessons learned:

We found that sharing with SP5 rough quantitative data instead of elaborated final data is very useful to avoid mistakes and accelerates the multiple steps of analysis required to generate the final data.

3D Reconstruction (Hippocampus): Automatic estimation of dendritic thickness is problematic, because the intensity of staining and therefore the apparent diameter in the scanned image depends on the depth within the section. Types should be assigned to dendritic segments and the same characteristic thickness (measured accurately) has to be assigned to the types.

Axonal projections analysis: Although a “negative result”, from our collaboration with LENS we have learned that laser-sheet microscopy and transparentisation methods (CLARITY and Scale) are not suitable for achieving our WP goals.

9.2.7 Changes to WP activities, structure or personnel:

Axonal projections analysis: We are emphasising the refinement of methods to increase the yield of controlled labelling of single physiologically identified neurons.

9.3 Connectome: Main Problems

9.3.1 Milestones missed by WP in period reported on (or reached >1 month late)

MS6, Scanning and counting methods, was not achieved in a timely manner. See section 8.3 for further information.

9.3.2 Other areas where WP is behind:

3D Reconstruction (Hippocampus): After about 11 months, we have not yet achieved the 12-Month target of 20 reconstructions.

9.3.3 Reasons and remedies

Fine-tuning the reconstruction process raised several difficult issues (e.g., optimising slice cutting and labelling in 8-week-old animals, improving penetration in staining, precise measurement of dendritic diameters). However, as more than 20 cells are currently in the pipeline, and the process has been optimised, we expect to be back on track within the next 3 months.

9.4 Connectome: The Next Six Months

We are on schedule for meeting the planned Milestone for this period: MS8: *Synaptic map and ultrastructural data*, which is expected to be delivered in M18.

9.4.1 Other major things WP should accomplish in this period:

3D Reconstruction (Hippocampus): Calibration of the measurements of dendritic diameters should be finished.

9.4.2 Work that can now start because necessary preliminaries have been completed:

Synapse Maps of the Mouse Brain: Finalising the validation of the 3D counting method and generation of the first synaptic map of different brain regions (M18).

3D Reconstruction (Hippocampus): As the first complete and fully calibrated reconstructions are now available, morphologies can be transferred for testing to SP5.

9.4.3 Any changes you will need to make to the WP's original Ramp-Up Phase plan:

3D Reconstruction (Hippocampus): Since the pipeline is optimised we will soon be able to increase the number of reconstructed neurons per month. We do not expect any large change (up or down) in our original estimation.

9.4.4 Major problems that the WP might encounter & your plans to address them:

3D Reconstruction (Neocortex): Using the image data of whole GFP mouse brain, it won't work for whole axon reconstruction of labelled pyramidal cells because the fine axonal collaterals in the cortex are not clear at all. In order to get whole-axonal clusters of PCs reconstructed using these image stacks, it is necessary 1) to get stronger labelling of PCs, 2) to improve the image quality. For the latter, a PI in HUST is working on for this purpose.

Axonal projections: As explained in point 1.1, the selective labelling and reconstruction of the long, complex axons of LRPN is a central challenge for brain connectomics for which no optimal neuroanatomical technology is yet available. From the interactions and focused discussions with other HBP laboratories is now clear that a key goal must be is obtaining a high-efficacy, fluorescent labelling of the whole axon of single physiologically identified neurons. To this end, we are exploring innovative single-cell in vivo transfection methods: we have established new collaborations, have mounted a new, dedicated experimental setup (with non-HBP funds) and hired an experienced electrophysiologist (with non-HBP funds) to reinforce the group of researchers in our lab that already had expertise in such techniques.

Besides, we have studied or tested several new optical microscopy methods for tracing and measuring terminal axonal branches and boutons without compromising the subsequent electronmicroscopic analysis of the synapses formed by the labelled axons, for their sensitivity and spatial resolution. We have not yet found the ideal one, but or search now is focused and informed, and we hope to come with a result in the coming year. Until then, we will keep labelling and reconstructing axons form serially sectioned brains, as originally proposed in our Work Package.

10. Neuro-vascular-glia (T1.2.1)

10.1 Neuro-vascular-glia: Overall Goals

Detailed Anatomical Map of Brain Vasculature (in T1.2.1)

3D reconstruction with X-ray tomographic microscopy. A method has been developed which allows for scanning the entire mouse brain vascular system at sub-micrometre resolution in only one day. Members of our Subproject met several times in Zurich and Villigen at the synchrotron beam line, which will be used to acquire synchrotron radiation, based X-ray tomographic microscopy (srXTM) images of the entire mouse brain. The focus of our initial efforts is on establishing a robust workflow from sample preparation and data acquisition to data analysis. The beamline headed by the Consortium member Marco Stampanoni) has recently been further improved, particularly with respect to the speed of acquisition. Currently, we are able to image 1x1x1 millimetre at 700 nanometre resolution within one minute. As a consequence, the time required to scan the entire mouse brain vascular system at sub-micrometre resolution dropped to approximately one day.

3D reconstruction with serial two-photon microscopy. A technique based on serial two-photon microscopy (s2pm) is currently being carried out to reconstruct large murine cortical samples successfully. As a parallel and complementary approach to srXTM, we will also work on serial two-photon microscopy (s2pm) together with David Kleinfeld, who has successfully reconstructed large murine cortical samples. A meeting was held in December 2012 in Zurich and a further meeting was held in February 2014 to set up a detailed workflow, which includes a comparison between srXTM and s2pm.

10.2 Neuro-vascular-glia: Main Achievements

10.2.1 Completion of Milestones:

MS12, *Informatics and data management set-up*, was achieved as scheduled. Imaging whole mouse brain samples generates huge amounts of data in the range of several terabytes. In order to be able to handle these excessively large amounts of data, we have designed and implemented a modular data processing pipeline. Customised C++ software tools can readily be integrated in a high-performance computing environment, which is a key requirement to make processing of whole mouse brain samples feasible. Furthermore, acquisition of whole mouse brain vasculature using synchrotron radiation X-ray tomographic microscopy requires partitioning of the sample into smaller regions of interest (ROIs) due to physical constraints of the imaging modality. For this, we have defined an acquisition protocol with our collaborators at the Swiss Light Source (PSI, Villigen, Switzerland) using partitioning with partially overlapping ROIs. This allows for more accurate stitching than purely relying on the coarse spatial alignment that can be recovered from the (world-) coordinates of the individual ROIs as provided by the scanner readouts. We have developed proper stitching tools that have been tested on small datasets. Finally, we have defined the key parameters and meta information of the acquisition protocol that is provided in a comprehensible data format, which readily allows for automatic integration of the acquired datasets into the HBP data registry and the HBP knowledge graph.

10.2.2 Other WP progress:

We have applied for beamtime access to the Swiss Light Source and have been rated with high priority (Ranking: 5 [compared to maximum: 5, average: 3.52]). The reviewing panel

has provided us with 3 days of 24h beamtime shifts throughout 2014 (04 Jul 2014, 18 Oct 2014, 09 Dec 2014). During the first shift, we have tested new contrast media based on hexadecane, dodecane, decane. In order to stabilise the intravascular contrast agents, scanning was performed in a cooling chamber at -20 degrees Celsius. Contrast obtained with the tested compound was not satisfactory and therefore alternative contrast media are currently tested in extra shifts provided by the group of Prof. Stampanoni at the Paul Scherrer Institute.

WP progress is on the schedule for meeting the scientific KPIs planned in this area according to D1.4.1. The generation and set up of methods to reconstruct the brain vascular system at submicron resolution is in principle finalised, however the group seeks to improve certain properties of the contrast agents that enhance the quality of large specimen tomograms (SP1_SKPI-17). The generation of maps of mouse vasculature is already started and number of maps generated in M12 is as scheduled, i.e. 4 (see Table 2 & 3 & 4 in D1.4.1) (SP1_SKPI-06).

10.2.3 Results from collaboration between Partners in different countries or with other WPs

Optical data obtained from two-photon microscopy was obtained from David Kleinfeld (UC San Diego, U.S.A.). The data contains a reconstructed and annotated vascular network from the mouse somatosensory cortex. We have programmed a parser to read the data and are now able to display the network. These data will be directly compared to those acquired using X-ray tomography.

10.2.4 Internal monitoring and quality control activities

As in previous areas of the cellular section, the internal communication flow, together with control points stated in the work plan and scientific KPIs defined in D1.4.1 have been used as the main tools for monitoring the work progress.

- SP1_SKPI-06 No. of maps of mouse vasculature – in time
- SP1_SKPI-17 Reconstruction of the brain vascular system:
  - Generation of methods. Planned: 2014/02/28 - 2014/03/31 – done
  - Set up of the methods for reconstruction. Planned: 2014/02/28 - 2014/09/30 – done
  - Maps of mouse vasculature generation. Planned: 2014/08/31 - 2016/03/31 – in progress

10.2.5 Changes to WP activities, structure or personnel

To accelerate sample preparation procedures, a technician was hired for the project (50% position to Mrs Tanja Mali, MSc, Biologist).

10.3 Neuro-vascular-glia: Main Problems

No problems have been detected in this area.

10.4 Neuro-vascular-glia: The Next Six Months

MS7: *Map of mouse vaculature*, which is associated to T1.2.1, is scheduled to be delivered in M18. We are on schedule for meeting this Milestone.

The Consortium is in the process of establishing collaboration with the group of Wuhan University who have successfully obtained vascular data in mouse brains using Micro-

Optical Sectioning Tomography (MOST). For this, the Task leader has contacted Sean Hill (SP5) and Felix Schürmann (SP6) and further steps will be taken in the upcoming weeks.

11. Cellome (T1.2.2)

11.1 Cellome: Overall Goals

Whole Brain Cell Distributions (in T1.2.2)

3D cell localisation with light sheet microscope. A novel state of the art approach for 3D cell localisation and analysis of data has been produced using new light sheet microscope with improved features: aberration correction, high-speed imaging, Bessel beam double-side illumination, multi-colour imaging. This new technology will be used to produce maps of cell distributions throughout the whole mouse brain. This new microscope will guarantee imaging of a whole mouse brain with submicron (~6-700 nm) resolution in 8-10 hours, producing a dataset in the 10 TeraBytes range per mouse brain. To store and analyse this huge amount of data, we set up a high-speed (10 Gbit/s) direct link to CINECA. Several tools to manage and inspect the data are already available (such as TeraStitcher and TeraFly) and can be used either in line modality or in remote desktop modality. Other tools for the extraction of information (like cell counting and localisation) are almost ready to be used. We are also preparing exhaustive documentation about the microscope and the data analysis in order to allow scientist within HBP to come and use the apparatus.

CLARITY. CLARITY protocol has been reproduced for mouse brain with intrinsic fluorescence (i.e. without immunohistochemistry). It seems quite stable in our hands, and we have found various alternatives to the very expensive FocusClear as imaging solution. We are in the meanwhile reproducing CLARITY immunohistochemistry on thick (~`500 microns) mouse brain slices. This will allow better integration with other data generated within the Consortium. The light sheet / CLARITY approach will be used starting from the first months of the next year to generate data about cell number and localisation: Purkinje cells in the cerebellum, pyramidal neurons in the cortex, GABAergic neurons in the whole brain. Mid-term measurements will concern long-range connectivity and blood vessels.

3D reconstruction with serial two-photon microscopy. A two-photon serial sectioning tomography method is currently being setting up for subregion reconstruction with high resolution. With this method the whole brain cannot be observed at full resolution (it will take months), but subregion can be reconstructed with high contrast. We have already some preliminary results, and we are also coupling this technique with mild clearing techniques (as Scale) to reduce the need for sectioning. The idea will be to use this method to image single thalamocortical axons. The whole brain does not need to be scanned, but only the region containing the axon branches. The contrast and resolution of the two-photon microscopy will allow detecting single synaptic boutons, and the speed we plan to achieve (1-2 samples per week) will allow massive data acquisition with respect to conventional neurolucida methods. We are also adapting our existing data management tool to this technique.

