SP1 Mouse Brain Organisation and Interspecies Comparisons: First Data Results package (update on implementation of the DMP) (D1.6.1 – SGA2)

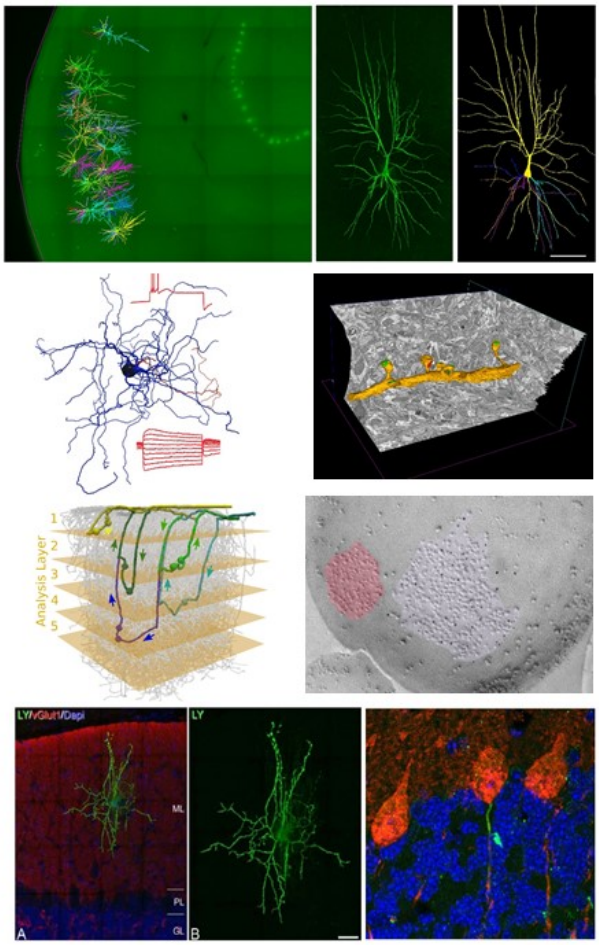

Figure 1: Examples of outputs contributing to SP1 Key Results in the period M1-M12 from different laboratories

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Description in GA:	This is the first deliverable report that includes the preliminary list of deliveries for the period M01-M12 including datasets, tools, and models. The results expected to be included are as follows: preliminary datasets generated in the SGA2 on whole brain and four major brain circuits (neocortex, cerebellum, basal ganglia, hippocampus); First draft on the integration of molecular, cellular and functional data (neocortex, cerebellum, basal ganglia, hippocampus) and its application for modelling; Preliminary datasets for the comparative study of cells and microcircuits in the rodent and human brain.
Abstract:	This version is the revised version of Deliverable D1.6.1. This Deliverable outlines the main SP1 outputs during the period M1-M12. These outputs are releases of the components planned in the SP1 roadmap. The SP1 studies have adopted all advanced techniques required to meet the needs of SP6 in particular, as well as those of SP4, SP10, CDP1 and CDP2. The SP1 main outputs outlined in this report are included in the five main SP1 Key Results which are based on the SP1 Data Strategy. In addition, the SP DMP has been updated in terms of outputs (releases) as planned.
Keywords:	Molecular and Subcellular, Cellular and Microcircuits, Whole-Brain, Datasets, IT tools, models, data integration, comparative studies
Target Users/Readers:	Consortium members, Neuroscience community, Computational neuroscience community.

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History of Changes made to this Deliverable (post Submission)

Date	Change Requested / Change Made / Other Action
3 Apr 2019	Deliverable submitted to EC
22 Jul 2019	Resubmission with specified changes requested in Review Report Main changes requested: • To improve the contents of the report in terms of the outputs displayed in KR1.1, KR1.2, KR1.3, KR1.4 and KR1.5 including the following aspects: (i) The **integration of the outputs in the HBP infrastructure** is included (ii) The **tools or methods developed** in the tasks in M1-M12 to be exploited are reported. (iii) The **integration and impact of the results from SP1 on the activities of other SPs** within HBP is included. Specify the SP/s and the activities. (iv) How **novel neuroscientific concepts arise from the generation of data** is visible from the report. (v) That **data generated in M1-M12 is accessible** for the reviewers to evaluate. (vi) The **datasets on the Collaboratory platform are in good shape** and reflect the resources spent. (vii) The information displayed refers to the HBP project. If collaborations with other projects are reported, ensure that **the benefit for the HBP is visible and clear.** • Clarification of the SP1 DMP Update by M12 • The information regarding the **Curation process** should be included
10 Jan 2020	Revised draft sent by SP1 to PCO. Main changes made, with indication where each change was made: • Revision of the 'Introduction': Data Strategy included; Access to the SP1 DMP updated (see Section Introduction) • Revision and update of KR1.1: Datasets available and well-structured. Links to data added to Outputs 1-8; Data integration in SP1, SP2, SP5, SP6 and CDP2 stated (see Section 3 Key Result KR1.1) • Revision and update of KR1.2: Datasets available and well-structured; Links to datasets added to Outputs 1-7; Datasets already in the KG stated; Data integration in modelling tasks of SP6 indicated; Collaboration with SP5 displayed (see Section 4 Key Result KR1.2) • Revision and update of KR1.3: Links to datasets added to Outputs 1 and 2; Data integration in other SPs, SP3, SP4, SP5 and SP10, stated; Datasets already in the KG stated; CDP1 strengthened (see Section 5 Key Result KR1.3) • Revision and update of KR1.4: Links to datasets added to Outputs 1-9; Data integration in other SPs, SP3, SP4, and SP6, stated; Link to the SGA3 indicated (see Section 6 Key Result KR1.4) • Revision and update of KR1.5: Links to datasets added to Outputs 1-6; Usage of data in the SP1 IT tools and models stated (see Section 7 Key Result KR1.5) • Revision and update of the SP1 DMP: Collab tidied up (see Section 8 'Update on implementation of the DMP) • Addition of the information regarding the coordination between SP1 and the KG Team for the curation process (see Section 8 'Update on implementation of the DMP) • Revision of the conclusions: more substantial and quantitative information added (see Section 9 Conclusion and Outlook) • Update of Components (see Table 3) • Addition of the summary with the main action undertaken by SP1 after the M1-M12 review (see Section Annex B)
13 Jan 2020	Revised version resubmitted to EC by PCO via SyGMa

1. Overview

The objective of SP1 is to generate neuroscientific concepts, knowledge, experimental datasets and tools, which will be used to build standalone and multi-scale models of the rodent and human brain. An important role for SP1 is to provide data and knowledge to support activities undertaken by other SPs in the HBP. The idea is to promote interdisciplinary collaborations through the implementation of adequate neuroscience strategies to achieve a better understanding of the brain from different perspectives.

SP1 generates data mainly on the mouse brain and —to a limited degree— on human brain tissue. The mouse remains the model of choice for human brain function because it is possible to directly study genetic, molecular and cell biological processes including neuronal and glial physiology and cognitive processes in living animals, as well as in valid genetic models of human disease. From the outset of the HBP, it was recognised that it was not feasible to study all aspects of mouse biology at the same time. Hence, a phased strategy, with long-term milestones and objectives, was required and the project was designed to evolve accordingly.

From the beginning of the Project, SP1 scientists have established valid methods to be used for mapping the mouse brain and they have obtained strategic mouse brain datasets across the key domains of transcriptome, proteome, neuroanatomy, physiology, channel function and behaviour. They worked on data aggregation, integration and dissemination. These studies have established a strong foundation for the development of HBP platforms. Moreover, innovative technologies have been and are currently developed, that are expected to be of use worldwide

SP1 currently focuses on the whole brain and on four major brain circuits: neocortex (including the thalamocortical system), hippocampus, basal ganglia and cerebellum with as goal to examine the molecular, genetic and anatomical patterns separately in these particular regions. In addition, in this project phase, SP1 also tackles the integration of multilevel data and includes human-rodent comparative studies. This is particularly important, because due to ethical limitations, not all necessary datasets can be acquired directly from human brains. Therefore, choosing appropriate experiments to obtain strategic data that could be extrapolated to the human brain is another major goal.

2. Introduction

As outlined in the HBP roadmap, the overarching objective of Subproject 1 (SP1) is to generate neuroscientific concepts, knowledge, experimental datasets, methods and tools, to build models for the simulation of the brain. In addition, SP1 provides data and knowledge to support activities undertaken by other Subprojects (SPs) and Co-Design Projects (CDPs), mainly SP6, but also SP4, SP10, CDP1 and CDP2. SP1 generates data mainly on the rodent brain, but also on human brain tissue. During SGA2, SP1 also tackles the integration of multilevel data and includes human-rodent comparative studies.

This report outlines the main SP1 outputs during the first year of SGA2. Most of them are the releases of the components planned in the SP1 roadmap in line with the SP1 data strategy. In this strategy, the SP1 studies have all adopted advanced techniques required by SP6 in particular, as well as by SP4, SP10, CDP1 and CDP2. This includes (i) performing inter-domain analyses and across-scale investigations encompassing molecular, anatomical and functional data integration in rodents, and (ii) carrying out comparative studies of cells and microcircuits in the rodent and human brain. Specifically, SP1 has focused on the SP1 data strategy, which aims to achieve the five Key Results (KRs) defined in its roadmap.

The data strategy is focused on achieving the SP1 five main Key Results (KRs) defined in the SP1 roadmap. The main SP1 outputs during the first project year are contributing to these KRs, which are listed below:

1) KR1.1: High-quality multilevel datasets at molecular and subcellular level of single molecules, single synapses and single cells.
2) KR1.2: High-level multi-scale datasets at cellular and microcircuit level on selected brain regions: neocortex (including thalamus), hippocampus, basal ganglia and cerebellum.
3) KR1.3: Structural and functional datasets on a brain-wide scale by using cutting-edge imaging technologies. These technologies are used to obtain functional and structural measurements. The technological development is one of the key issues in this KR. The technical advances produced will be of worldwide utility.
4) KR1.4: Multilevel datasets generated by integrating neuroanatomical data with genetic, molecular and physiological data using advanced technologies. A new approach is being developed by combining advanced technologies to perform recordings at different scales. A new framework including novel tools for integration, visualisation and analysis of anatomical and functional data is being implemented across different scales.
5) KR1.5: Strategic datasets on single neurons and circuits to be used in comparative studies on human and rodent.

The rationale for these strategic choices in KRs for SGA2 were agreed with SP2, SP4, SP5, SP6, SP9, SP10, CDP1 and CDP2, in order to guarantee the feasibility of an integrated experimental-modelling effort.

Finally, the list of major contributions and outputs by M12 is displayed in detail in the SP1 SGA2 Data Management Plan (DMP), version HBP-SGA2-SP1DMP-M12-v2.2, (https://collab.humanbrainproject.eu/#/collab/5972/nav/46222; Storage/Workspace section '**SGA2**'/ Subsection '**HBP-SGA2-SP1DMP-M12-v2.2**'). This Collab is only accessible to the EC and SGA2 reviewers. This data management plan has been updated in line with the Section 'completeness' displayed in the DMP for the SP1 outputs, and has been updated by M3, M6,M12 and M18 according to the time schedule planned for the releases. The description of this update is displayed in Section 8.

3. Key Result KR1.1: High-quality multilevel datasets at molecular and subcellular level of single molecules, single synapses and single cells

3.1 Outputs

3.1.1 Overview of Outputs

KR1.1 aims at generating high-quality molecular, subcellular, cellular data needed for imaging, mapping, proteomics and physiology to inform brain modelling of HBP (mostly by SP5, SP6, CDP1, and CDP2). The research strategy of WP1.1 and the common rationale underlying data generation in KR1.1, is to generate these data, exploiting the development of novel methods (i.e. high resolution of channel densities, direct labelling of nanobodies, Synactive reporters) and exploitable tools (i.e. nanobodies, SynActive toolbox).

At M12, KR1.1 comprises 8 outputs containing results towards the achievement of SO1.1.

Main outputs and components involved (in brackets) are as follows:

1) Imaging of Amyloid Beta Oligomers (C1767)
2) Imaging of Neuroligin2 (C1767)
3) New nanobodies against Neurexin 3β (C1767)

4) Development of AAV vectors for SynActive mapping of potentiated synapses (C1767)
5) Interfering with transsynaptic signalling in the hippocampus severely impairs rhythmogenesis and hippocampal-dependent social memory (C1770)
6) Mapping of potentiated dendritic spines (C1770)
7) Proteomics of potentiated spines: the PSD-95 interactome (C1770)
8) New datasets on channel densities and first attempt of visualising tagged molecules with a resolution of a few nanometres (C1886)

See Annex A: Component Details for further information on components

3.1.2 Output 1

Imaging of Amyloid Beta Oligomers

Amyloid Beta Oligomers (AbetaOs) are crucial soluble molecular targets in Alzheimer's Disease (AD) pathogenesis. Their imaging in human and mouse brains is still difficult due to the lack of selective probes. We developed a unique panel of conformation-sensitive and sequence-specific nanobodies, exploited in pilot studies in SGA1, allowing to cover some experimental gaps existing in literature. Here we show: a) data on human brain cortex from additional Alzheimer's cases; b) new data on mouse brains from Alzheimer's model (5xFAD) and controls.

Methods: use of anti-AbetaOs nanobodies as primary antibodies in confocal microscopy following previously established protocols, in combination with different commercial anti-Abeta antibodies.

Data location: https://collab.humanbrainproject.eu/#/collab/44109/nav/303324 (Folder path: i) Nanobodies for imaging / Anti-Amyloid Oligomers in human brains / DATASET#1 C1767.pdf; ii) Nanobodies for imaging / Anti-Amyloid Oligomers in mouse brains / DATASET#2 C1767.pdf)

3.1.3 Output 2

Imaging of Neuroligin2

Neuroligin2 (NLG2) is a post-synaptic transmembrane scaffolding protein involved in trans-synaptic signalling via its interactions with neurexin family members. NLG2 plays a role in synapse function and synaptic signal transmission, especially via GABA (A) receptors in inhibitory synapses. In SGA1 we selected new anti-NLG2 nanobodies, which are under validation as intrabodies and as new imaging tools. Here we show: new data of Neuroligin2 imaging in mouse brains.

Methods: first use of anti-NLG2 nanobody #1 as primary antibody in confocal microscopy by optimisation of new protocols.

Data location: https://collab.humanbrainproject.eu/#/collab/44109/nav/303324 (Folder path: Nanobodies for imaging / Anti-Neuroligin2 in mouse brains / DATASET#3 C1767.pdf).

3.1.4 Output 3

New nanobodies against Neurexin 3β

Neurexins (NRXNs) are pre-synaptic transmembrane molecules involved in trans-synaptic signalling via interactions with members of the Neuroligin family. Neurexins encoded by three genes (NRXN1–NRXN3) can generate more than 1300 transcripts by using alternative promoters (α and β), they undergo alternative splicing at up to six alternatively spliced segments (AS1-6), which provide a barcode fingerprint of cell and synapse identities in the brain ("neurexin code"). Selective nanobodies against various isoforms of NRXN are inestimable tools to dissect this complex "neurexin code" by different experimental approaches. We chose to select nanobodies against splice site 5

(AS5) of NRXN3, recently shown to regulate synaptic network and epileptic activity through interaction with GluK2 kainate receptors.

Methods: The intracellular antibody capture technology (IACT) was used to isolate nanobodies that are specific for the spliced extracellular part of NRXN3β: the NRXN3β AS5^{25b} isoform.

Data location: https://collab.humanbrainproject.eu/#/collab/44109/nav/303324 (Folder path: New Nanobodies / DATASET#4 C1767.pdf)

3.1.5 Output 4

Development of AAV vectors for SynActive mapping of potentiated synapses

The SynActive platform provides a toolbox for imaging and the molecular characterisation of potentiated synapses *in vivo* (Gobbo *et al.,* 2017, Nat Comm). In the current version, SynActive mapping constructs are delivered *in vivo* via *in utero* electroporation. To overcome this limitation, we have developed a pair of AAV constructs which can be delivered to any adult brain region of interest via stereotaxic injections. In addition, the temporal window for activity-dependent tagging of potentiated synapses can be controlled by doxycycline injection.