11.2 Cellome: Main Achievements

A new light sheet microscope has been developed and tested allowing fast, high-contrast and high-resolution volumetric imaging of whole mouse brains and of macroscopic portions of human brain. The CLARITY protocol has also been set up with high stability both on mouse brains and on post-surgery human brain tissue, and found a new index-matching agent. This agent can also be used to improve the penetration depth of two-photon microscopy in non-clarified samples. A serial-sectioning two-photon microscope, compatible with clearing agents has been developed, which allows reconstructing large portion of mouse brain (e.g. the hippocampus) with high sensitivity. A fully automated

software tool for cell body localisation in large-scale images has been set up (SP1_SKPI-07).

WP progress is on the schedule for meeting the scientific KPIs planned in this area according to D1.4.1. The generation of methods to acquire whole brain scans for labelled cells as well as their set-up for the 3D cell localisation and reconstruction have been successfully finalised (SP1_SKPI-18). These methods are already tested and validated (see Table 2 & 3 in D1.4.1).

11.2.1 Results from collaboration between Partners in different countries or with other WPs

We have performed some preliminary experiment with the group Seth Grant to assess the feasibility of reconstructing the full distribution of synaptic puncta in the mouse hippocampus using two-photon serial sectioning microscopy. We have also interacted with the group of Javier DeFelipe to compare different analysis methods in cell counting. We found that our method affords a precision of 3% in detection cell nuclei. Recently, we have set up of work plan with Katrin Amunts from SP2 to perform a multimodal investigation of portions of human brain.

11.2.2 Results from combination of data and ICT

In collaboration with computer scientists from the University of Florence (Prof. Frasconi) and from the University Campus Bio-Medico in Rome (Prof. Iannello) we developed a fully automated tool for localisation of cell bodies in 3D light sheet whole-brain images [Frasconi et al., Bioinformatics 2014]. We demonstrated the method by counting all Purkinje cells in the cerebellum of an L7-GFP mouse.

11.2.3 Internal monitoring and quality control activities

As in previous areas of the cellular section, the internal communication flow, together with control points stated in the work plan and scientific KPIs defined in D1.4.1 have been used as the main tools for monitoring the work progress.

- SP1_SKPI-07 No. of cell distribution maps of the whole mouse brain – in time
- SP1_SKPI-18 Acquisition of whole brain scans for labelled cells:
  - Generation of methods. Planned: 2014/02/28 - 2014/03/31 - done
  - Set up of the methods for 3D cell localisation. Planned: 2014/02/28 - 2014/09/30 - done
  - Generation of cell distribution maps. Planned: 2015/02/28 - 2016/03/31

11.2.4 Lessons learned

Data management and analysis are becoming an important part of our Task, almost as important as imaging techniques. We want to be ready to deal with the 10TB datasets that the new light sheet microscope is producing, and to extract information from them.

11.2.5 Changes to WP activities, structure or personnel

We hired a computer scientist (Dr. Leonardo Onofri) working full time on the development of tools for data management, analysis and visualisation.

11.2.6 Outstanding contributions to WP work

The combination of our imaging and analysis methods allow us to perform for the first time quantitative description of cellular distribution on brain-wide scale.

11.3 Cellome: Main Problems

We need to improve our data management and processing pipeline to enter into a real production mode.

We focused our efforts mostly on the development of imaging technologies and of clearing methods. We are now concentrating on the data processing issues by setting up collaborations and hiring internal personnel. We would anyway benefit from help from the Consortium to better deploy acquired data into the HBP mouse brain atlas.

11.4 Cellome: The Next Six Months

This area will improve user-friendliness and reliability of the imaging methods to be able to go into a massive data production.

Pilot full data acquisition pipeline: tissue processing, imaging (both with light sheet microscopy and serial two-photon microscopy), data analysis, data deployment into the HBP data space.

12. Data Aggregation, Analysis and Dissemination (WP1.3)

12.1 Data aggregation: Overall Goals

Deposit data in HBP Mouse Brain Atlas (in T1.3.1)

Establishment of informatics analysis and data management pipeline for linking SP1 molecular and cellular data.

Data sources and tools for molecular and cellular informatics (in T1.3.2)

Development and maintenance of informatics tools.

12.2 Data aggregation: Main Achievements

A review of data to be generated within SP1 and datasets available within the wider community was held at a meeting of key Partners in Edinburgh in February 2014. Together with representatives from SP5 we walked through Use Case scenarios for Data Space and how modellers would access data. The ontologies, where they are used by the molecular groups, were found to be appropriate. However the coverage is incomplete. In particular, the specificity of anatomical location for biological tissue samples used for functional genomic and proteomic analysis varies widely. It was agreed that the WP1.3 groups would interact with the data generating groups to ensure these are appropriate for later modelling use.

A total of ~40 neuronal proteomics datasets (a mix of published and pre-published) have been obtained. For each of these the molecular IDs have been extracted, checked and orthologues mapped across human, mouse, rat and fly. Functional annotation including disease association and genome sequence variation has been obtained for each. These are currently held in both MySQL tables and flat files awaiting the finalisation of the data structure (T1.3.2). Mapping of molecular interaction data for these proteins is mostly complete. Cellular location will be annotated post-hoc using the ontology selected in T1.3.2.

Also, Partner UT has continued to upgrade GeneNetwork 2.0 database by improving mapping routines so that Factored Spectrally Transformed Linear Mixed Models (FaST-LMM) and Geometric Modeling and Multimedia algorithms (GEMMA) are now available. Substantial improvements of interface and code in GeneNetwork 2.0 database have been done, making the data more accessible and useful for a person without bioinformatics background. The code is open source and can be used by all HBP Partners.

The proteomics and functional genomics work completed to date form the internal tools and pipeline for handling the molecular datasets from SP1. This meets MS14 on time.

The molecular groups at EPFL (Telefont and Zwahlen) are developing a data model for interfacing between molecular database activities and molecular modelling. We are expecting this to be released for comment/revision imminently and assuming it is fit for purpose, conversion of existing datasets to this structure should be trivial. We do expect some additional annotation will be required post-hoc.

The proteomics data assembled represented the single most comprehensive resource for neuroproteomics in the field. A public resource and an accompanying publication are under development.

In addition to the core data aggregation work we have also extended the kappa modelling framework. In brief this allows us to integrate proteomic datasets more readily into

cellular simulations. This work is in press and the code required has been make available open source.

12.2.1 Internal monitoring and quality control activities:

- SP1_SKPI-24 Establishment of informatics analysis and data management pipeline:
  - Generation of methods. Planned: 2014/02/28 - 2014/03/31 - done
  - Pipeline development. Planned: 2014/03/31 - 2014/09/30 - done
  - Molecular and Cellular data integration. Planned: 2014/07/31 - 2016/03/31 in progress
- SP1_SKPI-25 Development and maintenance informatics tools allowing integration of molecular data from multiple data sources:
  - Generation of methods. Planned: 2014/07/31 - 2015/03/31 - in progress
  - Tools development. Planned: 2014/04/30 - 2014/09/30 - done
  - Data integration. Planned: 2014/08/31 - 2016/03/31 – in progress

12.3 Data aggregation: Main Problems

We are waiting for the modelling groups to finalise their requirements (i.e. data model) so that we can ensure that exchange will happen smoothly. However meetings to coordinate this have been happening and more are scheduled. We also note that the data the modellers will require are a subset of those we already manage, therefore risk or additional work is likely to be negligible.

12.4 Data aggregation: The Next Six Months

We are preparing a public version of the neuroproteomics data and are working with the data generating groups in SP1 to prepare for the first HBP generated datasets.

13. Challenging Future Research Topics

13.1 Modelling pyramidal neurons for neuromorphic computation

Neuromorphic computation brings a radical change in current computer architectures in order to mimic the way our brain processes information. The more detailed brain circuit diagrams become available, the more we will learn with computer simulations about the role of each element of the circuit. We are working together with computer scientists to bridge between brain structure and function and computer architectures.

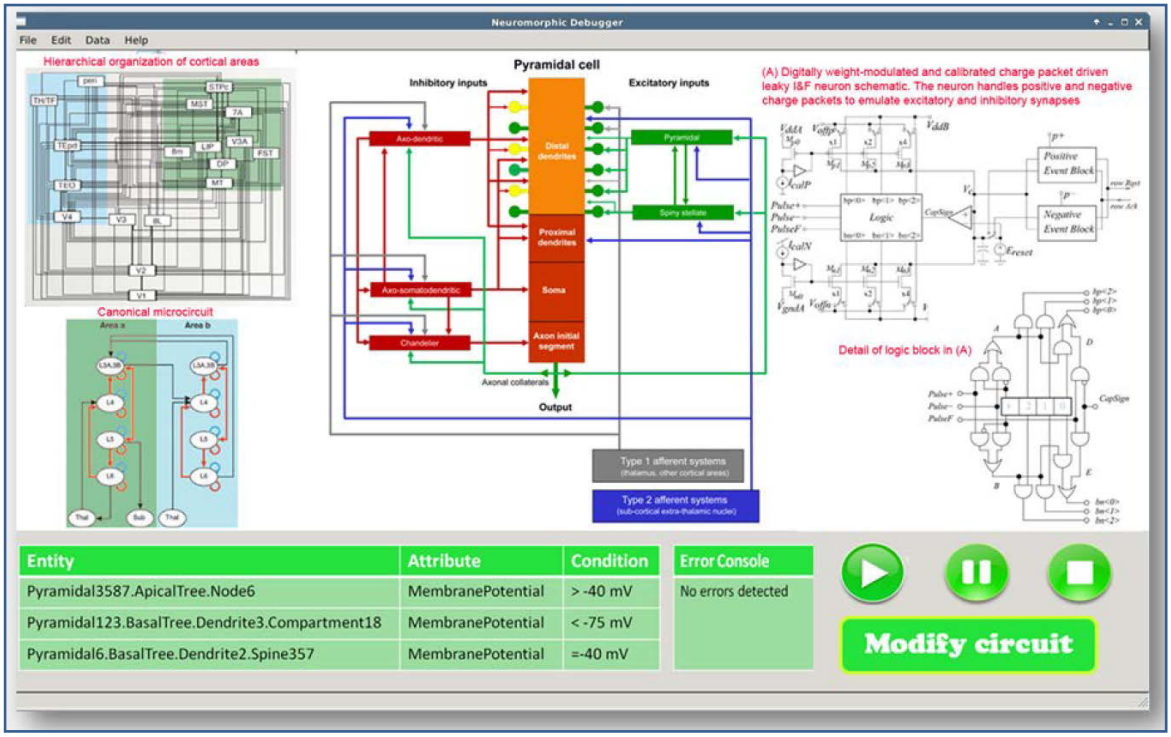


Figure 5: An interactive visualisation development tool & debugger for neuromorphic computation

13.2 Electron microscopy of the human brain

We are trying to improve the ultra-structural preservation of human tissue using microwave oven fixation and embedding protocols