Methods: A pair of AAVs for (i) constitutive expression (under control of the synapsin promoter) of the reverse tetracyclin transactivator (rtTA), along with the TdTomato fluorescent reporter and (ii) Tetracyclin-responsive element (TRE), SynActive-dependent expression of Venus fluorescent protein.

Data location: https://collab.humanbrainproject.eu/#/collab/44109/nav/303324 (Folder paths SynActive tools & methodological advancements/Protocol for usage of SynActive-based AAVs for imaging of potentiated spines & sample image; SynActive tools & methodological advancements/SynActive optogenetics)

3.1.6 Output 5

Mapping of potentiated dendritic spines

Mapping the distribution of potentiated synapses is a step towards deciphering the code for memory acquisition and storage in the brain. To pursue this goal, we developed a toolbox, SynActive (see Output 4), to tag potentiated synapses via activity-dependent expression of a fluorescent reporter protein. Moreover, the time window for expressing this construct was controlled via a "TetON" inducible system. We used this approach to provide the first cartography of potentiated synapses in the hippocampus following an *in vivo* learning and memory task.

Methods: After *in vitro* validation, a gene construct encoding the TetON-SynActive fluorescent reporter was electroporated *in utero* for expression in the mouse hippocampus. Then, mice were exposed to contextual fear conditioning, while being treated with doxycycline. Tissue sections were prepared and the distribution of potentiated synapses was imaged and quantified.

Data: https://collab.humanbrainproject.eu/#/collab/44102/nav/303276 (Main folder path: SynActive, containing validation of constructs, schematics of the protocol and preliminary data analysis (see respective file descriptions). Sub-folder path: SynActive/imaging data, containing raw confocal microscopy data, namely: HC_1_dSR.lif; HC_1_pSR.lif; HC_1_SLM.lif; HC_1_SO.lif; HC_3_dSR.lif; HC_3_pSR.lif; HC_3_SLM.lif; HC_3_SO.lif; FC_1_dSR.lif; FC_1_pSR.lif; FC_1_SLM.lif; FC_1_SO.lif; FC_3_dSR.lif; FC_3_pSR.lif; FC_3_SLM.lif; FC_3_SO.lif; key to file names: HC, control animals maintained in their Home Cage; FC, animals exposed to contextual Fear Conditioning; dSR, distal Stratum Radiatum; pSR, proximal Stratum Radiatum; SLM, Stratum Lacunosum Molecolare; SO, Stratum Oriens).

3.1.7 Output 6

Proteomics of potentiated spines: the PSD-95 interactome

Understanding how memory storage can be implemented at synapses requires defining the molecular signature of potentiated dendritic spines. To this aim, we have exploited the SynActive toolbox (see Output 4) to immunoprecipitate the interactome of PSD-95 – a hub that organises postsynaptic proteins, such as neurotransmitter receptors, scaffolds, and signal transducers. We have performed analysis of datasets generated using this tool, improved the signal-to-noise ratio and enhanced the detection of potentiated synapse-specific proteins. The acquired proteomic datasets are being analysed bioinformatically in collaboration with D. ARMSTRONG (UEDIN).

Methods: An AAV encoding FLAGged PSD-95 under the control of SynActive regulatory sequences (SA::PSD-FLAG) was injected into the CA area of mice; the control AAV was represented by the same protein, expressed under the control of the neuron-specific, constitutive promoter of synapsin (syn::PSD-FLAG). Mice expressing SA::PSD-FLAG were exposed to the association phase of contextual fear conditioning, then the hippocampi were dissected. Hippocampi were also dissected from syn::PSD-FLAG-injected mice. Then, protein extracts were processed for immunoprecipitation using paramagnetic beads conjugated to M2 anti-FLAG antibodies. Finally, immunoprecipitates were analysed by mass spectrometry (MS-MS). The raw datasets were normalised using the FLAG tag as common parametric anchor.

Data: https://collab.humanbrainproject.eu/#/collab/44102/nav/303276 (Folder path: hbp-01770-Synactive-Proteomics-ESARE and hSyn normalized on FLAG.xlsx).

3.1.8 Output 7

Interfering with transsynaptic signalling in the hippocampus severely impairs rhythmogenesis and hippocampal-dependent social memory

We investigated whether network oscillations and learning behaviour are affected in mice with alterations in transsynaptic signalling, in particular in mice lacking the synaptic adhesion molecule neuroligin 3 (NLG3 knock-out). Low field potentials (LFP) were recorded in the stratum radiatum of CA2 and CA3 regions of the hippocampus from *in vivo* anaesthetised adult animals. Data analysis (with Complete Ensemble Empirical Mode Decomposition with Adaptive Noise) revealed a significant reduction of rhythms occurring in the theta (5-14 Hz), low (25-55 Hz) and high (56-120 Hz) gamma frequencies in both somatic and dendritic recordings. Furthermore, hippocampal-dependent social memory, investigated with the three-chamber test, was impaired.

Data: https://collab.humanbrainproject.eu/#/collab/44102/nav/303276 (**Folder** "In vivo Oscillations- Hippocampus" [**Subfolders:** Controls NLG3 KI (Wild Type), Controls NLG3 KO (Wild Type), NLG3 KI, NLG3 KO]; **Folder** "Behavioral Data/Sociability Data" [**Subfolders:** Control mice (wild-type littermates of NLG3 KO mice)**,** NLG3 KO mice]; **Folder** "Behavioral Data/Social Novelty Data" [**Subfolders:** Control mice (wild-type littermates of NLG3 KO mice)**,** NLG3 KO mice]).

3.1.9 Output 8

New datasets on channel densities and first attempt of visualising tagged molecules with a resolution of a few nanometres

Data on channel densities were generated and we did a first attempt of visualising tagged molecules with a resolution of a few nanometres with reaction tag system. Current tagging methods in the field are mostly for Light Microscopy (LM) and not comparable with ours in terms of resolution. A publication on the first EM visualization of receptors with the chemical labelling is now under revision and another work on the second tag-probe pair has been published (see Section 3.2.3).

Data location:

- AMPAR subunits: Freeze-fracture replica labelling for panAMPAR, GluA1, GluA2 and GluA3 in the CA1 area of the hippocampus and the cerebellum: https://collab.humanbrainproject.eu/#/collab/52838/nav/361777
- P/Q-type calcium channel: Freeze-fracture replica labelling for Cav2.1 subunit in the hippocampus and cerebellum https://collab.humanbrainproject.eu/#/collab/52839/nav/361782
- 2D maps of receptors and ion channels, by replica labelling obtained in hippocampus, neocortex and cerebellum: https://collab.humanbrainproject.eu/#/collab/52909/nav/362229
- 2D spatial relationship between receptors and ion channels, by replica double-labelling in the hippocampus, neocortex and cerebellum: https://collab.humanbrainproject.eu/#/collab/52910/nav/362234

3.2 Validation and Impact

3.2.1 Actual Use of Output(s)

Data generated in Outputs 1-8 have been uploaded in the HBP Collaboratory portal and have already been shared with the curation team. New data will be uploaded and shared with the curation team, between M18-M24 to complete the current partial status.

Output 1: As actual use of this output, we mapped AbetaOligomers in human and mouse cortex (in post-mortem brain slices) with high resolution. Interestingly, a differential and new distribution of antigen is observed in comparison to immunostaining with other anti-Abeta antibodies tested, in cortical areas related to plaques and in pyramidal neurons. Extensive maps of antigen distribution in different brain areas, cell types and subcellular compartments are in progress. We plan to release nanobodies to Task T1.1.3 for EM uses, and to T1.3.2, T2.3.1, and T2.3.2 for whole brain studies. Data will be used in HBP by the end of SGA2 by SP5 atlasing (images analysis by T5.6.1)

Output 2: As actual use of this output, we detected for the first time NLG2 in mouse brains with nanobodies. Nanobodies are raised against different epitopes in comparison to commercially available IgG, and due to their small size and increased permeability in tissues in comparison with full IgG show a different and new pattern of NLG2 immunostaining in hippocampal area. We plan to release the nanobodies to T1.1.3 for Electron Microscopy (EM) uses, and to T1.3.2, T2.3.1, and T2.3.2 for whole brain studies.

Output 3: Anti-NRXN nanobodies were newly generated and their actual use is in different experimental settings, such as immunoblot analysis, upon expression and purification as recombinant proteins.

Output 4: These AAVs were validated *in vitro* by infecting hippocampal primary cultures, which have been subsequently treated with doxycycline, followed by KCl to induce chemical LTP. Finally, cultures were histologically fixed and imaged using confocal microscopy. Representative images have been obtained, showing enrichment of SynActive-PSDVenus at dendritic spines following induction of chemical LTP.

Output 5: Data are used to build the map of potentiated spine distribution in the hippocampus. This is the first map of a synaptic engram correlated to a behavioural task. The dataset is currently under analysis to include the recall phase of memory (i.e. hippocampal sections obtained from animals that were re-exposed to the conditioning stimulus – context – in the absence of the unconditioned stimulus – electric shock).

Output 6: Two datasets, corresponding to the PSD-95 interactomes of (i) hippocampal potentiated spines and (ii) hippocampal total constitutive spines (not potentiated) have been generated. These datasets are used to extract synaptic proteins showing differential expression at potentiated synapses.

Output 7: This output provides new insights on the functional role of transsynaptic signalling in brain oscillations and hippocampal memory. About half of the planned data have been collected.

Output 8: No use of the output yet for modelling proposes. Data were sent out to the curation team and development of the second reaction tag-probe pair has been published (see Section 3.2.3).

3.2.2 Potential Use of Output(s)

Output 1: Planned release of nanobodies to Task T1.1.3 for EM uses, and to T1.3.2, T2.3.1, and T2.3.2 for whole brain studies. Data will be used in HBP by the end of SGA2 by SP5 atlasing (images analysis by T5.6.1). Nanobody tools, also thanks to the possibility of direct and site-directed labelling and imaging data will have a strong impact in the field of AD, for anatomical and physiopathological research in human brains, as well as for future diagnosis strategies such as *in vivo* imaging approaches alternatively to current Amyloid PET. Applications will be useful also for researchers and users out of HBP. Future extended data on the mouse should allow a comparative analysis of mouse brains versus human brains (in line with KR1.5).

Output 2: Nanobodies could be best for imaging (data under investigation). We planned to release the nanobodies to T1.1.3 for Electron Microscopy (EM) uses, and to T1.3.2, T2.3.1, and T2.3.2 for whole brain studies. Nanobody tools and imaging data will have utility and impact also for researchers and users out of HBP.

Output 3: Selective functional interference with AS5 variants of NRXN3 for transsynaptic signalling and modelling. New potential selective tools for imaging and dissection of the NRXN code.

Output 4: The AAVs will be delivered in the hippocampus of adult mice for mapping of potentiated synapse distribution following a behavioural task triggering learning and memory (i.e. contextual fear conditioning).

Output 5: The datasets will be used to (i) generate a cartography of synaptic engrams of hippocampus; (ii) implement a comparison of potentiated spines in different memory phases; correlation between cellular(c-fos positive neurons, cellular engram) and synaptic engrams to implement and/or validate the hippocampal model performing memory tasks in the Brain Simulation Platform (Task T6.1.5 and CDP2); (iii) bridge different scales of investigation (from synapses to the whole hippocampus, WP4.1); and (iv) to implement multi-scale simulations of spatio-temporal dynamics and plasticity (CDP2).

Output 6: The datasets will be used for molecular models of synapses in the context of compartmental models of neurons (Task T1.4.3).

Output 7: The datasets will be used to (i) implement and/or validate the hippocampal model performing memory tasks in the Brain Simulation Platform (Task T6.1.5 and CDP2); (ii) bridge different scales of investigation (from synapses to the whole hippocampus, WP4.1); and (iii) implement multi-scale simulations of spatio-temporal dynamics and plasticity (CDP2).

Output 8: Data of channel densities are mature. The reaction tag system should be verified by generating knock-in mice. If visualisation in brain is successful, potential results would be useful for quantification of any ion channel subunits that have no useful antibodies.

3.2.3 Publications

- P2219: Zenmyo N, Tokumaru H, Uchinomiya S, Fuchida H, Tabata S, Hamachi I, Shigemoto R, Ojida A, Optimized Reaction Pair of the CysHis Tag and Ni(II)-NTA Probe for Highly Selective Chemical Labeling of Membrane Proteins, Bulletin of the Chemical Society of Japan, Vol. 92, No. 5 https://doi.org/10.1246/bcsj.20190034

  Significance: This publication optimised a pair of new tag and chemical probe combination for specific labelling of membrane proteins, supporting Output 8.

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

Outputs contributing to this KR have been presented to internal HBP meetings, to scientific conferences and to as part of outreach activity to the community. The main three dissemination activities are as follows:

- "Neuroscience 2018" Society for Neuroscience, oral presentation at the nanosymposium "444.04 – LTP: Intracellular Signaling, Pre- and Postsynaptic Mechanisms", Nov 2018, San Diego, USA. Outputs 6 and 7.
- HBP Internal Meeting "Hippocampus", 28-30 Jan 2019, Paris, France. This meeting was aimed at strengthening links between groups with different expertise working on the hippocampus and to plan future experiments to increase the impact of the outputs. Outputs 5, 6 and 7.
- The "proMEMO 2019" meeting, 5-8 March 2019, Copenhagen, Denmark. "Comparison of synaptic and cellular engrams in the hippocampus during acquisition and consolidation of a contextual fear memory" (oral presentation) Output 6, and "Molecular fingerprinting of *in vivo* potentiated synapses via isolation of the PSD-95 interactome from mouse hippocampus" (poster presentation), Output 7.

4. Key Result KR1.2: High-level multi-scale datasets at cellular and microcircuit level on selected brain regions: neocortex (including thalamus), hippocampus, basal ganglia and cerebellum

4.1 Outputs

4.1.1 Overview of Outputs

Objective SO1.2 in SP1 is the generation of high-quality cellular and microcircuit level data needed for hypothesis and data-driven brain modelling, mostly by SP6, CDP1, and CDP2, and the integration of the anatomical and functional studies. The KR1.2 outputs are needed to meet this Objective. In the period M1-M12, the outputs achieved for this KR include high-level multi-scale datasets, preliminary or final, at cellular and microcircuit level on selected brain regions (neocortex including thalamus, hippocampus, basal ganglia and cerebellum).

Specifically, a total of 8 main outputs have been contributing to this KR:

1) New datasets from reconstructions of the dendritic trees and from morphological characteristics of the axon initial segments (C1744 and C 1743)
2) Interneurons of the cerebellar cortex (C1771, C1772)
3) Neuronal subtypes in the basal ganglia (striatum) (C1774)
4) Single-cell 3D reconstruction and measurement of thalamocortical nuclei and quantitative ultrastructural analysis of the synapses (C1866, C1867)
5) Morphological characterisation of hippocampal neurons (C1796)
6) Hippocampal circuitry (C1768, C1795)
7) Synapse maps on inhibitory neurons (C1738)

See Annex A: Component Details for more details

4.1.2 Output 1

New datasets from reconstructions of the dendritic trees and from morphological characteristics of the axon initial segments

This output has been achieved by two component releases. On the one hand, a release of C1744 consisting in a data set from reconstructions of the dendritic trees from mouse stellate (n=22), basket (n=24), granular (n=7) and Golgi (n=6) cerebellar neurons and from D1 striatal medium spiny projection neurons type (n=12). On the other hand, a release of C1743 (Task T1.2.1) consisting in data sets from morphological characteristics of the axon initial segments from stellate (n=64), basket (n=62) and Purkinje (n= 42) cerebellar neurons; and from striatal D1-type medium spiny projection neurons (n=87) and PV (n=81), Chat (n=51), CR (n=28) and nNOS (n=73) striatal interneurons.

Data location:

- AIS geometric characteristics: https://collab.humanbrainproject.eu/#/collab/54050/nav/369778
- Preliminary data on dendrites of striatal and cerebellar neurons: https://collab.humanbrainproject.eu/#/collab/54050/nav/369778

4.1.3 Output 2

Interneurons of the cerebellar cortex

Patch clamp recordings were performed and used for modelling the main interneurons of the cerebellar microcircuit. More specifically, cerebellum cells have been recorded as planned (25%): Stellate cells (15 cells); Purkinje cells (5 cells); granule cells (25 cells recorded); Golgi cells (2 cells recorded). Also, the cerebellar network has been recorded as scheduled (25%): MEA recordings (25); VSD recordings (12); optogenetics (4 recordings); and cerebellar neurons have been recorded *in vivo* as planned (25%): single unit recordings (7). All data were continuously updated by the curation team. The final release is planned for the end of the second year of the project (M24).

Several datasets have already been linked to the KG:

https://doi.org/10.25493%2F6R48-E3V

https://doi.org/10.25493%2FMDAR-XEB

https://doi.org/10.25493%2F4AF6-WSD

https://doi.org/10.25493%2FF2VK-MB4

https://doi.org/10.25493%2FG07Q-K87

https://doi.org/10.25493%2FMVHQ-4YA

https://doi.org/10.25493%2FJQH3-0A4

4.1.4 Output 3

Neuronal subtypes in the basal ganglia (striatum)

Different neuronal subtypes in the basal ganglia (striatum) were described. The focus was initially at describing the two types of striatal projection neurons with D1 and D2 dopamine receptors at the origin of the direct and indirect pathways, this was used as background for simulation of the processing in the globus pallidus externa. A further great detailed characterisation of the cholinergic interneurons is ready and the process of analysing the LTS (Low Threshold Striatal) interneurons is

ongoing. The membrane properties of the different neuron subclasses, the expressed ion channels and the detailed morphology were described in detail. This information is required for the detailed simulations of each neuronal subtype, where each morphological subtype is matched versus the membrane properties (input resistance, plateau properties, action potential shape, after hyperpolarisation etc.).

These data are partially available in the Collab and will be reported by M20 as planned.

Basal Ganglia: https://collab.humanbrainproject.eu/#/collab/376/nav/3413

'Model Inventory: Basal Ganglia': https://collab.humanbrainproject.eu/#/collab/7840/nav/59622

4.1.5 Output 4

Single-cell 3D reconstruction and measurement of thalamocortical nuclei and quantitative ultrastructural analysis of the synapses

This output involves the following achievements:

The first 3D measurement of a presynaptic and postsynaptic element structure in a statistically significant sample of the Ventroposterior nucleus (a Sensory / First-Order thalamic nucleus) axon terminals in primary vibrissal sensory cortex was completed. This work was performed in collaboration with other SP1 teams (UPM and others outside the HBP (J. LUBKE Lab.) 3D datasets (serial TEM and FIB-SEM image stacks) from this study will be made available to the public at M18, as planned.

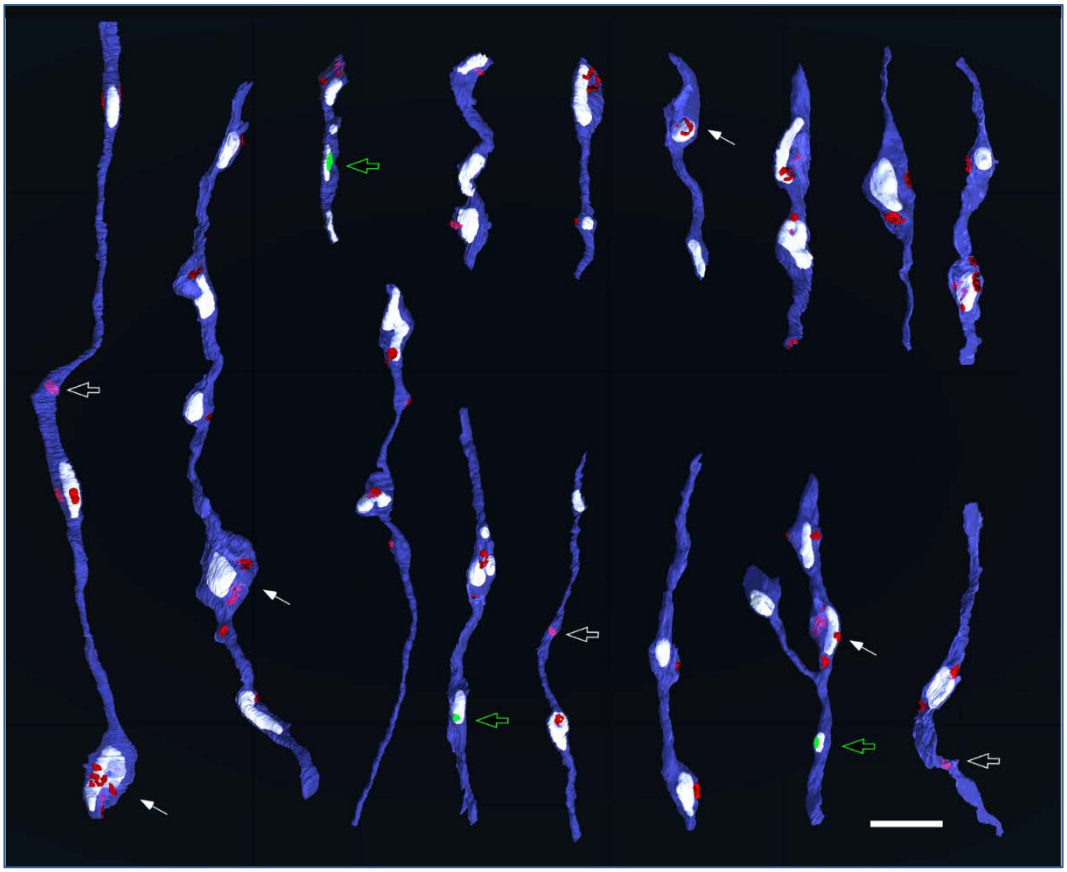

Figure 2: Ventroposterior thalamic axonal segments 3D-reconstructed from FIB/SEM image stacks

Preliminary results were obtained for the first 3D measurement of presynaptic and postsynaptic elements of the Posterior nucleus (an Association/Higher-Order thalamic nucleus) in two different layers (5 and 1) of the primary vibrissal sensory cortex. This work was performed in collaboration with other SP1 teams (UPM and others outside the HBP (J. LUBKE Lab.)

The first demonstration, that synaptic terminals from the Posterior nucleus (an Association/Higher-Order thalamic nucleus) axons in the primary motor cortex are significantly bigger and structurally more complex than those of in the primary somatosensory cortex, was completed. This achievement has been carried out in collaboration with another team outside of HBP (J. LUBKE Lab.) See Figure 2.Reconstructions and metadata from 16 complete thalamocortical axon morphologies were made available in the HBP Knowledge Graph (although reconstructions are still under embargo).

Data location: https://collab.humanbrainproject.eu/#/collab/56535/nav/386213; https://kg.ebrains.eu/instances/Dataset/6dee616062025ddf5fd6341ee2b865ad (under embargo).

Metadata location: https://kg.ebrains.eu/instances/Dataset/6dee616062025ddf5fd6341ee2b865ad (accessible in the Knowledge graph).

This output also contributes to KR1.4 and KR1.5 (see section 6 and 7).

4.1.6 Output 5

Morphological characterisation of hippocampal neurons

Functionally important classes of hippocampal interneuron (basket cells, dendritic inhibitory cells, neurogliaform cells, interneuron-specific interneurons, and hippocampo-septal (HS) cells) were characterised in a targeted manner, using genetically expressed markers (parvalbumin, VIP, calretinin) and retrograde tracers (for HS cells), providing 3D morphological reconstructions and correlated electrophysiological data from the hippocampal slice preparation. A total of 21 new reconstructions of hippocampal neurons were obtained. Further, in addition, to the axonal bouton clouds which are provided for all reconstructions, the detailed axonal branching pattern was reconstructed for 20 neurons (including pyramidal cells and different types of interneurons). The data are continuously integrated into the full morphological and electrophysiological database that forms the basis of single cell and network modelling in SP6.

Data location: https://collab.humanbrainproject.eu/#/collab/63498/nav/432079

4.1.7 Output 6

Hippocampal circuitry

The physiological effects of cholinergic input on pyramidal cells was studied. Specifically, the effects of the cholinergic agonist carbachol on the electrophysiological characteristics of CA3 pyramidal cells (n=8) were measured. These data are required to model the effects of subcortical neuromodulatory input on hippocampal single cell properties and emergent network dynamics.

In vivo electrophysiological recordings: whole-cell patch-clamp recordings were executed in the CA1 region, along with the measurement of the contralateral local field potential, in head-restrained, awake mice (n=7), while subcortical inputs from the MR region were stimulated. Glutamatergic terminals from MR were optogenetically activated (hChR2(H134)) in dorsal CA1, while the subthreshold activity of CA1 pyramidal cells and the local field potential were monitored. These data allow us to characterise the properties of hippocampal neurons in the intact animal and how these properties are changed by subcortical input, and provide important constraints for network models of the hippocampus.

Data location:

- Blockface scanning EM data from mouse hippocampal neurons:
  https://collab.humanbrainproject.eu/#/collab/63494/nav/432054
- *In vitro* electrophysiological data from mouse hippocampus:
  https://collab.humanbrainproject.eu/#/collab/63508/nav/432140

4.1.8 Output 7

Synapse maps on inhibitory neurons

The whole dendrite (95,7 μm (the ratio of loss sections: 1174/34; 2.9%) of a Calbindin-D28K immuno-positive GABAergic interneuron from layer 5 in mouse V1 has been traced at the electron microscope level and reconstructed in 3D, as shown in Figure 3.

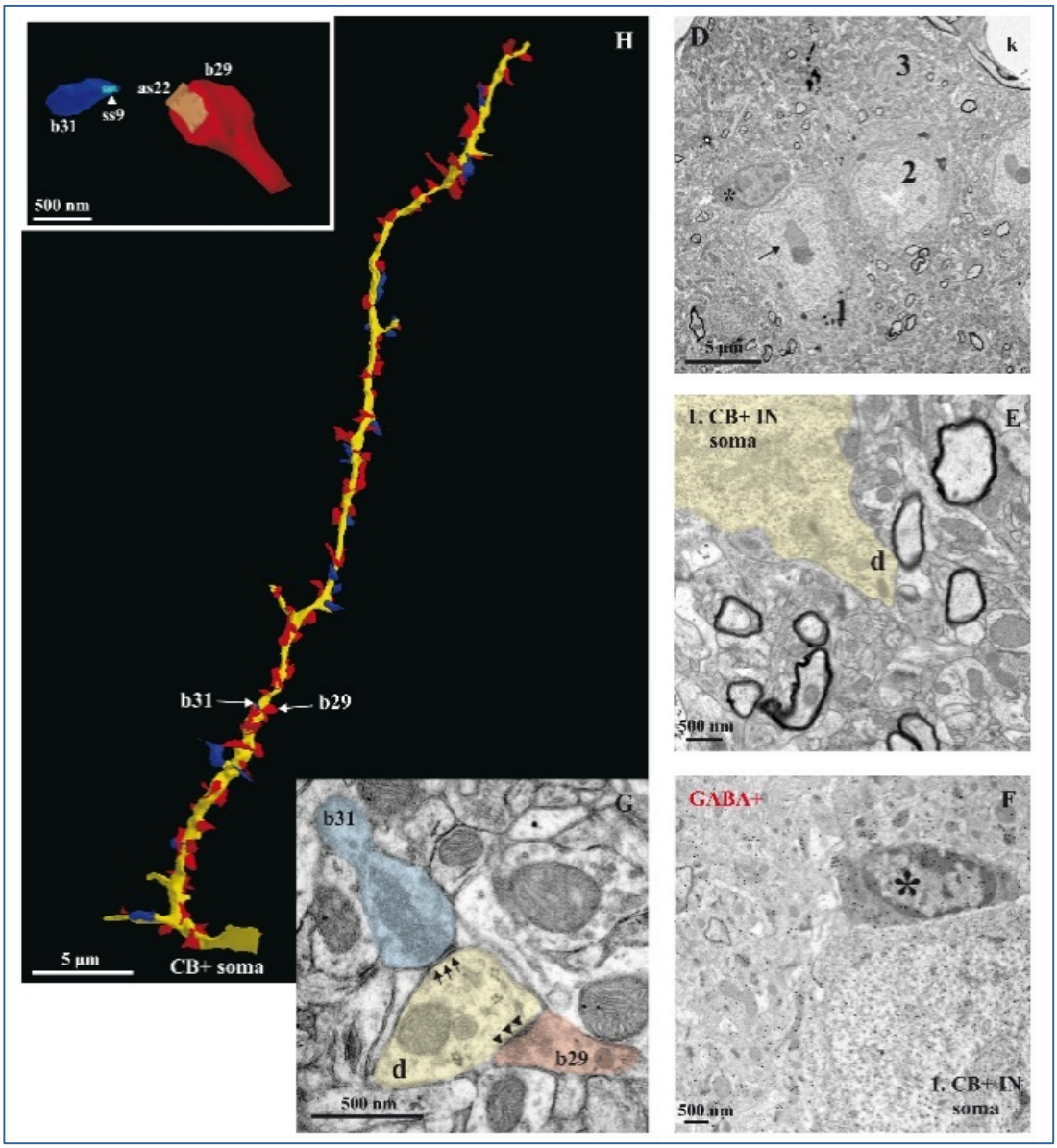

Figure 3: Synapse maps on inhibitory neurons

A concise description of the raw data in addition to the resulting EM reconstruction (only for internal use for the time being) is located at https://collab.humanbrainproject.eu/#/collab/6280/nav/48469?state=uuid%3D6f35e165-dbc4-4c11-8a5d-282fd4a04605

4.2 Validation and Impact

4.2.1 Actual Use of Output(s)

Output 1: These data are used by SP6 (T6.2.2: 'Models of Cerebellum and Community' and T6.2.4: 'Models of basal ganglia')

Output 2: The data were incorporated into models of Cerebellum and used for simulations on the Brain Simulation Platform and on the Neurorobotics Platform and for mouse brain atlasing.

Output 3: These data have already been used in the simulations of striatum in SP6, (T6.2.4: 'Models of basal ganglia'), in which populations of the different subtypes of neurons have been represented including the variability observed within each class. There is a close interaction with SP6 ('Models of basal ganglia'), we have weekly meetings to build compartmental models of each neuron with properties as similar as possible to their biological counterparts. The neuron populations are simulated with the observed variability, and with the correct density of neurons and dendritic arborisations. The properties of the excitatory synapses from cortex and thalamus are simulated as well as the inhibitory GABAergic interactions between the subtypes of striatal neurons and LTS interneurons, and the cholinergic interneurons.

Output 4: 3D-reconstructions of thalamocortical cells are shared with P. TIESINGA (SP5) and R BAKKER at SKU and used as reference for developing new capabilities (segmentation, non-linear transformations of selected axonal domains to map them to their correct terminal neuropils) in the HBP morphology viewer: https://neuroinformatics.nl/HBP/morphology-viewer-dev/. 3D-reconstruction datasets of thalamocortical cells are shared with T BRAUNS and M. PUCHADES (UIO) to develop atlasing tools for positioning single-cell reconstructions in the Allen Bran Mouse Atlas space. 3D-reconstruction datasets of thalamocortical cells are shared with the Southeastern University-Allen Brain Institute Center for Neuronal Morphology (Hanchuan PENG, non-HBP) for a joint project aiming at the evaluation of the accuracy of Vaa3 virtual reality software tools for neuronal reconstruction from volumetric datasets. Finally, 3D reconstruction datasets (morphology, length, spatial distribution of boutons) of axon collateral branches from somatosensory cortex-projecting thalamic neurons in the Reticular thalamic nucleus are used to model the reticulo-thalamic circuit loop (E. IAVARONE and S. HILL, Blue Brain group EPFL non-HBP)

Output 5: Morphological and physiological data are shared with Task T6.2.3 (Models of hippocampus), and are used to build morphologically and biophysically detailed single cell models through parameter optimisation. The morphological data are also necessary to build detailed 3-dimensional models of the hippocampal network, and to predict the hippocampal connectome. The data are also shared with the HBP neuroinformatics team, and curation will be completed when the dataset is finalised.