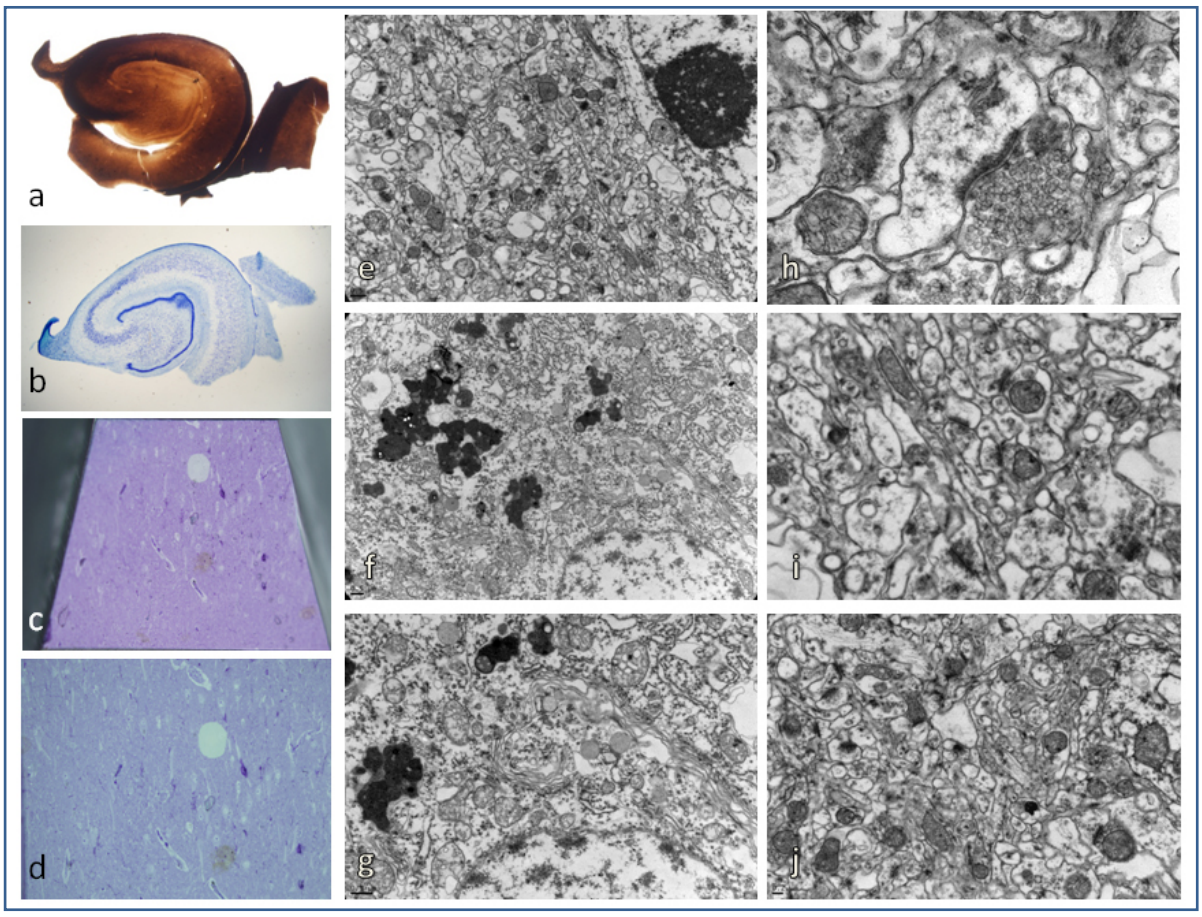


Figure 6: Electron microscopy of the human brain

Human brain tissue (autopsy material) fixed by immersion in aldehydes, using a microwave oven. After post-fixation, tissue sections are embedded in Araldite for electron microscopy (a). Semithin sections are obtained and stained with tolouidin blue (b). The region of interest is selected, trimmed (c) and prepared for ultrathin sectioning (d). Ultrathin sections are stained with Lead citrate and uranyl acetate and photographed under the transmission electron microscope (e-j).

14. Scientific Coordination (WP1.4)

14.1 Scientific Coordination: Internal Meetings

Date	Description	Location	Participants	Comments
5-6 /5/2013	First SP1 meeting	Madrid (Spain)	All SP1 Members	This meeting was held ahead of the official launch of the Project.
July 2013	SP1 meeting	Firenze (Italy)	SP1 Members (LENS, UAM UEDIN)	This meeting was held ahead of the official launch of the Project.
24-25/2/2014	SP1 meeting	Edinburgh (UK)	All SP1 Members from the Molecular section & UPM	
16/7/2014	SP1 Competitive Call molecular meeting	VC	Grant and all Partners from T1.1.4-1.1.7	
29/9/2014	SP1 Meeting	Heidelberg (Germany)	SP1 Participants & HBP STO & members from the HBP Editing Team	

Table 1: Meetings between SP staff

14.2 Scientific Coordination: HBP Meetings

Date	Description	Location	Participants	Comments
5/11/2014	HBP Wiki	VC	SP1 & SP5	
15/7/2014	Cellular section of SP1: Thalamocortical projections	VC	SP1 & SP5	
7/7/2014	FENS		SP1 & SP5	
17/7/2014	Data Meeting	VC	SP1,2,3,4,5, etc	
12/08/2014	HBP efforts in single cell transcriptome data generation.	KI (Stockholm Sweden)	SP1 & SP5	Meeting in Stockholm linked to the Single Cell Genomics meeting
19/08/2014	Cross- SP meeting: Modeling pyramidal neurons using neuromorphic technologies	Madrid (Spain)	SP1 & SP5 & SP7 (Visualisation) & SP9	
21/8/2014	Data management and annotation	Zurich (Switzerland)	SP1,SP5	
30/9/2014	Cross- SP meetings	Heidelberg (Germany)	SP1 & SP2 (slot I)	
30/9/2014	Cross- SP meetings	Heidelberg (Germany)	SP1 & SP3 (slot II)	
1/10/2014	Cross- SP meetings	Heidelberg (Germany)	SP1,SP2, SP5, SP6	
1/10/2014	MGT Workshop	Heidelberg (Germany)	SP1 (P.F.Romero) & SP13 & Others	

Table 2: Meetings between this SP and other SPs

14.3 Scientific Coordination: External Meetings

Date	Description	Location	Participants	Comments
1/10/2013	External Meeting	Madrid (Spain)	SP1 (DeFelipe) & Scientific Community & Private sector	Desarrollo Clínico
28/10/2013	Meeting about HBP Spanish Participation	Madrid (Spain)	SP1, SP2, SP3, SP4, SP5, SP7, SP11 [DeFelipe (UPM); F. Romero (UPM); León (UPM, HBP national representative); Merchán (UPM); Barrera (UPM); Clascá (UAM); Slater (UB); Deco (UPF); Peña (UPM); Larrañaga (UPM); Labarta (BSC); Girona (BSC); Arcara (BSC); Martín (UPM); Pastor (URJC); Ros (UG)]	
4/11/2013	Meeting with the Spanish Funding Agency	Madrid (Spain)	SP1 (DeFelipe) & Representatives of the Spanish Funding Agency	
13/11/2013	Meeting about scanning workflow	PSI (Switzerland)	SP1 (Weber) & Group of Prof. M. Stampanoni	
28/11/2013	Meeting with the Spanish Funding Agency	Madrid (Spain)	SP1 (DeFelipe) & León (UPM, HBP national representative) & Representatives of the Spanish Funding Agency	
12/12/2013	Meeting with the Spanish Funding Agency	Madrid (Spain)	SP1 (DeFelipe) & León (UPM, HBP national representative) & Representatives of the Spanish Funding Agency	
28/1/2014	Meeting with the Spanish Funding Agency	Madrid (Spain)	SP1 (DeFelipe) & León (UPM, HBP national representative) & Representatives of the Spanish Funding Agency & Representatives of some companies from the private sector	
18-19/2/2014	FLAGERA 2nd Meeting	Rome (Italy)	SP1 (DeFelipe) & others HBP members & FLAGERA members	

Date	Description	Location	Participants	Comments
22/4/2014	Meeting with the Spanish Funding Agency	Madrid (Spain)	SP1 (DeFelipe) & León (UPM, HBP national representative) & Representatives of the Spanish Funding Agency & Representatives of some companies from the private sector	
6/5/2014	Meeting of Transfer about software licensing issues	ETH (Switzerland)	SP1 (Weber) & ETH members	
11/6/2014	Meeting about contrast media	PSI (Switzerland)	SP1 (Weber) & Group of Prof. M. Stampanoni	

Table 3: Meetings between this SP and Partners outside the HBP

14.4 Scientific Coordination: Monitoring and Quality Control

As stated above, from the beginning of the Project, a regular internal communication flow has been established to ensure the information exchange among the WP participants as well as with the Molecular Section of the SP1. This communication flow has been used to ensure that work has been performed as scheduled and to detect and correct deviations, where appropriate. Specifically, we have been in direct contact on a weekly basis with the laboratories involved in the WP by electronic means (email, Skype calls, etc.), In addition, the Milestones scheduled in the Project work plan, together with the scientific KPIs defined in the report D1.4.1 have been used as internal tools for monitoring the work progress in the Tasks involved in the WP.

14.5 Scientific Coordination: Additional Comments

WP1.4 is progressing well and as scheduled as reported in EMDESK. However, in the first Project Year there was a deviation – the delay in submitting this report, which was due in M12. The reason for this was timeline constraints. SP1 directors and managers are aware of the impact that this issue could have on the HBP. SP1 management will focus on avoiding delays in the future, while ensuring that other activities also receive adequate attention.

14.6 Scientific Coordination: The Next Six Months

WP1.4 will continue working on Task T1.4.1 to ensure that work within SP1 is carried out according to the planned objectives concerning coordination of SP1 Partners, coordination of both SP1 sections, molecular & cellular, reporting activity, communication, disseminations and ethical issues in SP1. In addition, WP1.4 will be particularly focused on the achievement of Milestones planned for M13-M30 together with the monitoring of KPIs for SP1 activities. Finally, another crucial issue that WP1.4 will be dealt with is the drawing up of the Deliverables scheduled for M13-M30. These Deliverables are as follows:

- D1.4.3: Strategic Mouse Brain Data for the HBP Mouse Atlas: Package one. To be delivered in M18.

- D1.4.4: Strategic Mouse Brain Data for the HBP Mouse Atlas and modelling: Package two. To be delivered in M30.

Annex A: Milestones

No.	Milestone Name	WP	Month Due	Month Achieved	See Page
MS1	Proof of concept for cell-type transcriptomic analysis; set-up of cellular resolution whole brain ultramicroscopic scanning.	1.1	6	6	13
MS2	Strategy established for mapping distribution of specific proteins and proteomes across the whole mouse brain.	1.1	12	12	16
MS6	Scanning and counting methods (set up of the methods for 3D counting of cells and puncta (immunoreactive or GFP puncta).	1.2	6	12	29
MS12	Informatics and data management set-up.	1.2	6	6	36
MS13	Data format and ontology specifications for molecular experiments.	1.3	6	6	8
MS14	Informatics tools v1 ready for managing molecular and cellular data.	1.3	12	12	41
MS252	Automated homology models (T1.1.5).	1.1	12	12	20

Annex B: Scientific Key Performance Indicators (SKPIs)

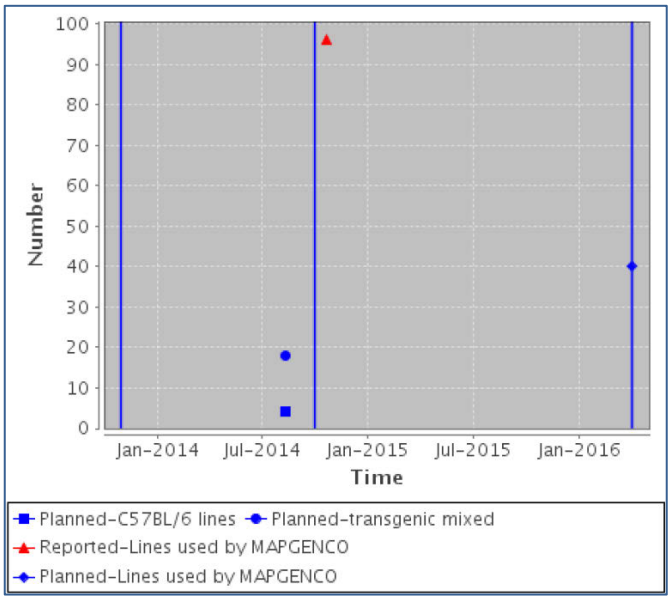
This SP's current KPI information can be seen in the STO's KPI website via this link:

https://flagship.kip.uni-heidelberg.de/jss/CollectKPI?ui=268&s=UJuR3AgTezrb&um=sPO&oSP=1