Output 6: The data have been used to build simplified models of hippocampal neurons under baseline conditions and in the presence of neuromodulatory input, with the aim of understanding the transitions between different types of network dynamics in response to subcortical modulatory influences. Curation of the *in vivo* dataset is also in progress.

Output 7: Data generation was only completed recently. No use of the output yet

4.2.2 Potential Use of Output(s)

Output 1: Data can be used to improve understanding of the anatomy of the basal ganglia and cerebellum, and to generate models in these brain structures.

Outputs 2: A potential further use is for spiking robotic controllers (Voucher programme #47), neuromorphic hardware design (Voucher programme #49) and for incorporation into the mouse virtual brain (partnering project CEREBNEST).

Output 3: These data are new morphological and physiological correlations that could be used in models of the basal ganglia. With regard to the basal ganglia the material of the rodent cell types (medium spiny D1R and D2R subtype and LTS and aspiny (Ach) interneurons) will be further extended to achieve detailed simulations of the striatal microcircuit.

Output 4: These structural and functional data are important to biologically constrain models of the rodent somatic sensory cortex and motor cortex circuits. The neuron reconstructions, produced from counterstained serial histological sections, constitute the gold-standard dataset against which semi-automated software tools for neuronal morphology analysis will be compared.

Output 5: In addition to being essential for building both detailed and simplified models of hippocampal cells and circuits, this large database of morphological and physiological data also allows an improved classification of hippocampal neurons, and better characterisation of structure-function relationships in various hippocampal cell types.

Output 6: The data provide constraints for building detailed models of hippocampal neurons and circuits in different neuromodulatory states, which may also lead to a mechanistic understanding of the effects of subcortical inputs at various levels of description.

Output 7: These data could be used for data-driven modelling of neocortical cells and circuits, as well as to carry out comparative studies between ultrastructural features of morphologically identified and physiologically characterised GABAergic sub-types in human and mouse neocortex.

4.2.3 Publications

The three main publications of this Key Result are:

- P1634: Romano V, De Propis L, Bosman LVJ, Warnaar P, ten Brinken MM, Lindeman S, Ju C, Velauthapillai A, Spanke JK, Middentorp Guerra E, Hoogland TM, Negrello M, D'Angelo E, De Zeeuw CI. Potentiation of cerebellar Purkinje cells facilitates whisker reflex adaptation through increased simple spike activity. eLIFE, 18 December 2018
- P1764: von Twickel A, Kowatschew D, Saltürk M, Schauer M, Robertson B, Korsching S, Walkowiak W, Grillner S, Pérez-Fernández J Individual Dopaminergic Neurons of Lamprey SNc/VTA Project to Both the Striatum and Optic Tectum but Restrict Co-release of Glutamate to Striatum Only. Curr Biol. 2019 Feb 18; 29(4):677-685.e6. doi: 10.1016/j.cub.2019.01.004. Epub 2019 Jan 31.
- P1110: Rodriguez Moreno J., J, Rollenhagen A., Arlandis J, Santuy A, Merchán-Pérez A, DeFelipe J, Lubke JHR, Clasca F (2018) Quantitative 3D ultrastructure of thalamocortical synapses from the 'lemniscal' ventral posteromedial nucleus in mouse barrel cortex. Cerebral Cortex. 28:3159-3175. https://doi.org/10.1093/cercor/bhx187

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

Outputs contributing to this KR have been presented to scientific conferences and to as part of outreach activity to the community. The three main activities are the following:

- 11th FENS Forum of Neuroscience, Berlin, Germany. 7-11 Jul 2018, the following posters:
  - "A Realistic Model of Cerebellar Stellate Neurons Predicts Intrinsic Excitability and the Impact of Synaptic Inputs", Martina Francesca Rizza, Francesca Locatelli, Stefano Masoli, Francesca Prestori, Diana Sanchez Ponce, Alberto Muñoz, Egidio D'Angelo (Output 1)
  - "The primordial vertebrate cortex – sensory and motor maps in the layered lamprey lateral pallium", Suryanarayana SM, Pérez-Fernández J, Wallén P, Robertson B, Grillner S. (Output 3)
  - "SNc direct control of lamprey superior colliculus modulates visuomotor responses by salient stimuli", Pérez-Fernández J, Kardamakis AA, Robertson B, Grillner S. (Output 3)

  - "Investigating action selection in the basal ganglia – computational approaches at different levels of biological description", Suryanarayana SM, Kozlov A, Hjorth J, Frost Nylén J, Hellgren Kotaleski J, Gurney K, Grillner S. (Output 3)

- School of Brain Cells & Circuits "Camillo Golgi" Ettore Majorana Foundation and Centre for Scientific Culture, 11-15 Dec 2018, Erice (Italy): *The Neural Bases of Action – from cellular microcircuits to large-scale networks and modelling,* the following poster*:*
  - Montagna, L. Moscato, L. DE Propris, S. Tritto, L. Mapelli, E. D'Angelo "Long-lasting changes following theta-patterned tactile stimulation in deep cerebellar nuclei correlate with low-frequency oscillations *in vivo*." (Output 2)
- Hjorth JJ, Suryanarayana SM, Kozlov A, Frost Nylén J, Silberberg G, Gurney KN, Hellgren Kotaleski J, Grillner S. Investigating action selection in the basal ganglia - computational approaches at different levels of biological description. Soc Neurosci Abstr, 147.13., Neuroscience Meeting, San Diego, CA, Society for Neuroscience, 2018. Output 3.

5. Key Result KR1.3: Structural and functional datasets on a brain-wide scale by using cutting-edge imaging technologies

5.1 Outputs

5.1.1 Overview of Outputs

The SP1 objective SO1.3 is to obtain strategic structural and functional data on a brain-wide scale, to provide a systemic -rather than a regional- view of the mouse brain and to obtain multi-scale and multilevel integration, from microcircuitry up to whole-brain level. Therefore, KR1.3 generates structural and functional datasets on a brain-wide scale, using cutting-edge imaging technologies for functional and structural measurements. Our rationale is to generate datasets that provide a valuable reference for HBP simulations at brain-wide scale, and that complement data obtained in other parts of SP1. More specifically, we focus on three main outputs that address brain structure and function, and the vascular system which is the main energy supply of the brain itself. Our outputs are thus:

1) Whole-brain datasets at sub-cellular resolution (C1745, C1742)
2) Functional connectivity of cortical neurons on GCamMP6f mice (C1765)
3) Brain Vascular Network Reconstruction (C1732)

See Annex A: Component Details for more component details.

5.1.2 Output 1

Whole brain datasets at sub-cellular resolution

Additional to datasets generated in SGA1, whole-brain datasets of inhibitory interneurons have been generated, together with activation datasets (via *c-fos* labelling) under different behavioural conditions. Further, whole-brain vasculature datasets have been generated. QC by visual inspection has been performed on each dataset by at least 2 researchers of the same lab. Dataset curation is still ongoing, (data are being transferred from Collab (https://collab.humanbrainproject.eu/#/collab/299/nav/2265) to CSCS repository), and we forecast to finish it by M22.

These whole-brain datasets at cellular resolution are definitely beyond the state of the art: indeed, serial sectioning histology (e.g. Allen) provides only partial volumetric data. Available light-sheet data of whole mouse brains are at coarser resolution, with the exception of few proof-of-principle studies. fMOST brain reconstruction has comparable resolution across the whole brain, but that technology is in general less scalable (1 brain reconstructed in 10 days vs. 1 day with our system). We have collected dozens of datasets that demonstrate that this approach is fully scalable to cohort studies.

The whole-brain datasets have been generated using methods for imaging and image processing developed during SGA1 and SGA2 (Table 1). These methods include RAPID (http://dx.doi.org/10.1101/170555), a real-time image-based autofocus system which has been patented by LENS and whose commercial exploitation is ongoing with optics companies. Another method is the ZetaStitcher (https://github.com/lens-biophotonics/ZetaStitcher), which is a free open-source software for image stitching and efficient access that is currently used by several labs in the world.

Table 1: Whole-Brain Datasets.

Name of dataset	Description	DOI	Status	Consistency of datasets
Whole brain images of selected neuronal types	Spatial distribution of different cell types (parvalbumin interneurons, somatostatin interneurons, VIP interneurons and pyramidal cells) across the entire brain	10.25493/68S1-9R1	Under embargo until 31/01/2020	26 subjects (11 from SGA1: PV 23-26; SST 09, 11, 15; VIP 16, 19-21. 15 from SGA2: PV 27-31; SST 32,38-40,42; VIP 12, 17, 21, 22, 37)
Whole brain images of resting state brain activation	Whole-brain images of neuronal activation in mouse brain acquired with light-sheet microscopy. Animal models will be used to detect immediate early genes (IEGs) expression	10.25493/77F8-7B4	Under embargo until 31/01/2020	61 subjects (2 from SGA1: control1, VisDep2; 59 from SGA2: FT26-86)

Collab link: https://collab.humanbrainproject.eu/#/collab/299/nav/2265 (Storage/Workspace/ Folder: 'Whole-Brain datasets_Output1)

5.1.3 Output 2

Functional connectivity of cortical neurons on GCaMP6f mice

In SGA2, we extended the library of imaging datasets in awake mice performing a goal-directed motor task by performing high-resolution (two-photon) imaging. The first characterisation of the high-resolution (two-photon) microscope was performed by imaging brains from anaesthetised and quite-awake mice. These datasets proved to be extremely useful to our collaborators in SP3 and SP4 for building and validating the models of calcium–associated spiking activity and large-scale propagation of cortical waves. Indeed, state-of-the art high-resolution cortical recording techniques like electrophysiological recordings lack cell specificity and, when targeting a group of cells, can hardly disentangle single cells contribution. On the other hand, our high-resolution measures can provide highly detailed information on the contribution of excitatory neurons to a specific brain state and, importantly, to the transitions between states. To target these two issues, we performed additional (not originally planned) data acquisitions under different anaesthesia levels and using different anaesthetics, with both wide-field and two-photon fluorescence imaging (Table 2).

The phenomenon of slow cortical waves (delta waves) is a regime of brain activity that is observed in all mammals in a state of deep sleep or under anaesthesia, and has been traditionally addressed with electrophysiological techniques. In order to investigate the spatiotemporal pattern of

propagation of slow wave activity, we took advantage of large-scale wide-field fluorescence microscopy technique coupled to transgenic mice expressing genetically encoded calcium indicators (GECIs) in excitatory neurons. These fluorescent indicators enable to visualise fluctuations in calcium concentration, which is an indirect reporter of neuronal spiking activity. Here, the highly sensitive GECI named GCaMP6 was used in combination with two-photon and wide-field microscopy to investigate neuronal activity of anaesthetised and awake mice at the micro- and meso-scale. Despite the lower temporal resolution, this approach is beyond the state of the art, since it is endowed with (i) increased spatial resolution and (ii) specificity of the recorded neuronal population compared to electrophysiological methods. Here, calcium imaging was used as a measure of cortical activity in the brains of C57BL/6J-Tg (Thy1GCaMP6f) GP5.17Dkim/J (here referred to as GCaMP6f) mice. To visualise cortical waves, we acquired functional data *in vivo* under different anaesthesia states, using different anaesthetics. Resolution is at cell level (preliminary - using two-photon microscopy) and meso-scale (using wide-field microscopy). EEG signal was recorded simultaneously with optical imaging (both two-photon and wide-field) to have a more conventional reading on the level of anaesthesia (Table 2).

Table 2: Functional Datasets.

Name of dataset	Description	DOI	Status	Consistency of datasets
Fluorescence cortical recording of mouse activity after stroke	We recorded the activity in the right hemisphere while the mouse is performing a passive extension-active retraction of the left forelimb in a robotic device, pre-stroke and post-stroke (one month after stroke on the right M1, without treatment). The forces applied and the position of the forelimb is also recorded simultaneously.	10.25493/Z9J0-ZZQ	Under embargo (until 31 Jan 2020)	5 subjects (pre-stroke, post-stroke, from SGA1)
Cellular resolution calcium activity maps over wide regions of the cortex	Cellular resolution calcium activity maps over wide regions of the cortex (mm2) in resting state and during voluntary movement (pulling and grasping).	N/A	Resting state dataset (2P and WF): shared in the Collab, curation in progress Pulling dataset: planned by M21-22 Grasping dataset (only with WF imaging): shared in the Collab, curation in progress	Resting state dataset: 3 subjects TPM, 3 WF Pulling dataset: to be acquired Grasping dataset: 2 subjects WF

Collab link: https://collab.humanbrainproject.eu/#/collab/299/nav/2265

Functional datasets (T1.3.4): Folders "2P_Cellular resolution calcium activity - MS1.3.10 (SGA2)", "WF_Calcium activity maps over wide regions of the cortex" and "WF - Calcium imaging of cortical activity during Reach-to-Grasp". Containing resting state datasets (SP1/CDP1) acquired with Two Photon and Wide-Field imaging and reach-to-Grasp dataset (CDP1). Curation of these datasets has started and data are expected to be available on the KG at M22. The acquisition of pulling dataset is still in progress.

5.1.4 Output 3

Brain Vascular Network Reconstruction

An alternative segmentation pipeline for creating high quality segmentation maps of brain vasculature, based on state-of-the-art deep learning methods, was implemented.

A big issue with learning methods (especially those that work with 3D imaging data) is a lack of training data. A solution for gathering training data for these deep learning methods was sought. When manually done, acquiring ground truth data is very time consuming. Imaging modalities and technologies change frequently and therefore, newly acquired datasets from different origins no longer can be successfully segmented by our trained networks. For new datasets, new ground truth annotations need to be acquired. Understanding this, we started working towards generating good quality synthetic datasets, based on conditional generative adversarial network approaches. We aimed at generating synthetic images that are looking realistic, style/texture-wise similar to the dataset we want to mimic and which follow the pattern of the underlying structure (input segmentation from synthetic trees that are based on mathematical models) as well. A sample of a generated image and its underlying segmentation map can be seen in Figure 4. This example tries to mimic a synchrotron radiation X-Ray image.

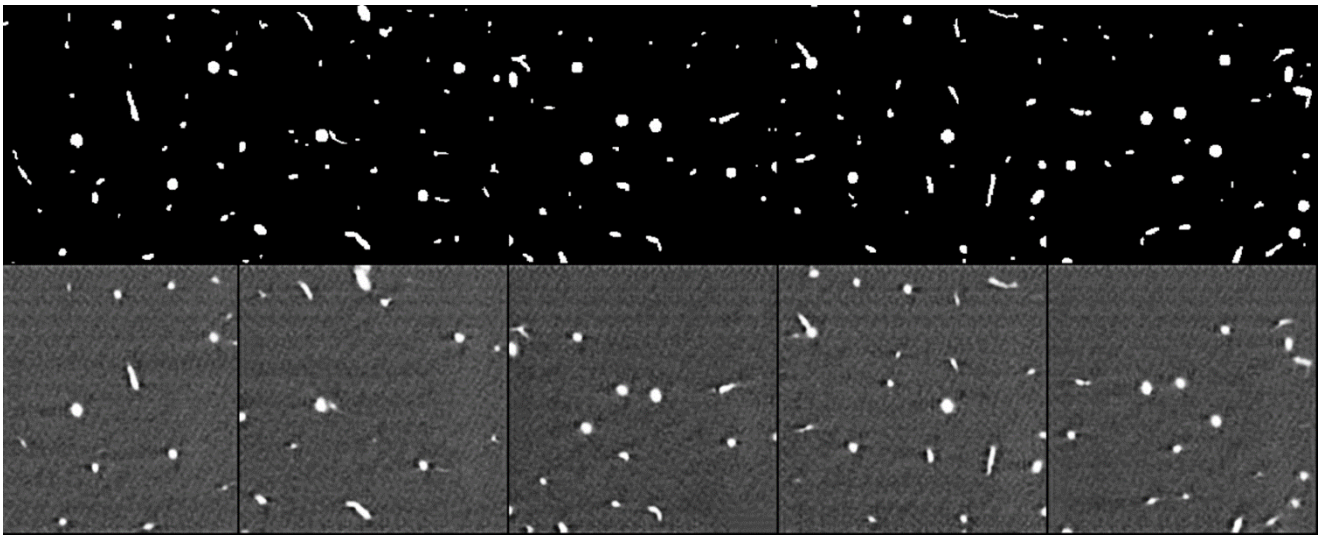

Figure 4: GAN Approach

GAN Approach, top row: segmentation maps, bottom row: synthesized SRX-Ray images

Further developments scheduled by the end of SGA2 include:

1) Improvement of the performance of the conditional generative adversarial network
2) Using our deep learning-based pipeline to fully segment this and any future full-brain data. Note that the resolution is available down to capillary level.

As part of component C1732, we will make the segmentation of the full-brain (see Figure 5) as a vtk formatted graph network and the source code of our methods available. This should make the output of this component testable by other scientists on other datasets with different modalities and resolutions.

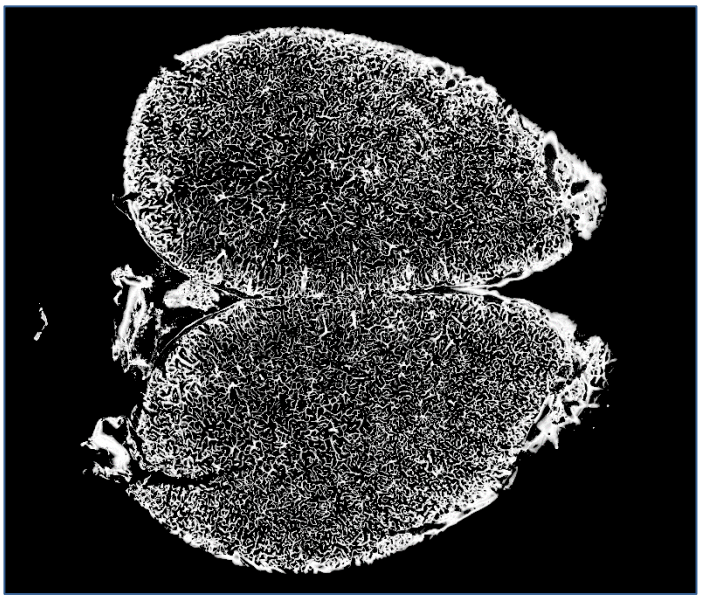

Figure 5: Blood vessels of the dorsal aspects of a whole mouse brain

Maximum intensity projection of the segmentation of a stack showing the blood vessels of the dorsal aspects of a whole mouse brain

5.2 Validation and Impact

5.2.1 Actual Use of Output(s)

Output 1: Since 2018, images are used by FUA lab (EPFL, T5.3.6) and KRESHUK lab (UHEI, then EMBL, T5.6.4) within HBP to test and develop analysis pipelines.

Output 2: Functional imaging data have been shared with our SP3 and SP4 collaborators (PAOLUCCI at INFN, T3.2.5 and DESTEXHE at CNRS, T4.1.4 and T4.4.1) to model fluorescence signals from of calcium concentration fluctuations and eventually from spiking activity. In addition, they are working on modelling calcium activity under different anaesthesia levels. The data have been shared in a Collab (https://collab.humanbrainproject.eu/#/collab/299/nav/2265 - only accessible to SGA2 reviewers) but they are not yet curated. We expect to finalise curation in the next months (M20).

In detail, our SP3 collaborators (PAOLUCCI, DE BONIS, INFN) developed a pipeline of data analysis that can extract the main features of wave propagation across the cortex from wide-field calcium images. This analysis tool and the model of the propagation of calcium waves, described in this pre-print article: https://arxiv.org/abs/1811.11687, will be essential for the next experiments on stroke, where the peri-infarct region shows slow-wave activity that could be modulated by rehabilitative treatment.

In parallel, our collaborators in SP4 (DESTEXHE, TORT COLET) developed a spiking network model capable of reproducing the spontaneous activity of a cortical network during anaesthesia. They decreased the strength of adaptation to reproduce the increase in frequency of the slow oscillations observed from two-photon calcium signals when decreasing the level of anaesthesia in Thy1-GCaMP6f mice (see a detailed description of results is in the CDP1 Collab (https://collab.humanbrainproject.eu/#/collab/299/nav/330284)).

In addition, new longitudinal fluorescence imaging data on rehabilitated stroke mice have been fully curated and shared with our collaborators in SP4 (JIRSA, PETKOSKI at AMU, T4.5.2) to validate their mean-field models on whole brain activity before and after stroke.

During SGA2, the longitudinal imaging data on rehab mice were used for model implementation and validation by the HBP groups of DECO (UPF, T4.5.1), JIRSA (AMU, T4.5.2) and LASCHI (SSSA, T10.1.1).

These collaborations are consolidated in the framework of CDP1.

Finally, data on rehabilitated mice are shared with our external collaborators, FANELLI's group (UNIFI), outside HBP, to validate their model on brain network plasticity.

Output 3: As of now, the output is not actively used until curation is complete.

5.2.2 Potential Use of Output(s)

Output 1: Whole brain datasets at sub-cellular resolution

Raw images are ready to use. Maps (point clouds) still require further refinement, especially because of the need to align them reliably to the atlas – a quite challenging task since the brains are a bit distorted by the clearing process.

Raw images can be used to validate a novel image analytics framework, both in academia and in industry. Several labs asked for our datasets (e.g. BLINDER's lab in Tel Aviv), as well as small companies working on machine vision (Bioretics Srl, Cesena, Italia).

Whole brain maps of different types can be used to generate more realistic brain models. We are already in contact with Michele MIGLIORE (CNR) to transfer interneuron distribution types.

Whole-brain activation maps can be used to validate brain activity models, also in the context of drug discovery.

Output 2: Functional connectivity of cortical neurons on GCamMP6f mice

Raw images are ready to use. Curated images, aligned within the Allen Mouse Reference Atlas, can be accessed on the NIP.

Longitudinal imaging data on stroke and rehabilitated mice will provide a framework to further develop a brain model with predictive capability, ready to be applied in clinical settings to define more effective treatments.

Fluorescence imaging data on slow wave activity, used to validate brain activity models, will be essential to unravel the mechanisms of brain state transitions, consciousness and stroke recovery.

Output 3: The source code can be used by any researcher to segment vasculature datasets. The methods are not just optimised for mice data, they can be used on any other dataset from other sources, including humans. The segmentation networks can be used for different types of simulations, since they also contain vessel diameters. Both segmentation maps and networks can be used to make statistical analysis and comparisons by interested researchers.

5.2.3 Publications

The 2 main publications for this KR are:

- P1387: Di Giovanna, A. P., Tibo, A., Silvestri, L., Müllenbroich, M. C., Costantini, I., Allegra Mascaro, A. L., Sacconi, L., Frasconi, P. and Pavone, F. S., Whole-Brain Vasculature Reconstruction at the Single Capillary Level, Scientific reports, 2018, 8(1), 12573. doi:10.1038/s41598-018-30533-3.

  Significance: Important example of whole-brain reconstruction of vascular system. Output 1.

- P1834: Di Giovanna A. P., Credi C., Franceschini A., Muellenbroich M. C., Silvestri L. and Pavone F. S., Tailored Sample Mounting for Light-Sheet Fluorescence Microscopy of Clarified Specimens by Polydimethylsiloxane Casting, Front. Neuroanat., 27 March 2019 | https://doi.org/10.3389/fnana.2019.00035

- P1762: Conti, E., Allegra Mascaro, A. L., Pavone, F. S., Large Scale Double-Path Illumination System with Split Field of View for the All-Optical Study of Inter-and Intra-Hemispheric

Functional Connectivity on Mice, Methods Protoc. 2019, 2(1), 11; https://doi.org/10.3390/mps2010011.

Significance: technology development to investigate brain plasticity in healthy subjects and after stroke. Output 2.

5.2.4 Measures to Increase Impact of Output(s): disseminations

Outputs contributing to KR1.3 have been presented to science conference and other dissemination events. Main examples are as follows:

- Neuron Reconstruction and Applications (NRA') Nanjing, China, 8-10 September 2018. L. Silvestri, "Effective management and analysis of ultra-terabyte brain images" (conference).

  Significance: we presented our results in an international workshop with scientists from HBP, Allen Institute, NIH and other major institutions (Output 1)

- OSA Optics and the Brain, Hollywood (FL), USA, 3-6 April 2018, L. Silvestri, A. P. Di Giovanna, G. Mazzamuto, T. Leergard, F. Orsini, I. Costantini, J. Bjaalie, P. Frasconi, F. S. Pavone, "Towards a Full Volumetric Atlas of Cell-specific Neuronal Spatial Organization in the Entire Mouse Brain" (poster)

  Significance: we presented our results in an international conference about optical methods in neuroscience (Output 1)

- SfN San Diego, 4 November, 2018. F. Resta, E. Conti, E. Montagni, G. De Vito, A. Scaglione, L. Sacconi, A. Allegra Mascaro, F. Pavone, "Simultaneous all-optical stimulation and readout of neuronal activity during optogenetically-evoked motor task", https://abstractsonline.com/pp8/#!/4649/presentation/41327. (presentation)

  Significance: We presented our results in the largest neuroscience audience at the Neuroscience congress, SFN 2019, during the nanosymposium "Voluntary movements". (Output 2)

6. Key Result KR1.4: Multilevel datasets generated by integrating neuroanatomical data with genetic, molecular and physiological data using advanced technologies

6.1 Outputs

6.1.1 Overview of Outputs

SP1 Objective SO1.4 aims at the integration of neuroanatomical information with genetic, molecular and physiological data to build models, make predictions and suggest new hypotheses to discover new aspects of the structural and functional organisation of the brain. To accomplish this Objective, outputs expected in KR1.4 include multilevel datasets generated by integrating neuroanatomical data with genetic, molecular and physiological data, using advanced technologies. In addition, novel tools for integration, visualisation and analysis of anatomical and functional data is being implemented across different scales.

A total of seven main outputs have been contributing to this KR in the first project year, these are the following:

1) New quantitative ultrastructural data of the rodent hippocampus and neocortex (C1746, C1749)
2) A setup for dual-area laminar probe recordings and optogenetics (C1831)
3) Interactive tools for the analysis of anatomical and functional data (C1869)
4) Vishnu Tool for the preparation of data to be loaded in different applications (C1870)
5) A new version of the KappaNEURON software released (C1612)
6) First update of proteomic dataset (C1611)
7) Parameter collection for model and disease molecular interactions and catalogue of molecular rule-based model Components (C17776, C1777)

See Annex A: Component Details for further component details.

6.1.2 Output 1

New data on quantitative ultrastructural data from the rodent hippocampus and neocortex

Quantitative ultrastructural data from the rodent hippocampus and neocortex were obtained. Five out of 21 FIB-SEM samples of the neocortex and three out of 12 FIB-SEM samples of the hippocampus were acquired. Two papers were published. Data have already been shared with the curation team. This output also contributes to KR1.5 (see Section 7).

Data location: https://collab.humanbrainproject.eu/#/collab/54052/nav/369794

6.1.3 Output 2

A setup for dual-area laminar probe recordings and optogenetics

A setup for dual-area laminar probe (32 channels per probe) recordings and optogenetics in awake and anaesthetised head-fixed mice has been developed. A new functional dataset was generated (30 experiments in 10 mice, 3 recordings per mouse: 4 VIP-ChR2 mice, 2 PV-ChR2 mice and 4 SST-ChR2 mice, targeting the 3 major interneuronal subpopulations). Data have been published and already been shared with the curation team.

Data location: https://collab.humanbrainproject.eu/#/collab/54057/nav/369818 (Access: SGA2 reviewers). This Collab (component C1831) has been updated recently in touch with the curation team. All data collected so far and the metadata necessary to read the data itself have been updated.

6.1.4 Output 3

Interactive tools for the analysis of anatomical and functional data

Preliminary versions of several tools, developed in SGA1, with a subset of the planned functionalities have been made publicly available. These tools are as follows: 'Intool Explorer' to provide a user-designed canvas for data visualisation and interaction and to perform specific exploratory tasks according to the user needs; 'Pyramidal Explorer' to explore the data through content retrieval operations (see Figure 6 and Figure 7); 'DC Explorer', a WEB-based application designed to compare populations of micro-anatomical data; and 'Clint Explorer' that uses supervised and unsupervised learning techniques to cluster neurobiological dataset. A paper was published (see Section 6.2.3).

The upgraded tools have been added to the Collab:

- Pyramidal Explorer: https://collab.humanbrainproject.eu/#/collab/57641/nav/393533
- Clint Explorer: https://collab.humanbrainproject.eu/#/collab/54219/nav/370882
- DC Explorer: https://collab.humanbrainproject.eu/#/collab/31935/nav/222567

- InTool Explorer: https://collab.humanbrainproject.eu/#/collab/31936/nav/222572

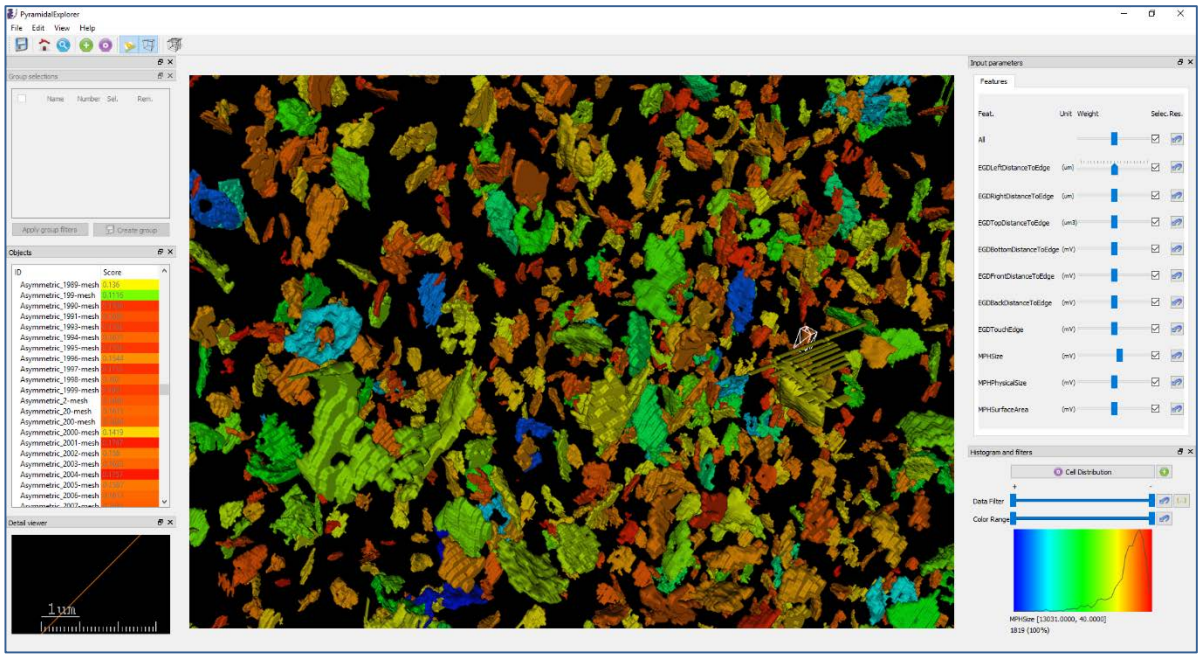

Figure 6: Synaptic information from 1819 asymmetric synapses visualised with Pyramidal Explorer

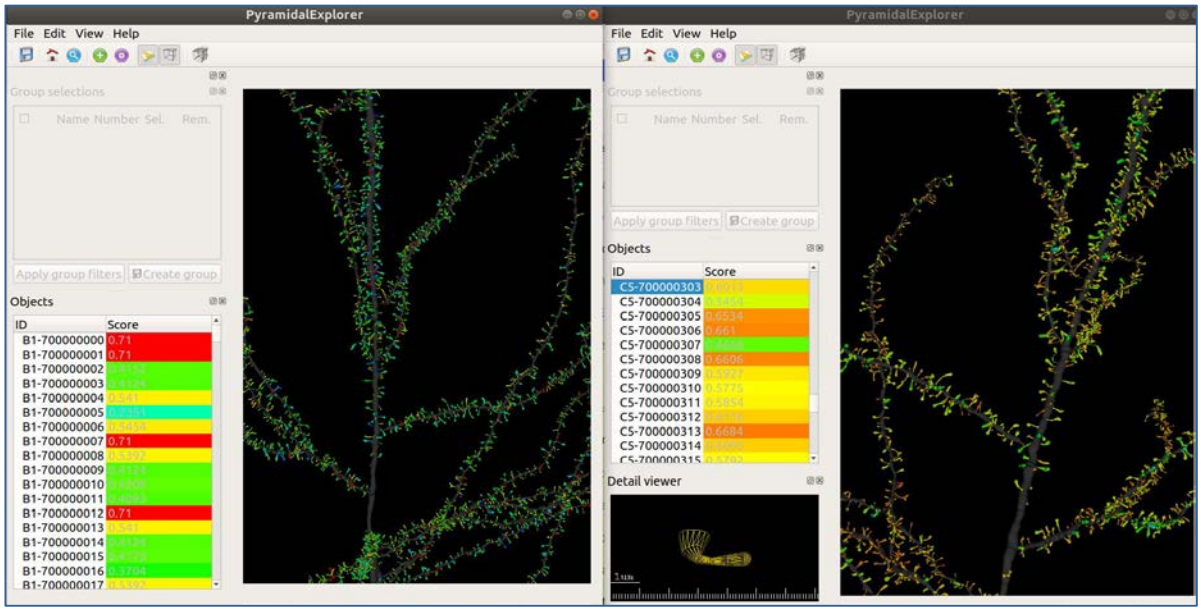

Figure 7: Pyramidal Explorer visualisation of 2 different neurons

6.1.1 Output 4

Vishnu Tool for the preparation of data to be loaded in different applications. The tool Vishu was developed as communication module for the information exchange between data analysis tools. This tool prepares data to be loaded in different applications. It defines a communication protocol that allows exploratory analysis applications to interact in real time. The developers are working on adapting the applications of the interactive tools for the analysis of anatomical and functional data to make use of this new communication protocol. The first prototype has been released to the target user group at the end of M14.