SP1_SKPI-01 No of suitable mouse lines for cell-type studies

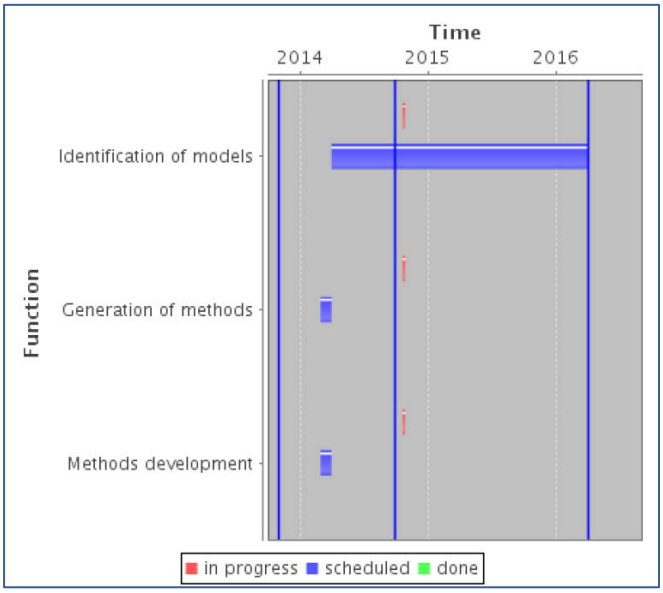
Responsible: labwilliams@gmail.com

2014/10/20 (value 96): All available from UTHSC and/or the Jackson Laboratory



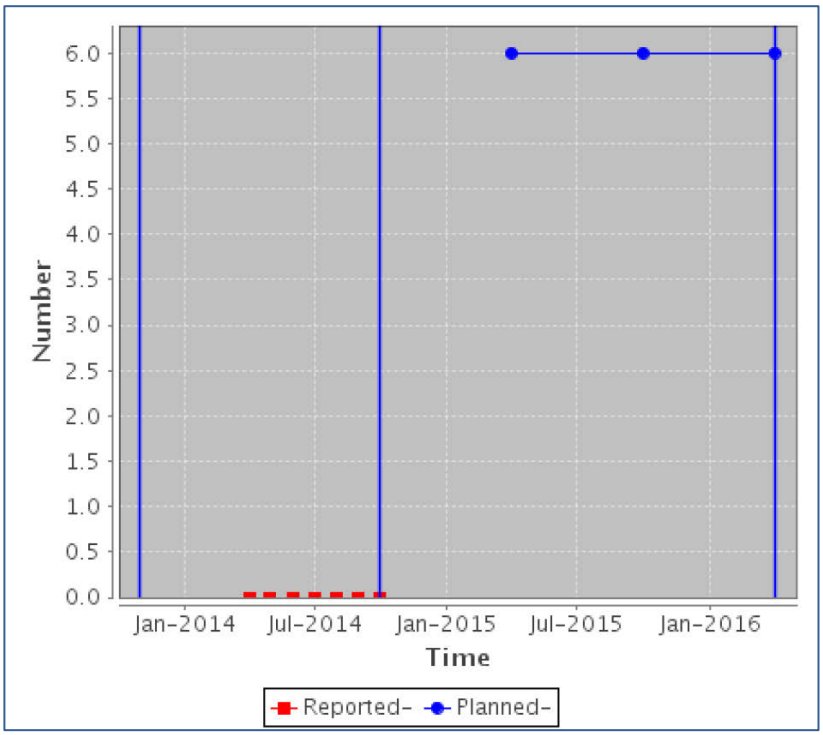
SP1_SKPI-14 Identification of mouse lines

- Identification of models. Planned: 2014/03/31 - 2016/03/31
- Generation of methods. Planned: 2014/02/28 - 2014/03/31
- Methods development. Planned: 2014/02/28 - 2014/03/31
- Responsible: labwilliams@gmail.com



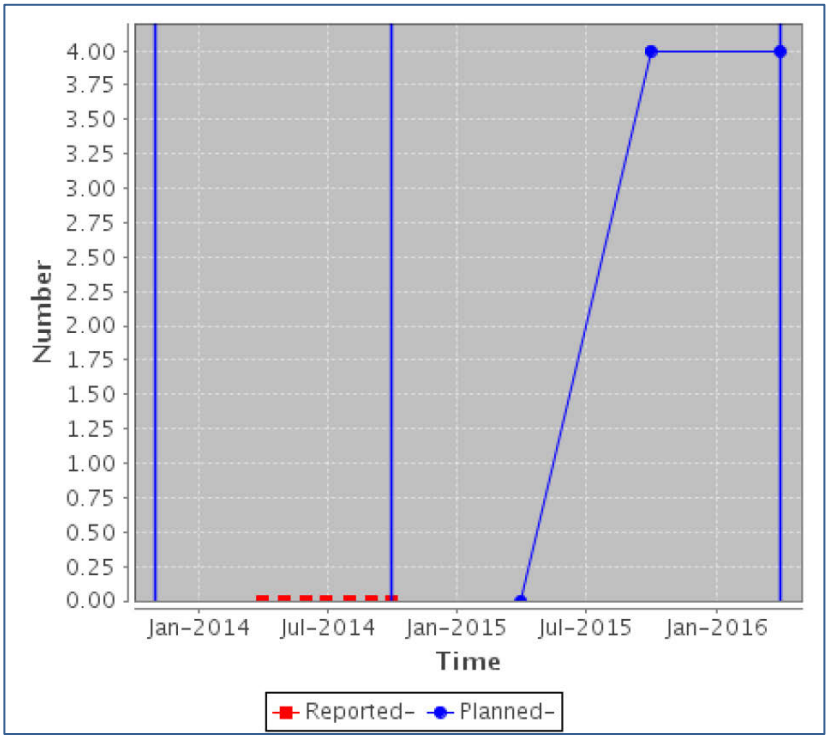
SP1_SKPI-02 No. of neocortical neuron types with first draft SCTs

Responsible: chris.ponting@dpag.ox.ac.uk



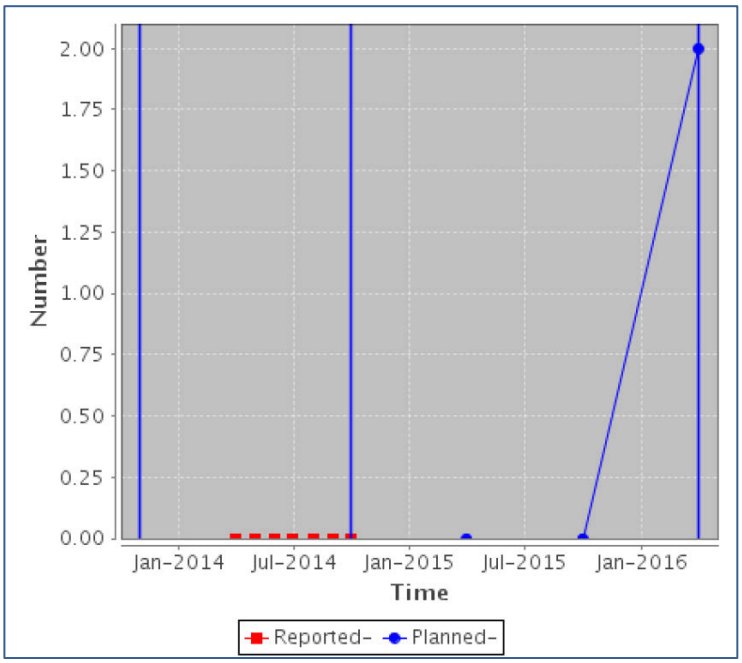
SP1_SKPI-03 No. of hippocampal neuron types with first draft SCTs

Responsible: chris.ponting@dpag.ox.ac.uk



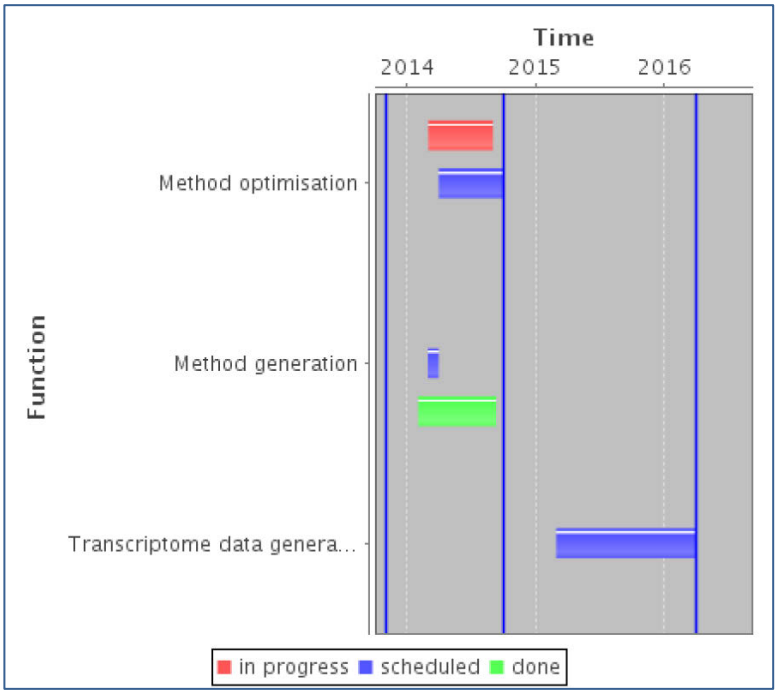
SP1_SKPI-04 No. of cerebellum neuron types with first draft SCTs

Responsible: chris.ponting@dpag.ox.ac.uk



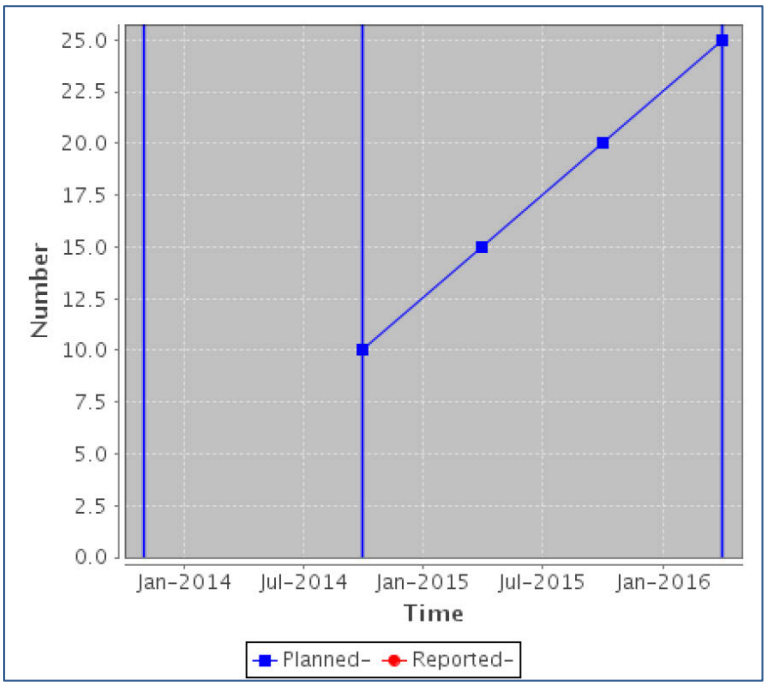
SP1_SKPI-15 Establishment of method for single cell transcriptomics (SCTs) from well characterised neuron-types

- Method generation. Planned: 2014/02/28 - 2014/03/31
- Method optimisation. Planned: 2014/03/31 - 2014/09/30
- Transcriptome data generation. Planned: 2015/02/28 - 2016/03/31
- Responsible: chris.ponting@dpag.ox.ac.uk



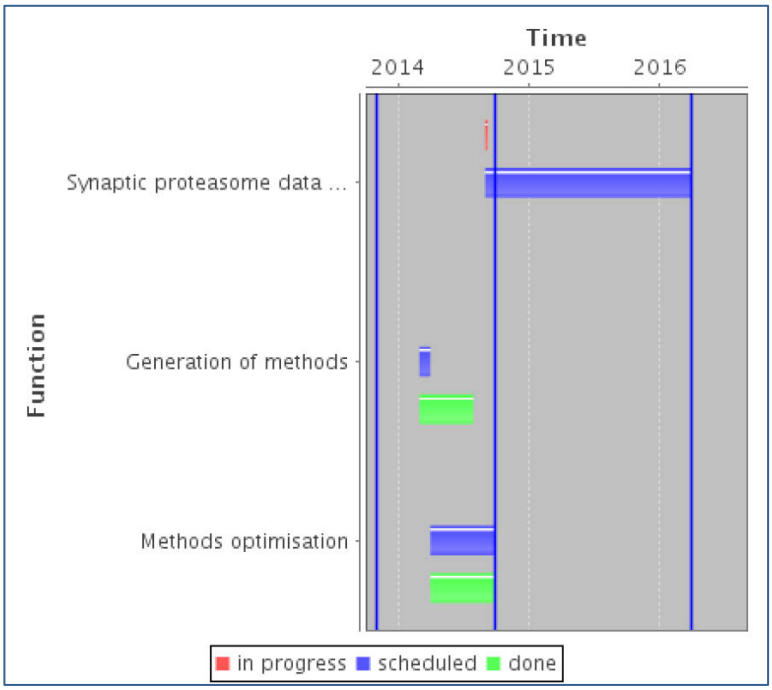
SP1_SKPI-05 No. of brain region synapse proteomes