Collab link: https://collab.humanbrainproject.eu/#/collab/54221/nav/370894

6.1.2 Output 5

A new version of the KappaNEURON software released

A new version of the KappaNEURON software was released (https://github.com/davidcsterratt/KappaNEURON). New features in the KappaNEURON v0.3.0 release include: passing the voltage to the Kappa submodel, allowing, e.g., modelling of NMDAR Mg-unblock; and installation using pip. KappaNEURON has been used to build a detailed model of signalling pathways in the context of a synapse on a CA1 hippocampal cell. This M12 release is available in the KappaNEURON Collab and at the above link). KappaNEURON is integrated in the Brain Simulation Platform as an online use case at https://collab.humanbrainproject.eu/#/collab/1655/nav/362935.

6.1.3 Output 6

First update of proteomic dataset

28 postsynaptic proteome studies were curated, containing a total of 5387 proteins, 9 presynaptic proteome studies (1951 proteins) and 7 whole synaptosome studies (5962 proteins). This constitutes the release ("First draft of proteomic dataset") released at M10. At M12 the corresponding numbers were: Postsynaptic proteome - 28 studies, 5387 proteins; presynaptic proteome 9+14=23 studies, 1951+342=2293 proteins; synaptosome – 7+3=10 studies, 5962+131= 6093 proteins. These numbers exceed the planned progress at the M12 release: ~5600 proteins - >95% complete. Data are available at the "Synaptic proteins and molecular interactions" collab (this collab is only accessible to SGA2 reviewers). A manuscript presenting this work is in preparation.

6.1.4 Output 7

Parameter collection for model and disease molecular interactions and catalogue of molecular rule-based model Components

Feedback has been incorporated to the draft schema for curating kinetic data by including mappings onto standard identifiers via the https://identifiers.org/ resolution service (cf MIRIAM standards), and the M12 version was released at the "Synaptic proteins and molecular interactions" collab (see Output 6) (this collab is only accessible to SGA2 reviewers). The schema is being used to describe both the catalogue of 250 molecular rule-based model components (C1777) and the detailed parameter collection for model- and disease-relevant molecular interactions (C1776). As planned in our M12 release of C1777, it includes a table of definitions of 100 out of a planned 250 "agents" (proteins or ions). It also includes a table of 130 "rules" (generalised reactions), curated from the literature, with reaction rates, as well as a table with data on abundance of the proteins. As planned in the M12 release of C1776, it contains curated parameters from 10 more reactions. The rules from this database form part of the KappaNEURON model (C1612). A manuscript presenting this work is in preparation.

6.1.5 Output 8

Multi-scale organization of circuit activity and plasticity in the mouse cerebellum following pattern sensory stimulation

Electrophysiological recordings and optogenetic stimulation *in vivo* revealed that synaptic plasticity could be induced into the deep cerebellar nuclei by naturally patterned sensory stimulation. This is the last form of plasticity that we needed to parameterize the cerebellar changes during learning. The unique dependency of this plasticity on circuit oscillations discloses a potential relationship

between cerebellar learning and activity patterns generated in the cerebro-cerebello-cortical loops. A paper was published (see publications Section 6.2.3).

Several datasets have already been linked to the KG:

https://doi.org/10.25493%2F6R48-E3V

https://doi.org/10.25493%2FMDAR-XEB

https://doi.org/10.25493%2F4AF6-WSD

https://doi.org/10.25493%2FF2VK-MB4

https://doi.org/10.25493%2FG07Q-K87

https://doi.org/10.25493%2FMVHQ-4YA

https://doi.org/10.25493%2FJQH3-0A4

6.1.6 Output 9

Single-cell 3D reconstruction and measurement of thalamocortical nuclei and quantitative ultrastructural analysis of the synapses

This is also Output 4 from KR1.2 (Section 4.1.5). It has also contributed to this KR. In particular, this is the first demonstration that axons from the Posterior nucleus (an Association/Higher-Order thalamic nucleus), which branch to innervate both the primary motor and somatosensory cortices, form in each area contacts with different synaptic structure and glutamatergic receptor mechanisms (Complete). This study combines *in vivo* extracellular electrophysiology and electron microscopy of selectively labelled thalamocortical synapses. This has been done in collaboration with other groups outside the HBP (NUÑEZ and LUBKE labs.). These structural and functional data are relevant for biologically constraining models of the rodent somatic sensory cortex and motor cortex circuits. A paper is under review in Brain Structure and Function.

6.2 Validation and Impact

6.2.1 Actual Use of Output(s)

Output 1: This Output is not actively used until curation is complete.

Output 2: A data sharing protocol with Maurizio MATTIA (SP3, T3.5.2) has been initiated. Data collected at different levels of anaesthesia will be used to investigate how cortical slow oscillations travel across areas. This expands their current setup to explore how slow oscillations travel between cortical layers. A collaboration with the partnering project CANON (and in particular with Zoltan ZSOMOGYVARI and Laszlo NEGYESSY, Hungarian Academy of Science) is carried out. In this collaboration we are expanding the frame of reference of the CANON project (focused on V1 and association cortices) to S1HL. Finally, the integration of physiological data with anatomical characterisation of S1HL has already started with other SP1 members.

Output 3: Although the final version of the data analysis tools has not been released, the current prototypes of Intool Explorer and Explorer implement useful functionalities in TRL4 and have been validated by its TUG. Both applications are publicly available at http://cajalbbp.es/intoolexplorer_web/ and http://cajalbbp.es/dcexplorer_web/. At those websites, users can choose between a WEB-based environment and a standalone application compiled for Microsoft Windows. The Pyramidal Explorer version developed in SGA1 has been validated by the users, is in TRL4 and binaries are publicly available at http://gmrv.es/gmrvvis/pyramidalexplorer/ for Microsoft Windows, macOS and Ubuntu operative system. The new version of Pyramidal Explorer has been sent to the TUG for its validation but it is not publicly available. This output is currently being used by SP1 Tasks (T1.4.1 and T1.5.1).

Output 4: the first prototype of the integrated environment is currently under development and not available yet.

Output 5: it has been shared with SP6, T6.2.7 (HELLGREN, KTH and KELLER, EPFL), feeding into the ongoing collaboration about using rule-based modelling for computational neuroscience models. In UEDIN we have been using KappaNEURON to model calcium signalling in spines, and to model tetanic Long Term Potentiation.

Outputs 6 and 7: Several HBP groups (e.g. CARLONI's group (JUELICH) have started to explore the protein-protein interactions (PPIs) for on-going detailed molecular dynamics. The datasets developed in both outputs will underpin molecular activities in SGA3 by HELLGREN-KOTALESKI, CARLONI, ROSSETTI and CICHON. The rule-based components are currently being used in a simulation/model, which we hope to publish.

Output 8: Cross-validation of data and models is ongoing. The single cell data obtained here are transformed into models and incorporated into local network and large-scale network simulators on the Brain Simulation Platform. The data on the multi-scale organisation of circuit activity are used as a fundamental validation test-bench for large-scale closed-loop simulations. The impact is on the generation of models, workflows and robotic SNN.

6.2.2 Potential Use of Output(s)

Output 1: Data will be used in models of the hippocampus and neocortex, as well as in comparative studies between human and rodent brains as planned. In addition, the data would provide new knowledge about the structural design of these neurons.

Output 2: A conversation with SP6 (Vishal SOOD) was initiated to use the collected data in S1HL to validate the model of the mouse neocortex. This activity will start at the end of the curation process. The focus is on comparing spontaneous (i.e. not sensory-evoked) activity *in vivo* and *in silico*.

Outputs 3: The released version of the three data analysis tools could be used in any Use Case where data visualisation and exploration is needed, inside the HBP and outside the HBP and not only in the neuroscience domain.

Output 4: The final version of this tool could be used with anatomical data and will be able to be extended to other types of data.

Output 5: This could be used for rule-based modelling for computational neuroscience models.

Outputs 6 and 7: These outputs will be used to underpin and direct molecular dynamics simulations of key molecular interactions in SGA3. Output 6 will provide a broad contextual map of interactions and Output 7 the critical elements for selected interactions to feed into molecular dynamic simulations.

Output 8: The potential further use is for spiking robotic controllers (voucher program #47), neuromorphic hardware design (voucher program #49) and incorporation into the mouse virtual brain (partnering project CEREBNEST).

6.2.3 Publications

The three main publications of this KR are:

- P1691: Meijer, G. T., Mertens, P. E., Pennartz, C. M., Olcese, U., & Lansink, C. S. (2019). The circuit architecture of cortical multisensory processing: distinct functions jointly operating within a common anatomical network. Progress in neurobiology, 174.

  Significance: This publication provides a review of the current knowledge of how sensory modalities interact in neocortical circuits, and indicates which open questions remain. This publication is the foundation indicating how our output (datasets related to component ID 1831) goes beyond the state of the art.

- P1767: Furcila D, García M, Toader C, Morales J, LaTorre A, Rodríguez A, Pastor L, DeFelipe J,Alonso-Nanclares L. InTool Explorer: an interactive exploratory analysis tool for versatile visualizations of neuroscientific data. Frontiers in Neuroanatomy 2019 Mar 11;13:28. doi: 10.3389/fnana.2019.00028

  Significance: This publication shows the usefulness of providing a user configurable and flexible environment to develop custom visual data analysis workflow in the neurobiological domain.

- P1750: Moscato L, Montagna I, De Propris L, Tritto S, Mapelli L and D'Angelo E (2019) Long-Lasting Response Changes in Deep Cerebellar Nuclei in vivo Correlate With Low-Frequency Oscillations. Front. Cell. Neurosci. 13:84. doi: 10.3389/fncel.2019.00084.

6.2.4 Measures to Increase Impact of Output(s): disseminations

Outputs contributing to this KR have been presented to scientific conferences and to as part of outreach activity to the community.

The main three dissemination activities for this KR are:

- SfN Neuroscience, minisymposium 'Species differences in the morphology and neurochemical features of cortical interneurons', 6 November, 2018. San Diego (USA). DeFelipe, J. "Multidimensional Neuronal Cell Type Classification in the Cerebral Cortex". Output 1
- Fall Brain Conference "The Necessity of Cell Types for Brain Function", 7-10 October, 2018, Moltkes Palæ. Copenhagen (Denmark). DeFelipe, J. "Cortical cell type differences and similarities in different species: human cortical circuit specializations." Output 1
- II Jornadas CNS Exeltis Day, 18 January 2019, Madrid, Spain. DeFelipe, J. Key Lecture: "Nuevas tecnologías para el estudio del cerebro: Human Brain Project." Output 1

7. Key Result KR1.5: Strategic datasets on single neurons and circuits to be used in comparative studies on human and rodent

7.1 Outputs

7.1.1 Overview of Outputs

KR1.5 is aiming at generating key datasets on single neurons and circuits to be used in comparative studies on human and rodent brains for modelling by using ground-breaking techniques and new statistical models. The aim is to obtain critical information about differences and similarities in brain organisation across species (Objective SO1.5). In the first project year, preliminary datasets were generated and comparative analyses were carried out. The main outputs contributing to KR1.5 are as follows:

1) New data of the human neuropil at the ultrastructural level (C1747)
2) Comparative study of 3D reconstructions of human and mouse pyramidal cells (C1740, C1741)
3) New data on quantitative ultrastructural data from the rodent hippocampus and neocortex (C1746, C1749)
4) New datasets to implement comparative studies of mouse and human brains circuits (C1729, C1731)

5) Morphology comparative models (C1804)
6) A novel way for multivariate comparison and analysis of neuron morphology and electrophysiology (C1802, C1803, C1804)

See Annex A: Component Details for more details.

7.1.2 Output 1

New human data of the neuropil at the ultrastructural level

3D Quantitative data of the neuropil at the ultrastructural level from human mesial temporal cortex (neocortex and hippocampus) were obtained. Specifically, the acquisition of 9 FIB/SEM samples of the temporal neocortex, and 12 FIB/SEM samples from the CA1 region of the human hippocampus (including stratum oriens, stratum pyramidale, stratum radiatum and stratum lacunosum-moleculare) was accomplished.

Data location: https://collab.humanbrainproject.eu/#/collab/54053/nav/369799

7.1.3 Output 2

Comparative study of 3D reconstructions of pyramidal cells between species and between cortical areas

A comparative study of 3D reconstructions of pyramidal cells using Neurolucida software from CA1 hippocampus of mice (n=25) and humans (n=25) was performed.

3D reconstructions of pyramidal cells in the human visual cortex (area 17; n= 11), the motor cortex (area 4; n= 7), the middle temporal cortex (area 21; n= 17), the superior temporal cortex (area 22; n= 7), the CA1 transition to subiculum (n=8) and the subiculum (n= 20) of the hippocampal formation, were obtained using Neurolucida software from a 3D confocal stack of images.

Data location:

- Comparative study of 3D reconstructions of pyramidal cells using Neurolucida software from CA1 hippocampus of mice (n=25) and humans (n=25: https://collab.humanbrainproject.eu/#/collab/54055/nav/369809
- 3D reconstructions of pyramidal cells in the human visual cortex, the motor cortex, the middle temporal cortex, the superior temporal cortex, the CA1 transition to subiculum (and the subiculum of the hippocampal formation: https://collab.humanbrainproject.eu/#/collab/54056/nav/369814

7.1.4 Output 3

New data on quantitative ultrastructural data from the rodent hippocampus and neocortex

This Output contributes to both this KR and KR1.4. It is described in detail in Section 6.1.2.

7.1.5 Output 4

New datasets to implement comparative studies of mouse and human

New datasets to implement comparative studies were obtained.

Comparative physiology of mouse and human neocortical excitatory synapses: excitatory synapse properties received by pyramidal neurons and somatostatin interneurons types were determined, and compared to their counter parts in the rodent neocortex. Similar to the rodent neocortex, PYR-SOM glutamatergic synapses are facilitating and can drive interneurons to action potential firing.

Comparative physiology of neuromodulation of neocortical circuits in mouse and human brains: neuromodulation of cortical microcircuits by acetylcholine, adenosine and metabotropic glutamate receptors was quantified, in particular of excitatory and inhibitory synapses as well as Martinotti cells, and compared to neuromodulation in rodent neocortex. An article was published (see Section 7.2.3 Publications).

Access to the data is in the publications:

- P1962: Kroon T, Dawitz J, Kramvis I, Anink J, Obermayer J, Verhoog MB, Wilbers R, Goriounova NA, Idema S, Baayen JC, Aronica E, Mansvelder HD, Meredith RM. (2019) Group I mGluR-Mediated Activation of Martinotti Cells Inhibits Local Cortical Circuitry in Human Cortex. Frontiers Cell Neurosci. 2019 Jul 11;13:315. doi: 10.3389/fncel.2019.00315.
- P1641: Obermayer J, Heistek TS, Kerkhofs A, Goriounova NA, Kroon T, Baayen JC, Idema S, Testa-Silva G, Couey JJ, Mansvelder HD. (2018) Lateral inhibition by Martinotti interneurons is facilitated by cholinergic inputs in human and mouse neocortex. Nature Commun. 2018 Oct 5;9 (1):4101

7.1.6 Output 5

A novel way for multivariate comparison and analysis of neuron morphology and electro-physiology

A novel way for multivariate comparison and analysis of neuron morphology and electro-physiology data has been introduced. The groundwork for such comparisons has been laid by defining useful metrics, such as the dendritic branching regularity index (P1724: Anton-Sanchez et al., "A regularity index for dendrites – local statistics of a neuron's input space", ***PLoS Computational Biology***, vol. 14, 11, e1006593), and the NeuroSTR software (https://computationalintelligencegroup.github.io/neurostr/) to compute them (e.g., P1722: Mihaljevic et al., "Towards a supervised classification of neocortical interneuron morphologies", ***BMC Bioinformatics***, vol. 19, issue 1, pp. 511, 2018)), and the use of circular statistics to analyse dendritic field orientation (P1723: Leguey et al., "Patterns of dendritic basal field orientation of pyramidal neurons in the rat somatosensory cortex", ***eNeuro***, vol. 5, issue 6, 2018).

7.1.7 Output 6

Single-cell 3D reconstruction and measurement of thalamocortical nuclei and quantitative ultrastructural analysis of the synapses

This Output is the same as Output 4 from KR1.2 that has also contributed to this KR. It is described in detail in Section 4.1.5.

7.2 Validation and Impact

7.2.1 Actual Use of Output(s)

Output 1: Data generation was only completed recently. These preliminary data are planned to be used to develop maps of functional properties of the synapses in T1.5.2, and are being used in SP1 to develop tools for the analysis of micronatomical data in T1.4.4. Final datasets are planned to be released in M24.

Output 2: 3D reconstructions of pyramidal cells from CA1 hippocampus of mice and humans have been used to carry out comparative studies planned in SP1 (see SP1 publication P1963).

Output 3: see section 6.2.1

Output 4: Data have been used to implement comparative studies planned in SP1 (see SP1 publications P1343, P1641 and P1962).

Output 5: This model has been used by neuroanatomists from SP1 (T1.5.1) for comparisons of the dendritic structure of pyramidal neurons.

7.2.2 Potential Use of Output(s)

Output 1: data on synapses in the neuropil are necessary in detailed models of the hippocampus and neocortex, as well as in comparative studies between human and rodent brains. In addition, this Output could be used in the Human Brain Atlas.

Output 2: data on 3D reconstructions of pyramidal cells could be used in comparative modelling and integration of anatomical data with functional studies in different brain regions. In addition, this Output could be used in the Human Brain Atlas.

Output 3: Data could be used in models of the hippocampus and neocortex, as well as in comparative studies between human and rodent brains as planned. In addition, the data would provide new knowledge about the structural design of these neurons.

Output 4: data have been used to implement the comparative studies planned in SP1 (see publications section below). These studies could be critical to better understanding the similarities and differences of the structural and functional organisation of cortical circuits.

Output 5: this model could be applied for multivariate comparison and analysis of neuron morphology and electro-physiology in other brain regions.

7.2.3 Publications

The main three publications of this KR are:

- P1963: Ruth Benavides-Piccione, Mamen Regalado-Reyes, Isabel Fernaud-Espinosa, Asta Kastanauskaite, Silvia Tapia-González, Gonzalo León-Espinosa, Concepcion Rojo, Ricardo Insausti, Idan Segev, Javier DeFelipe (2019) Differential structure of hippocampal CA1 pyramidal neurons in the human and mouse. Cerebral Cortex, 2019; 00: 1–23. doi: 10.1093/cercor/bhz122
- P1641: Obermayer J, Heistek TS, Kerkhofs A, Goriounova NA, Kroon T, Baayen JC, Idema S, Testa-Silva G, Couey JJ, Mansvelder HD. (2018) Lateral inhibition by Martinotti interneurons is facilitated by cholinergic inputs in human and mouse neocortex. Nature Commun. 2018 Oct 5;9 (1):4101
- P1962: Kroon T, Dawitz J, Kramvis I, Anink J, Obermayer J, Verhoog MB, Wilbers R, Goriounova NA, Idema S, Baayen JC, Aronica E, Mansvelder HD, Meredith RM. (2019) Group I mGluR-Mediated Activation of Martinotti Cells Inhibits Local Cortical Circuitry in Human Cortex. Frontiers Cell Neurosci. 2019 Jul 11;13:315. doi: 10.3389/fncel.2019.00315.

7.2.4 Measures to Increase Impact of Output(s): disseminations

Outputs contributing to KR1.5 have been presented at science conferences and other dissemination events. The main three dissemination events are:

- FENS Forum 2018, 7-11 July 2018 Berlin (Germany). Marta Dominguez, Marta Montero-Crespo, Ricardo Insausti, Lidia Blazquez-Llorca, Lidia Alonso-Nanclares, Javier DeFelipe. Poster (Abstract: 1425) C019: “Synaptology of the mesial temporal cortex in Alzheimer’s disease”. Outputs 1-3)
- Volga Neuroscience Meeting, 22-25 July 2018, Nizhny Novgorod – Samara – Nizhny Novgorod, Russia. Huib MANSVELDER. Plenary Lecture “Organization and function of cortical microcircuits in mouse and human brain”. Output 4.

- 3rd HBP Student Conference on "Interdisciplinary Brain Research", 6-7 February 2019, Ghent, Belgium, the following posters:
  - Mihaljevic, B., C. Bielza, and P. Larrañaga "Multivariate comparison of human and mouse pyramidal cell dendritic morphologies." (Output 5)
  - Nicolás Cano-Astorga, Javier DeFelipe, Lidia Alonso-Nanclares "Three dimensional analyses of synapses in the human temporal neocortex." (Outputs 1-3)

8. Update on implementation of the SP1 DMP

The SP1 DMP has been updated as planned. In particular, the completeness of releases has been assessed according to the timeline planned: at M3, M6 and M12.

In general, the releases planned in the period M1-M12 have been achieved as scheduled, except for a minor delay in the first data analysis of the cross-species map of striatal neurons. The procedure to process monkey, rodent & human frozen tissue to perform single-cell RNA-seq has been set-up, but a dataset cannot be provided by M12 as planned. However, we expect to release it in the next few months and no major deviations are expected in the progress of the activities scheduled to achieve the relevant Key Result (i.e. KR1.5).

These releases contribute to the 5 SP1 Key Results. In particular:

- At molecular level, new datasets on quantification of densities of AMPA-type glutamate receptor in hippocampal CA1 pyramidal cells have been generated (Output 8, see Section 3.1.9) to contribute to KR1.1
- At cellular and microcircuits level, morphological and functional datasets have been obtained in the cerebellum, hippocampus, basal ganglia and neocortex (Outputs 1-7 in Section 4.1). These datasets contribute to KR1.2.
- In the period M1-M12, releases planned at whole-brain level include datasets of inhibitory interneurons together with activation datasets (via c-fos labelling), of vasculature, and of functional data of fluorescence imaging of cortical activity (Outputs 1-3 in Section 5.1). These releases contribute to KR1.3.
- Releases achieved for the integration studies include new anatomical and functional datasets in the neocortex and cerebellum, as well as new advances for the molecular integration (a new proteomic dataset, parameter collection and a new version of the KappaNEURON software were released). These releases contribute to KR1.4 (Outputs 1, 2, 5, 6 and 7 in Section 6.1).
- Releases planned to carry out the comparative studies have also been achieved, including new datasets on quantitative data of the neuropil at the ultrastructural level in human brain, quantitative ultrastructural data of the mouse hippocampus and 3D reconstructions of human neocortical pyramidal cells. In addition, a first comparison of morphological parameters of pyramidal neurons between rodents and humans has been implemented, and morphology comparative models are complete. These releases contribute to KR1.5 and are reported as Outputs 1-6 in Section 7.1 of this report.

The detailed information on these achievements can be found in the SP1 DMP version HBP-SGA2-SP1DMP-M12-v2.2 (https://collab.humanbrainproject.eu/#/collab/5972/nav/46222; Navigation menu: Data Management Plan/Workspace section 'SGA2'/ Subsection 'HBP-SGA2-SP1DMP-M12-v2.2'). This Collab is only accessible to SGA2 reviewers. This Collab has been updated and reorganised in line with the reviewers' comments to ensure that the updates are easily accessible. The official update for M12 is now being stored separately from the other updates carried out during the first SGA2 project year. The updates, for M16, M18 and M24, are and will also be stored in separate sections.

The generation of the remaining datasets, models and tools is expected to continue as planned. The final update on the implementation will include the progress of the components achieved until M24. These updates will be uploaded in the above Collab.

Coordination with the Curation Team (CT) to register the SP1 data in the Knowledge Graph (KG)

In response to one of the main concerns raised by the reviewers, the data curation process followed by SP1 in collaboration with the CT is outlined below.

At the beginning of the SGA2, the SP1 DMP was shared with the CT to keep them informed about the SP1 planning in terms of datasets. This planning includes the storage of the outputs in HBP repositories, data curation and the integration of the datasets in the KG. At this stage, while some data generated have been uploaded in HBP repositories such as the Collab or CSCS containers to be curated and integrated into the KG, others are currently being processed and will be uploaded. Finally, the curation team regularly provides SP1 with an update on the status of the curation process and the availability of the data in the KG, when ready, as occurred in the previous project phase.

All datasets that have been curated and published in the HBP KG are stored at CSCS (HBP storage). Transferring the data to CSCS is a prerequisite for datasets to be published in the HBP KG. Datasets that have not been curated are stored locally at partner institutions or in other repositories (e.g., HBP Collaboratory). These datasets need to be transferred to CSCS as soon as the dataset is finalised/released and ready for entering the curation process. Even if entered in the curation process, datasets can be embargoed by the PIs.

In general, partially released datasets are not submitted to the curation process since the Curation team waits for full releases of datasets before proceeding with curation to avoid repeated curation steps. According to the information received, some proposals are being reviewed by this team to see how partially released data sets may be curated and made available in the KG.

As mentioned above, SP1 data generated in the period M1-M12 in the SGA2 are stored either in the CSCS containers or on the HBP Collaboratory, in specific SP1 Collabs. Specifically, the information regarding the SP1 datasets stored in the Collabs, as well as the IT tools and their relevant links, can be found in this Deliverable at output level and also in the Collab: SGA2 - Management and Coordination activities overview/SPs/SP1 Mouse Brain Organization. The information added to this Collab is copied to the Collab reviewers. Finally, it can be also found in the SP1 Collab https://collab.humanbrainproject.eu/#/collab/5972/nav/46222 (Navigation menu: Data Management Plan/Workspace section 'SGA2'/ Subsection 'SP1 Datasets and IT tools M1-M18').All the SP1 SGA2 Collab links are accessible via the account 'SGA2reviewers'.

9. Conclusion and Outlook

A total of 30 Outputs are outlined in the present Deliverable. These outputs show the advances that are being carried out in SP1, in line with the data plan defined for the five Key Results which are related to research on single molecules, synapses and cells, to build up the selected microcircuits, to research on brain-wide scale, and for comparative studies of single neurons and circuits. No major changes have been made to the Work Plan, and progress has been as planned. At the end of SGA2, it is expected that the scientific sub-objectives proposed to fulfil the overall objective of this SP will be met.

KR1.1 Outputs include the development of novel methods and exploitable tools for mapping and imaging, new software for automatic detection and clustering analysis, as well as new protocols to study plasticity at the molecular level. It is expected that the generation of molecular and subcellular datasets, coordinated and integrated with anatomical and functional studies, will provide the foundation for both key Platform Deliverables and co-designed drivers for advanced Platform capabilities.

For KR1.2, a unique contribution to the categorisation and standardisation of multi-scale brain data is being released, using high resolution analysis of neurons and neuronal microcircuits, both

structurally and functionally. New characterisation methods are being upgraded. Multilevel approaches are being carried out for the construction of models. Thus, the Outputs contributing to KR1.2 will help provide a better understanding of the microcircuits of the neocortex, cerebellum, hippocampus and the basal ganglia. The obtained morphological data are integrated with electrophysiological data to generate computational models that reproduce the functioning of these structures at single-cell and microcircuit levels. Therefore, the results are expected to represent major advances in neuroscience.

Outputs contributing to KR1.3 are providing structural and functional datasets on a brain-wide scale by using cutting-edge imaging technologies. These technologies are being used to obtain functional and structural measurements. As stated in the SP1 Work Plan, the technological development is one of the key issues in this KR and it is expected that the resulting technical advances will be of use worldwide. Data obtained are fundamental for validation of simulation of brain functionality and connectivity developed within other SPs, such as SP4 and SP6. In addition, data from the brain-wide studies are being used to build and validate the simulations of the same experimental paradigms within the NRP, together with SP10. This KR is directly linked to CDP1.

For KR1.4, the datasets obtained, and also those planned, are and will be unique datasets of simultaneous recordings of all the major subpopulations of cortical neurons in multiple areas. This is key for understanding how the brain works, in terms of single areas and in terms of interactions between multiple areas. The datasets that are being developed will be unique for the HBP Multilevel Atlas of the Mouse Brain and will be of value for the scientific community inside and outside the HBP.

Outputs for KR1.5 provide critical information about differences and similarities in brain organisation across species. Similarities between human and mouse brains at the level of microanatomy and physiology of pyramidal cells and synapses may be considered as basic building blocks of cortical organisation, building a bridge toward the SP2 analysis of the human brain at the integrative level.

To maximise the visibility of the Outputs achieved in SP1, they were shown in different dissemination events as planned in the dissemination plan. This plan was the main tool to facilitate the use of SP1 results internally and to maximise the impact of these results outside the HBP during the period M1-M12 and it will also be used in the remaining phase. The main dissemination activities carried out by SP1 in this period include publications (31, and 5 more that are accepted, *in press* or under review); participation in international conferences; seminars and other events; and media exposure. Another aspect related to results dissemination is the SP1 DMP for the SGA2 (see Section 8). The SP1 DMP describes the data management life cycle for the data that are generated by SP1, as well as the use of these data for modelling purposes. In addition, this plan also includes the planning of the methods, models and tools that are being developed in this SP. The DMP is a useful system to organise the outputs generated internally and to facilitate further use of them by other SPs, maximising their impact.

In summary, the following highlights might be considered:

1) In addition to the generation of data, SP1 participates in curation, annotation and interpretation of data, which is fundamental to generate densely annotated atlas. This is important because this kind of annotated atlas is basically lacking and it could be very useful for students and expert researchers.

2) Interactions across levels and sub-disciplines between different groups of SP1 is a major aim. Indeed, we have shown some cases how productive are such interactions (e.g. cross-SPs publications such as P1343, P1963, and P1110).

3) The updated and new tools developed by SP1 are highly relevant to facilitate the study of the brain. Currently, SP1 is trying to expand the use of these tools by the scientific community outside the HBP.

4) The comparative studies performed in SP1 are important for a better understanding of the human brain as they serve to fill gaps of knowledge which are impossible to obtain in human brains and allow predictions about what to expect in human brains. Furthermore, it facilitates interpretation of similarities and differences. For example, in P1963, the results show that

human CA1 pyramidal cells are not a stretched version of mouse CA1 cells. These results indicate that there are some morphological parameters of the pyramidal cells that are conserved, whereas others are species-specific.