Responsible: elaine.marshall@ed.ac.uk



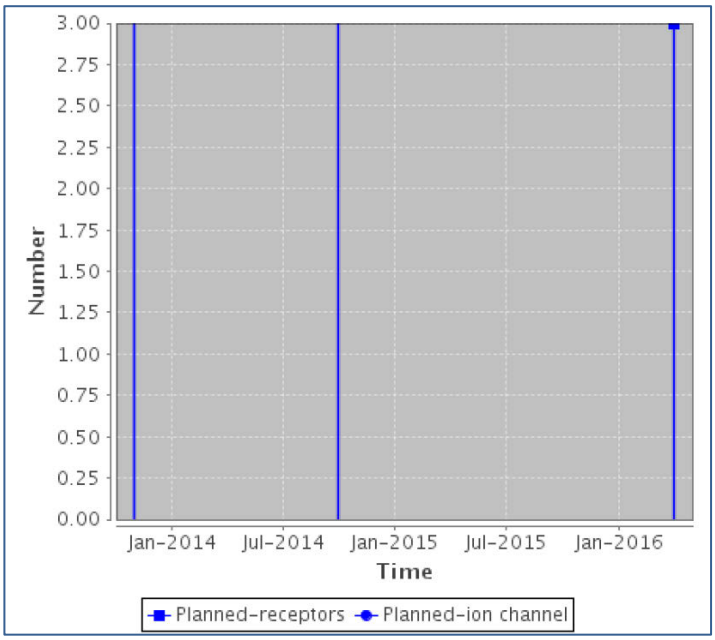
SP1_SKPI-16 Development of proteomic techniques

- Generation of methods. Planned: 2014/02/28 - 2014/03/31
- Methods optimisation. Planned: 2014/03/31 - 2014/09/30
- Synaptic proteasome data generation. Planned: 2014/08/31 - 2016/03/31
- Responsible: elaine.marshall@ed.ac.uk



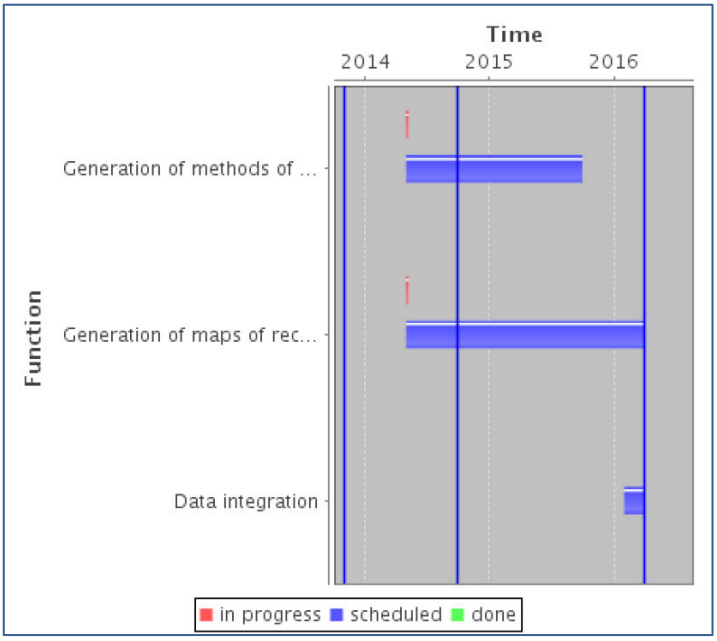
SP1_SKPI-32 No of maps for receptors and ion channels

Responsible: rafael.lujan@uclm.es



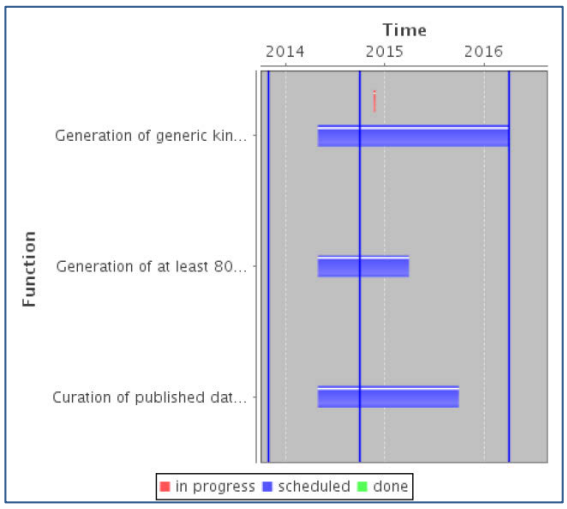
SP1_SKPI-31 Maps: Neural channelomics and receptomics

- Generation of methods of 2D and 3D ion channel mapping. Planned: 2014/04/30 - 2015/09/30
- Generation of maps of receptor and ion channel distribution. Planned: 2014/04/30 - 2016/03/31
- Data integration. Planned: 2016/01/31 - 2016/03/31
- Responsible: rafael.lujan@uclm.es



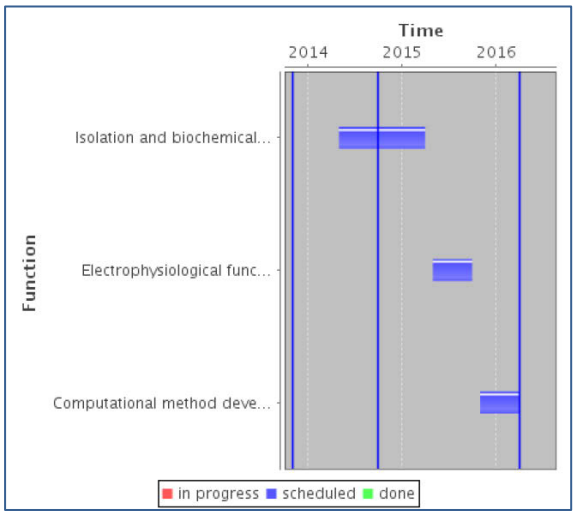
SP1_SKPI-34 Potassium channels kinetics

- Generation of at least 80 3-dimensional homology models. Planned: 2014/04/30 - 2015/03/31
- Curation of published data for a representative member of 5 subfamilies. Planned: 2014/04/30 - 2015/09/30
- Generation of generic kinetic models for subgroups of channels to be defined. Planned: 2014/04/30 - 2016/03/31
- Responsible: ioannis.xenarios@isb-sib.ch



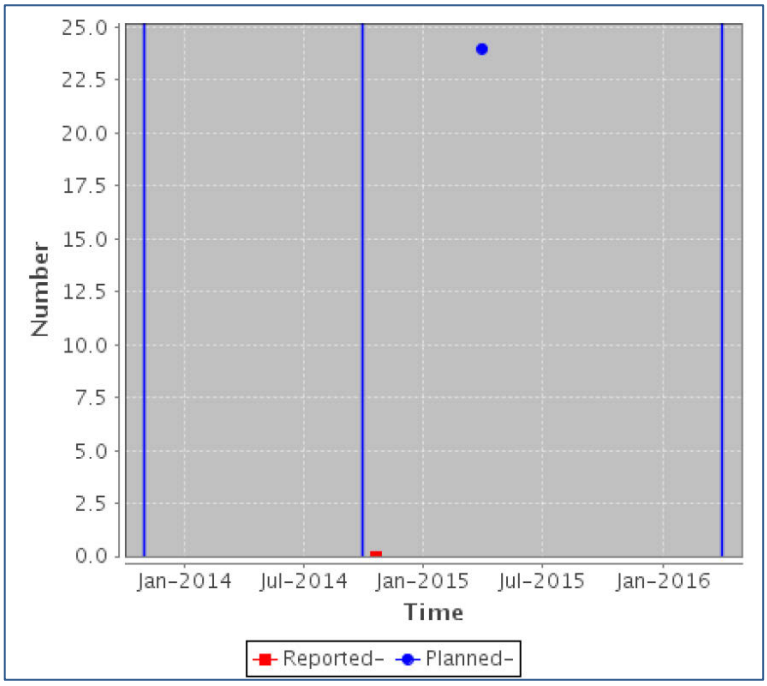
SP1_SKPI-33 Trans-synaptic signalling and receptor kinetics

- Isolation and biochemical validation of antibodies against gephyrin and neuroligin proteins. Planned: 2014/04/30 - 2015/03/31
- Electrophysiological functional validation of intrabodies against gephyrin and neuroligin protein. Planned: 2015/04/30 - 2015/09/30
- Computational method development for synaptic model. Planned: 2015/10/31 - 2016/03/31
- Responsible: antonino.cattaneo@sns.it



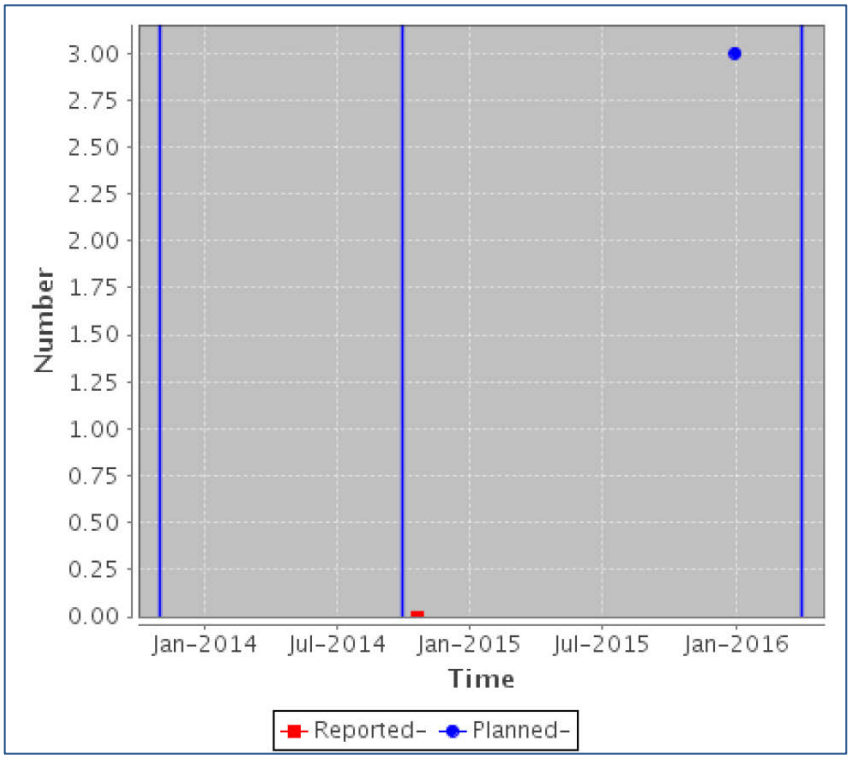
SP1_SKPI-27 Touchscreen analysis of recombinant inbred BxD lines, testing of 24 mouse lines

Responsible: max@synome.eu 2014/10/20 (value 0): Working



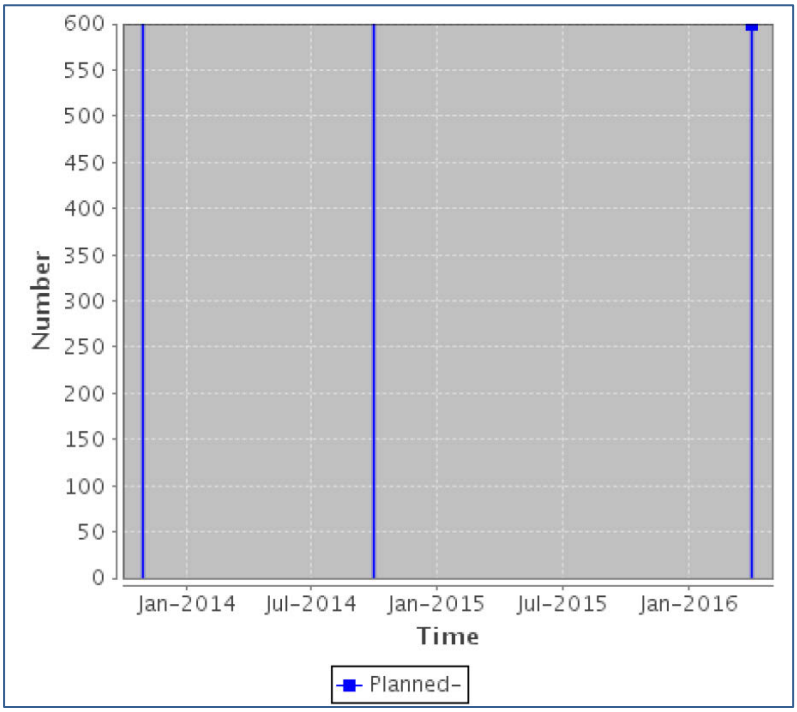
SP1_SKPI-28 Touchscreen analysis of gene-targeted mutant mice