Finally, we expect to continue with the progress of the SP as planned. Therefore, we are confident that the remaining outputs will be generated as scheduled to meet the scientific objectives proposed.

Annex A: Component Details

Table 3: Overview of releases and major updates related to Key Result KR1.1

ID	Component Name	Type	Contact	Info on releases and major updates
1767	Nanobodies and other strategies for Next-Generation Brain Imaging & Mapping	dataset	Antonino CATTANEO	Releases: • New nanobodies against members of the neurexin family and neuronal targeting of anti-neuroligins intrabodies • Brain imaging and mapping: new anatomical patterns of neurological relevant antigens obtained by immunodetection with nanobodies in different areas of mouse and human brains Effective date: M18/M20
1770	Functional *in vivo* interaction data between neuroligin and the neuroxin families, and their use for the computational modelling of trans-synaptic signalling	dataset	Antonino CATTANEO	Releases: • Functional characterization of short- and long-term synaptic plasticity in experimental models of synaptic interference • Models of Short and Long-Term synaptic plasticity including subcellular pathway • *In vivo* validation of synaptic interference and localization of activated dendritic spine following behavioural tasks Effective date: M18/M20/M20
1886	Density distribution of glutamate receptors and calcium channels in principal cells	dataset	Ryuichi SGHIGEMOTO	Release: Subcellular 2D & 3D distribution of GABAB receptors in the hippocampus Effective date: M18

Table 4: Overview of releases and major updates related to Key Result KR1.2

ID	Component Name	Type	Contact	Info on releases and major updates
C1743	Organisation of the axon initial segment (AIS) of striatal and cerebellar neurons	dataset	Alberto MUÑOZ	Release: AIS geometrical characteristics Effective date: M12
C1744	Organisation of dendritic trees of striatal and cerebellar neurons	dataset	Alberto MUÑOZ	Release: Preliminary data on dendrites of striatal and cerebellar neurons Effective date: M12
C1771	The micro-connectivity of the cerebellar glomerulus	dataset	Egidio D'ÁNGELO	Release: The cerebellar network recorded; Cerebellar neurons recorded. Effective date: M06/M12/M18/M24
C1772	Detailed reconstruction of inhibitory	dataset	Egidio D'ÁNGELO	Release:

	interneurons of the cerebellar cortex			The cerebellar network recorded; Cerebellar neurons recorded. Effective date: M06/M12/M18/M24
C1774	Structure and function of the striatal matrisomal microcircuit - interneurons and input organisation	dataset	Sten GRILLNER	Release: The striatal microcircuit – structure and function Effective date: M20
C1866	Quantitative ultrastructural analysis of the synapses established by identified thalamocortical axons in specific cortical layers	dataset	Francisco CLASCÁ	Release: Synaptic data on 2 type of thalamic nucleus axons Effective date: M18
C1867	Single-cell 3D reconstruction and measurement of thalamocortical ventral lateral, ventral anterior and parafascicular nuclei	dataset	Francisco CLASCÁ	Release: Single-cell 3D reconstruction and measurement Effective date: M12/M18/M24
C1795	*In vivo* electrophysiological recordings of single hippocampal neurons during activation of subcortical inputs	dataset	Szabolcs KÁLI	Release: *In vivo* recordings of hippocampal neurons in the presence of manipulations Effective date: M24
C1796	Morphological and electrophysiological characterization of hippocampal interneurons	dataset	Szabolcs KÁLI	Release: Database of morphological and electrophysiological data from hippocampal interneurons Effective date: M24
C1768	Characterisation of the physiological effects of cholinergic input in the mouse hippocampal slice preparation	dataset	Szabolcs KÁLI	Release: *In vitro* recordings of hippocampal neurons and synapses in the presence of manipulations Effective date: M24
C1738	Synaptic coverage and polyneuronal innervations of dendrites of inhibitory neuronal sub-types in mice and human neocortex	dataset	Zoltan KISVARDAY	Release: The 3D reconstruction of one dendrite of cell3 (Calbindin-D28K positive). 5 calretinin immunopositive GAGAergic Ins were identified, and designate one dendrite. The collecting of serial ultrathin sections (60 µm vibratome section → ~1200 sections at 50 nm thickness) was completed. Effective date: M12/M20

Table 5: Overview of releases and major updates related to Key Result KR1.3

ID	Component Name	Type	Contact	Info on releases and major updates
C1745	Whole-brain maps of different neuronal types	dataset	Francesco PAVONE	Release: First maps of different neuronal types across the entire mouse brain Effective date: M14 (data acquisition) / M20 (data available in the KG)
C1742	Whole-brain images of different molecular/transgenic markers	dataset	Francesco PAVONE	Release: First images of entire mouse brain labelled with selected markers Effective date: M10
C1765	Cellular resolution calcium activity maps over wide regions of the cortex C1.3.4.1	dataset	Anna L. ALLEGRA	Release: Cellular resolution calcium activity maps (Collab - https://collab.humanbrainproject.eu/#/collab/299/nav/2265) Effective date: M21
C1732	Brain Vascular Network Reconstruction	dataset	Efremov VELIZAR	3D reconstructions of the brain vascular system: a full-brain mice dataset Effective date: M12

Table 6: Overview of releases and major updates related to Key Result KR1.4

ID	Component Name	Type	Contact	Info on releases and major updates
C1746	Quantitative ultrastructural data of the mouse hippocampus	Quantitative ultrastructural data of the mouse hippocampus	Ángel MERCHÁN	Release: Volume fraction of mitochondria in mouse CA1 Effective date: M12
C1749	Quantitative ultrastructural data of the mouse neocortex	dataset	Javier DEFELIPE	Release: Volume fraction of mitochondria in mouse neocortex Effective date: M12
C1831	Dataset on cross-modal and brain state-dependent modulation of the activity and interaction between neuronal subtypes in S1HL	dataset	Umberto OLCESE	Release: Role of cross-modal interaction on S1HL activity Effective date: M12
C1869	Interactive tools for the analysis of anatomical and functional data	Software	Luis PASTOR	Release: First toolset prototype Effective date: M12 Links: http://gmrv.es/gmrvvis/pyramidalexplorer/ https://collab.humanbrainproject.eu/#/collab/31936/nav/222572 https://collab.humanbrainproject.eu/#/collab/31935/nav/222567

C1870	Integrated environment for acquisition and early analysis of anatomical and functional data	Software	Luis PASTOR	Release: Integrated environment first prototype Effective date: M14
C1612	KappaNEURON	Software	David STERRATT	Release: KappaNEURON accessible from Jupyter notebook in Collab Effective date: M3/M12 Link: https://github.com/davidcsterratt/KappaNEURON
C1611	Integrated Mouse and human synaptic proteome dataset with complete literature/public database coverage	dataset	David STERRATT	Release: First update of proteomic dataset; DMP release: ~5600 proteins > 95% complete Effective date: M10/M12
C1776	Detailed parameter collection for model and disease relevant molecular interactions	Dataset	David STERRATT	Release: Schema for curating kinetic data DMP release: In response to feedback from previous release, fields finalised, and parameters from 10 more reactions curated Effective date: M12
C1777	Catalogue of 250 molecular rule-based model Components	model	David STERRATT	DMP release: [Rules including] 100 out of 250 proteins Effective date: M12

Table 7: Overview of releases and major updates related to Key Result KR1.5

ID	Component Name	Type	Contact	Info on releases and major updates
C1747	Quantitative data of the neuropil at the ultrastructural level in human brain	dataset	Lidia ALONSO-NANCLARES	Release: Preliminary synaptic data Effective date: M12
C1740	Comparison of morphological parameters of pyramidal neurons between rodents and humans	dataset	Ruth BENAVIDES-PICCIONE	Release: Branching dendritic structure of human and mouse pyramidal cells (50 out of 100 cells traced) Effective date: M12
C1741	3D reconstructions human neocortical pyramidal cells	dataset	Ruth BENAVIDES-PICCIONE	Release: Dendritic arbor 3D reconstructions of human pyramidal cells (70 out of 150 3D reconstructions of cells) Effective date: M12
C1729	Comparative physiology of mouse and human neocortical excitatory synapses	dataset	Huib MANSVELDER	Release: Functional properties of human excitatory synapses Effective date: M24
C1731	Comparative physiology of neuromodulation of	dataset	Huib MANSVELDER	Release: Physiological data on neuromodulatory effects of acetylcholine and

	neocortical circuits in mouse and human brain			metabotropic glutamate receptor activation Effective date: M24
C1802	Principal cells morphology comparative models	model	Concha BIELZA	Release: Final release of the model Effective date: M16
C1803	Principal cells electro-physiology comparative models	Model	Concha BIELZA	Release: Final release of the model Effective date: M16
C1804	Principal cells joint electro-physiology and morphology comparative models	model	Concha BIELZA	Release: Final release of the model Effective date: M18

Annex B: Summary of the main actions undertaken for Year 2

The actions undertaken by SP1 in line with the reviewers' recommendations are outlined below.

SP1: Within one month, elaborate a plan to address the following requirements for the last part of SGA2:

1. The few datasets that are available so far are not well structured, nor stringent – this needs solving for all SP1 SGA2 datasets in the context of the second year. They also need to be computer-readable, to avoid that their broader utility in the HBP is severely limited. Data structure and data delivery to the HBP/Knowledge Graph needs to be significantly improved too.

Since SP1 is oriented toward 4 well identified microcircuits - CEREBRAL CORTEX, CEREBELLUM, HIPPOCAMPUS, BASAL GANGLIA - these will be considered separately to generate 4 data sets and then aggregated into a WHOLE.BRAIN dataset. An additional dataset will be identified for human neuron recordings.

All the datasets are accessible in the links displayed in this report. In addition, this information is also added to the Collab: https://collab.humanbrainproject.eu/#/collab/1260/nav/328274

Currently, all the SP1 teams are working in close collaboration with the Curation team to start the curation process once the datasets are completed. In addition, some SP1 datasets are already linked to the KG. These data sets are as follows:

- Cerebellum:
  - https://doi.org/10.25493%2F6R48-E3V
  - https://doi.org/10.25493%2FMDAR-XEB
  - https://doi.org/10.25493%2F4AF6-WSD
  - https://doi.org/10.25493%2FF2VK-MB4
  - https://doi.org/10.25493%2FG07Q-K87
  - https://doi.org/10.25493%2FMVHQ-4YA
  - https://doi.org/10.25493%2FJQH3-0A4
- Datasets at Whole Brain level:
  - 10.25493/68S1-9R1
  - 10.25493/77F8-7B4
  - 10.25493/Z9J0-ZZQ

2. More generally, SP1 partners need to ensure the legacy of the data from the mouse brain SPs, so that it is properly accessible for the remaining duration of the HBP. Given the planned shift in focus from mouse to exclusively human brain research in SGA3, it will be important to ensure that the datasets, novel tools and methods used to conduct comparative research should be available to all potential users into the future of the HBP. Plans for the curation of the data sets (structures, morphology, physiology, and function) should be firmly established in the next phase of SGA2 and into SGA3.

We agree. From the beginning of the SGA2, SP1 has been working in close collaboration with the Curation Team. We are further collaborating with SP5 to establish an effective curation plan to ensure the legacy of the data. In particular, we have met several times with the KG team after receiving the review report to ensure that the planning to register the data into the KG is being followed. Also, all the SP1 are informed about how they have to proceed to start the data curation process. Further details on the coordination process between SP1 and the KG team are displayed in this report (see Section 8).

3. It must be ensured that any Y1 delays to the start of the activities for any of the partners are offset by greater efforts in the second half of SGA2. In addition, the very significant deviations in the scRNA-seq data should be addressed, with efforts increased.

We agree that it would have been nice to already have these data, but we have been optimizing the protocol to generate the data in the first months of SGA2 and since a few weeks now have optimized and adopted the protocol in a fashion that we are now able to process the material we have collected over the last months. We are increasing efforts to provide these data. We have collected the non-human primate material over the last months and optimized the protocol for scRNA-seq so that in the next month we will generate the respective data and make them accessible' (received from the T1.5.7 Task Leader in charge of the scRNA-seq data).

4. For the future, it would be helpful if the information under "Objectives and Priorities for the Remainder of the Phase" would provide some more substantial and quantitative information. As it is now, the information is just that the aim is to complete the ongoing studies, which does not provide any information on the feasibility or efforts required to reach the goal.

We agree and this will be implemented in future reports.

5. Greater evidence of collaborative links between rodent and the human brain is required for the HBP to capitalise on models (multilevel, or at any level) of brain function. Notwithstanding the complexity of determining common structures, processes, and functions, to date efforts to combine these brain models have begun but these activities must continue to converge.

We agree as we recognize this is a fundamental point in the HBP strategy and SP1 plays an important role in the process. For the CEREBRAL CORTEX, recordings and models on human neurons have been pioneered by SP1 and are ongoing. For the other microcircuits (CEREBELLUM, HIPPOCAMPUS, BASAL GANGLIA), SP1 does not have any specific commitment for human data and model production in SGA2, while these actions are planned in WP1 during SGA3. It should however be noted that, for the CEREBELLUM, we have already started a careful comparison of the neuronal and microcircuit properties between mice and humans, that will likely lead to a review paper based on existing literature and possibly to a preliminary model to be presented by the end of Y2.

6. For the success of the HBP as a whole, SP1 should be proactive in the preparation of SGA3. One important undertaking being foreseen for SGA3 is to "progressively morphing mouse microcircuits onto humanised microcircuit models": this is unproven territory and the models and validation methodology need to be established. SP1 is in the best position to develop the models and validate the methodology in SGA2 Year 2.

We agree, even if, according to the SGA3 work plan, not all members of SP1 are expected to continue their activity in SGA3. Actually, the SP1 leadership has played a crucial role in shaping the SGA3 work plan by taking part to a different degree to the writing teams of the scientific work packages and tasks. A substantial part of the pipeline for human brain modeling in WP1 will be morphed from mice studies and will continue and extend data collection form human neurons successfully initiated by SP1 in SGA2. The whole-brain imaging techniques of SP1 will substantially contribute to WP2. The models of WP3 have, in many cases, their foundation into the data and the theoretical concepts derived from the SP1-SP6-CDP2 interaction that occurred in SGA1 and SGA2. And SP1 members are engineering, within SP1, the open call on Mouse Brain Modeling that will run in tandem with Human Brain Modeling. We have no doubt that the contribution of SP1 to SGA3 is not just proactive but substantial.

7. More generally, SP1 and CDP1 need to focus on what will be usable for the HBP infrastructure or part of the SGA3 so that efforts can be concentrated on the success of the HBP as a whole. Every aspect of the project needs to find an entry in approachable exemplars or proof-of-concept for preparing the success of the final phase of HBP.

In the remainder of the phase, SP1 is focused on the SP1 Data Strategy set up at the beginning of the SGA2 for the integration of the data in the HBP infrastructure to ensure that data are key to the success of the HBP. In addition, SP1 members foreseen in the SGA3 have been and being very proactive in the preparation of the SGA3 to contribute to the success of the final phase of HBP.

8. Mitigation for the departure of one of the key collaborations from SP6 and SP10 should be implemented to ensure that co-activities which are still relevant continue for the remaining duration of the SGA.

SP1/CDP1 have put mitigation measures in place to continue the relevant activities planned under the SP1-CDP1-SP6 and SP10 collaboration. Unfortunately, the team developing the scaffold model (Marc-Oliver GEWALTIG and his group at EPFL) left the HBP in April 2019 and the work of CDP1 on whole brain modelling has been redirected. Two different strategies are being applied to simulate functional behaviour of the brain in the virtual experiments: a functional model of the motor cortex will be developed by Egidio FALOTICO and his team in SP10, in order to drive the activities of the spinal cord model of the embodied mouse in the virtual environment. In parallel, the whole brain network model developed by Viktor JIRSA and his team in SP4 will be used for the same purpose. This second option will face the compatibility of mean-field (brain) and spiking neuron (spinal cord) models, a well-known challenge in HBP. Moreover, Egidio FALOTICO, the new implementation leader of CDP1, seamlessly took over the coordination role related to SP10 activities previously fulfilled by Marc-Oliver GEWALTIG.