Responsible: max@synome.eu 2014/10/20 (value 0): NotStarted



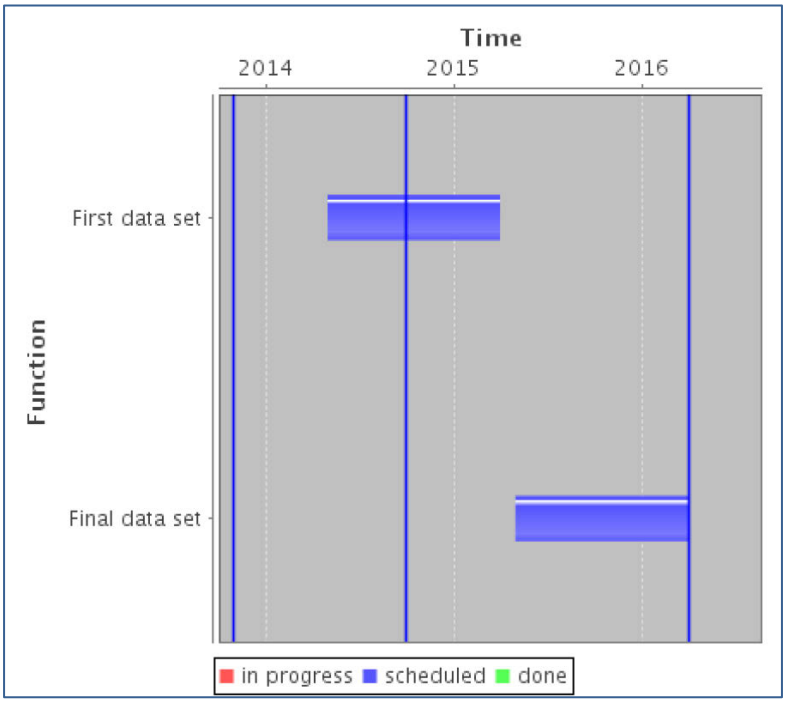
SP1_SKPI-30 Proteomic analysis of BxD prefrontal cortex specimen, total around 600 proteins

Responsible: maarten.loos@sylics.com



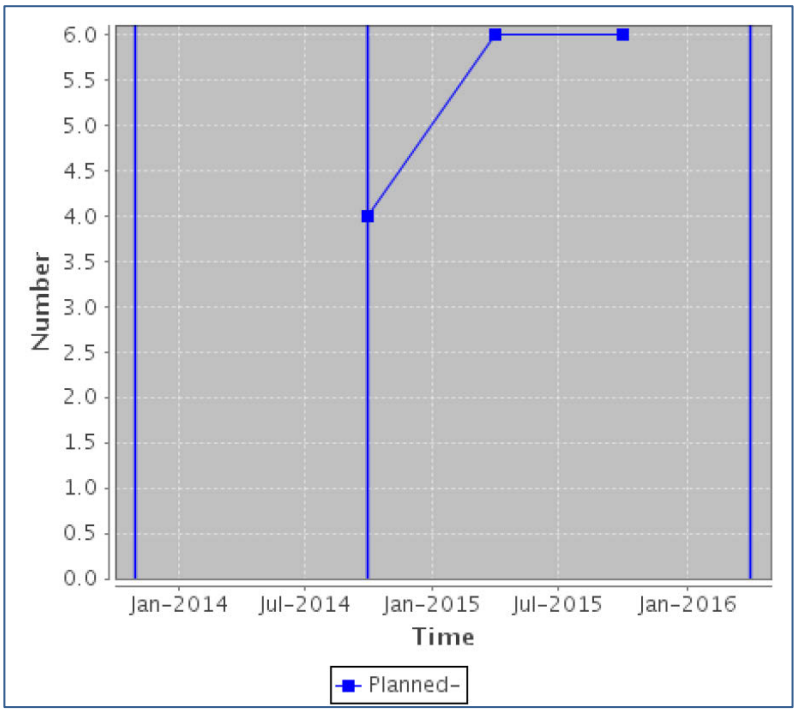
SP1_SKPI-29 Behavioural analysis of BxD mice at Synaptologics

- First data set. Planned: 2014/04/30 - 2015/03/31
- Final data set. Planned: 2015/04/30 - 2016/03/31
- Responsible: max@synome.eu



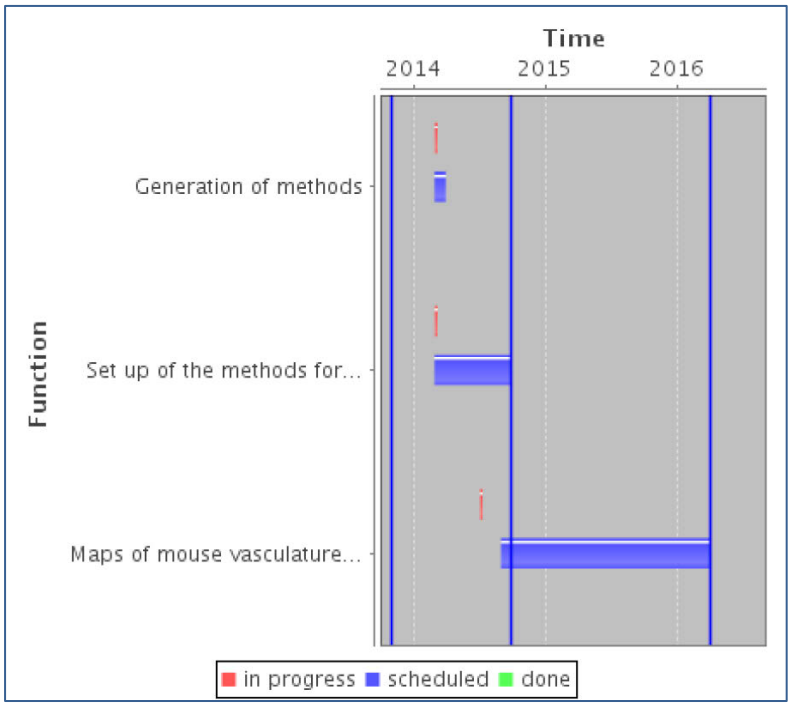
SP1_SKPI-06 No. of maps of mouse vasculature

Responsible: bweber@pharma.uzh.ch



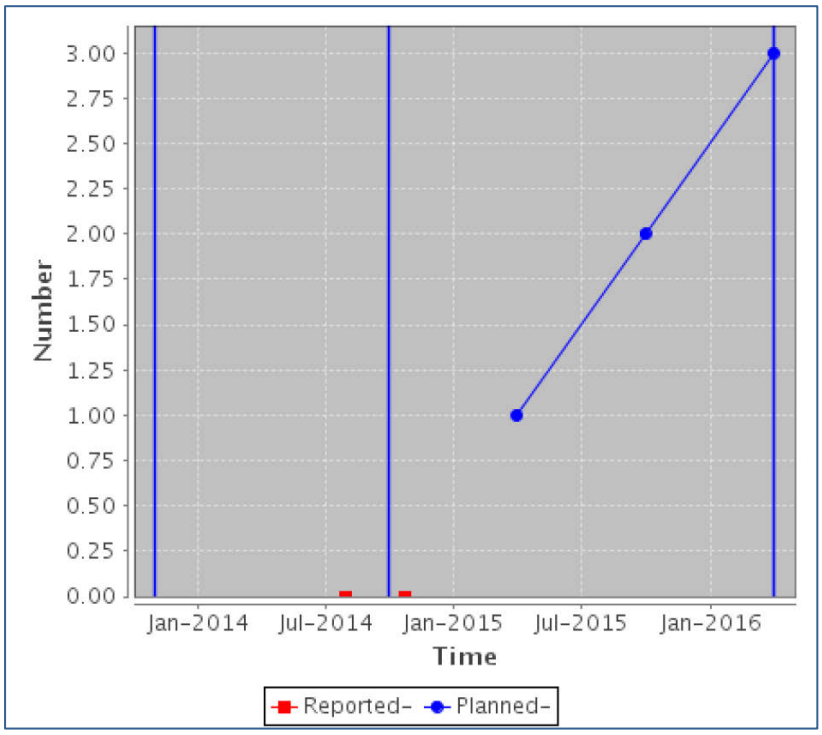
SP1_SKPI-17 Reconstruction of the brain vascular system

- Generation of methods. Planned: 2014/02/28 - 2014/03/31
- Set up of the methods for reconstruction. Planned: 2014/02/28 - 2014/09/30
- Maps of mouse vasculature generation. Planned: 2014/08/31 - 2016/03/31
- Responsible: bweber@pharma.uzh.ch



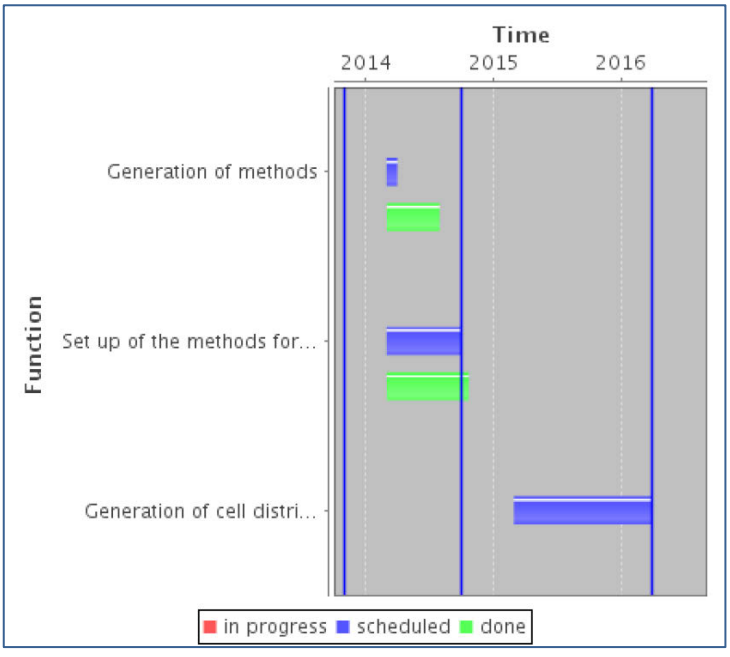
SP1_SKPI-07 No. of cell distribution maps of the whole mouse brain

Responsible: francesco.pavone@unifi.it



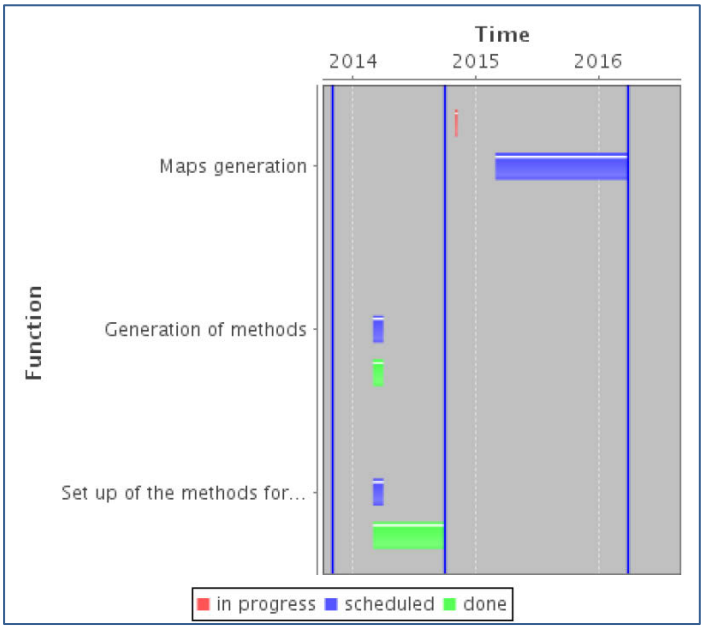
SP1_SKPI-18 Acquisition of whole brain scans for labelled cells

- Generation of methods. Planned: 2014/02/28 - 2014/03/31
- Set up of the methods for 3D cell localisation. Planned: 2014/02/28 - 2014/09/30
- Generation of cell distribution maps. Planned: 2015/02/28 - 2016/03/31
- Responsible: francesco.pavone@unifi.it



SP1_SKPI-19 Establishment of automated methods to generate maps of inhibitory and excitatory neurons and glia

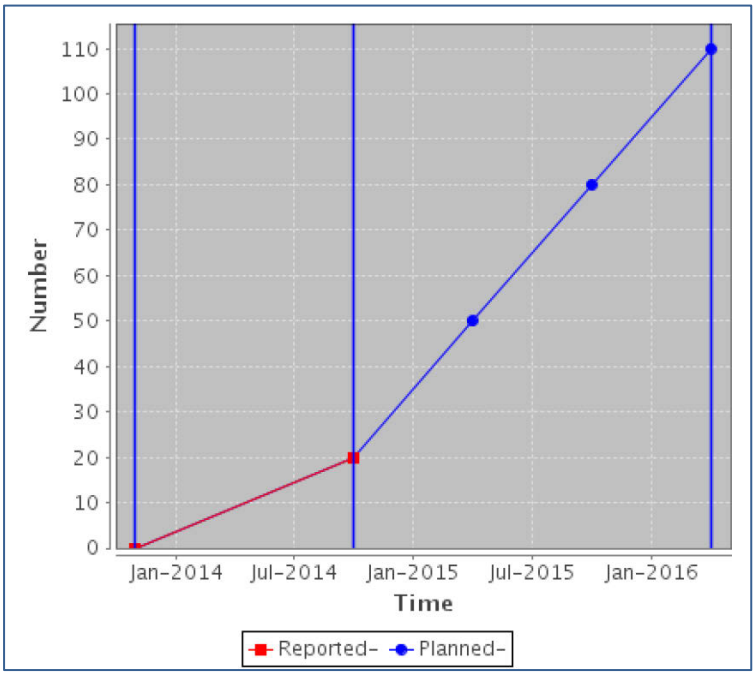
- Generation of methods. Planned: 2014/02/28 - 2014/03/31
- Set up of the methods for 3D counting. Planned: 2014/02/28 - 2014/03/31
- Maps generation. Planned: 2015/02/28 - 2016/03/31
- Responsible: pilarfr@cesvima.upm.es



T1.2.4 Morphological analysis of neurons and glia

SP1_SKPI-09 No. of mouse cell morphologies reconstructed (hippocampus)

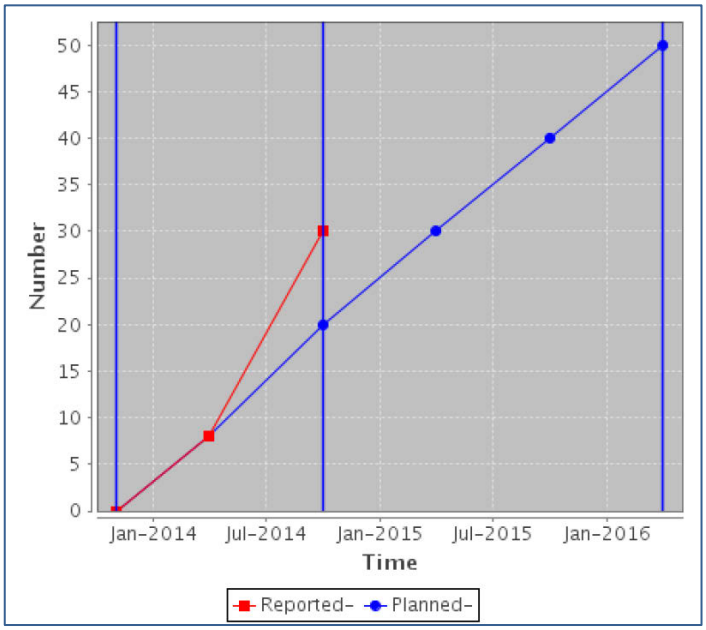
Responsible: yun.wang@tufts.edu



SP1_SKPI-10 No. of mouse cell morphologies reconstructed (neocortex)

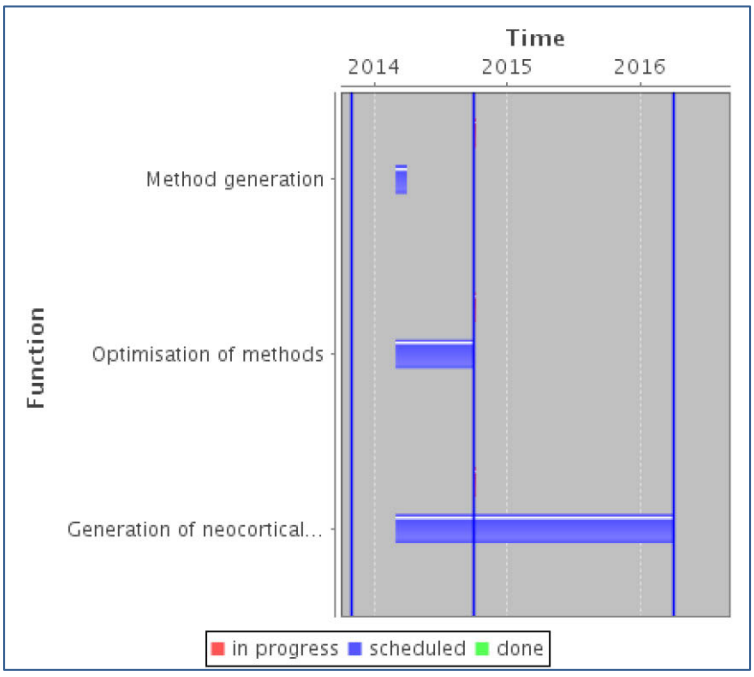
Responsible: yun.wang@tufts.edu 2014/09/30

SSC, 19 from image stack of a whole rat brain; 3 whole-structure PCs from image stack of a GFP whole mouse brain (in progress)



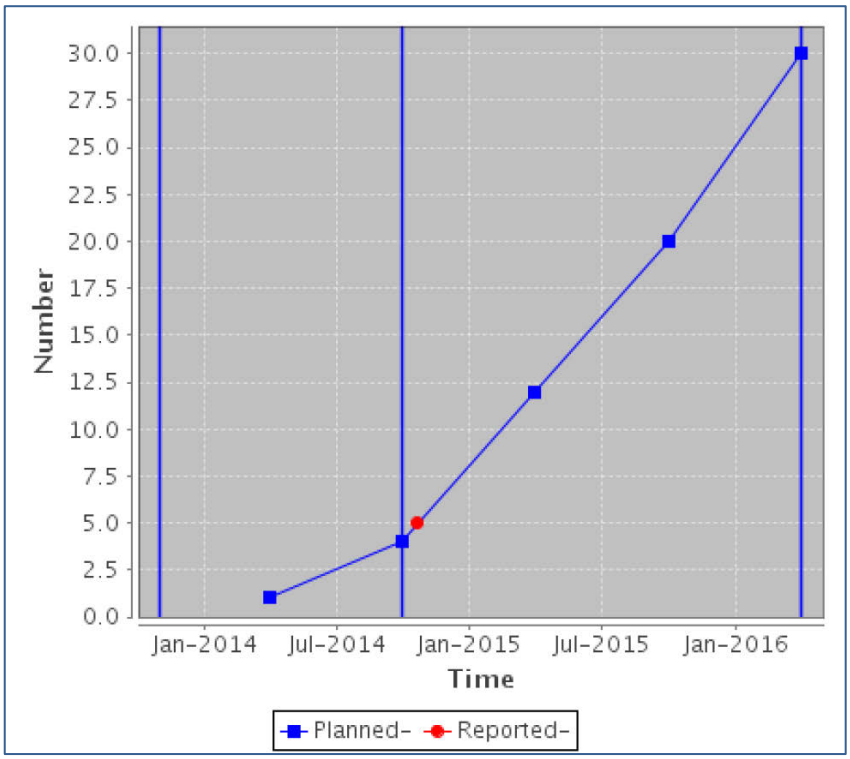
SP1_SKPI-20 Reconstruction and classification of mouse neurons and glia

- Method generation. Planned: 2014/02/28 - 2014/03/31
- Optimisation of methods. Planned: 2014/02/28 - 2014/09/30
- Generation of neocortical and hippocampal cell morphologies. Planned: 2014/02/28 - 2016/03/31
- Responsible: kali@koki.hu



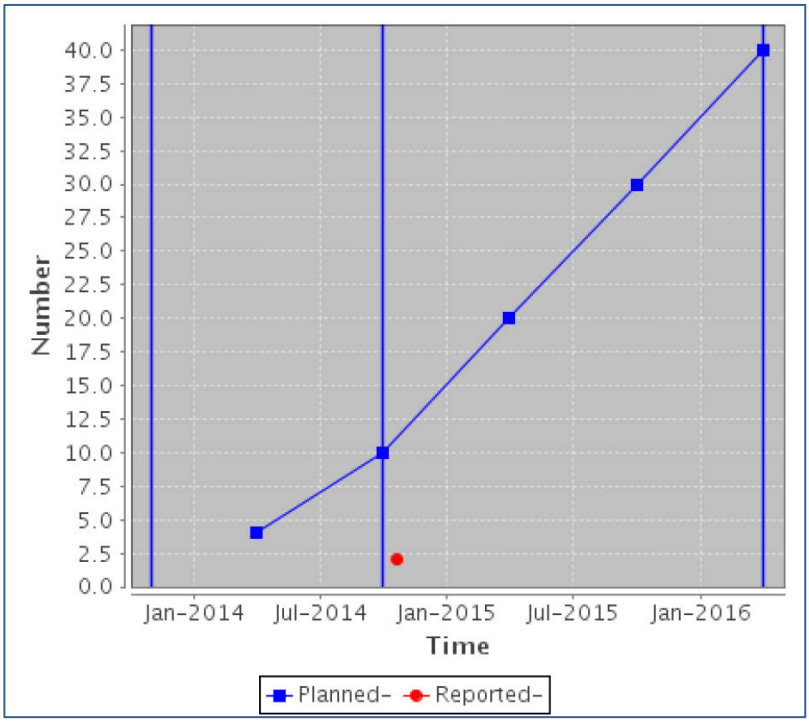
SP1_SKPI-11 No. of axonal projections traced

Responsible: francisco.clasca@uam.es



SP1_SKPI-12 No. of terminal axon branches and monosynaptic targets identified

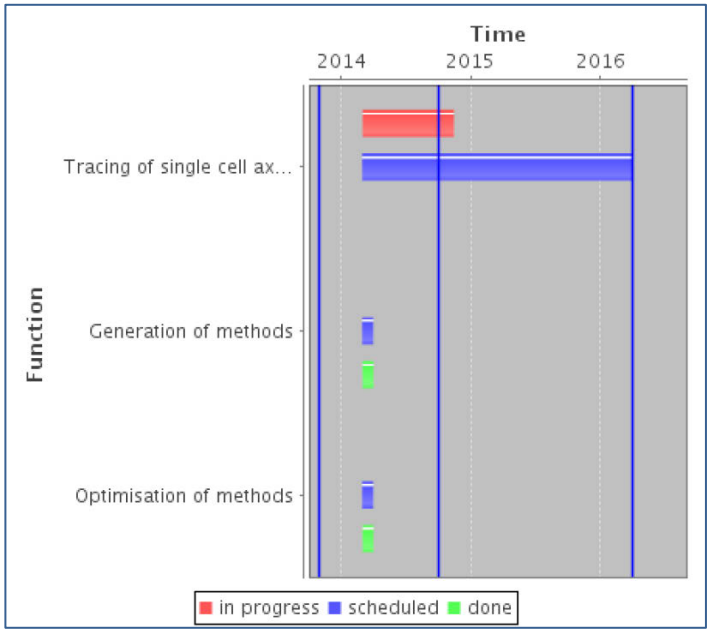
Responsible: francisco.clasca@uam.es



SP1_SKPI-21 Tracing of axonal projections

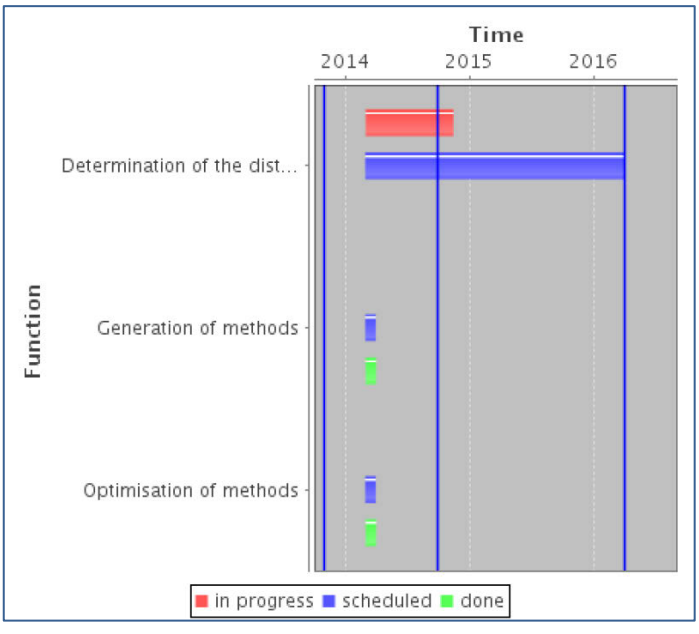
- Generation of methods. Planned: 2014/02/28 - 2014/03/31

- Optimisation of methods. Planned: 2014/02/28 - 2014/03/31
- Tracing of single cell axonal projections. Planned: 2014/02/28 - 2016/03/31
- Responsible: francisco.clasca@uam.es



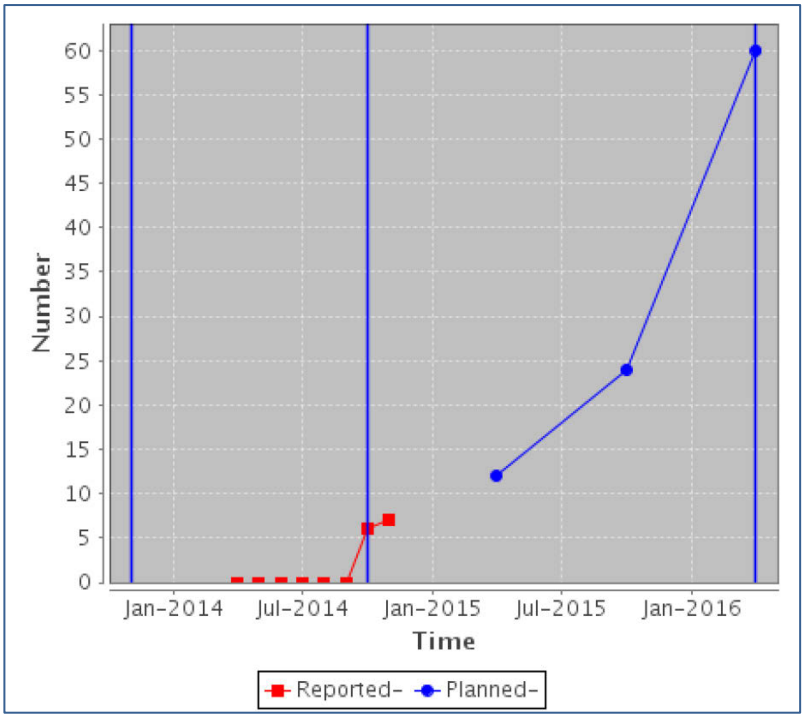
SP1_SKPI-22 Identification and distributions of terminal axon branches and monosynaptic targets

- Generation of methods. Planned: 2014/02/28 - 2014/03/31
- Optimisation of methods. Planned: 2014/02/28 - 2014/03/31
- Determination of the distribution of terminal axons branches. Planned: 2014/02/28 - 2016/03/31
- Responsible: francisco.clasca@uam.es



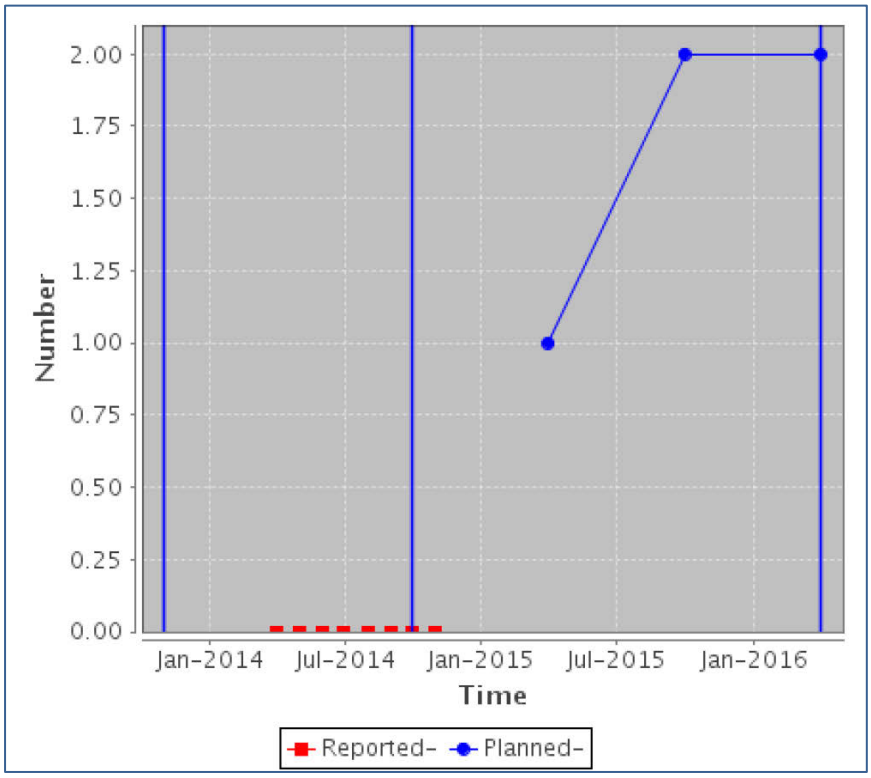
SP1_SKPI-08 No. of synaptic maps of individual identified neurons

Responsible: pilarfr@cesvima.upm.es



SP1_SKPI-13 No. of synaptic maps of mouse brain

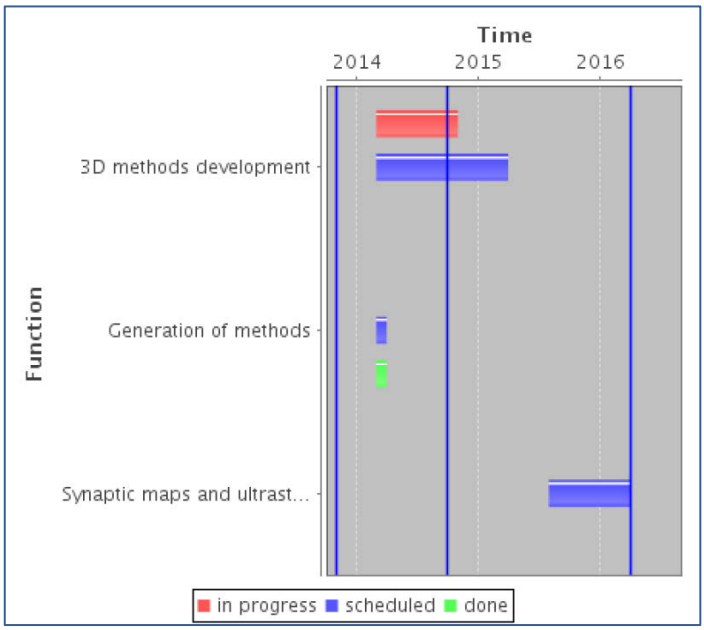
Responsible: pilarfr@cesvima.upm.es



SP1_SKPI-23 Generation of synaptic maps of brain regions

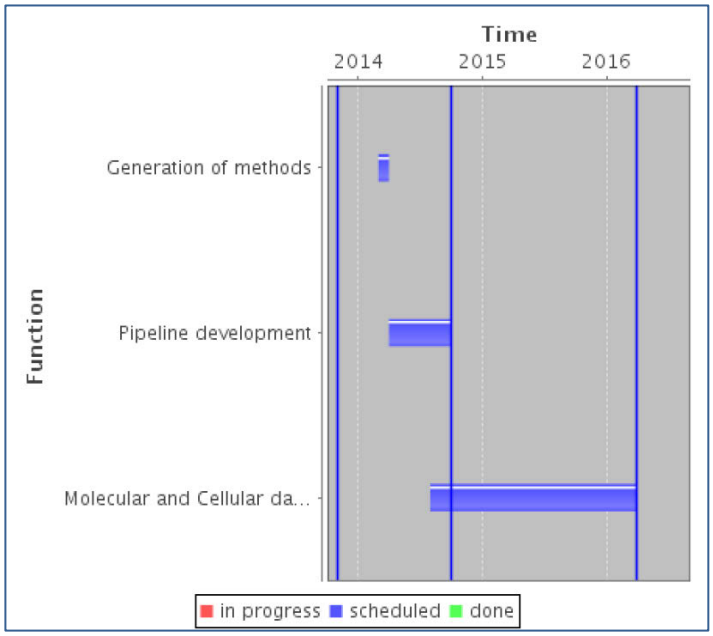
- Generation of methods. Planned: 2014/02/28 - 2014/03/31
- 3D methods development. Planned: 2014/02/28 - 2015/03/31

- Synaptic maps and ultrastructural data. Planned: 2015/07/31 - 2016/03/31
- Responsible: pilarfr@cesvima.upm.es



SP1_SKPI-24 Establishment of informatics analysis and data management pipeline

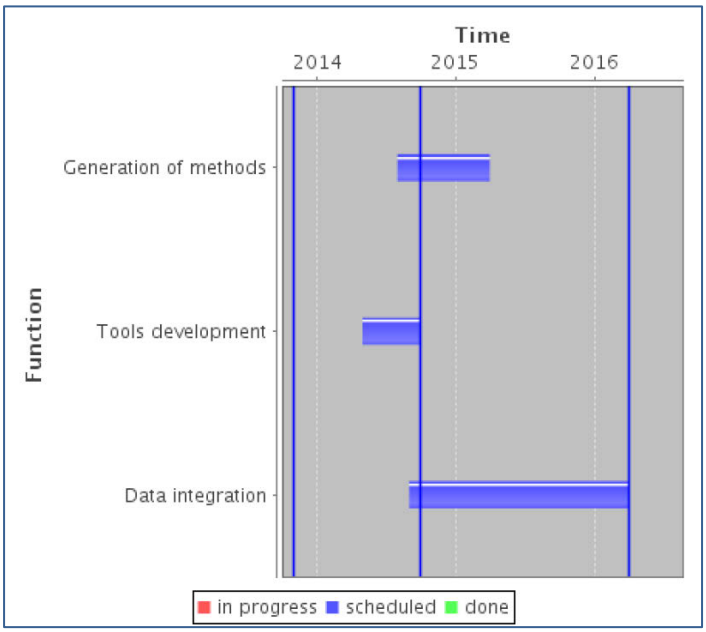
- Generation of methods. Planned: 2014/02/28 - 2014/03/31
- Pipeline development. Planned: 2014/03/31 - 2014/09/30
- Molecular and Cellular data integration. Planned: 2014/07/31 - 2016/03/31
- Responsible: jda@inf.ed.ac.uk



SP1_SKPI-25 Development and maintenance informatics tools allowing integration of molecular data

- Generation of methods. Planned: 2014/07/31 - 2015/03/31
- Tools development. Planned: 2014/04/30 - 2014/09/30
- Data integration. Planned: 2014/08/31 - 2016/03/31

- Responsible: jda@inf.ed.ac.uk



SP1_SKPI-26 SP1 Meetings, Responsible: pilarfr@cesvima.upm.es